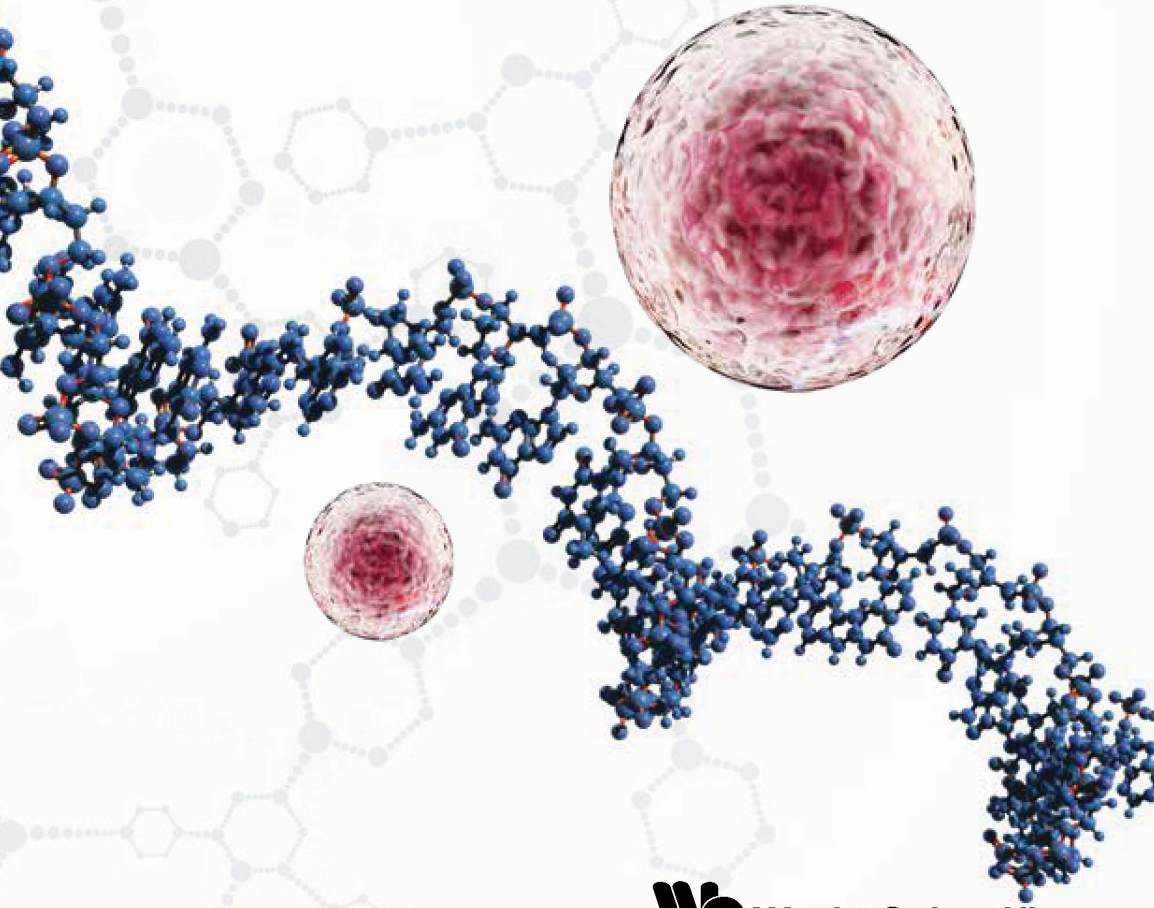
RNA-based Mechanisms in Cancer

Editors Gary Brewer | Gerald M Wilson



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Editors

Gary Brewer
Rutgers Robert Wood Johnson Medical School

Gerald M Wilson
University of Maryland School of Medicine

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About the Editors

Gary Brewer, Ph.D., received his B.S. in Physics from Auburn University after which he was a computer programmer at NASA's Marshall Space Flight Center in Huntsville, Alabama. A career epiphany led to him receiving his Ph.D. in Biochemistry from Vanderbilt University School of Medicine. As a postdoctoral fellow at the McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, he worked with Dr. Jeffrey Ross. There, he utilized a novel, cell-free mRNA decay system in quest of protein factors involved in the decay of human proto-oncogene *MYC* mRNA. Following postdoctoral training, Dr. Brewer moved to Wake Forest University School of Medicine in Winston-Salem, North Carolina as an Assistant Professor. He continued his research into *MYC* mRNA-binding proteins. Many hours spent in the cold room to fractionate cell extracts led to the purification and later, cloning of mRNA decay and translation regulatory factor, AUF1. This protein is a key regulator of many mRNAs encoding oncoproteins, cytokines, growth factors, and signaling proteins. Dr. Brewer later moved to the Rutgers Robert Wood Johnson Medical School where he is now Professor of Biochemistry and Molecular Biology. His laboratory continued the biochemical and biophysical characterization of AUF1-RNA interactions. Recently, his research has taken him into the world of host RNA-binding proteins' roles in translation of enterovirus and coronavirus RNA genomes. In response to the global COVID-19 pandemic, his research has pursued RNA-targeted antivirals for these viruses (with outstanding collaborators).

To this day, he remains a long-time RNA aficionado, gym rat, and avid runner.

Gerald Wilson, Ph.D., earned his B.Sc. and Ph.D. degrees in Biochemistry from Queen's University in Kingston, Ontario, Canada. It was during his Ph.D. studies that he caught the RNA bug, identifying novel mechanisms regulating the decay kinetics of human LDL receptor mRNA. To expand his training into the emerging field of *trans*-acting posttranscriptional regulatory factors, he joined Dr. Brewer's lab at the Wake Forest University School of Medicine in North Carolina, and later at Rutgers Robert Wood Johnson Medical School, as a postdoctoral fellow. Together, they developed quantitative fluorescence-based approaches to describe the energetics and structural consequences of protein recruitment to RNA substrates and mechanisms by which posttranslational protein modifications can regulate these processes. Dr. Wilson subsequently joined the University of Maryland School of Medicine where he now serves as Professor of Biochemistry and Molecular Biology. There, he has continued his studies on the biophysics of RNA-protein recognition and contributions of posttranscriptional gene regulation to human diseases including cancer. When not working in the lab or on his next grant application, Dr. Wilson is an accomplished guitarist, unapologetic oenophile, and budding woodworker.

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Chapter 1

Introduction to RNA and Cancer

Gary Brewer[1,*] and Gerald M. Wilson[2,**]

[1]*Department of Biochemistry and Molecular Biology, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA*
[2]*Department of Biochemistry and Molecular Biology and Marlene and Stewart Greenebaum NCI Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA*

RNAs play central roles in virtually all steps of gene expression. Messenger RNAs (mRNA), ribosomal RNAs (rRNAs), and transfer RNAs (tRNAs) are the intermediaries for the transfer of genetic information from DNA to proteins. Within this central dogma of molecular biology, there are numerous other RNAs essential for creating protein diversity and regulating gene expression. For example, small nuclear RNAs (snRNAs) organize the machinery for pre-mRNA splicing, which contributes to protein diversity, and small nucleolar RNAs (snoRNAs) participate in maturation of ribosomal RNAs. Other RNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) orchestrate regulatory mechanisms that control protein synthesis rates and mRNA abundance. Of course, these RNAs often act in concert with proteins, some of which bind

* Corresponding author's email: brewerga@rwjms.rutgers.edu
** Corresponding author's email: gwilson@som.umaryland.edu

to specific RNA sequences and/or structures to effect gene expression timing, kinetics, and dynamics. Moreover, RNAs are often subject to post-transcriptional nucleobase modifications, referred to as the epitranscriptome; these modifications alter RNA recognition by proteins and hence their regulation. As one might expect, numerous signal transduction pathways exert exquisite control over all these aforementioned processes to control the levels and timing of gene expression.

Mutations within coding regions of mRNAs encoding key proteins important for transcription, signal transduction, proliferation, differentiation, and apoptosis, to name a few, have long been known to serve as driver mutations of cancer pathogenesis [1]. However, over the years, we have come to appreciate that the 5′- and 3′-untranslated regions (UTRs) can likewise harbor driver mutations that affect the levels and timing of gene expression during cancer pathogenesis [2]. The importance of UTRs in general, and cancer in particular, was revealed by observations that they contain sequence and/or structural elements that regulate pre-mRNA processing, mRNA turnover rates, and translation initiation rates. Deregulation of these regulatory elements is a hallmark of numerous cancers. We thus devote this opus to molecular links between RNAs and cancer.

The chapters in the book focus on four areas: 1) RNA processing and cancer, 2) mRNA turnover and cancer, 3) translation and cancer, and 4) *trans*-acting and modified RNAs in cancer. The first two chapters examine the roles of aberrant RNA splicing and alternative 3′-RNA processing in cancer. Chapter 2 by Leclair *et al*. examines dysregulated alternative RNA splicing, which leads to pro-oncogenic RNA isoforms encoding proteins that impact cell proliferation, decrease apoptosis, enhance cell migration and metastatic potential, resist chemotherapy, or promote immune surveillance evasion. These changes often result from mutations in, or altered expression of, splicing factors, defined as RNA-binding proteins that act as upstream regulators of RNA splicing. These include numerous heterogenous nuclear ribonucleoprotein (hnRNP)-family proteins, SF3B1 (splicing factor 3b), which is the most frequently mutated spliceosomal component in cancer, and myriad SR (serine-arginine) protein family members. The authors also explore strategies designed to reprogram RNA splicing in tumor cells by targeting the splicing machinery, including RNA-based therapeutics. Of particular note are

approaches to target cancer-specific "neo-antigens". These proteins derive from mutations or alterations in splicing that lead to cryptic splice products unique to tumor cells. As such, these unique polypeptides can be used to generate a robust T_h-cell response that can, for example, selectively kill malignant B cell clones. Chapter 3 by Masamha examines the effects of disrupted alternative 3′-RNA processing in tumors. This process allows regulated formation of different 3′ ends for mRNAs in normal cells. While some alternative polyadenylation (APA) arises from alternative splicing of exons or intron inclusion, the most common form of APA results in 3′UTR length and content variation without alteration of the protein coding sequence. The functional consequences of APA observed in tumors is that key regulatory sequences and/or structural elements in the 3′UTR may be aberrantly gained or lost. For example, a tumor-specific, shortened 3′UTR may eliminate critical miRNA or regulatory protein binding sites that would normally limit the abundance or disrupt proper synthesis rates of the encoded protein product. mRNA structural alterations such as these can impact tumor formation, progression, invasion, and metastasis. Of course, identifying the protein drivers of APA in different tumor cells will permit identification of potential therapeutic targets. Perhaps not surprisingly, these driver proteins include the cleavage factors and cleavage and polyadenylation specificity factors (CPSFs) that are the principal effectors of 3′-RNA processing.

The next three chapters comprise the second section, which examines mRNA turnover and cancer. The levels of mRNAs, and often the abundance of their encoded proteins, are a consequence of mRNA synthesis/processing rates and mRNA turnover (degradation) rates. Cells degrade mRNAs at rates specific to each mRNA, specified by *cis*-acting sequence and/or structural elements in the mRNA. These elements are most often located in the 3′UTR but are also found in the 5′UTR and coding region. Their interactions with RNA-binding proteins and/or non-coding RNAs dictate their decay rates, most often by initially removing the 3′-poly(A) tail of the mRNA (deadenylation) followed by 5′-to-3′ and/or 3′-to-5′ degradation of the mRNA body. Perhaps the best characterized mRNA turnover sequences are AU-rich elements (AREs). There are a number of ARE-binding proteins (AUBPs) that promote mRNA degradation or stability via their interactions with the deadenylation and mRNA decapping

machineries. As many ARE-mRNAs encode transcription factors, oncoproteins, signaling proteins, growth factors, cytokines, and apoptotic factors, it is not surprising that their dysregulation often plays significant roles in cancer. Chapter 4 by Stemberger *et al*. describes the roles of CCCH zinc finger RNA-binding proteins in tumor progression. These proteins contain an internal domain of two tandemly arrayed CCCH-class zinc fingers important for ARE binding. One of the earliest identified members of this protein class also contains three stretches of four contiguous prolines (tetra-proline), hence the protein name Tristetraprolin (TTP) [3]. Other groups identified TTP-related proteins. As noted above, these proteins act by recruiting the deadenylation machinery, particularly the CNOT1 (CCR4-NOT transcription complex subunit 1) scaffolding subunit of the CCR4-NOT (carbon catabolite repression-negative on TATA-less) deadenylase complex, to select ARE-containing mRNA substrates [4]. As with many AUBPs, each CCCH zinc finger protein has its preferred mRNA targets, though many AUBPs have overlapping mRNA targets as well. Nonetheless, many of their mRNA targets encode pro-tumorigenic factors, including MYC (human myelocytomatosis transcription factor), Cyclin D1, E2F1 (E2F transcription factor 1), and PIM1 (proviral integration site for Molony murine leukemia virus 1). Moreover, suppression of TTP and other CCCH zinc finger proteins occurs frequently in cancer and indicates poor patient outcomes. The authors also note several therapeutic opportunities to restore CCCH zinc finger protein expression or function. These include histone deacetylase inhibitors to increase their transcription, inhibiting specific kinases or other signaling pathways, or by direct protein delivery. Chapter 5 by Sobolewski *et al*. examines proteins that present the flip side of mRNA turnover — mRNA stabilization. A paradigm of this subset of AUBPs is the Hu/ELAV protein family (Human antigen/embryonic lethal abnormal vision) [5–7]. Unlike the CCCH zinc finger family of AUBPs, the Hu/ELAV proteins bind AREs via three non-identical RNA recognition motifs (RRMs). While these proteins promote mRNA stabilization, they can affect translation as well. HuB, HuC, and HuD are largely neural specific, while HuR is expressed ubiquitously. As such, HuR presents elevated expression across a much broader range of tumor types, indicating its putative role as a tumor promotor via ARE-mRNA stabilization. Additionally, tumor aggressiveness and poor

outcomes are associated with elevated HuR expression in numerous tumor types. Interestingly, elevated expression of the neuronal HuB, HuC, and HuD member proteins can exert a protective effect and associate with lower risk, for example, in low grade glioma. To close out the mRNA turnover and cancer section, Lee and Wilson devote Chapter 6 to the pro- and anti-apoptotic activities of the AUBP AUF1 (ARE/poly(U)-binding/degradation factor) [8]. This protein, which has four isoforms produced by alternative pre-mRNA splicing, can promote mRNA turnover, mRNA stabilization, or modulate translation efficiency, depending on the mRNA target and cellular context. It also binds select miRNAs and long noncoding RNAs to effect control over a broad range of gene expression networks associated with inflammation and cancer, among others. AUF1 binds cellular RNA targets (as well as some RNA virus structures) via two non-identical RRMs common to all four protein isoforms. AUF1 expression is often dysregulated in cancer, which in turn alters expression of a plethora of proteins to allow both pro- and anti-tumorigenic control of gene expression. The authors detail examples of each including its pro-tumorigenic increase in translation of *MYC* mRNA, stabilization of *TWIST1* (Twist family BHLH transcription factor 1) and *IL6* (Interleukin 6) mRNAs, and degradation of *Dicer* mRNA, and its anti-tumorigenic degradation of *MYC* and *BCL2* (B-cell lymhoma-2) mRNAs, as well as *ANRIL* (antisense noncoding RNA in the *INK4* locus) and other oncogenic lncRNAs. Interestingly, the authors posit that the AUF1 isoform-specific diversity of context-dependent regulatory functions may be explained by its RNA chaperone activity — remodeling local RNA conformational landscapes to enhance or mask *trans*-acting factor binding to overlapping or proximal sites on its target RNAs.

The next three chapters comprise the third section examining translation and cancer. While gene expression is manifest as transcription, pre-mRNA processing in all its forms, nucleocytoplasmic transport and localization of the mature mRNA, nucleotide modifications, and its turnover or stabilization, the “end game” of gene expression is translation of the mRNA to produce the encoded protein product. Additionally, analyses reveal that mRNA levels can only explain approximately 40% of the variability in cellular protein levels, it is thus translation rates that play a large part in determining protein abundance [9]. As every story has a

beginning, middle, and end, the translation process has its three phases of initiation, elongation, and termination. Not surprisingly, the bulk of translational control occurs at the initiation phase, and as such, many oncogenic signals rewire translational output by affecting this phase of translation. Chapter 7 by Kayastha *et al*. examines the oncogenic consequences of dysregulated translation initiation. The authors underscore the importance of translation initiation as it provides for rapid, reversible, and spatial control of gene expression. Moreover, it allows tumor cells to respond rapidly to local signaling and chemotactic stimuli in their microenvironments. As the authors note, while tumor cells typically present higher rates of overall protein synthesis, the abundance of subsets of oncogenic proteins appear to be especially controlled by translational regulation of their mRNAs. These mRNAs encode the usual suspect proteins affecting signaling, proliferation, angiogenesis, invasion and metastasis, and chemoresistance, for example. Many oncogenic signals converge at the initiation phase of translation, particularly at the eIF4F complex of proteins, including eIF4G, eIF4E, and eIF4A, all critical for initiation. The authors review these protein factors as well as some of their supporting cast of characters. Specifically, they describe the roles of eIF4F in chemoresistance and its potential for therapeutic targeting by small molecules. However, the authors note the importance of studying the gamut of translation initiation factors, including the eIF3 complex and eIF1, -2, -5, and -6, all of which are perturbed in cancer. Finally, they note the importance of integrating translation factors and signaling cascades, particularly the PI3K-mTOR (phosphoinositide 3-kinase–mammalian target of rapamycin) pathway, which regulates translation rates of the cancer cell. This takes us directly to Chapter 8 by Patel and Jacinto, who review the extensive literature on the role of mTOR complexes for coordinating metabolism and translation. mTOR has an interesting history. A team of scientists visited Easter Island (also known as Rapa Nui) to collect soil samples that led to isolation of an antifungal macrolide from *Streptomyces hygroscopicus* later named rapamycin. Rapamycin also has powerful immunosuppressive and anti-proliferative properties. This led to identification of the yeast TOR1 and TOR2 proteins, which in turn led to discovery of mTOR in mammals. mTOR is an atypical serine/threonine kinase in the PI3K-related kinase family of lipid kinases. It forms the catalytic

subunit of two biochemically and functionally distinct multiprotein complexes named TORC1 and TORC2 (mTOR complex-1, -2). The mTOR signaling pathway is a major regulator of cellular metabolism and growth and is often dysregulated in diabetes and in many cancers. Among its functions is controlling translation rates in a number of ways. For example, mTORC1 regulates translation via its targets S6K1 (p70 ribosomal S6 kinase) and 4E-BP1 (eIF4E-binding protein 1). S6K1 phosphorylates ribosomal protein S6 and 4E-BP1 binds the eIF4E cap-binding protein to block translation initiation. Phosphorylation of 4E-BP1 by mTOR inhibits its ability to bind eIF4E, thereby promoting initiation. It thus acts as a rheostat to tune translation rates. mTORC1 and mTORC2 may both be involved in controlling protein synthesis by regulating other translation regulators, such as RNA-binding proteins and RSK (p90 ribosomal S6 kinase). mTOR also plays a role in ribosome biogenesis and it controls the processes that provide the raw materials for protein synthesis — amino acids, ribose, and nucleotides. Given its preponderance in signaling and growth in normal and tumor cells, it is a major focus of efforts to identify therapeutics. Chapter 9 by Jovanovic and Topisirovic details the therapeutic potential of inhibiting mTOR signaling in cancer. The first generation of mTOR inhibitors was rapamycin and its derivatives called rapalogs. Rapamycin binds its intracellular receptor protein, immunophilin FKBP12 (FK 506-binding protein of 12 kDa), to create a complex that targets the FRB (FKBP12-rapamycin binding) domain of mTOR. This blocks the interaction of rapamycin with the mTORC1-specific subunit Raptor (regulatory-associated protein of mTOR). Thus, acute treatment with rapamycin does not block mTORC2 activity. Additionally, rapamycin does not block all mTORC1 outputs; for example, the small 4E-BPs preferentially escape inhibition. Nonetheless, its effectiveness as an anticancer drug requires greater water solubility. Hence the development of rapalogs. While showing modest promise, they led to unwanted resistance, thus requiring a second generation of mTOR inhibitors. Second generation inhibitors were designed to directly target the kinase active site and thus inhibit both mTORC1 and TORC2. Unfortunately, like rapalogs, TOR kinase inhibitors (TORKi) have also led to unwanted drug resistance. Beginning in 2016, the latest generation of mTOR inhibitors, represented by Rapalink-1, were designed to overcome the resistance mechanisms of

the prior generation drugs. Rapalink-1 has shown promise to significantly delay growth of patient-derived xenografts in mouse models of bone-metastatic prostate cancer and to alter tumor heterogeneity. However, there is clearly much work still to be done.

The final four chapters comprise the last section, and examine *trans*-acting and modified RNAs in cancer. The human genome is replete with information, much of which, it turns out, is not junk DNA, but rather, is actively transcribed. A significant percentage of transcription produces nonprotein-coding RNAs, one class of which is 17-24 nt and called microRNAs (miRNAs) due to their diminutive lengths following processing of their precursor transcripts. These single-stranded RNAs act via their (usually) partial annealing to a 3′UTR to repress translation and/or promote degradation of its mRNA target. Another class of non-coding RNA is >200 nt called long noncoding RNAs (lncRNAs). These RNAs interact with other RNAs, DNA, lipids, and proteins in the nucleus and cytoplasm. Like miRNAs, subsets of lncRNAs can act as proto-oncogenes and others as tumor suppressors. Chapter 10 by Kim *et al*. reviews the roles of miRNAs in hematologic malignancies. Hematopoiesis generates all of the mature functional cells in blood and the immune system throughout life. Hematopoietic cells are generally short-lived and thus require constant replenishment from hematopoietic stem-progenitor cells (HSPCs). Regulation of their quiescence, self-renewal, proliferation, and differentiation is essential to maintain homeostasis of blood and to respond to acute and chronic challenges, such as bleeding and infection. Given the plethora of cell types in blood, it is not surprising that there are many types of hematopoietic cancers. Nonetheless, these are classified into the three main categories of leukemias, lymphomas, and myelomas. Each of these contains additional subclasses depending on the cell types involved. miRNAs regulate virtually all phases of hematopoiesis. As such, hematologic cancers are associated with altered miRNA expression patterns. The authors describe the numerous mechanisms by which their expression goes awry and the consequences for altered gene expression networks. The authors also detail a fascinating mechanism by which miRNAs transfer to different cell types in hematopoietic cancers. This mechanism involves intercellular exosomal transfer of miRNAs. Exosomes are membrane-bound vesicles that most cells release as a means of

extracellular communication. They can contain proteins, lipids, mRNAs, tRNAs, and miRNAs, the last being referred to as exomiRs. Two sources important for cancer are cancer-derived exosomes and stromal cell-derived exosomes. Recipient cells of these exosomes include both normal and cancer cells, normal HSPCs, and bone marrow stromal cells (BMSCs). The effects of exomiRs are to regulate cancer development, progression, and response to therapy. The authors conclude with a brief discussion of miRNA-based therapeutic drugs. As of early 2022, there were 1,226 funded miRNA-related clinical studies including active and already completed/terminated studies. The drugs include miRNA mimics and miRNA inhibitors. However, problems with this approach include their target specificity and delivery issues. Chapter 13 in this book will further address miRNA-based drug strategies and their delivery issues.

As noted above, miRNA is not the only class of non-coding RNA involved in cancer. LncRNAs also play important roles, as addressed in Chapter 11 by Price *et al*. In addition to epigenetically modulating protein-coding genes, lncRNAs can also modulate miRNA functions. For example, over half of 140 lncRNAs analyzed in one study affected their targets in a miRNA-dependent manner [10]. LncRNAs often function as competitive endogenous RNA (ceRNA) causing derepression of miRNA targets, acting as sponges to functionally dilute a miRNA's concentration. Dysregulated lncRNAs can thus affect gene expression programs involved in proliferation, cell motility, and viability. As such, there are thousands of lncRNAs aberrantly expressed in many tumor types often in a tumor-specific fashion. They are also relatively stable and readily detected in body fluids of cancer patients. LncRNAs thus hold promise as diagnostic/prognostic markers and therapeutic targets. Additionally, lncRNA expression profiles of cancer patients can help predict whether the patient will respond favorably to a specific treatment. For example, a study indicated that the lncRNA HOTAIR (Hox transcript antisense intergenic RNA) may identify patients who will benefit from combination fluorouracil and platinum therapy. However, as the authors note, the structures and functions of the ~60,000 known lncRNAs have yet to be characterized and they are poorly conserved. This complicates development of novel *in vitro* and animal models to understand the effects of their elevated or reduced expression levels. Nonetheless, the authors posit that a crucial step is to

continue the quest to develop databases of functional roles for lncRNAs in cancer and other diseases.

The next chapter by Rawn and Wilson examines the epitranscriptome and cancer. Primary transcripts are initially limited to the four canonical nucleobases adenine, cytosine, guanine, and uracil. However, as we have learned over the past 60 or so years, there are over 170 distinct modified nucleobase forms that decorate all major types of RNA, collectively known as the "epitranscriptome". For example, in mRNA, posttranscriptional nucleobase modifications regulate pre-mRNA processing, nucleocytoplasmic transport, mRNA turnover, and translation efficiency. Nucleobase modifications of rRNAs and tRNAs also impact translation efficiency. Nucleobase modifications of lncRNAs and miRNAs modulate their synthesis and gene regulatory functions. As one would expect, the epitranscriptome is heterogenous and dynamic, thus providing modulation of RNA functions. As such, there are three classes of interacting proteins, separately responsible for producing, removing, and mediating their regulatory functions, referred to as writers, erasers, and readers, respectively. Given the importance of RNA modifications in gene expression, it is no wonder that disruption of these three regulatory processes impacts the development and progression of cancers. Thus, modified RNAs may serve as cancer biomarkers and RNA-modifying enzymes as targets for anticancer therapeutics. The authors nicely survey the major nucleobase modifications, e.g., N^6-methyladenosine (m^6A) and N^1-methyladenosine (m^1A), in different RNA classes together with their respective reader, writer, and eraser enzymes. The authors conclude with a survey of the roles of RNA modifications in cancer. Interestingly, while writer, eraser, and reader proteins are not considered to be oncogenic drivers, many are functionally linked to tumor survival, proliferation, growth, and differentiation. One particularly interesting example demonstrating how RNA modifications (or the lack thereof) can alter expression of multiple genes is via the effects of m^6A on miRNAs in cancer. In hepatocellular carcinoma (HCC), abundance of the m^6A writer METTL14 (methyltransferase-like 14) is downregulated. As a result, one target of METTL14, pri-miR-126, presents reduced m^6A modifications, which reduces its processing to mature miR-126. This miRNA is a tumor suppressor that inhibits synthesis of several proteins, enhancing cell proliferation,

stemness, and angiogenesis. As such, the decrease in functional miR-126 resulting from m^6A diminution in HCC leads to enhanced production of several pro-tumorigenic factors, which in turn facilitate aggressive oncogenic phenotypes.

The final chapter in the section on *trans*-acting RNA and cancer, by Biswas and Menon, reviews miRNA-based therapeutic strategies. Treatment of cancer patients with a drug against selective targets often leads to drug resistance. Thus, there is a growing demand for new strategies and molecules to replace traditional ones. Given the ability of miRNAs to regulate multiple mRNA targets and cellular processes simultaneously, they present immense potential as next generation therapeutics. The authors thus describe in detail four approaches for the design of miRNA-based molecules: 1) RNAi inhibition strategies, 2) mimetic agents, 3) miRNA inhibitors, and 4) small molecule inhibitors. Of course, the efficacy of miRNA requires that the molecule be delivered to its target calls. Thus, the delivery vehicle must protect locked nucleic acid (LNA) anti-miRNAs, antagomirs, miRNA masks and sponges, or small molecule inhibitors from degradation while in circulating fluids, must bring the molecule to its desired location, must support cellular uptake of the molecule, should elicit a minimal immunologic response, and be easily degraded or excreted without any toxicity. The authors then discuss the pros and cons of current miRNA delivery strategies, including viral and nonviral delivery systems. The authors close with a list of 15 trials as a sampling of those currently in various stages of development (from https://clinicaltrials.gov) as a sign of encouragement that miRNA-based therapeutics likely have a prolonged future in the decades ahead.

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References

1. Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A, Colaprico A, Wendl MC, Kim J, Reardon B, Ng PK, Jeong KJ, Cao S, Wang Z, Gao J, Gao Q, Wang F, Liu EM, Mularoni L, Rubio-Perez C, Nagarajan N, Cortés-Ciriano I, Zhou DC, Liang WW, Hess JM, Yellapantula VD, Tamborero D, Gonzalez-Perez A, Suphavilai C, Ko JY, Khurana E, Park PJ, Van Allen EM, Liang H, MC3 Working Group, Cancer Genome Atlas Research Network, Lawrence MS, Godzik A, Lopez-Bigas N, Stuart J, Wheeler D, Getz G, Chen K, Lazar AJ, Mills GB, Karchin R and Ding L. (2018) Comprehensive characterization of cancer driver genes and mutations. *Cell*, **173**, 371–385.
2. Schuster SJ and Hsieh AC. (2019) The untranslated regions of mRNA in cancer. *Trends in Cancer*, **5**, 245–262.
3. Lai WS, Stumpo DJ and Blackshear PJ. (1990) Rapid insulin-stimulated accumulation of an mRNA encoding a proline-rich protein. *Journal of Biological Chemistry*, **265**, 16556–16563.
4. Sandler H, Kreth J, Timmers HT and Stoecklin G. (2011) Not1 mediates recruitment of the deadenylase Caf1 to mRNAs targeted for degradation by tristetraprolin. *Nucleic Acids Research*, **39**, 4373–4386.
5. Szabo A, Dalmau J, Manley G, Rosenfeld M, Wong E, Henson J, Posner JB and Furneaux HM. (1991) HuD, a paraneoplastic encephalomyelitis antigen, contains RNA-binding domains and is homologous to Elav and Sex-lethal. *Cell*, **67**, 325–333.
6. Levine TD, Gao F, King PH, Andrews LG and Keene JD. (1993) Hel-N1: An autoimmune RNA-binding protein with specificity for 3′ uridylate-rich untranslated regions of growth factor mRNAs. *Molecular and Cellular Biology*, **13**, 3494–3504.
7. Ma WJ, Cheng S, Campbell C, Wright A and Furneaux H. (1996) Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein. *Journal of Biological Chemistry*, **271**, 8144–8151.
8. Zucconi BE and Wilson GM. (2011) Modulation of neoplastic gene regulatory pathways by the RNA-binding factor AUF1. *Frontiers in Bioscience*, **16**, 2307–2325.
9. Schwanhausser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W and Selbach M. (2011) Global quantification of mammalian gene expression control. *Nature*, **473**, 337–342.
10. Tan JY, Sirey T, Honti F, Graham B, Piovesan A, Merkenschlager M, Webber C, Ponting CP and Marques AC. (2015) Extensive microRNA-mediated crosstalk between lncRNAs and mRNAs in mouse embryonic stem cells. *Genome Research*, **25**, 655–666.

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Chapter 2

Aberrant RNA Splicing and Therapeutic Reprogramming in Cancer

Nathan K. Leclair[1,2,*], Brittany L. Angarola[1,*], and Olga Anczukow[1,2,3,4,#]

[1]*The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA*
[2]*Graduate Program in Genetics and Development, UConn Health, Farmington, CT, USA*
[3]*Department of Genetics and Genome Sciences, UConn Health, Farmington, CT, USA*
[4]*Institute for Systems Genomics, UConn Health, Farmington, CT, USA*

Abstract

Dysregulation of alternative RNA splicing is a hallmark of cancer that leads to the expression of pro-oncogenic RNA isoforms which can impact cell proliferation, decrease apoptosis, enhance migration and metastatic potential, resist chemotherapy, or allow evasion of immune surveillance. Cancer-associated aberrant RNA splicing is driven by alterations in splicing factors, a class of RNA-binding proteins that act as upstream regulators of RNA splicing. Molecular inhibitors of oncogenic splicing factors or core spliceosome components have been

* equal contributions
# Corresponding author's email: olga.anczukow@jax.org

developed as anti-cancer therapeutics. In recent years, a better understanding of individual spliced isoforms that play critical roles in cancer cell transformation have led to the development of novel approaches to target these individual events. Here, we discuss splicing changes in cancer cell transcriptomes and review the current and emerging approaches at targeting alternative splicing as a cancer therapeutic.

1. Introduction — splicing machinery and mechanism

RNA splicing is a post-transcriptional mechanism that mediates the removal of introns from an immature pre-mRNA transcript and the ligation of exons to form a mature mRNA molecule. RNA splicing is a highly regulated process performed by the spliceosome — a very large complex consisting of both RNA and protein components, along with additional regulatory proteins, termed splicing factors (SFs), that fine-tune its activity. The spliceosome recognizes core regulatory sequences in the pre-mRNA including the 5′ and 3′ splice sites that mark intron-exon boundaries, the branch point site, and the polypyrimidine tract [1] (Fig. 1). Two spliceosomal complexes carry out RNA splicing reactions — the U2-dependent (major spliceosome) and the U12-dependent (minor spliceosome). They differ mainly in a subset of RNA components used during their respective splicing reactions and in the splice site sequences they recognize [2]. The major U2-dependent spliceosome, which has a preference for GT-AG splice sites and is responsible for the removal of ~99% of introns, contains over 300 components, including small nuclear RNA (snRNA) molecules that interact with Sm core proteins and additional proteins to form small nuclear ribonucleoprotein (snRNP) particles that undergo dynamic remodeling during the splicing reaction [3–5]. The Sm proteins are less than 20kDa in size and contain a conserved amino acid motif, the Sm motif. They associate with each other to form a ring-shaped complex that binds to U1, U2, U4, and U5 snRNAs. The minor U12-type spliceosome, which has a preference for AT-AC splice sites but also recognizes GT-AG sites, is involved in the removal of less than 1% of introns and regulates a distinct set of splicing events and utilizes different spliceosomal snRNA and protein components, including ZRSR2 [2, 6]. The

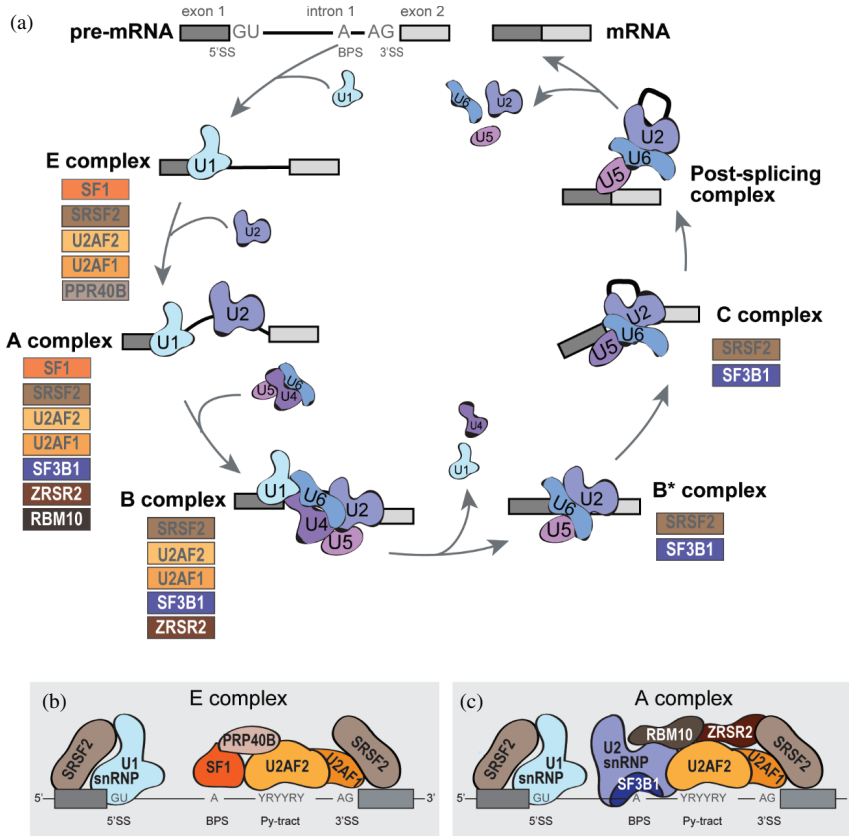

Figure 1. The dynamic splicing cycle of the major U2-dependent spliceosome.

(a) Stepwise assembly of spliceosomal complexes on a pre-mRNA molecule and catalysis of the splicing reaction to generate mature spliced mRNA. See text for details. (b, c) Spliceosomal factors that exhibit alterations in human tumors are listed next to each complex (colored boxes) and are shown in more details for complexes E and A. Abbreviations: 5′/3′ASS: 5′/3′ splice site; BPS: branch point site; Py-tract: polypyrimidine tract; snRNP: small nuclear ribonucleoprotein.

U2-specific snRNPs are U1, U2, U4, and U6, while the U12-dependent snRNPs are U11, U12, U4atac, and U6atac. U5 snRNP is shared between the two spliceosomal complexes [2].

Briefly, during the first step of the splicing reaction, the ATP-independent binding of U1 snRNP to the 5′ splice site initiates the assembly of the "Early" or E complex. SF1 and U2AF2 bind to the branch point

site and polypyrimidine tract (Py-tract), respectively. Splicing factor PRPF40B binds to SF1 and U2AF2/F1 and bridges U1 at the 5′ splice site and SF1 at the branch site [7] (Fig. 1a, b). Another key splicing factor, SRSF2, assembles during E complex formation and will later mediate specific interactions between U1 and U2 snRNPs at the 5′ and 3′ splice sites [8]. In the second step, the ATP-dependent interaction of U2 snRNP with the branch point site, stabilized by the U2AF2/U2AF1 complex and the spliceosome factor 3a and 3b (SF3a/b) complex, leads to A complex formation, followed by SF1 displacement from the branch point site (Fig. 1a, c). SF3B1 is the largest subunit of the SF3b complex and is a core component of both U2 and U12 spliceosomes. Splicing factor RBM10 is found in the A complex and interacts with both SF1 and U2AF2/F1m as well as U2 snRNA [9]. The formation of the catalytically inactive B complex is marked by the recruitment of the U4/U6/U5 tri-snRNP complex. Formation of the active B* complex formation occurs following major conformational changes, including release of U1 and U4 (Fig. 1a). The first catalytic step generates the C complex and results in lariat formation. Complex C performs the second catalytic step, which results in the joining of the two exons. The spliceosome then disassembles in an orderly manner, releasing the mRNA and the lariat bound by U2/U5/U6. The detailed the compositions and structures of each spliceosomal complexes have been reviewed extensively [1, 3, 10, 11]. Several spliceosomal components are found altered in human tumors (Fig. 1a), including recurrent hotspot mutations in components of complexes E and A (Fig. 1b, c), and will be further discussed below.

With the exception of dinucleotides found within the 5′ and 3′ splice sites, the core-regulatory sequences recognized by the spliceosome are rather degenerate in humans and allow for a huge diversity in their sequences [12–14]. This allows for an additional layer of regulation that utilizes both cis-acting regulatory sequences and trans-acting proteins that can strengthen or weaken the spliceosome's recognition of the splice sites [14, 15]. Together, this layer of regulation allows for the process of alternative RNA splicing (AS) — the differential inclusion of sequences from the pre-mRNA into the mature mRNA that allows for a single gene to encode multiple different and functionally distinct RNA isoforms with the ability to be translated into different protein isoforms (Fig. 2a). For example,

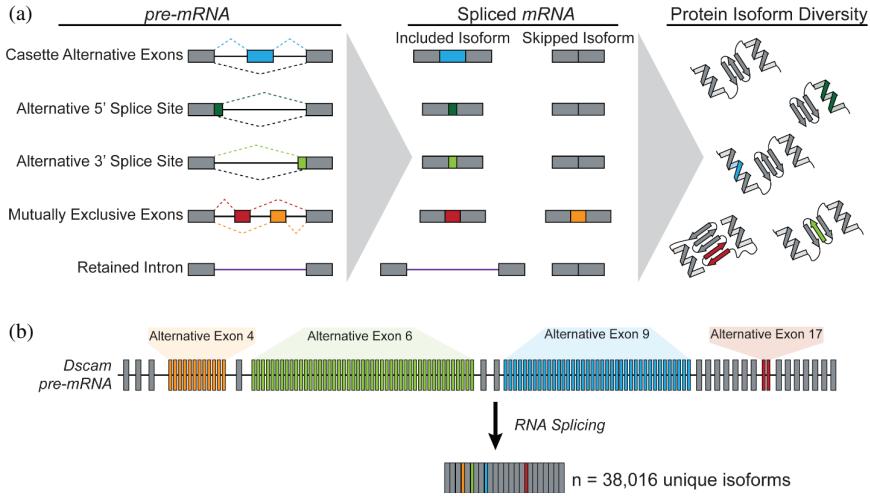

Figure 2. Alternative RNA splicing creates transcriptomic and proteomic diversity.

(a) Alternative splicing patterns are classified into cassette alternative exon splicing, alternative 5′ and 3′ splice site usage, mutually exclusive exons (either one or another is included), and intron retention. These splicing patterns lead to distinct spliced mRNA isoforms that can be translated into protein isoforms with distinct sequences and functions. (b) The *Drosophila melanogaster Dscam* gene is an extreme example of alternative RNA splicing, whereby a single gene can encode up to 38,016 unique isoforms via the combinatorial inclusion of exons from four regions of mutually exclusive exons (symbolized by distinct colors).

alternatively spliced RNA isoforms can differ in their coding potential, stability, localization, and translation efficiency [16]. It is currently estimated that each human protein coding gene encodes on average 7.43 RNA isoforms, however more extreme examples of alternative splicing have been described [17]. For example, the drosophila *Dscam* gene can encode 38,016 different spliced mRNAs through different combinations of alternatively spliced exons 4, 6, 9, and 17 [18] (Fig. 2b).

Regulatory trans-acting SFs that modulate alternative splicing are a class of RNA-binding proteins (RBPs) that recognize and bind cis-regulatory elements on the pre-mRNA, namely intronic or exonic splicing silencer or enhancer sequences, and correspondingly promote or repress AS of that exon (Fig. 3). The serine-arginine rich (SR) proteins and heterogenous nuclear ribonucleoproteins (hnRNP) are two SF families that are

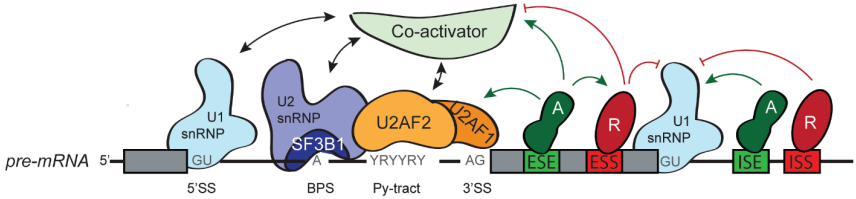

Figure 3. Cis-acting regulatory sequences and trans-acting regulatory splicing factors promote or inhibit spliceosome assembly during alternative RNA splicing.

Spliceosome assembly is regulated by cis-acting sequences encoded into the pre-mRNA that act as splicing enhancers or splicing silencers. These can be within exon sequences (Exonic splicing enhancer/silencer; ESE/ESS) or intron sequences (Intronic splicing enhancer/silencer; ISE/ISS). Enhancers are recognized and bound by splicing activator (A) proteins that recruit other co-activators and core subunits of the spliceosome, ultimately inducing exon inclusion. Splicing silencer sequences are recognized and bound by splicing repressor (R) proteins that inhibit spliceosome recruitment. Abbreviations: 5′/3′ASS: 5′/3′ splice site; BPS: branch point site; Py-tract: polypyrimidine tract; snRNP: small nuclear ribonucleoprotein.

well known to regulate AS by binding regulatory elements in the pre-mRNA and regulating AS in a concentration-dependent manner [19–21]. SR proteins contain an RNA-recognition motif (RRM) that binds RNA and a serine/arginine rich (RS) domain that mediates protein-protein and protein-RNA interactions. The hnRNPs comprise a large and structurally diverse family of RBPs, which contain one or multiple RRMs, along with a glycine-rich and/or a arginine/glycine-rich region, and/or one or multiple K Homology (KH)-domain [21]. hnRNPs play diverse roles in AS, mRNA transport, and translation, often functioning as antagonists to SR-protein-regulated AS events [21]. The distinct RNA binding motifs of SR proteins and hnRNPs suggest that these SFs can work antagonistically or cooperatively, and the intricate interplay of these regulatory SFs is only beginning to be unraveled. Recent findings demonstrate extensive cross-regulation within and across SFs families. For example, all 14 SR proteins modulate each other's levels via the regulation of their non-coding poison exons, coupling AS to nonsense mediated decay (NMD) [22].

AS is critical for a wide-range of key biological processes including normal development, cell-type specific mechanisms, stem cell biology [23–27], and its misregulation and the resulting aberrant protein isoforms

have been implicated in numerous pathologies, including cancer [28]. In disease, AS can be misregulated via mutations in splice site sequences or splicing regulatory elements, as well as alterations in genes encoding regulatory SFs and splicing machinery components [29]. More recently, alterations in epigenetics have been appreciated as a cause of altered splicing profiles in tumors. Since splicing is a co-transcriptional process, chromatin and transcriptional regulators can impact the final splicing outcome [30]. In particular, nucleosome positioning and histone marks within or near exon bodies, as well as transcription kinetics, can alter RNA polymerase dynamics and the accessibility of the spliceosome to the nascent RNA, or even directly recruit splicing factors to chromatin to influence exon inclusion [31–33]. Finally, epigenetic modifications of SF genes are detected in blood cancer and could lead to downstream changes in their splicing targets [34].

2. Splicing alterations in human tumors

Mutations in or expression changes in splicing machinery components or SFs are critical in cancer initiation and progression. These alterations have the potential to not only alter one protein isoform, but also to disrupt a network of downstream products and cancer pathways. Several key examples are highlighted in the following sections.

2.1. *Mutations in spliceosomal components*

Recurrent somatic mutations in *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2* occur frequently in hematological malignancies, including myelodysplastic syndromes (MDS), chronic myelomonocytic leukemia (CMML), chronic myeloid leukemia (CML), acute myeloid leukemia (AML), and chronic lymphocytic leukemia (CLL) [35, 36].

Splicing factor 3b (*SF3B1*) is the most frequently mutated spliceosomal component in cancer [37]. Recurrent somatic mutations in *SF3B1* play a role in tumor development and progression and are detected in approximately 30% of all MDS patients, including 83% of cases of MDS-subtype refractory anemia with ringed sideroblasts (RARS) and 76% of cases of MDS-subtype refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS) [35, 38–41] (Fig. 4). *SF3B1*

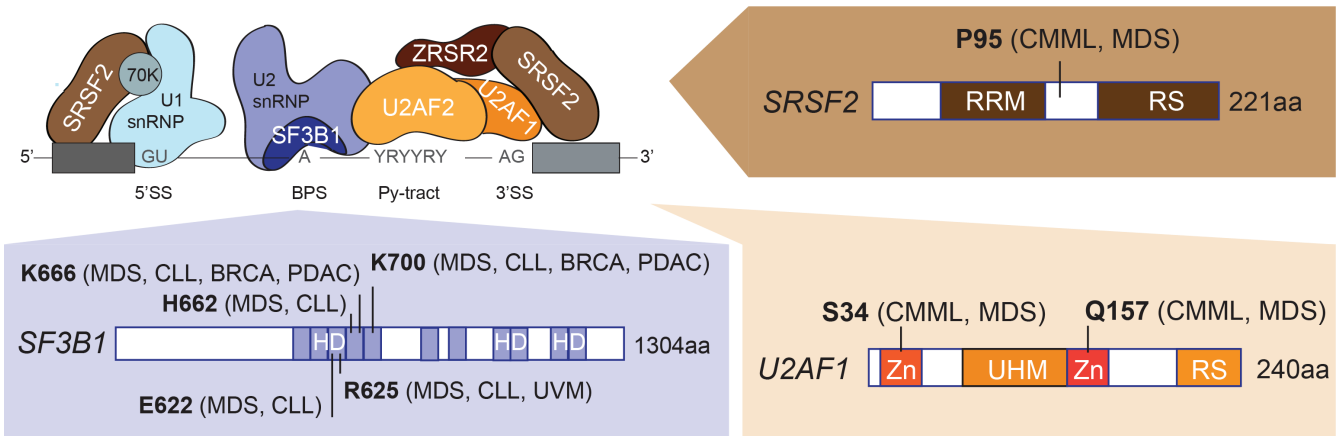

Figure 4. Recurrent hotspots cancer-associated mutations in components from the spliceosomal A complex.

Hotspot mutations in spliceosomal components *U2AF1*, *SRSF2*, and *SF3B1* frequently detected in human malignancies. Positions of recurrent mutations are indicated along with the protein structures. Tumor abbreviations: BRCA: breast cancer; CLL: chronic lymphocytic leukemia; CLL: chronic myelomonocytic leukemia; MDS: myelodysplastic syndromes; PDAC: pancreatic adenocarcinoma; UVM: uveal melanoma. Domain abbreviations: Zn: zinc finger domain; UHM: U2AF homology motif domain; RS: arginine/ serine-rich domain; RRM: RNA-recognition motif; HD: heat domain.

mutations are also detected in other cancers including, but not limited to, 15% of CLL, 3% of pancreatic cancer, 1.8% of breast tumors (4% of luminal breast tumors), 1% of cutaneous melanomas, and 20% of uveal melanomas [42, 43–47]. SF3B1 is a core component of the U2 snRNP, involved in branch point site recognition and spliceosomal complex A assembly [48] (Fig. 1). *SF3B1* point mutations are frequently found in the C-terminal HEAT (Huntington, Elongation Factor 3, PR65/A, TOR) domain and are associated with decreased splicing fidelity and branch point site recognition, leading to cryptic 3′ splice site selection, increased NMD, and differential AS of transcripts [49–52]. The prognostic value of a *SF3B1* mutation is debated as there is evidence for good and poor prognostic outcomes. For example, in MDS, the frequent K700E missense mutation is associated with a better prognosis, possibly due to its role in promoting AS of an erythroid lineage transcription factor TAL1 isoform that reduces erythroid differentiation *in vitro* [35, 36, 53]. However, in CLL, *SF3B1* mutations are associated with anti-apoptotic effects and correlate with poor overall prognosis [40, 54, 55]. The exact role of mutant *SF3B1* in carcinogenesis is still actively investigated, but likely impacts numerous cellular pathways critical in tumorigenesis [41].

Recurrent mutations in SR protein *SRSF2* have been observed in 10% of all MDS patients, including in 31–47% of CMML, and 2% of AML tumors [36] (Fig. 4). Currently, data suggests that *SRSF2* mutations are linked with poor clinical outcomes in MDS and increased progression from MDS to AML [36]. Required for both constitutive and AS, SRSF2 mediates recognition of the 5′ and 3′ splice sites by interacting respectively with U1 and U2 snRNPs [8, 56] (Fig. 1). Heterozygous mutations in the sequence linking the RRM and RS region of *SRSF2* (almost exclusively at P95) alter its RNA binding preference in a sequence-specific manner, favoring AS at C-rich sequences (CCNG motif) while having reduced affinity for G-rich consensus sequences (GGNG motif) [57, 58]. This alters the efficiency of SRSF2-mediated exon inclusion and results in mis-splicing of its targets. For example, this alteration in binding preference results in downregulation of *EZH2*, a histone methyltransferase implicated in the pathogenesis of MDS, due to increased inclusion of an exon containing a premature termination codon [57]. Potentially linked, *EZH2* loss-of-function mutations in CMML are reported to be mutually

exclusive with *SRSF2* mutation [59]. Interestingly, *SRSF2* mutations frequently co-occur with additional oncogenic somatic mutations [60]. Further, mutant *SRSF2* leads to AS alterations in additional SFs, causing more global splicing dysregulation [61]. Yet, mutations in *SF3B1, SRSF2, and U2AF1* occur as heterozygous missense point mutations (suggesting gain-of-function alterations) and are mutually exclusive as they elicit either redundant and/or distinct overwhelming effects on AS and are synthetically lethal due to the cumulative impact on hematopoietic stem cell survival and quiescence [62].

U2AF1 is mutated in 5–15% of MDS, 5–17% of CMML, and 3% of lung adenocarcinomas [37, 63] (Fig. 4). The U2AF1/U2AF2 heterodimer recognizes the 3′ SS (U2AF1 binding to the AG dinucleotide and U2AF2 to the polypyrimidine tract) and is critical for U2 snRNP binding (Fig. 1) [64]. Hotspot S34 and Q157 mutations in *U2AF1* occur in two zinc finger domains, thus affecting RNA binding, splice site recognition, and RNA splicing patterns [65–68]. Transgenic mice expressing the S34 mutant exhibited altered hematopoiesis and AS [69] and overexpression of the S34 mutant in HeLa cells led to increased expression of NMD components and upregulation of unspliced introns [36]. Pathways dysregulated by *U2AF1* include DNA damage response, RNA localization and transport, cell cycle, epigenetic regulation, and pre-mRNA splicing [70]. The prognostic value of a *U2AF1* mutation is still debated, however, poor prognosis may be linked with mutation type and mutational load [71].

ZRSR2, an X-linked gene, is mutated in 1–11% MDS without ring sideroblasts, 0.8–8% CMML, and at lower rates amongst other hematological cancers [37]. ZRSR2 comprises a heterodimer with ZRSR1 and is important for recognition of 3′ splice site in both major U2-dependent and minor U12-dependent classes of introns [2] (Fig. 1). *ZRSR2* loss results in improper retention of U12-type minor introns, without affecting splicing of U2-type major introns [72]. In contrast to the hotspot alterations described above, mutations in *ZRSR2* occur across the whole gene, creating loss-of-function mutants, and are not mutually exclusive with *SF3B1*, *SRSF2* or *U2AF1* mutations [28, 70]. Leukemia patients with *ZRSR2* mutations show poor tumor-free survival compared to unmutated individuals [73].

Finally, mutations in other spliceosomal components (e.g., *SF3A1*, *PRPF8*, *SF1*, *HNRNPK*, *U2AF2*, *SRSF6*, *SRSF1*, *SRSF7*, *TRA2β*, *SRRM2*) occur but are less frequent in both hematological and solid tumor malignancies [37]. The role of SF mutations in cancer is still being uncovered and could be more broadly significant. Recently, it was suggested that across multiple cancer types, >100 genes encoding spliceosomal components contain putative driver mutations [74]. However, the frequency of these mutations is minor compared to the recurrent mutations in core spliceosomal components discussed above and therefore their role in disease pathogenesis must be further investigated.

2.2. *Expression changes in splicing-factor levels*

SF levels and activity are tightly controlled epigenetically, transcriptionally, post-transcriptionally via AS coupled with NMD, translationally, and post-translationally including via phosphorylation by specific kinases [20, 21]. Changes to any of these regulatory pathways may lead to altered SF expression and altered AS patterns of their downstream targets. Importantly, while the aforementioned recurrent mutations in SFs are evident in hematological malignancies, altered SF expression levels and copy number changes seem to be more prominent in solid tumors [28, 75–80]. SFs regulate AS of downstream mRNA targets in a concentration-dependent manner [19]. Therefore, changes in SF expression levels alone can induce AS deregulation in tumors [20, 21]. Causal links have been identified between SF misregulation and multiple cancers (Table 1). Of note, several SFs upregulated in breast tumors exhibit oncogenic functions and are sufficient to promote tumor initiation in breast cancer models [75, 77, 78, 80]. SFs can also serve as tumor suppressors and therefore downregulation of SFs can contribute to tumor development [76].

Highlighting an example of SF expression changes in cancer, the SR protein SRSF1 is frequently upregulated in breast, lung, colon, and bladder tumors, in part due to an amplification of Chr.17q23, but also in tumors with transcription factor MYC amplifications [75, 77–79, 81, 82] (Table 1). SRSF1 promotes oncogenesis via mechanisms directly and indirectly related to its AS role. Its overexpression enhances splicing of isoforms

Table 1. Examples of splicing-factor alterations in primary human tumors.

Splicing factor gene	Alteration type*	Tumor type**	Reference
ESRP1,2	Up/Down	BRCA, OSCC	[90 , 91]
HNRNPA1	Up	BRCA, COAD, GBM, OVCA,	[75, 92–95]
HNRNPA2/B1	Amp, Up	GBM	[95]
HNRNPH	Up	GBM	[96]
HNRNPI (PTB)	Up	BRCA, COAD, GBM, OVCA,	[97–100]
HNRNPK	Down	AML, BRCA, COAD, ESCA, PDAC, OSCC, SKCM	[58, 101–104]
HNRNPM	Up	BRCA	[105–108]
QKI	Down	LUAD, OSCC, PRAD	[74, 109–111]
RBFOX2	Down Localization change	BRCA, OVCA, COAD	[80, 112, 113]
RBM5	Down Up	BRCA, LUAD, PRAD, BRCA	[111, 114–116]
RBM6	Up	BRCA	[114, 116]
RBM10	Up Mutations	BRCA LUAD	[63, 74, 114, 116–120]
SF3B1	Mutations	RARS, RCMD-RS, BRCA, UVM, PRL	[35, 38–47, 121, 122]
SRSF1	AMP, Up	BLCA, BRCA, COAD, GBM, KI, LUAD, OVCA, THCA, SI,	[75, 77–79, 81, 82, 123–127]
SRSF2	Mutations Up	MDS, CLL OVCA	[35, 36, 128]
SRSF3	Up Down	BLCA, BRCA, CESC, COAD, GBM, LIHC, LUAD, KI, OSCC, OVCA, STAD, THCA, SKCM LIHC	[87, 98, 125, 129–132]
SRSF4	Up	BRCA	[80]
SRSF5	Up	BRCA, LUAD, OSCC	[133–135]
SRSF6	Amp, Up	BRCA, COAD, LUAD	[75, 80, 136, 137]
SRSF10	Up	COAD	[138–140]
TRA2β	Up	BRCA, CESC, COAD, LUAD, OVCA	[22, 80, 123, 125, 128, 141]

Table 1. (*Continued*)

Splicing factor gene	Alteration type*	Tumor type**	Reference
U2AF1	Mutations	MDS, CMML, LUAD	[35, 38, 39, 63, 71]
ZRSR2	Mutations	MDS, CMML	[35, 38, 39, 63]

*Alterations abbreviations: Amp: copy number amplification; Down: gene expression downregulation; Up: gene expression upregulation.

**Tumor abbreviations: AML: acute myeloid leukemia; BRCA: breast carcinoma, BLCA: bladder carcinoma; CESC: cervix carcinoma; COAD: colon adenocarcinoma; ESCA: esophageal carcinoma; GBM: glioblastoma; KI: kidney carcinoma; LIHC: liver hepatocellular carcinoma; LU: lung adenocarcinoma; PDAC: pancreatic adenocarcinoma; PRAD: prostate adenocarcinoma; PRL: prolactinomas; OSCC: oral carcinoma; OVCA: ovarian carcinoma; RARS: refractory anemia with ringed sideroblasts; RCMD-RS: refractory cytopenia with multilineage dysplasia and ringed sideroblasts; STAD: stomach adenocarcinoma; SI: small intestine carcinoma; SKCM: skin cutaneous melanoma; THCA: thyroid carcinoma; UVM: uveal melanoma.

associated with decreased cell death (i.e., *BIN1*, *BCL2L11*, *MCL1*, and *CASC4*), increased cell proliferation (i.e., *RON*, *MKNK2*, *S6K1*, *CASC4*, and *PRRC2C*), or resistance to DNA damage (i.e., *PTPMT1* and *DBF4B*), resulting in the transformation of cells *in vivo* and *in vitro* [77, 79, 81, 83–86]. Higher expression of SRSF1 can also lead to mRNA stabilization via RNA binding of cancer-related transcripts such as *LIG1* [87]. SRSF1 can act synergistically with MYC, often resulting in higher tumor grade and decreased survival in breast and lung cancer patients, in part by potentiating the activation of eIF4E, a translational regulator of cell growth signaling pathways [77, 79]. Further, SRSF1 can activate mTORC1 growth signaling and promote translation initiation in part via interactions with both the phosphatase PP2A and mTOR and enhancing phosphorylation of 4E-BP1 [88, 89].

Another SR protein family member, *SRSF3*, is found overexpressed in lung, breast, ovarian, stomach, bladder, colon, bone, liver, brain, and oral tumors, in part due to copy number changes on Chr.6p21 [125, 130, 131, 142, 143] (Table 1). Conversely, decreased expression of SRSF3 is also found in tumors, for example in human hepatocellular carcinoma [144], suggesting a more complex role in tumorigenesis. Targets of SRSF3 do play roles in cellular metabolism, growth, cytoskeleton organization, and splicing [132, 142, 144, 145]. For example, SRSF3 regulates the AS switch between *PKM2* and *PKM1*, two isoforms of pyruvate kinase, a key

metabolic enzyme underlying the Warburg effect on cancer cells [145]. Another SRSF3 target, the Δ8 isoform of *HIPK2*, has been associated with cell death [146]. SRSF3 also controls AS of target genes involved in glucose and lipid metabolism [132], and its conditional knockout in mouse hepatocytes causes fibrosis and the development of metastatic hepatocellular carcinoma with aging [144]. Finally, SRSF3 regulates the production of spliced isoforms of interleukin enhancer binding factor 3 (*ILF3*) and production of this double-strand RNA-binding protein [147].

Additional upregulated SFs include members of the SR protein family, such as SRSF4, SRSF6, or the SR-like protein TRA2β, or members of the hnRNP protein family, such as hnRNPA1, hnRNPA2/B1, HNRNPM, or PTB (also known as hnRNPI), as well as other SFs including ESPR1 and 2, RBM5, 6, and 10 (Table 1).

Alternatively, a number of SFs are downregulated in human tumors, including HNRNPK, ESPR1 and 2, RBFOX2, RBM5, or QKI (Table 1). Decreased levels of Quaking (QKI), a KH-domain containing RNA binding protein, are detected in several solid tumors, such as lung, oral, or prostate cancers, and are associated with poor prognosis [109–111]. QKI regulates AS of *NUMB*, a membrane-associated inhibitor of Notch signaling, leading to an isoform that decreases cell proliferation and prevents Notch signaling [109]. QKI also regulates the expression of transcription factor SOX2 by binding a cis-element in its 3′UTR [110]. In addition, gene fusions of *QKI* with *MYB* have been described in angiocentric gliomas, a subtype of pediatric low-grade brain tumors, and have shown to promote transformation *in vitro* and *in vivo* [148].

2.3. *Aberrantly spliced RNA isoforms*

Tumors often exhibit a more complex AS repertoire compared to normal tissues [149] and tumorgenicity may be associated with cancer-specific AS events that arise during the transformation process [150]. These AS switches impact thousands of genes and are often specific to a given tumor type [149], or even subtype, likely because the baseline splicing profiles differ between normal tissues. Yet several AS events are detected across multiple tumor types (Table 2), suggesting shared splicing regulatory networks across tissue types.

Table 2. Spliced isoforms in human primary tumors and their associated cancer hallmarks.

Spliced gene	Splicing event*	Tumor type**	Associated cancer hallmark	Reference
AR	CA	PRAD	Enable drug resistance	[154–161]
BCL2L1	A5′SS	HEM	Escape cell death Enable drug resistance	[162–168]
BCL2L11 (BIM)	CA	BRCA, HEM, LUAD	Escape cell death Enable drug resistance	[77, 169–174]
BIN1	CA	BRCA, SKCM	Escape cell death	[75, 77, 95, 175, 176]
***BIRC5* (SURVIVIN)**	CA	BRCA, LU, KI	Escape cell death Increase cell growth	[177–180]
CASP2	CA	CESC, HEM, OVCA	Escape cell death	[181–183]
CCND1	CA/ALE	BLCA, BRCA, CESC, HEM, HNSCC, LU, OVCA	Increase cell growth Enable cell invasion	[184–189]
CD44	CA	BLCA, BRCA, CESC, COAD, KI, PRAD, OVCA, STAD	Enable cell invasion	
CEACAM1	CA	BRCA, COAD, KI	Enable cell invasion	[190, 191]
ENAH	CA	BRCA, LUAD	Enable cell invasion	[192–197]
FAS	CA	BLCA, CESC, KI, HEM, OVCA	Escape cell death	[198–204]
FBLN2	CA	BRCA, COAD, HNSC, LUAD, THCA	Escape cell death	[29, 76, 205, 206]
FGFR2	MXE	BLCA, BRCA, CESC, COAD, KI, PDAC, PRAD OVCA	Increase cell growth Enable cell invasion	[97, 207–210]
FN1	CA	BRCA, HNSCC, KIRC, LIHC, LUAD, THCA	Enable cell invasion	[205, 211–214]
HER2	CA	BRCA	Increase cell growth Enable cell invasion	[215–218]
HRAS	CA	PRAD	Increase cell growth	[219–223]
IL1RAP	CA	CESC	Alter immune response	[205, 224]

(*Continued*)

Table 2. (*Continued*)

Spliced gene	Splicing event*	Tumor type**	Associated cancer hallmark	Reference
KLF6	A5′SS	BRCA, CESC, LIHC, LU, PDAC, PRAD, OVCA	Enable cell invasion	[225–230]
MCL1	CA	HNSCC, SKCM	Escape cell death	[83, 231–233]
MKNK2	CA/ALE	BRCA, COAD, LUAD	Increase cell growth Escape cell death	[75, 77, 79, 80, 152, 234, 235]
MSTR1 (RON)	CA	BRCA, COAD, GBM, HNSCC, LU, STAD, OVCA	Enable cell invasion	[77, 81, 236–240]
NUMB	CA	BRCA, HCC, LU, UCEC	Increase stemness Enable cell invasion	[109, 116, 119, 241, 242]
PKM	MXE	BRCA, CESC, COAD, HNSCC, LIHC, LUAD, PDAC, OVCA, STAD	Rewire metabolism	[243–251]
RAC1	CA	BRCA, COAD, HNSCC, LUAD, STAD	Increase cell growth Enable cell invasion	[252–256]
RPS6KB1	CA	BRCA, LUAD, PRAD	Increase cell growth	[75, 77, 257–259]
TERT	CA	BRCA, LU, PDAC	Alter cell replication Escape cell death	[260–262]
TNC	CA	BRCA, PDAC	Enable cell invasion	[137, 263, 264]
VEGFA	CA/ALE	COAD, HNSCC, KI, PRAD, SKCM	Alter angiogenesis	[265–268]

*Splicing event abbreviations: CA: Cassette alternative exon; A5SS: Alternative 5′SS; MXE: Mutually exclusive exons; ALE: Alternative last exon.

**Tumor abbreviations: AML: acute myeloid leukemia; BRCA: breast carcinoma; BLCA: bladder carcinoma; CESC: cervix carcinoma; COAD: colon adenocarcinoma; ESCA: esophageal carcinoma; GBM: glioblastoma; HEM: hematological; HNSCC: head and neck squamous cell carcinoma; KI: kidney carcinoma, LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; MED: medulloblastoma; PDAC: pancreatic adenocarcinoma; PRAD: prostate adenocarcinoma; OSCC: oral carcinoma; OVCA: ovarian carcinoma; STAD: stomach adenocarcinoma; SI: small intestine carcinoma; SKCM: skin cutaneous melanoma; THCA: thyroid carcinoma; UCEC: uterine carcinoma.

These spliced isoforms often impact the so-called "Cancer Hallmarks," a series of biological capabilities acquired during the development of human tumors, as defined originally by Hanahan and Weinberg, and which are used as an organizing principle for rationalizing cancer complexity [151]. These isoforms can provide a proliferative advantage, improve cell migration and metastasis, enable escape from cell death, rewire cell metabolism or cell signaling, promote an abetting microenvironment, alter immune response, or even enable drug resistance (Table 2). These cancer-associated AS switches are often due to changes in SF-levels or activity, or can arise due to mutations at specific splice site. Functional studies in model systems have demonstrated that alterations in a single isoform can impact tumor growth but are often not sufficient to fully recapitulate SF-mediated transformation [77, 78, 80, 152, 153], and that the combination of multiple AS isoform switches is likely required to promote the different steps of tumorigenesis [149, 150].

3. Targeting the splicing machinery in cancers

AS has arisen as a new therapeutic target to combat cancer. A variety of approaches have been adopted that range from broadly targeting key spliceosomal proteins, regulatory SFs, or specific oncogenic AS events. The following discusses these approaches starting from the broadest spectrum to specific isoform level approaches and ends with a discussion of novel techniques that show great potential in pre-clinical settings (Fig. 5).

3.1. *Targeting the core spliceosome*

One therapeutic option for targeting aberrant splicing is to target the spliceosome itself. SF3B1 is a spliceosome component critical for branch point and 3′ splice site selection [64] (Fig. 1), and therefore compounds limiting its function disrupt splicing at very early stages in spliceosome assembly. Multiple small molecules have been developed that target SF3B1, including FR901464 and its derivatives (e.g., Spliceostatin A, Meayamycin, thailanstatins), sudemycin, pladienolides (e.g., E7107, H3B-8800, FD-895), and herboxidienes (Fig. 5).

FR901464 was first isolated from a *Pseudomonas* strain and found to have antitumor activity in mouse models [269]. Later it was chemically

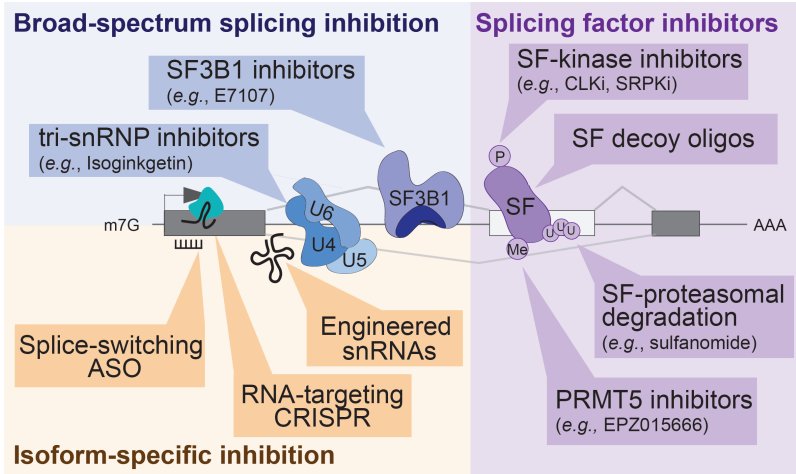

Figure 5. Therapeutic splicing reprogramming strategies.

Current strategies to target alternative RNA splicing in cancer include broad-spectrum inhibition, splicing factor specific inhibition both directly or through inhibition of upstream post-translational modification regulators, and modulation of specific isoforms.

synthesized [270] and eventually demonstrated to inhibit pre-mRNA splicing by binding to SF3B1 [271]. Spliceostatin A, meayamycin, 1-methoxy, and 1-methyl analogues of FR901464, demonstrated similar effects as FR901464 but at much lower concentrations [272, 273]. The thailanstatins were discovered through a search for gene clusters homologous to the pathway components responsible for the synthesis of FR901464 in *Pseudomonas* [274]. These compounds have a similar mechanism as FR901464 but lack the hydroxyl group and contain an extra carboxyl group that greatly enhances their stability [274]. Mechanistically, SF3B1 inhibition prevents branch point recognition and leads to AS changes in transcripts involved with cell proliferation and death [275]. Interestingly, only a fraction of AS events (~10%) are affected by SF3B1 inhibition, suggesting that some splice sites, likely weak sites, are more sensitive than others to spliceosomal inhibitors [275, 276]. Cancer cells bearing mutations in spliceosomal genes are particularly sensitive to SF3B1 inhibitors, both *in vitro* and *in vivo*, compared to wild-type [276, 277].

Several SF3B1 inhibitors have been taken into clinical trials. E7107 entered into phase I trials for solid tumors and resulted in dose-related AS changes in patient cells but did not demonstrate broad efficacy and carried significant toxicities that eventually led to discontinuation of the study [278, 279]. H3B-8800 has also undergone phase I clinical trials as a treatment for myeloid neoplasms with an oral dosing strategy [280]. Patients in this cohort experienced decreased need for blood transfusions with minor adverse events [280].

Another broad spectrum spliceosome inhibitor is isoginkgetin, which prevents recruitment of the U4/U5/U6 tri-snRNP and leads to stalling at the presplicesomal A complex [281]. Isoginketin treatment influences a number of cancer relevant-pathways including cell death [282], invasion [283], and immune response [284].

3.2. *Targeting alternative splicing factors*

The development of inhibitors targeting specific RBPs and SFs has been challenging, in part due to the lack of a catalytically active site which is targeted by most classical small molecule inhibitor approaches. One of these compounds targets the RNA-binding motif protein 39 (RBM39), a regulatory SF that works with U2AF65 and SF3B1 in the initial stages of spliceosome assembly and splice site recognition [285–287]. Additionally, RBM39 coordinates the action of other regulatory SFs including SR proteins [288]. RBM39 knockdown globally impacts AS events, including a 20% overlap with U2AF65-regulated AS events [289, 290]. A series of aryl sulfonamides compounds (e.g., E7820, indisulam, tasisulam, chloroquinoxaline) have been developed to target RBM39 for degradation [291–293] (Fig. 5). Numerous clinical trials of aryl sulfonamides have been undertaken [290], including one phase III trial comparing tamsisulam to paclitaxel for metastatic melanoma that was stopped early due to myeloid toxicity and lack of evidence that tamsisulam was superior to the standard of care [294].

3.3. *Targeting upstream regulatory proteins*

The protein arginine methyltransferase 5 (PRMT5) is critical for regulation of both constitutive and AS through its methylation of Sm proteins

and regulatory SFs [295, 296]. In addition to its role in regulating splicing, PRMT5 impacts transcription through methylation of histones and transcription factors such as p53 [297–299]. *PRMT5* itself is a direct target of the MYC oncogene, providing a link between MYC driven tumors and AS [153]. Large scale small molecule screens identified EPZ015666, a PRMT5 inhibitor that has anti-tumor activity against lymphoma and leukemias [300] (Fig. 5). Currently, PRTM5 inhibitors are in early phase 1 clinical trials to determine safety and efficacy in treatment of multiple tumor types.

Many SFs, and particularly SR proteins, are heavily phosphorylated. These phosphorylation events alter SF activity and localization and are ultimately required for the activation of their splicing activity. Inhibition of the upstream kinases that regulate these phosphorylation events are therefore a viable strategy to diminish the activity of oncogenic SR proteins (Fig. 5). Serine-rich protein kinase-1 (SRPK1) inhibitors, including SRPKIN-1, SRPIN340, MVRL09, and SHPINX, lead to decreased phosphorylation of multiple SR proteins and have antiangiogenic effects through SRSF1-mediated AS of *VEGF* [301–303]. Another compound, TG003, influences SR protein phosphorylation by inhibiting CDC2-like kinase (CLK1) [304] and exhibits anti-cancer effects in prostate and gastric cancer models [305, 306]. Additional inhibitors targeting CLK1, CLK2, and CLK4, impair viability of colorectal cancer models by impacting the interaction of SRSF10 with these kinases [140]. Yet most of these kinase inhibitors impact the activity of multiple SR proteins at the same time and it remains to be determined whether greater selectivity is required to limit toxicity in cancer patients.

4. RNA-based therapeutics in oncology

4.1. *Splicing modulation using RNA-based approaches*

RNA-based therapeutics offer the specificity of virtually any pre-mRNA sequence for the purpose of targeted gene knockdown or alteration of pre-mRNA splicing. Splice-switching antisense oligonucleotides (ASOs) are short chemically modified RNA oligos designed to bind a reverse complimentary sequence in a target pre-mRNA, preventing its interaction with the splicing machinery (Fig. 6a). Splice-switching ASOs can be designed to

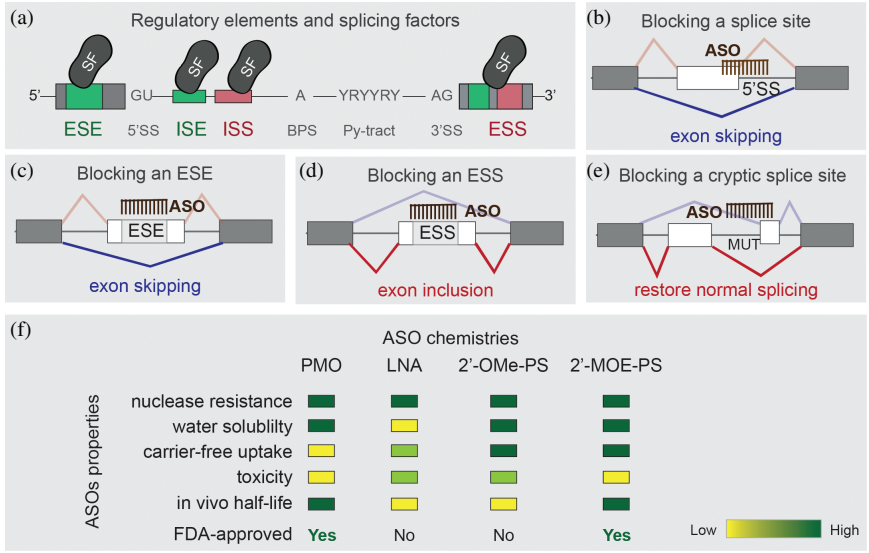

Figure 6. Splice-switching antisense oligonucleotide approaches.

(a) pre-mRNA contains regulatory regions encoded in both the exon and intron sequences termed splicing enhancers (ESE, ISE) and splicing silencers (ESS, ISE) that recruit specific splicing factors (SF) to promote or inhibit exon inclusion, respectively. (b–e) Mechanism of ASO-mediated skipping or inclusion of splicing events. (f) The different chemistries of ASOs allow for differences in nuclease resistance, water solubility, carrier-free uptake, toxicity, and half-life. ASO chemistry abbreviations: PMO: Phosphordiamidate Morpholino Oligomers; LNA: Locked Nucleic Acid; 2′-OMe-PS: 2′-O-Methyl Phosphorothiaote backbone; 2′-MOE-PS: 2′-O-Methoxyethly Phosphorothioate backbone.

specifically target: 1) a 5′ or 3′ splice site, thus blocking its usage (Fig. 6b), 2) a splicing enhancer sequence, thus preventing binding of a SF activator and promoting exon skipping (Fig. 6c), 3) a splicing silencer sequence, thus preventing binding of a SF repressor and promoting exon inclusion [307] (Fig. 6d) or blocking a cryptic splice site that arises due to a mutation, thus restoring the wild splice site (Fig. 6e). Chemical modifications to the phosphate backbone and/or the ribose ring have generated highly stable ASOs with high substrate specificity, low toxicity, low immunogenicity, and that limit ribonuclease (RNAse) H degradation [308] (Fig. 6f).

Several distinct ASO chemistries are currently used for AS modulation (Fig. 6f). A common backbone modification uses phosphorothioates (PS) at the nucleotide link [308]. PS-ASOs are more hydrophobic, more

nuclease resistant, and bind with higher affinity than ASOs with unmodified phosphodiester linkages [309]. PS-ASOs are often combined with ribose modifications such as 2′-O-(2-methoxyethyl) (2′-MOE) or 2′-O-methyl (2′-OMe) [308]. Uniformly modified 2′-MOE/PS ASOs are effective when administered in saline by nearly all routes of administration and their tissue half-lives ranges from 2–4 weeks, but can even achieve six months in the central nervous system [309]. Another type of modification uses locked nucleic acid (LNA), which increases binding affinity and reduces off-target effects by allowing the usage of shorter sequences that are less likely to partially hybridize to nontarget sequences [307]. A distinct class of backbone chemistry uses phosphorodiamidate linkages in morpholino oligomers (PMO or morpholino) [307]. PMOs are neutrally charged and provide better specificity and display lower toxicity than PS-ASOs. However, PMOs often need to be conjugated to a delivery moiety for *in vivo* delivery. Finally, peptide nucleic acid (PNA) offer specificity similar to PMO, but their low water solubility limits their use [307].

Delivery of ASOs to a target tissue and then to the correct intracellular compartment, mainly the nucleus, remains a significant challenge to their widespread therapeutic usage. Current FDA-approved ASOs are delivered directly to their target location or systemically. For example, nusinersen, an ASO directed against *SMN2* transcripts and used to treat spinal muscular atrophy, is injected intrathecally and achieves 4–6 months half-life in cerebrospinal fluid [310]. Eteplirsen, an ASO used to treat Duchenne Muscular Dystrophy, is delivered systemically by intravenous infusion and is subject to high renal clearance [311]. Novel approaches to ASO delivery involve packaging formulations that enhance cellular uptake or targeted approaches like aptamer- or antibody-conjugation that direct the ASO to specific tissues or cell types [311].

4.2. *Applications of antisense oligonucleotides in oncology*

Regardless of their *in vivo* delivery challenges, the catalogue of ASOs targeting cancer pathways has grown. In many cases, ASOs correcting cancer-associated AS events have led to promising anti-cancer phenotypes in cell line and animal models (Table 3). For example, the apoptotic

Table 3. Splicing modulating antisense oligonucleotides in oncology.

Target gene	ASO chemistry*	Induced event	Tumor type**	Cell line data	*In vivo* data	References
ATM	2′-OMe/PS	blocks NMD exon inclusion	LE	yes	no	[314, 315]
BCL-X	2′-MOE/PS	switches Bcl-xL to Bcl-xS	BRCA, GBM, LUAD, PRAD	yes	yes	[167, 316–318]
BIM	2′-MOE/PS	exon 4 inclusion	LE	yes	no	[319]
BRCA2	2′-OMe/PS	cryptic exon skipping	BRCA	yes	no	[320]
BRD9	PMO	exon 14a skipping	SCKM	yes	yes	[312]
ERBB4 (HER4)	LNA	exon 26 skipping	BRCA	yes	yes	[321]
ERG	PMO	exon 4 skipping	PRAD	yes	yes	[322]
EZH2	2′-OMe/PS	poison exon skipping	LE	Yes	no	[323]
FGFR1	PMO	exon alpha inclusion	GBM	yes	no	[324]
GLDC	2′-MOE/PS	exon 7 skipping	LUAD	yes	yes	[325]
IL5R	2′-MOE/PS	exon 5 skipping	LE	yes	no	[326]
MCL1	PMO	exon 2 skipping	SCKM	yes	no	[327]
MDM2	PNA	exon 4 skipping	UCEC	yes	no	[328]
MDM4	PMO	exon 6 skipping	DLBC, SCKM	yes	yes	[329]
MKNK2	2′-OMe/PS and 2′-MOE/PS	3′ UTR intron retention	GBM	yes	yes	[152]
MSTR1	PMO	exon 11 skipping	BRCA, STAD	yes	no	[330]
PKM2	2′-MOE/PS	exon 9 inclusion	GBM	yes	no	[145]
SRSF3	2′-MOE/PS	poison exon inclusion	BRCA, OSCC	yes	no	[313]
STAT3	PMO	exon 23 skipping	BRCA	yes	yes	[331, 332]

(*Continued*)

Table 3. (*Continued*)

Target gene	ASO chemistry*	Induced event	Tumor type**	Cell line data	*In vivo* data	References
TRA2β	2′-MOE/PS	poison exon inclusion	BRCA	yes	no	[22]
USP5	PMO	alternative 5′SS	GBM	yes	no	[333]

* Chemistry abbreviations: Phosphorodiamidate Morpholino Oligomer (PMO), Locked Nucleic Acid (LNA), Peptide Nucleic Acid (PNA), 2′-O-Methly phosphorothioated oligo (2′-OMe/PS), 2′-O-Methoxyethyl phosphorothioated oligo (2′-MOE/PS).

** Tumor type abbreviations: breast carcinoma (BRCA), diffuse large B-cell lymphoma (DLBC), glioblastoma (GBM), leukemia (LE), lung adenocarcinoma (LUAD), prostate adenocarcinoma (PRAD), oral carcinoma (OSCC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD).

protein Bcl-x can be alternatively spliced into a pro-apoptotic isoform, *Bcl-xS*, and an antiapoptotic isoform, *Bcl-xL*, and an ASO that promotes the formation of *Bcl-xS,* induces apoptosis in glioma cell models [167]. The bromodomain containing 9 (*BRD9*) gene encodes a "poison" exon that leads to the degradation of its mRNA when included. PMO-based ASO that forces skipping of this exon, results in increased BRD9 protein levels and decreased tumor volume in melanoma mouse models [312]. A similar approach has been taken to target poison exons in transcripts encoding oncogenic SFs. ASOs that promote inclusion of these exons in *SRSF3* [313] and *TRA2β* [22] lead to AS changes in their target transcripts and decreases proliferation of cancer cells. Additional targets include regulators of p53 (e.g., *MDM2, MDM4, USP5*), cell signaling (e.g., *ERRB4, IL5R, STAT3, FGFR1, MSTR1*), cell death (e.g., *BCL2L1, BIM, Bcl-X, MCL1*), DNA damage (e.g., *BRCA2, ATM*), and chromatin remodeling and transcription (e.g., *BRD9, ERG*) (Table 3).

5. Novel strategies targeting alternative RNA splicing

In recent years, new approaches aimed at targeting either SFs or specific AS events have emerged to widen the repertoire of RNA targeting tools.

One example of SF inhibitors are the decoy oligonucleotides. These oligonucleotides are designed to represent a short sequence corresponding to the SFs mRNA binding motif and thereby attenuate their activity by competing for their natural binding targets [334] (Fig. 5). These decoy oligonucleotides induce transcriptome changes similar to knockdown of the target SF, and SRSF1 decoys can limit the growth of glioma cells in mouse models [334]. Another approach is the use of engineered U7 snRNA's as a potential therapeutic aimed at correcting a specific AS event. This approach alters U7's specificity for histone mRNA processing and reengineers it to block specific pre-mRNA sequences, effectively acting as an antisense molecule [335]. However, stable expression of these constructs overcomes the limitation of conventional antisense therapeutics, in that they would not require multiple rounds of administration [335]. So far, this approach has been utilized in models of myotonic dystrophy, Duchenne muscular dystrophy, amyotrophic lateral sclerosis, β-thalassemia, HIV infection, and spinal muscular atrophy [335]. Additionally, alterations in the sequence recognition of the U1 snRNA allows for specific targeting of exons to promote their inclusion. This approach has been applied to multiple RNA targets including *SMN2* (Spinal Muscular Atrophy) and *SPINK5* (Netherton Syndrome) [336–339].

The idea of engineering programmable SFs started with the use of RNA binding domains from Pumilio1, that allows for targeting of specific pre-mRNA sequences when combined in different combinations [340]. When designed to target *Bcl-X*, these pumilio engineered SFs could promote the formation of pro-apoptotic *Bcl-xS* and sensitize cancer cells to chemotherapy [340]. Later, CRISPR entered the RNA processing field initially with the adaptation of Cas9 to target RNA (rCas9) [341] and then with the discovery of Cas family proteins that naturally target RNA [342]. RNA-targeting Cas13 has been adapted to induce RNA interference [342, 343], base edit target RNA [344], allow for live cell imaging of transcripts [345], and alter splicing of pre-mRNA [343]. Building on the RNA-targeting capability of Cas13, CRISPR Artificial Splicing Factors (CASFx) were developed to direct the splicing activity of individual SFs to a target pre-mRNA using a guide RNA [346] (Fig. 5). So far, the CASFx system

has been used to target splicing of *SMN2* in models of spinal muscular atrophy [346] and regulatory exons of oncogenic SFs in breast cancer models [22].

6. Targeting splicing-derived neo-antigens

Peptides translated from aberrant cancer-associated alternatively spliced RNA isoforms are promising targets for immunotherapies. These cancer-specific so called "neo-antigens" arise from mutations in splice junctions, intron retention, and dysregulation of the splicing machinery leading to cryptic splice products [347]. For example, AS of *CD20* that occurs in B-cell lymphomas produces a T_h-cell response that can selectively kill malignant B-cell clones and vaccination of humanized mice with the corresponding peptide from *CD20* splice variants can produce a robust T-cell response [348]. Large scale analysis of sequencing and proteomic data has uncovered splice variant neo-antigens capable of binding to major histocompatibility complexes (MHC) in over half of tumor samples analyzed [349]. Additionally, studies using long-read RNA-sequencing accurately identified aberrant tumor-specific splice variants that correlated with NMD deficient samples and SF mutations. About half of these splice variants were immunogenic in mice and represented putative splicing-derived neo-antigens [350]. These AS-derived neo-antigens represent a rich source of potential targets for cancer vaccination [347, 351–353].

Potentially more promising is the synergistic effect of splice-modulating drugs and immune checkpoint inhibitors [354]. Therapeutic modulation of AS resulted in the formation of neo-antigens that produced robust tumor immune responses and enhanced response to checkpoint inhibition [354]. Indeed, the amount of tumor mutations, a genomic level source of neo-antigens, does not always correlate with an individual patient's response to immune checkpoint inhibitors [355], and discovery of AS-derived neo-antigens may compliment genomic analysis to determine which patients will respond to immune checkpoint therapy [352].

7. Concluding remarks

Cancer cells exhibit an extensive rewiring of the splicing machinery that induces the aberrant expression pro-oncogenic isoforms driving the hallmarks of cancer. While this dysregulation of AS is favorable for cancer survival, proliferation, and dissemination, it may also be an Achille's heel ripe for therapeutic exploits. Current strategies to target AS in cancer employ anywhere from broad inhibition of core spliceosome components to very specific individual AS events. As these strategies are further developed, we can begin to examine how they synergize with current therapies and interact with immunotherapy. Modulation of AS presents a promising strategy for the development of novel cancer therapeutics.

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Conflict of interest

None.

References

1. Wilkinson ME, Charenton C and Nagai K. (2020) RNA splicing by the spliceosome. *Annual Review of Biochemistry*, **89**, 359–388.
2. Turunen JJ, *et al.* (2013) The significant other: Splicing by the minor spliceosome. *Wiley Interdisciplinary Reviews RNA*, **4**(1), 61–76.
3. Hegele A, *et al*. (2012) Dynamic protein-protein interaction wiring of the human spliceosome. *Molecular Cell*, **45**(4), 567–580.
4. Shi Y. (2017) The spliceosome: A protein-directed metalloribozyme. *Journal of Molecular Biology*, **429**(17), 2640–2653.
5. Bonnal SC, ILópez-Oreja I and Valcárcel J. (2020) Roles and mechanisms of alternative splicing in cancer — Implications for care. *Nature Reviews Clinical Oncology*, **17**(8), 457–474.

6. Akinyi MV and Frilander MJ. (2021) At the intersection of major and minor spliceosomes: Crosstalk mechanisms and their impact on gene expression. *Frontiers in Genetics*, **12**(1311).
7. Padgett RA. (2012) New connections between splicing and human disease. *Trends in Genetics*, **28**(4), 147–154.
8. Fu XD and Maniatis T. (1992) The 35-kDa mammalian splicing factor SC35 mediates specific interactions between U1 and U2 small nuclear ribonucleoprotein particles at the 3′ splice site. *Proceedings of the National Academy of Sciences of the United States of America*, **89**(5), 1725–1729.
9. Inoue A. (2021) RBM10: Structure, functions, and associated diseases. *Gene*, **783**, 145463.
10. Yan C, Wan R and Shi Y. (2019) Molecular mechanisms of pre-mRNA splicing through structural biology of the spliceosome. *Cold Spring Harbor Perspectives in Biology*, **11**(1).
11. Kastner B, *et al*. (2019) Structural insights into nuclear pre-mRNA splicing in higher eukaryotes. *Cold Spring Harbor Perspectives in Biology*, **11**(11).
12. Wong MS, Kinney JB and Krainer AR. (2018) Quantitative activity profile and context dependence of all human 5′ splice sites. *Molecular Cell*, **71**(6), 1012–1026.
13. Roca X, Krainer AR and Eperon IC. (2013) Pick one, but be quick: 5′ splice sites and the problems of too many choices. *Genes & Development*, **27**(2), 129–144.
14. Lee Y and Rio DC. (2015) Mechanisms and regulation of alternative pre-mRNA splicing. *Annual Review of Biochemistry*, **84**, 291–323.
15. Chen M and Manley JL. (2009) Mechanisms of alternative splicing regulation: Insights from molecular and genomics approaches. *Nature Reviews Molecular Cell Biology*, **10**(11), 741–754.
16. Nilsen TW and Graveley BR. (2010) Expansion of the eukaryotic proteome by alternative splicing. *Nature*, **463**(7280), 457–463.
17. Tung KF, *et al.* (2020) Top-ranked expressed gene transcripts of human protein-coding genes investigated with GTEx dataset. *Scientific Reports*, **10**(1), 16245.
18. Celotto AM and Graveley BR. (2001) Alternative splicing of the Drosophila Dscam pre-mRNA is both temporally and spatially regulated. *Genetics*, **159**(2), 599–608.
19. Long JC and Caceres JF. (2009) The SR protein family of splicing factors: Master regulators of gene expression. *Biochemical Journal*, **417**(1), 15–27.
20. Howard JM and Sanford JR. (2015) The RNAissance family: SR proteins as multifaceted regulators of gene expression. *Wiley Interdisciplinary Reviews RNA*, **6**(1), 93–110.
21. Geuens T, Bouhy D and Timmerman V. (2016) The hnRNP family: Insights into their role in health and disease. *Human Genetics*, **135**(8), 851–867.
22. Leclair NK, *et al*. (2020) Poison exon splicing regulates a coordinated network of SR protein expression during differentiation and tumorigenesis. *Molecular Cell*, **80**(4), 648–665.

23. Baralle FE and Giudice J. (2017) Alternative splicing as a regulator of development and tissue identity. *Nature Reviews Molecular Cell Biology*, **18**(7), 437–451.
24. Blech-Hermoni Y and Ladd AN. (2013) RNA binding proteins in the regulation of heart development. *The International Journal of Biochemistry & Cell Biology*, **45**(11), 2467–2478.
25. Colegrove-Otero LJ, Minshall N and Standart N. (2005) RNA-binding proteins in early development. *Critical Reviews in Biochemistry and Molecular Biology*, **40**(1), 21–73.
26. Chen K, Dai X and Wu J. (2015) Alternative splicing: An important mechanism in stem cell biology. *World Journal of Stem Cells*, **7**(1), 1–10.
27. Park JW, *et al*. (2020) Alternative splicing in mesenchymal stem cell differentiation. *Stem Cells*.
28. Urbanski LM, Leclair N and Anczukow O. (2018) Alternative-splicing defects in cancer: Splicing regulators and their downstream targets, guiding the way to novel cancer therapeutics. *Wiley Interdisciplinary Reviews RNA*, **9**(4), e1476.
29. Climente-Gonzalez H, *et al*. (2017) The functional impact of alternative splicing in cancer. *Cell Reports*, **20**(9), 2215–2226.
30. Luco RF, *et al*. (2011) Epigenetics in alternative pre-mRNA splicing. *Cell*, **144**(1), 16–26.
31. Yoshimi A, *et al.* (2019) Coordinated alterations in RNA splicing and epigenetic regulation drive leukaemogenesis. *Nature*, **574**(7777), 273–277.
32. Gimeno-Valiente F, *et al*. (2022) Alternative splicing, epigenetic modifications and cancer: A dangerous triangle, or a hopeful one? *Cancers (Basel)*, **14**(3).
33. Agirre E, *et al.* (2021) Splicing-associated chromatin signatures: A combinatorial and position-dependent role for histone marks in splicing definition. *Nature Communication*, **12**(1), 682.
34. Wong JJ, *et al.* (2014) Epigenetic modifications of splicing factor genes in myelodysplastic syndromes and acute myeloid leukemia. *Cancer Science*, **105**(11), 1457–1463.
35. Papaemmanuil E, *et al*. (2011) Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *The New England Journal of Medicine*, **365**(15), 1384–1395.
36. Yoshida K, *et al.* (2011) Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature*, **478**(7367), 64–69.
37. Anczuków O and Krainer AR. (2016) Splicing-factor alterations in cancers. *RNA*, **22**(9), 1285–1301.
38. Malcovati L, *et al.* (2011) Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Blood*, **118**(24), 6239–6246.
39. Rossi D, *et al*. (2011) Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: Association with progression and fludarabine-refractoriness. *Blood*, **118**(26), 6904–6908.

40. Wang L, *et al*. (2011) SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *The New England Journal of Medicine*, **365**(26), 2497–2506.
41. Zhou Z, *et al*. (2020) The biological function and clinical significance of SF3B1 mutations in cancer. *Biomarker Research*, **8**(1).
42. Biankin AV, *et al*. (2012) Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature*, **491**(7424), 399–405.
43. Maguire SL, *et al*. (2015) SF3B1 mutations constitute a novel therapeutic target in breast cancer. *The Journal of Pathology*, **235**(4), 571–580.
44. Harbour JW, *et al*. (2013) Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. *Nature Genetics*, **45**(2), 133–135.
45. Kong Y, Krauthammer M and Halaban R. (2014) Rare SF3B1 R625 mutations in cutaneous melanoma. *Melanoma Research*, **24**(4), 332–324.
46. Martin M, *et al*. (2013) Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. *Nature Genetics*, **45**(8), 933–936.
47. Robertson AG, *et al.* (2017) Integrative analysis identifies four molecular and clinical subsets in uveal melanoma. *Cancer Cell*, **32**(2), 204–220.
48. Gozani O, Potashkin J and Reed R. (1998) A potential role for U2AF-SAP 155 interactions in recruiting U2 snRNP to the branch site. *Molecular and Cellular Biology*, **18**(8), 4752–4760.
49. Alsafadi S, *et al*. (2016) Cancer-associated SF3B1 mutations affect alternative splicing by promoting alternative branchpoint usage. *Nature Communications*, **7**, 10615.
50. Darman RB, *et al*. (2015) Cancer-associated SF3B1 hotspot mutations induce cryptic 3′ splice site selection through use of a different branch point. *Cell Reports*, **13**(5), 1033–1045.
51. Carrocci TJ, *et al*. (2017) SF3b1 mutations associated with myelodysplastic syndromes alter the fidelity of branchsite selection in yeast. *Nucleic Acids Research*, **45**(8), 4837–4852.
52. Tang Q, *et al.* (2016) SF3B1/Hsh155 HEAT motif mutations affect interaction with the spliceosomal ATPase Prp5, resulting in altered branch site selectivity in pre-mRNA splicing. *Genes & Development*, **30**(24), 2710–2723.
53. Jin S, *et al*. (2017) Splicing factor SF3B1K700E mutant dysregulates erythroid differentiation via aberrant alternative splicing of transcription factor TAL1. *PLoS One*, **12**(5), e0175523.
54. Quesada V, *et al.* (2012) Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nat Genetics*, **44**(1), 47–52.
55. Te Raa GD, *et al*. (2015) The impact of SF3B1 mutations in CLL on the DNA-damage response. *Leukemia*, **29**(5), 1133–1142.
56. Fu XD, *et al*. (1992) General splicing factors SF2 and SC35 have equivalent activities in vitro, and both affect alternative 5′ and 3′ splice site selection. *Proceedings*

of the National Academy of Sciences of the United States of America, **89**(23), 11224–11228.
57. Kim E, *et al.* (2015) SRSF2 mutations contribute to Myelodysplasia by mutant-specific effects on exon recognition. *Cancer Cell*, **27**(5), 617–630.
58. Gallardo M, *et al*. (2015) hnRNP K Is a haploinsufficient tumor suppressor that regulates proliferation and differentiation programs in hematologic malignancies. *Cancer Cell*, **28**(4), 486–499.
59. Aujla A, *et al*. (2018) SRSF2 mutations in myelodysplasia/myeloproliferative neoplasms. *Biomark Research*, **6**, 29.
60. Todisco G, *et al.* (2021) Co-mutation pattern, clonal hierarchy, and clone size concur to determine disease phenotype of SRSF2P95-mutated neoplasms. *Leukemia*, **35**(8), 2371–2381.
61. Liang Y, *et al.* (2018) SRSF2 mutations drive oncogenesis by activating a global program of aberrant alternative splicing in hematopoietic cells. *Leukemia*, **32**(12), 2659–2671.
62. Lee SC, *et al*. (2018) Synthetic lethal and convergent biological effects of cancer-associated spliceosomal gene mutations. *Cancer Cell*, **34**(2), 225–241.
63. Imielinski M, *et al*. (2012) Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. *Cell*, **150**(6), 1107–1120.
64. Wahl MC, Will CL and Luhrmann R. (2009) The spliceosome: Design principles of a dynamic RNP machine. *Cell*, **136**(4), 701–718.
65. Yoshida H, *et al*. (2015) A novel 3′ splice site recognition by the two zinc fingers in the U2AF small subunit. *Genes & Development*, **29**(15), 1649–1660.
66. Kielkopf CL. (2018) Insights from structures of cancer-relevant pre-mRNA splicing factors. *Current Opinion in Genetics & Development*, **48**, 57–66.
67. Okeyo-Owuor T, *et al*. (2015) U2AF1 mutations alter sequence specificity of pre-mRNA binding and splicing. *Leukemia*, **29**(4), 909–917.
68. Ilagan JO, *et al*. (2015) U2AF1mutations alter splice site recognition in hematological malignancies. *Genome Research*, **25**(1), 14–26.
69. Shirai CL, *et al*. (2015) Mutant U2AF1 expression alters hematopoiesis and pre-mRNA splicing in vivo. *Cancer Cell*, **27**(5), 631–643.
70. Taylor J and Lee SC. (2019) Mutations in spliceosome genes and therapeutic opportunities in myeloid malignancies. *Genes Chromosomes Cancer*, **58**(12), 889–902.
71. Wang H, *et al.* (2020) Differential U2AF1 mutation sites, burden and co-mutation genes can predict prognosis in patients with myelodysplastic syndrome. *Scientific Reports*, **10**(1), 18622.
72. Madan V, *et al.* (2015) Aberrant splicing of U12-type introns is the hallmark of ZRSR2 mutant myelodysplastic syndrome. *Nature Communications*, **6**, 6042.
73. Wang X, Song X and Yan X. (2019) Effect of RNA splicing machinery gene mutations on prognosis of patients with MDS: A meta-analysis. *Medicine (Baltimore)*, **98**(21), e15743.

74. Seiler M, *et al*. (2018) Somatic mutational landscape of splicing factor genes and their functional consequences across 33 cancer types. *Cell Reports*, **23**(1), 282–296.
75. Karni R, *et al*. (2007) The gene encoding the splicing factor SF2/ASF is a proto-oncogene. *Nature Structural & Molecular Biology*, **14**(3), 185–193.
76. Sebestyén E, *et al*. (2016) Large-scale analysis of genome and transcriptome alterations in multiple tumors unveils novel cancer-relevant splicing networks. *Genome Research*, **26**(6), 732–744.
77. Anczukow O, *et al*. (2012) The splicing factor SRSF1 regulates apoptosis and proliferation to promote mammary epithelial cell transformation. *Nature Structural & Molecular Biology*, **19**(2), 220–228.
78. Anczukow O, *et al*. (2015) SRSF1-regulated alternative splicing in breast cancer. *Molecular Cell*, **60**(1), 105–117.
79. Das S, *et al*. (2012) Oncogenic splicing factor SRSF1 is a critical transcriptional target of MYC. *Cell Reports*, **1**(2), 110–117.
80. Park S, *et al*. (2019) Differential functions of splicing factors in mammary transformation and breast cancer metastasis. *Cell Reports*, **29**(9), 2672–2688.
81. Ghigna C, *et al*. (2005) Cell motility is controlled by SF2/ASF through alternative splicing of the Ron protooncogene. *Molecular Cell*, **20**(6), 881–890.
82. Sheng J, *et al.* (2017) Bioinformatics analysis of SRSF1-controlled gene networks in colorectal cancer. *Oncol Letters*, **14**(5), 5393–5399.
83. Gautrey HL and Tyson-Capper AJ. (2012) Regulation of Mcl-1 by SRSF1 and SRSF5 in cancer cells. *PLoS One*, **7**(12), e51497.
84. De Miguel FJ, *et al*. (2014) Identification of alternative splicing events regulated by the oncogenic factor SRSF1 in lung cancer. *Cancer Research*, **74**(4), 1105–1115.
85. Du J-X, *et al.* (2021) Splicing factor SRSF1 promotes breast cancer progression via oncogenic splice switching of PTPMT1. *Journal of Experimental & Clinical Cancer Research*, **40**(1).
86. Chen L, *et al*. (2017) SRSF1 prevents DNA damage and promotes tumorigenesis through regulation of DBF4B pre-mRNA splicing. *Cell Reports*, **21**(12), 3406–3413.
87. Martínez-Terroba E, *et al*. (2018) The oncogenic RNA-binding protein SRSF1 regulates LIG1 in non-small cell lung cancer. *Laboratory Investigation*, **98**(12), 1562–1574.
88. Michlewski G, Sanford JR and Caceres JF. (2008) The splicing factor SF2/ASF regulates translation initiation by enhancing phosphorylation of 4E-BP1. *Molecular Cell*, **30**(2), 179–189.
89. Karni R, *et al*. (2008) The splicing-factor oncoprotein SF2/ASF activates mTORC1. *Proceedings of the National Academy of Sciences*, **105**(40), 15323.
90. Ishii H, *et al*. (2014) Epithelial splicing regulatory proteins 1 (ESRP1) and 2 (ESRP2) suppress cancer cell motility via different mechanisms. *Journal of Biological Chemistry*, **289**(40), 27386–27399.

91. Yae T, *et al*. (2012) Alternative splicing of CD44 mRNA by ESRP1 enhances lung colonization of metastatic cancer cell. *Nature Communications*, **3**, 883.
92. Fielding P, *et al.* (1999) Heterogeneous nuclear ribonucleoprotein A2/B1 up-regulation in bronchial lavage specimens: A clinical marker of early lung cancer detection. *Clinical Cancer Research*, **5**(12), 4048–4052.
93. Zhou J, *et al*. (2001) Differential expression of the early lung cancer detection marker, heterogeneous nuclear ribonucleoprotein-A2/B1 (hnRNP-A2/B1) in normal breast and neoplastic breast cancer. *Breast Cancer Research Treatment*, **66**(3), 217–224.
94. Zhou J, *et al.* (2001) Expression of early lung cancer detection marker: hnRNP-A2/B1 and its relation to microsatellite alteration in non-small cell lung cancer. *Lung Cancer*, **34**(3), 341–350.
95. Golan-Gerstl R, *et al*. (2011) Splicing factor hnRNP A2/B1 regulates tumor suppressor gene splicing and is an oncogenic driver in glioblastoma. *Cancer Research*, **71**(13), 4464–4472.
96. Lefave CV, *et al*. (2011) Splicing factor hnRNPH drives an oncogenic splicing switch in gliomas. *The EMBO Journal*, **30**(19), 4084–4097.
97. Jin W, *et al*. (2000) Fibroblast growth factor receptor-1 alpha-exon exclusion and polypyrimidine tract-binding protein in glioblastoma multiforme tumors. *Cancer Research*, **60**(5), 1221–1224.
98. He X, *et al*. (2004) Alternative splicing of the multidrug resistance protein 1/ATP binding cassette transporter subfamily gene in ovarian cancer creates functional splice variants and is associated with increased expression of the splicing factors PTB and SRp20. *Clinical Cancer Research*, **10**(14), 4652–4660.
99. He X, *et al.* (2014) Involvement of polypyrimidine tract-binding protein (PTBP1) in maintaining breast cancer cell growth and malignant properties. *Oncogenesis*, **3**, e84.
100. Li X, *et al*. (2018) PTBP1 promotes tumorigenesis by regulating apoptosis and cell cycle in colon cancer. *Bulletin du Cancer*, **105**(12), 1193–1201.
101. Roychoudhury P and Chaudhuri K. (2007) Evidence for heterogeneous nuclear ribonucleoprotein K overexpression in oral squamous cell carcinoma. *The British Journal of Cancer*, **97**(4), 574–575.
102. Zhou R, *et al*. (2010) Increased expression of the heterogeneous nuclear ribonucleoprotein K in pancreatic cancer and its association with the mutant p53. *International Journal of Cancer*, **126**(2), 395–404.
103. Carpenter B, *et al*. (2006) Heterogeneous nuclear ribonucleoprotein K is over expressed, aberrantly localised and is associated with poor prognosis in colorectal cancer. *The British Journal of Cancer*, **95**(7), 921–927.
104. Mandal M, *et al*. (2001) Growth factors regulate heterogeneous nuclear ribonucleoprotein K expression and function. *Journal of Biological Chemistry*, **276**(13), 9699–9704.

105. Xu Y, *et al.* (2014) Cell type-restricted activity of hnRNPM promotes breast cancer metastasis via regulating alternative splicing. *Genes & Development*, **28**(11), 1191–1203.
106. Passacantilli I, *et al*. (2017) hnRNPM guides an alternative splicing program in response to inhibition of the PI3K/AKT/mTOR pathway in Ewing sarcoma cells. *Nucleic Acids Research*, **45**(21), 12270–12284.
107. Harvey SE, *et al*. (2018) Coregulation of alternative splicing by hnRNPM and ESRP1 during EMT. *RNA*, **24**(10), 1326–1338.
108. Hu X, *et al*. (2020) The RNA-binding protein AKAP8 suppresses tumor metastasis by antagonizing EMT-associated alternative splicing. *Nature Communications*, **11**(1), 486.
109. Zong FY, *et al*. (2014) The RNA-binding protein QKI suppresses cancer-associated aberrant splicing. *PLoS Genetics*, **10**(4), e1004289.
110. Lu W, *et al*. (2014) QKI impairs self-renewal and tumorigenicity of oral cancer cells via repression of SOX2. *Cancer Biology & Therapy*, **15**(9), 1174–1184.
111. Zhao Y, *et al.* (2014) The tumor suppressing effects of QKI-5 in prostate cancer: A novel diagnostic and prognostic protein. *Cancer Biology & Therapy*, **15**(1), 108–118.
112. Venables JP, *et al.* (2013) RBFOX2 is an important regulator of mesenchymal tissue-specific splicing in both normal and cancer tissues. *Molecular and Cellular Biology*, **33**(2), 396–405.
113. Choi S, *et al.* (2019) Rbfox2 dissociation from stress granules suppresses cancer progression. *Experimental & Molecular Medicine*, **51**(4), 1–12.
114. Rintala-Maki ND, *et al*. (2007) Expression of RBM5-related factors in primary breast tissue. *Journal of Cellular Biochemistry*, **100**(6), 1440–1458.
115. Sutherland LC, Wang K and Robinson AG. (2010) RBM5 as a putative tumor suppressor gene for lung cancer. *Journal of Thoracic Oncology*, **5**(3), 294–298.
116. Bechara EG, *et al.* (2013) RBM5, 6, and 10 differentially regulate NUMB alternative splicing to control cancer cell proliferation. *Molecular Cell*, **52**(5), 720–733.
117. Foggetti G, *et al.* (2021) Genetic determinants of EGFR-driven lung cancer growth and therapeutic response in vivo. *Cancer Discovery*, **11**(7), 1736–1753.
118. Witkiewicz AK, *et al*. (2015) Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. *Nature Communications*, **6**, 6744.
119. Hernandez J, *et al*. (2016) Tumor suppressor properties of the splicing regulatory factor RBM10. *RNA Biology*, **13**(4), 466–472.
120. Zhao J, *et al*. (2017) Functional analysis reveals that RBM10 mutations contribute to lung adenocarcinoma pathogenesis by deregulating splicing. *Scientific Reports*, **7**(1), 40488.
121. Hou C, *et al.* (2020) Mutations of GNAQ, GNA11, SF3B1, EIF1AX, PLCB4 and CYSLTR in uveal melanoma in Chinese patients. *Ophthalmic Research*, **63**(3), 358–368.

122. Li C, *et al.* (2020) Somatic SF3B1 hotspot mutation in prolactinomas. *Nature Communications*, **11**(1).
123. Watermann DO, *et al.* (2006) Splicing factor Tra2-beta1 is specifically induced in breast cancer and regulates alternative splicing of the CD44 gene. *Cancer Research*, **66**(9), 4774–4780.
124. Karni R, *et al.* (2008) The splicing-factor oncoprotein SF2/ASF activates mTORC1. *Proceedings of the National Academy of Sciences of the United States of America*, **105**(40), 15323–15327.
125. Iborra S, *et al*. (2013) Alterations in expression pattern of splicing factors in epithelial ovarian cancer and its clinical impact. *International Journal of Gynecological Cancer*, **23**(6), 990–996.
126. Ezponda T, *et al*. (2010) The oncoprotein SF2/ASF promotes non-small cell lung cancer survival by enhancing survivin expression. *Clinical Cancer Research,* **16**(16), 4113–4125.
127. Zhou X, *et al.* (2019) Splicing factor SRSF1 promotes gliomagenesis via oncogenic splice-switching of MYO1B. *Journal of Clinical Investigation*, **129**(2), 676–693.
128. Fischer DC, *et al.* (2004) Expression of splicing factors in human ovarian cancer. *Oncology Reports*, **11**(5), 1085–1090.
129. He X, *et al*. (2011) Knockdown of splicing factor SRp20 causes apoptosis in ovarian cancer cells and its expression is associated with malignancy of epithelial ovarian cancer. *Oncogene*, **30**(3), 356–365.
130. Jia R, *et al.* (2010) SRp20 is a proto-oncogene critical for cell proliferation and tumor induction and maintenance. *International Journal of Biological Sciences*, **6**(7), 806–826.
131. Peiqi L, *et al*. (2016) Expression of SRSF3 is correlated with carcinogenesis and progression of oral squamous cell carcinoma. *International Journal of Medical Sciences*, **13**(7), 533–539.
132. Sen S, Jumaa H and Webster NJ. (2013) Splicing factor SRSF3 is crucial for hepatocyte differentiation and metabolic function. *Nature Communications*, **4**, 1336.
133. Huang CS, *et al.* (2007) Increased expression of SRp40 affecting CD44 splicing is associated with the clinical outcome of lymph node metastasis in human breast cancer. *Clinica Chimica Acta*, **384**(1–2), 69–74.
134. Biselli-Chicote PM, *et al.* (2017) Overexpression of antiangiogenic vascular endothelial growth factor isoform and splicing regulatory factors in oral, laryngeal and pharyngeal squamous cell carcinomas. *Asian Pacific Journal of Cancer Prevention*, **18**(8), 2171–2177.
135. Kim HR, *et al.* (2016) SRSF5: A novel marker for small-cell lung cancer and pleural metastatic cancer. *Lung Cancer*, **99**, 57–65.
136. Cohen-Eliav M, *et al*. (2013) The splicing factor SRSF6 is amplified and is an oncoprotein in lung and colon cancers. *The Journal of Pathology*, **229**(4), 630–639.

137. Jensen MA, Wilkinson JE and Krainer AR. (2014) Splicing factor SRSF6 promotes hyperplasia of sensitized skin. *Nature Structural & Molecular Biology*, **21**(2), 189–197.
138. Zhou X, *et al*. (2014) BCLAF1 and its splicing regulator SRSF10 regulate the tumorigenic potential of colon cancer cells. *Nature Communications*, **5**, 4581.
139. Shkreta L, *et al.* (2016) SRSF10 connects DNA damage to the alternative splicing of transcripts encoding apoptosis, cell-cycle control, and DNA repair factors. *Cell Reports*, **17**(8), 1990–2003.
140. Sohail M, *et al*. (2021) A novel class of inhibitors that target SRSF10 and promote p53-mediated cytotoxicity on human colorectal cancer cells. *NAR Cancer*, **3**(2), zcab019.
141. Kuwano Y, *et al*. (2015) Transformer 2beta and miR-204 regulate apoptosis through competitive binding to 3′ UTR of BCL2 mRNA. *Cell Death & Differentiation*, **22**(5), 815–825.
142. Ajiro M, *et al*. (2016) A genome landscape of SRSF3-regulated splicing events and gene expression in human osteosarcoma U2OS cells. *Nucleic Acids Research*, **44**(4), 1854–1870.
143. Song X, *et al*. (2019) SRSF3-regulated RNA alternative splicing promotes glioblastoma tumorigenicity by affecting multiple cellular processes. *Cancer Research*, **79**(20), 5288–5301.
144. Sen S, *et al.* (2015) Deletion of serine/arginine-rich splicing factor 3 in hepatocytes predisposes to hepatocellular carcinoma in mice. *Hepatology*, **61**(1), 171–183.
145. Wang Z, *et al*. (2012) Exon-centric regulation of pyruvate kinase M alternative splicing via mutually exclusive exons. *Journal of Molecular Cell Biology*, **4**(2), 79–87.
146. Kurokawa K, *et al*. (2014) Downregulation of serine/arginine-rich splicing factor 3 induces G1 cell cycle arrest and apoptosis in colon cancer cells. *Oncogene*, **33**(11), 1407–1417.
147. Jia R, *et al.* (2019) Oncogenic splicing factor SRSF3 regulates ILF3 alternative splicing to promote cancer cell proliferation and transformation. *RNA*, **25**(5), 630–644.
148. Bandopadhayay P, *et al*. (2016) MYB-QKI rearrangements in angiocentric glioma drive tumorigenicity through a tripartite mechanism. *Nature Genetics*, **48**(3), 273–282.
149. Kahles A, *et al.* (2018) Comprehensive analysis of alternative splicing across tumors from 8,705 patients. *Cancer Cell*, **34**(2), 211–224.
150. He C, *et al.* (2009) A global view of cancer-specific transcript variants by subtractive transcriptome-wide analysis. *PLoS One*, **4**(3), e4732.
151. Hanahan D and Weinberg RA. (2011) Hallmarks of cancer: The next generation. *Cell*, **144**(5), 646–674.

152. Mogilevsky M, *et al*. (2018) Modulation of MKNK2 alternative splicing by splice-switching oligonucleotides as a novel approach for glioblastoma treatment. *Nucleic Acids Research*, **46**(21), 11396–11404.
153. Koh CM, *et al.* (2015) MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphomagenesis. *Nature*, **523**(7558), 96–100.
154. Antonarakis ES, *et al*. (2017) Clinical significance of androgen receptor splice variant-7 mRNA detection in circulating tumor cells of men with metastatic castration-resistant prostate cancer treated with first- and second-line abiraterone and enzalutamide. *Journal of Clinical Oncology,* **35**(19), 2149–2156.
155. Chan SC, Li Y and Dehm SM. (2012) Androgen receptor splice variants activate androgen receptor target genes and support aberrant prostate cancer cell growth independent of canonical androgen receptor nuclear localization signal. *Journal of Biological Chemistry*, **287**(23), 19736–19749.
156. Dehm SM, *et al*. (2008) Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Research*, **68**(13), 5469–5477.
157. Hu R, *et al*. (2009) Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Research*, **69**(1), 16–22.
158. Hu R, *et al.* (2012) Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. *Cancer Research*, **72**(14), 3457–3462.
159. Li Y, *et al*. (2011) Intragenic rearrangement and altered RNA splicing of the androgen receptor in a cell-based model of prostate cancer progression. *Cancer Research*, **71**(6), 2108–2117.
160. Liu LL, *et al*. (2014) Mechanisms of the androgen receptor splicing in prostate cancer cells. *Oncogene*, **33**(24), 3140–3150.
161. Yu Z, *et al.* (2014) Rapid induction of androgen receptor splice variants by androgen deprivation in prostate cancer. *Clinical Cancer Research*, **20**(6), 1590–1600.
162. Boise LH, *et al.* (1993) bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell*, **74**(4), 597–608.
163. Dole MG, *et al.* (1995) Bcl-xL is expressed in neuroblastoma cells and modulates chemotherapy-induced apoptosis. *Cancer Research*, **55**(12), 2576–2582.
164. Minn AJ, Boise LH and Thompson CB. (1996) Bcl-x(S) anatagonizes the protective effects of Bcl-x(L). *Journal of Biological Chemistry*, **271**(11), 6306–6312.
165. Wang Y, *et al.* (2014) The splicing factor RBM4 controls apoptosis, proliferation, and migration to suppress tumor progression. *Cancer Cell*, **26**(3), 374–389.
166. Xerri L, *et al.* (1996) Predominant expression of the long isoform of Bcl-x (Bcl-xL) in human lymphomas. *British Journal of Haematology*, **92**(4), 900–906.

167. Li Z, *et al.* (2016) Pro-apoptotic effects of splice-switching oligonucleotides targeting Bcl-x pre-mRNA in human glioma cell lines. *Oncology Reports*, **35**(2), 1013–1019.
168. Tu Y, *et al.* (1998) BCL-X expression in multiple myeloma: Possible indicator of chemoresistance. *Cancer Research*, **58**(2), 256–262.
169. Liu JW, *et al.* (2002) Identification and characterization of Bimgamma, a novel proapoptotic BH3-only splice variant of Bim. *Cancer Research*, **62**(10), 2976–2981.
170. O'Connor L, *et al.* (1998) Bim: A novel member of the Bcl-2 family that promotes apoptosis. *The EMBO Journal*, **17**(2), 384–395.
171. Juan WC, Roca X and Ong ST. (2014) Identification of cis-acting elements and splicing factors involved in the regulation of BIM Pre-mRNA splicing. *PLoS One*, **9**(4), e95210.
172. Isobe K, *et al.* (2016) Association of BIM deletion polymorphism and BIM-gamma RNA expression in NSCLC with EGFR mutation. *Cancer Genomics Proteomics*, **13**(6), 475–482.
173. Ng KP, *et al.* (2012) A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. *Nature Medicine*, **18**(4), 521–528.
174. Faber AC, *et al.* (2011) BIM expression in treatment-naive cancers predicts responsiveness to kinase inhibitors. *Cancer Discovery*, **1**(4), 352–365.
175. Sakamuro D, *et al.* (1996) BIN1 is a novel MYC-interacting protein with features of a tumour suppressor. *Nature Genetics*, **14**(1), 69–77.
176. Ge K, *et al.* (1999) Mechanism for elimination of a tumor suppressor: Aberrant splicing of a brain-specific exon causes loss of function of Bin1 in melanoma. *Proceedings of the National Academy of Sciences of the United States of America*, **96**(17), 9689–9694.
177. Atlasi Y, Mowla SJ and Ziaee SA. (2009) Differential expression of survivin and its splice variants, survivin-DeltaEx3 and survivin-2B, in bladder cancer. *Cancer Detection and Prevention*, **32**(4), 308–313.
178. Ling X, *et al.* (2005) Differential expression of survivin-2B and survivin-DeltaEx3 is inversely associated with disease relapse and patient survival in non-small-cell lung cancer (NSCLC). *Lung Cancer*, **49**(3), 353–361.
179. Mahotka C, *et al.* (1999) Survivin-deltaEx3 and survivin-2B: Two novel splice variants of the apoptosis inhibitor survivin with different antiapoptotic properties. *Cancer Research*, **59**(24), 6097–6102.
180. Ryan B, *et al.* (2005) Expression of survivin and its splice variants survivin-2B and survivin-DeltaEx3 in breast cancer. *The British Journal of Cancer*, **92**(1), 120–124.
181. Wang L, *et al.* (1994) Ich-1, an Ice/ced-3-related gene, encodes both positive and negative regulators of programmed cell death. *Cell*, **78**(5), 739–750.

182. Droin N, *et al.* (2000) Identification of a caspase-2 isoform that behaves as an endogenous inhibitor of the caspase cascade. *Cancer Research*, **60**(24), 7039–7047.
183. Fushimi K, *et al.* (2008) Up-regulation of the proapoptotic caspase 2 splicing isoform by a candidate tumor suppressor, RBM5. *Proceedings of the National Academy of Sciences of the United States of America*, **105**(41), 15708–15713.
184. Wang Y, *et al.* (2008) Cyclin D1b is aberrantly regulated in response to therapeutic challenge and promotes resistance to estrogen antagonists. *Cancer Research*, **68**(14), 5628–5638.
185. Li R, *et al.* (2008) Expression of cyclin D1 splice variants is differentially associated with outcome in non-small cell lung cancer patients. Hum Pathol, **39**(12), 1792–1801.
186. Burd CJ, *et al.* (2006) Cyclin D1b variant influences prostate cancer growth through aberrant androgen receptor regulation. *Proceedings of the National Academy of Sciences of the United States of America*, **103**(7), 2190–2195.
187. Lu F, Gladden AB and Diehl JA. (2003) *An alternatively spliced cyclin D1 isoform, cyclin D1b, is a nuclear oncogene. Cancer Research*, **63**(21), 7056–7061.
188. Millar EK, *et al.* (2009) Cyclin D1b protein expression in breast cancer is independent of cyclin D1a and associated with poor disease outcome. *Oncogene*, **28**(15), 1812–1820.
189. Wang N, *et al.* (2014) Cyclin D1b overexpression inhibits cell proliferation and induces cell apoptosis in cervical cancer cells in vitro and in vivo. *International Journal of Clinical and Experimental Pathology*, **7**(7), 4016–4023.
190. Gaur S, *et al.* (2008) Altered splicing of CEACAM1 in breast cancer: Identification of regulatory sequences that control splicing of CEACAM1 into long or short cytoplasmic domain isoforms. *Molecular Cancer*, **7**, 46.
191. Piekielko-Witkowska A, *et al.* (2010) Disturbed expression of splicing factors in renal cancer affects alternative splicing of apoptosis regulators, oncogenes, and tumor suppressors. *PLoS One*, **5**(10), e13690.
192. Di Modugno F, *et al.* (2007) Molecular cloning of hMena (ENAH) and its splice variant hMena+11a: Epidermal growth factor increases their expression and stimulates hMena+11a phosphorylation in breast cancer cell lines. *Cancer Research*, **67**(6), 2657–2665.
193. Di Modugno F, *et al.* (2012) Splicing program of human MENA produces a previously undescribed isoform associated with invasive, mesenchymal-like breast tumors. *Proceedings of the National Academy of Sciences of the United States of America*, **109**(47), 19280–19285.
194. Di Modugno F, *et al.* (2006) The cytoskeleton regulatory protein hMena (ENAH) is overexpressed in human benign breast lesions with high risk of transformation and human epidermal growth factor receptor-2-positive/hormonal receptor-negative tumors. *Clinical Cancer Research*, **12**(5), 1470–1478.

195. Tanaka N, *et al.* (2014) Relative expression of hMena11a and hMenaINV splice isoforms is a useful biomarker in development and progression of human breast carcinoma. *International Journal of Oncology*, **45**(5), 1921–1928.
196. Oudin MJ, *et al.* (2016) Characterization of the expression of the pro-metastatic Mena(INV) isoform during breast tumor progression. *Clinical & Experimental Metastasis*, **33**(3), 249–261.
197. Philippar U, *et al.* (2008) A Mena invasion isoform potentiates EGF-induced carcinoma cell invasion and metastasis. *Developmental Cell*, **15**(6), 813–828.
198. Bonnal S, *et al.* (2008) RBM5/Luca-15/H37 regulates Fas alternative splice site pairing after exon definition. *Molecular Cell*, **32**(1), 81–95.
199. Cheng J, *et al.* (1994) Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. *Science*, **263**(5154), 1759–1762.
200. Inaba H, *et al.* (1999) mRNA expression of variant Fas molecules in acute leukemia cells. *American Journal of Hematology*, **62**(3), 150–158.
201. Konno R, *et al.* (2000) Serum soluble fas level as a prognostic factor in patients with gynecological malignancies. *Clinical Cancer Research*, **6**(9), 3576–3580.
202. Liu JH, *et al.* (2002) Blockade of Fas-dependent apoptosis by soluble Fas in LGL leukemia. *Blood*, **100**(4), 1449–1453.
203. Nonomura N, *et al.* (2000) Soluble Fas in serum from patients with renal cell carcinoma. *Urology*, **55**(1), 151–155.
204. Papoff G, *et al.* (1996) An N-terminal domain shared by Fas/Apo-1 (CD95) soluble variants prevents cell death in vitro. *Journal of Immunology*, **156**(12), 4622–4630.
205. Danan-Gotthold M, *et al.* (2015) Identification of recurrent regulated alternative splicing events across human solid tumors. *Nucleic Acids Research*, **43**(10), 5130–5144.
206. Law EW, *et al.* (2012) Anti-angiogenic and tumor-suppressive roles of candidate tumor-suppressor gene, Fibulin-2, in nasopharyngeal carcinoma. *Oncogene*, **31**(6), 728–738.
207. Warzecha CC, *et al.* (2009) ESRP1 and ESRP2 are epithelial cell-type-specific regulators of FGFR2 splicing. *Molecular Cell*, **33**(5), 591–601.
208. Matsuda Y, *et al.* (2014) Inhibition of fibroblast growth factor receptor 2 attenuates proliferation and invasion of pancreatic cancer. *Cancer Science*, **105**(9), 1212–1219.
209. Oltean S, *et al.* (2006) Alternative inclusion of fibroblast growth factor receptor 2 exon IIIc in Dunning prostate tumors reveals unexpected epithelial mesenchymal plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, **103**(38), 14116–14121.
210. Ishiwata T, *et al.* (2012) Enhanced expression of fibroblast growth factor receptor 2 IIIc promotes human pancreatic cancer cell proliferation. *The American Journal of Pathology*, **180**(5), 1928–1941.

211. Menzin AW, *et al.* (1998) Identification of oncofetal fibronectin in patients with advanced epithelial ovarian cancer: Detection in ascitic fluid and localization to primary sites and metastatic implants. *Cancer*, **82**(1), 152–158.
212. Tavian D, *et al.* (1994) RT-PCR detection of fibronectin EDA+ and EDB+ mRNA isoforms: Molecular markers for hepatocellular carcinoma. *International Journal of Cancer*, **56**(6), 820–825.
213. Gardina PJ, *et al.* (2006) Alternative splicing and differential gene expression in colon cancer detected by a whole genome exon array. *BMC Genomics*, **7**, 325.
214. Khan ZA, *et al.* (2005) ED-B fibronectin in non-small cell lung carcinoma. *Experimental Lung Research*, **31**(7), 701–711.
215. Castiglioni F, *et al.* (2006) Role of exon-16-deleted HER2 in breast carcinomas. *Endocrine-Related Cancer*, **13**(1), 221–232.
216. Wada R, Yagihashi S and Naito Z. (2016) mRNA expression of delta-HER2 and its clinicopathological correlation in HER2-overexpressing breast cancer. *Molecular Medicine Reports*, **14**(6), 5104–5110.
217. Gautrey H, *et al.* (2015) SRSF3 and hnRNP H1 regulate a splicing hotspot of HER2 in breast cancer cells. *RNA Biology*, **12**(10), 1139–1151.
218. Mitra D, *et al.* (2009) An oncogenic isoform of HER2 associated with locally disseminated breast cancer and trastuzumab resistance. *Molecular Cancer Therapeutics*, **8**(8), 2152–2162.
219. Cohen JB, Broz SD and Levinson AD. (1989) Expression of the H-ras proto-oncogene is controlled by alternative splicing. *Cell*, **58**(3), 461–472.
220. Guil S, Darzynkiewicz E and Bach-Elias M. (2002) Study of the 2719 mutant of the c-H-ras oncogene in a bi-intronic alternative splicing system. *Oncogene*, **21**(36), 5649–5653.
221. Guil S, *et al.* (2003) Alternative splicing of the human proto-oncogene c-H-ras renders a new Ras family protein that trafficks to cytoplasm and nucleus. *Cancer Research*, **63**(17), 5178–5187.
222. Camats M, *et al.* (2009) P19 H-ras induces G1/S phase delay maintaining cells in a reversible quiescence state. *PLoS One*, **4**(12), e8513.
223. Phillips JW, *et al.* (2020) Pathway-guided analysis identifies Myc-dependent alternative pre-mRNA splicing in aggressive prostate cancers. *Proceedings of the National Academy of Sciences of the United States of America*, **117**(10), 5269–5279.
224. Liu F, *et al.* (2018) SRSF10-mediated IL1RAP alternative splicing regulates cervical cancer oncogenesis via mIL1RAP-NF-kappaB-CD47 axis. *Oncogene*, **37**(18), 2394–2409.
225. DiFeo A, *et al.* (2008) A functional role for KLF6-SV1 in lung adenocarcinoma prognosis and chemotherapy response. *Cancer Research*, **68**(4), 965–970.
226. Hartel M, *et al.* (2008) Increased alternative splicing of the KLF6 tumour suppressor gene correlates with prognosis and tumour grade in patients with pancreatic cancer. *European Journal of Cancer*, **44**(13), 1895–1903.

227. Hatami R, *et al.* (2013) KLF6-SV1 drives breast cancer metastasis and is associated with poor survival. *Science Translational Medicine*, **5**(169), 169ra12.
228. Liu X, *et al.* (2012) KLF6 loss of function in human prostate cancer progression is implicated in resistance to androgen deprivation. *The American Journal of Pathology*, **181**(3), 1007–1016.
229. Narla G, *et al.* (2005) Targeted inhibition of the KLF6 splice variant, KLF6 SV1, suppresses prostate cancer cell growth and spread. *Cancer Research*, **65**(13), 5761–5768.
230. Yea S, *et al.* (2008) Ras promotes growth by alternative splicing-mediated inactivation of the KLF6 tumor suppressor in hepatocellular carcinoma. *Gastroenterology*, **134**(5), 1521–1531.
231. Le Gouill S, *et al.* (2004) Mcl-1 regulation and its role in multiple myeloma. *Cell Cycle*, **3**(10), 1259–1262.
232. Mallick, S, *et al.* (2009) Human oral cancers have altered expression of Bcl-2 family members and increased expression of the anti-apoptotic splice variant of Mcl-1. *The Journal of Pathology*, **217**(3), 398–407.
233. Palve V, *et al.* (2014) Overexpression of Mcl-1L splice variant is associated with poor prognosis and chemoresistance in oral cancers. *PLoS One*, **9**(11), e111927.
234. Adesso L, *et al.* (2013) Gemcitabine triggers a pro-survival response in pancreatic cancer cells through activation of the MNK2/eIF4E pathway. *Oncogene*, **32**(23), 2848–2857.
235. Maimon A, *et al.* (2014) Mnk2 alternative splicing modulates the p38-MAPK pathway and impacts Ras-induced transformation. *Cell Reports*, **7**(2), 501–513.
236. Eckerich C, *et al.* (2009) RON receptor tyrosine kinase in human gliomas: Expression, function, and identification of a novel soluble splice variant. *Journal of Neurochemistry*, **109**(4), 969–980.
237. Krishnaswamy S, *et al.* (2015) Recepteur d'Origine nantais (RON) tyrosine kinase splicing variants lacking exons 18 and 19 occur ubiquitously in lung cancer. *International Journal of Clinical and Experimental Medicine*, **8**(11), 20778–20786.
238. Krishnaswamy S, *et al.* (2017) Splice variants of the extracellular region of RON receptor tyrosine kinase in lung cancer cell lines identified by PCR and sequencing. *BMC Cancer*, **17**(1), 738.
239. Mayer S, *et al.* (2015) RON alternative splicing regulation in primary ovarian cancer. *Oncology Reports*, **34**(1), 423–430.
240. Zhou YQ, *et al.* (2003) Altered expression of the RON receptor tyrosine kinase in primary human colorectal adenocarcinomas: Generation of different splicing RON variants and their oncogenic potential. *Oncogene*, **22**(2), 186–197.
241. Lu Y, *et al.* (2015) Alternative splicing of the cell fate determinant Numb in hepatocellular carcinoma. *Hepatology*, **62**(4), 1122–1131.
242. Karaczyn A, *et al.* (2010) Two novel human NUMB isoforms provide a potential link between development and cancer. *Neural Development*, **5**, 31.

243. Christofk HR, *et al.* (2008) The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature*, **452**(7184), 230–233.
244. Clower CV, *et al.* (2010) The alternative splicing repressors hnRNP A1/A2 and PTB influence pyruvate kinase isoform expression and cell metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, **107**(5), 1894–1899.
245. David CJ, *et al.* (2010) HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. *Nature*, **463**(7279), 364–368.
246. Israelsen WJ, *et al.* (2013) PKM2 isoform-specific deletion reveals a differential requirement for pyruvate kinase in tumor cells. *Cell*, **155**(2), 397–409.
247. Lu W, *et al.* (2016) Up-regulation of PKM2 promote malignancy and related to adverse prognostic risk factor in human gallbladder cancer. *Scientific Reports*, **6**, 26351.
248. Shiroki T, *et al.* (2017) Enhanced expression of the M2 isoform of pyruvate kinase is involved in gastric cancer development by regulating cancer-specific metabolism. *Cancer Science*, **108**(5), 931–940.
249. Wang W, *et al.* (2017) Pyruvate kinase M2 deregulation enhances the metastatic potential of tongue squamous cell carcinoma. *Oncotarget*, **8**(40), 68252–68262.
250. Yang Y, *et al.* (2017) Prognostic significance of metabolic enzyme pyruvate kinase M2 in breast cancer: A meta-analysis. *Medicine (Baltimore)*, **96**(46), e8690.
251. Zhu H, *et al.* (2017) Pyruvate kinase M2 (PKM2) expression correlates with prognosis in solid cancers: A meta-analysis. *Oncotarget*, **8**(1), 1628–1640.
252. Singh A, *et al.* (2004) Rac1b, a tumor associated, constitutively active Rac1 splice variant, promotes cellular transformation. *Oncogene*, **23**(58), 9369–9380.
253. Matos P and Jordan P. (2008) Increased Rac1b expression sustains colorectal tumor cell survival. *Molecular Cancer Research*, **6**(7), 1178–1184.
254. Goncalves V, *et al.* (2014) Phosphorylation of SRSF1 by SRPK1 regulates alternative splicing of tumor-related Rac1b in colorectal cells. *RNA*, **20**(4), 474–482.
255. Schnelzer A, *et al.* (2000) Rac1 in human breast cancer: Overexpression, mutation analysis, and characterization of a new isoform, Rac1b. *Oncogene*, **19**(26), 3013–3020.
256. Zhou C, *et al.* (2013) The Rac1 splice form Rac1b promotes K-ras-induced lung tumorigenesis. *Oncogene*, **32**(7), 903–909.
257. Ben-Hur V, *et al.* (2013) S6K1 alternative splicing modulates its oncogenic activity and regulates mTORC1. *Cell Reports*, **3**(1), 103–115.
258. Mei H, *et al.* (2016) Alternative splicing of S6K1 promotes non-small cell lung cancer survival. *Tumor Biology*, **37**(10), 13369–13376.
259. Amaral CL, *et al.* (2016) S6Ks isoforms contribute to viability, migration, docetaxel resistance and tumor formation of prostate cancer cells. *BMC Cancer*, **16**, 602.
260. Ludlow AT, *et al.* (2018) NOVA1 regulates hTERT splicing and cell growth in non-small cell lung cancer. *Nature Communications*, **9**(1), 3112.

261. Yi X, Shay JW and Wright WE. (2001) Quantitation of telomerase components and hTERT mRNA splicing patterns in immortal human cells. *Nucleic Acids Research*, **29**(23), 4818–4825.
262. Listerman I, *et al.* (2013) The major reverse transcriptase-incompetent splice variant of the human telomerase protein inhibits telomerase activity but protects from apoptosis. *Cancer Research*, **73**(9), 2817–2828.
263. Borsi L, *et al.* (1992) Expression of different tenascin isoforms in normal, hyperplastic and neoplastic human breast tissues. *International Journal of Cancer*, **52**(5), 688–692.
264. Hagiwara K, *et al.* (2020) High co-expression of large tenascin C splice variants in stromal tissue and annexin A2 in cancer cell membranes is associated with poor prognosis in pancreatic cancer. *Annals of Surgical Oncology*, **27**(3), 924–930.
265. Bates DO, *et al.* (2002) VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. *Cancer Research*, **62**(14), 4123–4131.
266. Pritchard-Jones RO, *et al.* (2007) Expression of VEGF(xxx)b, the inhibitory isoforms of VEGF, in malignant melanoma. *The British Journal of Cancer*, **97**(2), 223–230.
267. Varey AH, *et al.* (2008) VEGF 165 b, an antiangiogenic VEGF-A isoform, binds and inhibits bevacizumab treatment in experimental colorectal carcinoma: Balance of pro- and antiangiogenic VEGF-A isoforms has implications for therapy. *The British Journal of Cancer*, **98**(8), 1366–1379.
268. Woolard J, *et al.* (2004) VEGF165b, an inhibitory vascular endothelial growth factor splice variant: Mechanism of action, in vivo effect on angiogenesis and endogenous protein expression. *Cancer Research*, **64**(21), 7822–7835.
269. Nakajima H, *et al.* (1996) New antitumor substances, FR901463, FR901464 and FR901465. II. Activities against experimental tumors in mice and mechanism of action. *The Journal of Antibiotics (Tokyo)*, **49**(12), 1204–1211.
270. Thompson CF, Jamison TF and Jacobsen EN. (2001) FR901464: Total synthesis, proof of structure, and evaluation of synthetic analogues. *Journal of the American Chemical Society*, **123**(41), 9974–9983.
271. Kaida D, *et al.* (2007) Spliceostatin A targets SF3b and inhibits both splicing and nuclear retention of pre-mRNA. *Nature Chemical Biology*, **3**(9), 576–583.
272. Osman S, *et al.* (2011) Structural requirements for the antiproliferative activity of pre-mRNA splicing inhibitor FR901464. *Chemistry*, **17**(3), 895–904.
273. Albert BJ, *et al.* (2007) Total syntheses, fragmentation studies, and antitumor/antiproliferative activities of FR901464 and its low picomolar analogue. *Journal of the American Chemical Society*, **129**(9), 2648–2659.
274. Liu X, *et al.* (2013) Genomics-guided discovery of thailanstatins A, B, and C as pre-mRNA splicing inhibitors and antiproliferative agents from Burkholderia thailandensis MSMB43. *Journal of Natural Products*, **76**(4), 685–693.

275. Corrionero A, Minana B and Valcarcel J. (2011) Reduced fidelity of branch point recognition and alternative splicing induced by the anti-tumor drug spliceostatin A. *Genes & Development*, **25**(5), 445–459.
276. Obeng EA, *et al.* (2016) Physiologic expression of Sf3b1(K700E) causes impaired erythropoiesis, aberrant splicing, and sensitivity to therapeutic spliceosome modulation. *Cancer Cell*, **30**(3), 404–417.
277. Seiler, M, *et al.* (2018) H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers. *Nature Medicine*, **24**(4), 497–504.
278. Hong DS, *et al.* (2014) A phase I, open-label, single-arm, dose-escalation study of E7107, a precursor messenger ribonucleic acid (pre-mRNA) splicesome inhibitor administered intravenously on days 1 and 8 every 21 days to patients with solid tumors. *Investigational New Drugs*, **32**(3), 436–444.
279. Eskens FA, *et al.* (2013) Phase I pharmacokinetic and pharmacodynamic study of the first-in-class spliceosome inhibitor E7107 in patients with advanced solid tumors. *Clinical Cancer Research*, **19**(22), 6296–6304.
280. Steensma DP, *et al.* (2021) Phase I first-in-human dose escalation study of the oral SF3B1 modulator H3B-8800 in myeloid neoplasms. *Leukemia*.
281. O'Brien K, *et al.* (2008) The biflavonoid isoginkgetin is a general inhibitor of pre-mRNA splicing. *Journal of Biological Chemistry*, **283**(48), 33147–33154.
282. Vanzyl EJ, *et al.* (2020) The spliceosome inhibitors isoginkgetin and pladienolide B induce ATF3-dependent cell death. *PLoS One*, **15**(12), e0224953.
283. Yoon SO, *et al.* (2006) Isoginkgetin inhibits tumor cell invasion by regulating phosphatidylinositol 3-kinase/Akt-dependent matrix metalloproteinase-9 expression. *Molecular Cancer Therapeutics*, **5**(11), 2666–2675.
284. Darrigrand R, *et al.* (2021) Isoginkgetin derivative IP2 enhances the adaptive immune response against tumor antigens. *Communications Biology*, **4**(1), 269.
285. Tari M, *et al.* (2019) U2AF(65) assemblies drive sequence-specific splice site recognition. *EMBO Reports*, **20**(8), e47604.
286. Stepanyuk GA, *et al.* (2016) UHM-ULM interactions in the RBM39-U2AF65 splicing-factor complex. *Acta Crystallographica Section D: Structural Biology*, **72**(Pt 4), 497–511.
287. Loerch S, *et al.* (2014) Cancer-relevant splicing factor CAPERalpha engages the essential splicing factor SF3b155 in a specific ternary complex. *Journal of Biological Chemistry*, **289**(25), 17325–17337.
288. Kralovicova J, *et al.* (2018) PUF60-activated exons uncover altered 3′ splice-site selection by germline missense mutations in a single RRM. *Nucleic Acids Research*, **46**(12), 6166–6187.
289. Mai S, *et al.* (2016) Global regulation of alternative RNA splicing by the SR-rich protein RBM39. *Biochimica et Biophysica Acta*, **1859**(8), 1014–1024.
290. Xu Y, Nijhuis A and Keun HC. (2020) RNA-binding motif protein 39 (RBM39): An emerging cancer target. *British Journal of Pharmacology*.

291. Han T, *et al.* (2017) Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. *Science*, **356**(6336).
292. Ting TC, *et al.* (2019) Aryl sulfonamides degrade RBM39 and RBM23 by recruitment to CRL4-DCAF15. *Cell Reports*, **29**(6), 1499–1510 e6.
293. Bussiere DE, *et al.* (2020) Structural basis of indisulam-mediated RBM39 recruitment to DCAF15 E3 ligase complex. *Nature Chemical Biology*, **16**(1), 15–23.
294. Hamid O, *et al.* (2014) A randomized, open-label clinical trial of tasisulam sodium versus paclitaxel as second-line treatment in patients with metastatic melanoma. *Cancer*, **120**(13), 2016–2024.
295. Bezzi M, *et al.* (2013) Regulation of constitutive and alternative splicing by PRMT5 reveals a role for Mdm4 pre-mRNA in sensing defects in the spliceosomal machinery. *Genes & Development*, **27**(17), 1903–1916.
296. Radzisheuskaya A, *et al.* (2019) PRMT5 methylome profiling uncovers a direct link to splicing regulation in acute myeloid leukemia. *Nature Structural & Molecular Biology*, **26**(11), 999–1012.
297. Tarighat SS, *et al.* (2016) The dual epigenetic role of PRMT5 in acute myeloid leukemia: Gene activation and repression via histone arginine methylation. *Leukemia*, **30**(4), 789–799.
298. Jansson M, *et al.* (2008) Arginine methylation regulates the p53 response. *Nature Cell Biology*, **10**(12), 1431–1439.
299. Kim H and Ronai ZA. (2020) PRMT5 function and targeting in cancer. *Cell Stress*, **4**(8), 199–215.
300. Chan-Penebre E, *et al.* (2015) A selective inhibitor of PRMT5 with in vivo and in vitro potency in MCL models. *Nature Chemical Biology*, **11**(6), 432–437.
301. Gammons MV, *et al.* (2013) Topical antiangiogenic SRPK1 inhibitors reduce choroidal neovascularization in rodent models of exudative AMD. *Investigative Ophthalmology & Visual Science*, **54**(9), 6052–6062.
302. Hatcher JM, *et al.* (2018) SRPKIN-1: A covalent SRPK1/2 inhibitor that potently converts VEGF from pro-angiogenic to anti-angiogenic isoform. *Cell Chemical Biology*, **25**(4), 460–470 e6.
303. Batson J, *et al.* (2017) Development of potent, selective SRPK1 inhibitors as potential topical therapeutics for neovascular eye disease. *ACS Chemical Biology*, **12**(3), 825–832.
304. Sakuma M, Iida K and Hagiwara M. (2015) Deciphering targeting rules of splicing modulator compounds: Case of TG003. *BMC Molecular Biology*, **16**, 16.
305. Babu N, *et al.* (2020) Phosphoproteomic analysis identifies CLK1 as a novel therapeutic target in gastric cancer. *Gastric Cancer*, **23**(5), 796–810.
306. Uzor S, *et al.* (2021) CDC2-like (CLK) protein kinase inhibition as a novel targeted therapeutic strategy in prostate cancer. *Scientific Reports*, **11**(1), 7963.
307. Havens MA and Hastings ML. (2016) Splice-switching antisense oligonucleotides as therapeutic drugs. *Nucleic Acids Research*, **44**(14), 6549–6563.

308. Bennett CF and Swayze EE. (2010) RNA targeting therapeutics: Molecular mechanisms of antisense oligonucleotides as a therapeutic platform. *Annual Review of Pharmacology and Toxicology*, **50**, 259–293.
309. Crooke ST, *et al.* (2017) Cellular uptake and trafficking of antisense oligonucleotides. *Nature Biotechnology*, **35**(3), 230–237.
310. Chiriboga CA, *et al.* (2016) Results from a phase 1 study of nusinersen (ISIS-SMN(Rx)) in children with spinal muscular atrophy. *Neurology*, **86**(10), 890–897.
311. Juliano RL. (2016) The delivery of therapeutic oligonucleotides. *Nucleic Acids Research*, **44**(14), 6518–6548.
312. Inoue D, *et al.* (2019) Spliceosomal disruption of the non-canonical BAF complex in cancer. *Nature*, **574**(7778), 432–436.
313. Sun Y, *et al.* (2019) Downregulation of SRSF3 by antisense oligonucleotides sensitizes oral squamous cell carcinoma and breast cancer cells to paclitaxel treatment. *Cancer Chemotherapy and Pharmacology*, **84**(5), 1133–1143.
314. Kralovicova J, *et al.* (2016) Antisense oligonucleotides modulating activation of a nonsense-mediated RNA decay switch exon in the ATM gene. *Nucleic Acid Therapeutics*, **26**(6), 392–400.
315. Kralovicova J, *et al.* (2016) Exon-centric regulation of ATM expression is population-dependent and amenable to antisense modification by pseudoexon targeting. *Scientific Reports*, **6**, 18741.
316. Bauman JA, *et al.* (2010) Anti-tumor activity of splice-switching oligonucleotides. *Nucleic Acids Research*, **38**(22), 8348–8356.
317. Mercatante DR, *et al.* (2001) Modification of alternative splicing of Bcl-x pre-mRNA in prostate and breast cancer cells. Analysis of apoptosis and cell death. *Journal of Biological Chemistry*, **276**(19), 16411–16417.
318. Taylor JK, *et al.* (1999) Induction of endogenous Bcl-xS through the control of Bcl-x pre-mRNA splicing by antisense oligonucleotides. *Nature Biotechnology*, **17**(11), 1097–1100.
319. Liu J, *et al.* (2017) Overcoming imatinib resistance conferred by the BIM deletion polymorphism in chronic myeloid leukemia with splice-switching antisense oligonucleotides. *Oncotarget*, **8**(44), 77567–77585.
320. Anczuków O, *et al.* (2012) BRCA2 deep intronic mutation causing activation of a cryptic exon: Opening toward a new preventive therapeutic strategy. *Clinical Cancer Research*, **18**(18), 4903–4909.
321. Nielsen TO, *et al.* (2013) Directing HER4 mRNA expression towards the CYT2 isoform by antisense oligonucleotide decreases growth of breast cancer cells in vitro and in vivo. *The British Journal of Cancer*, **108**(11), 2291–2298.
322. Li L, *et al.* (2020) Targeting the ERG oncogene with splice-switching oligonucleotides as a novel therapeutic strategy in prostate cancer. *The British Journal of Cancer*, **123**(6), 1024–1032.
323. Fong JY, *et al.* (2019) Therapeutic targeting of RNA splicing catalysis through inhibition of protein arginine methylation. *Cancer Cell*, **36**(2), 194–209 e9.

324. Bruno IG, Jin W and Cote GJ. (2004) Correction of aberrant FGFR1 alternative RNA splicing through targeting of intronic regulatory elements. *Human Molecular Genetics*, **13**(20), 2409–2420.
325. Lin J, *et al.* (2017) Induced-decay of glycine decarboxylase transcripts as an anti-cancer therapeutic strategy for non-small-cell lung carcinoma. *Molecular Therapy — Nucleic Acids*, **9**, 263–273.
326. Karras JG, *et al.* (2000) Antisense inhibition of membrane-bound human interleukin-5 receptor-alpha chain does not affect soluble receptor expression and induces apoptosis in TF-1 cells. *Antisense Nucleic Acid Drug Development*, **10**(5), 347–357.
327. Shieh JJ, *et al.* (2009) Modification of alternative splicing of Mcl-1 pre-mRNA using antisense morpholino oligonucleotides induces apoptosis in basal cell carcinoma cells. *Journal of Investigative Dermatology*, **129**(10), 2497–2506.
328. Shiraishi T, Eysturskarth J and Nielsen PE. (2010) Modulation of mdm2 pre-mRNA splicing by 9-aminoacridine-PNA (peptide nucleic acid) conjugates targeting intron-exon junctions. *BMC Cancer*, **10**, 342.
329. Dewaele M, *et al.* (2016) Antisense oligonucleotide-mediated MDM4 exon 6 skipping impairs tumor growth. *Journal of Clinical Investigation*, **126**(1), 68–84.
330. Ghigna C, *et al.* (2010) Pro-metastatic splicing of Ron proto-oncogene mRNA can be reversed: Therapeutic potential of bifunctional oligonucleotides and indole derivatives. *RNA Biology*, **7**(4), 495–503.
331. Zammarchi F, *et al.* (2011) Antitumorigenic potential of STAT3 alternative splicing modulation. *Proceedings of the National Academy of Sciences of the United States of America*, **108**(43), 17779–17784.
332. Tano V, Jans DA and Bogoyevitch MA. (2019) Oligonucleotide-directed STAT3 alternative splicing switch drives anti-tumorigenic outcomes in MCF10 human breast cancer cells. *Biochemical and Biophysical Research Communications*, **513**(4), 1076–1082.
333. Izaguirre DI, *et al.* (2012) PTBP1-dependent regulation of USP5 alternative RNA splicing plays a role in glioblastoma tumorigenesis. *Molecular Carcinogenesis*, **51**(11), 895–906.
334. Denichenko P, *et al.* (2019) Specific inhibition of splicing factor activity by decoy RNA oligonucleotides. *Nature Communications*, **10**(1), 1590.
335. Gadgil A and Raczynska KD. (2021) U7 snRNA: A tool for gene therapy. *The Journal of Gene Medicine*, **23**(4), e3321.
336. Rogalska ME, *et al.* (2016) Therapeutic activity of modified U1 core spliceosomal particles. *Nature Communications*, **7**, 11168.
337. Fernandez Alanis E, *et al.* (2012) An exon-specific U1 small nuclear RNA (snRNA) strategy to correct splicing defects. *Human Molecular Genetics*, **21**(11), 2389–2398.
338. Dal Mas A, *et al.* (2015) Improvement of SMN2 pre-mRNA processing mediated by exon-specific U1 small nuclear RNA. *The American Journal of Human Genetics*, **96**(1), 93–103.

339. Dal Mas A, *et al.* (2015) Exon-specific U1s correct SPINK5 Exon 11 skipping caused by a synonymous substitution that affects a bifunctional splicing regulatory element. *Human Mutation*, **36**(5), 504–512.
340. Wang Y, *et al.* (2009) Engineering splicing factors with designed specificities. *Nature Methods*, **6**(11), 825–830.
341. O'Connell MR, *et al.* (2014) Programmable RNA recognition and cleavage by CRISPR/Cas9. *Nature*, **516**(7530), 263–266.
342. Abudayyeh OO, *et al.* (2017) RNA targeting with CRISPR-Cas13. *Nature*, **550**(7675), 280–284.
343. Konermann S, *et al.* (2018) Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors. *Cell*, **173**(3), 665–676 e14.
344. Cox DBT, *et al.* (2017) RNA editing with CRISPR-Cas13. *Science*, **358**(6366), 1019–1027.
345. Yang LZ, *et al.* (2019) Dynamic imaging of RNA in living cells by CRISPR-Cas13 systems. *Molecular Cell*, **76**(6), 981–997 e7.
346. Du M, *et al.* (2020) CRISPR artificial splicing factors. *Nature Communications*, **11**(1), 2973.
347. Smith CC, *et al.* (2019) Alternative tumour-specific antigens. *Nature Reviews Cancer*, **19**(8), 465–478.
348. Vauchy C, *et al.* (2015) CD20 alternative splicing isoform generates immunogenic CD4 helper T epitopes. *International Journal of Cancer*, **137**(1), 116–126.
349. Kahles A, *et al.* (2018) Comprehensive analysis of alternative splicing across tumors from 8,705 patients. *Cancer Cell*, **34**(2), 211–224 e6.
350. Oka M, *et al.* (2021) Aberrant splicing isoforms detected by full-length transcriptome sequencing as transcripts of potential neoantigens in non-small cell lung cancer. *Genome Biology*, **22**(1), 9.
351. Slansky JE and Spellman PT. (2019) Alternative splicing in tumors — A path to immunogenicity? *The New England Journal of Medicine*, **380**(9), 877–880.
352. Frankiw L, Baltimore D, and Li G. (2019) Alternative mRNA splicing in cancer immunotherapy. *Nature Reviews Immunology*, **19**(11), 675–687.
353. Hoyos LE and Abdel-Wahab O. (2018) Cancer-specific splicing changes and the potential for splicing-derived neoantigens. *Cancer Cell*, **34**(2), 181–183.
354. Lu SX, *et al.* (2021) Pharmacologic modulation of RNA splicing enhances anti-tumor immunity. *Cell*, **184**(15), 4032–4047 e31.
355. Snyder A, *et al.* (2014) Genetic basis for clinical response to CTLA-4 blockade in melanoma. *The New England Journal of Medicine*, **371**(23), 2189–2199.

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Chapter 3

Alternative 3′-RNA Processing in Cancer

Chioniso Patience Masamha

Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Butler University, 4600 Sunset Avenue, Indianapolis, Indiana 46208

Abstract

With the exception of histone coding transcripts, the formation of polyadenylated 3′ends through 3′end cleavage and polyadenylation is a crucial maturation step for all mammalian pre-mRNAs. This facilitates nucleocytoplasmic translocation and translation of the mRNA to protein. Formation of different 3′ends through alternative polyadenylation (APA) gives rise to diverse mRNA transcripts. There are different forms of APA, some involving alternative splicing of exons, and in some cases, inclusion of intronic sequences. The most common form of APA involves changes in the length and content of the 3′untranslated region (3′UTR) with no effect on the protein coding sequence (3′UTR APA). Different lengths of the 3′UTR may affect transcript localization, function, and metabolism. 3′UTR APA occurs during organismal development and in different physiological and pathological processes. Hence, 3′UTR APA is an evolutionarily conserved, comprehensive form of

Corresponding author's email: cmasamha@butler.edu

post-transcriptional gene regulation. Dysregulation of 3′UTR APA has been detected during cellular transformation, cancer progression, and metastasis. This chapter is a review of our current understanding of 3′UTR APA in cancer as well as its potential implications in cancer prognosis.

1. Introduction

After transcription, most eukaryotic precursor mRNAs (pre-mRNAs) undergo capping at the 5′end, splicing together of exons, and 3′end cleavage and polyadenylation. In mammalian cells, 3′end formation is directed by several *cis*-elements found within the 3′UTR of pre-mRNAs (Fig. 1). The core elements include the polyadenylation signal (PAS), the cleavage site, and a U/GU rich downstream sequence element [1]. The formation of the 3′end is mediated by the core 3′end processing machinery which consists of four multi-subunit complexes: the cleavage and polyadenylation specificity factors (CPSFs), the cleavage stimulation factors (CstF), and the mammalian cleavage factors I (CFIm) and II (CFIIm) [1]. CPSFs

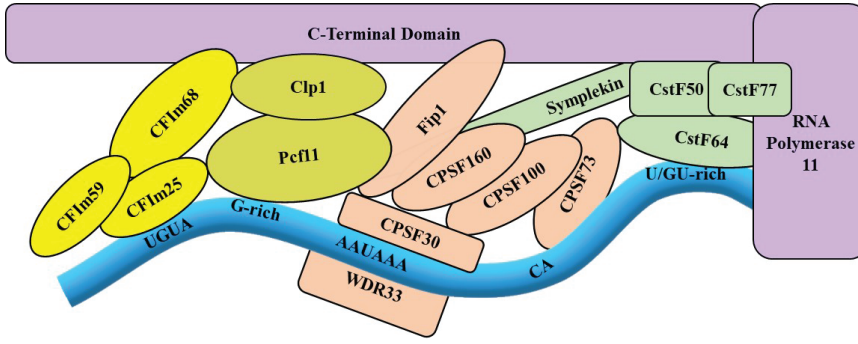

Figure 1. Cis-acting sequence elements and 3′end processing factors involved in 3′end formation.

Shown are the main cis-sequence elements within the pre-mRNA and the different proteins that are known to bind to the different sequences. Abbreviations: CFIm25, CFIm59, and CFIm68: CFIm-mammalian cleavage factor 1; Pcf11, Clip1: CFIIm-mammalian cleavage factor 11; CPSF30, CPSF73, CPSF100, CPSF160, WDR33, and Fip1: CPSF-cleavage and Polyadenylation Specificity Factors; CstF50, CstF64/ CstF64tau, and CstF77 with Symplekin: CstF-Cleavage Stimulation Factor.

consist of the CPSF30, CPSF73 (*CPSF3*), CPSF100 (*CPSF2*), CPSF160 (*CPSF1*), and FIP1 (*CPSF4*) subunits. The CstF complex consists of the CstF50 (*CSTF1*), CstF77 (*CSTF3*), CstF64 (*CSTF2*) and its paralog CstF64tau (*CSTF2T*). In the CFIm complex, CFIm25 (*NUDT21*) forms a complex with either the CFIm59 or the CFIm68 subunits. The CFIIm complex consists of PCF11 and CLP1. Another 3′end processing factor is symplekin, a large protein that forms a scaffold for different proteins. Some of these 3′end processing factors bind directly to *cis*-elements within the pre-mRNA. Binding of CFIm25 to UGUA sequences is believed to be one of the earliest steps in 3′end formation and may promote the recruitment and assembly of the rest of the 3′end processing machinery [2–4]. The CstF64 subunit binds to the U/GU rich downstream sequence element on the pre-mRNA. CPSF30 and WDR33 bind to the canonical PAS hexameric sequence (AAUAAA), with CPS160 (which was previously believed to bind to the polyadenylation signal) acting as a scaffold [5, 6]. Recruitment of the rest of the 3′end processing factors brings the pre-mRNA cleavage site into close proximity with the endoribonuclease CPSF73 (which forms a heterodimer with CPSF100), an endoribonuclease, which cleaves the pre-mRNA 15-30 nucleotides downstream of the PAS. Optimal cleavage occurs after the adenosine of the CA dinucleotide. This is followed by the addition of untemplated Adenines by poly(A) polymerase to generate the poly(A) tail [1, 7, 8]. There are other proteins and factors that have also been co-purified with the core members of the 3′end processing complex. These proteins are involved in other cellular processes including splicing and transcription [9]. Polyadenylation of the 3′end enables the recruitment of poly(A) binding proteins which protect the mRNA from degradation and facilitate nuclear to cytoplasmic shuttling and translation [1, 7].

Since the PAS plays a major role in 3′end processing, there has been a lot of focus on identifying the PAS used for different gene transcripts. In almost three quarters of the annotated genome, the PAS used consists of the hexameric A(A/U)UAAA sequence [1]. Up to 70% of human genes contain multiple PASs [10–12]. Hence 3′end cleavage and polyadenylation can occur at multiple sites, through a process called alternative polyadenylation (APA). This greatly increases the diversity of the transcriptome.

2. Types of alternative polyadenylation (APA)

There are several different types of APA. In some cases, APA involves alternative splicing (splicing APA) where the PAS used is within the terminal spliced exon of each transcript. This gives rise to different protein products with different C-terminal amino acids (Fig. 2). APA may also involve the use of an internal cryptic PAS (iPAS) found within introns (intronic APA), giving rise to truncated protein products. The most common type of APA occurs within the same terminal exon (3′UTR APA), resulting in transcripts that contain the same open reading frame but with 3′UTRs of different length and sequence content. The transcripts result in the same protein product [13].

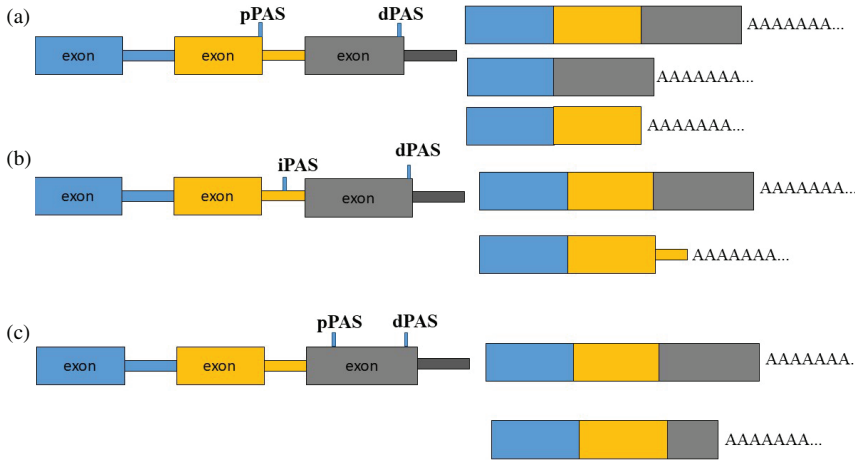

Figure 2. Different types of alternative polyadenylation (APA).

(a) Splicing APA. Alternative 3′ends resulting from exon skipping and hence alternative splicing using distinct polyadenylation signals within each exon. (b) Intronic APA. The formation of alternative 3′ends may involve use of cryptic polyadenylation signals within an intron (iPAS). (c) 3′UTR APA/Tandem exon APA. The generation of alternative 3′ends depends on the use of different polyadenylation signals within the same terminal exon. Abbreviations: pPAS: proximal polyadenylation signal; iPAS: intronic polyadenylation signal; dPAS: distal polyadenylation signal.

2.1. *Models for 3′UTR APA*

In the simplest scenario, selection of a PAS more proximal to the stop codon (pPAS) results in a shortened 3′UTR while use of a more distal PAS (dPAS) results in a longer 3′UTR. What is currently unclear is the mechanism involved in the selection of either the pPAS or dPAS for 3′end formation during 3′UTR APA (Fig. 3). To explain this, a number of different models have been put forth. These include the effects of sequence motifs

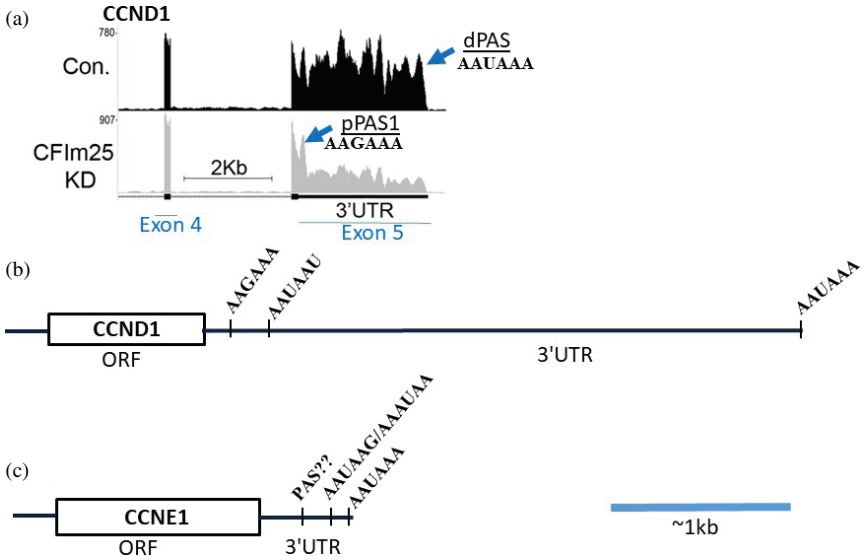

Figure 3. 3′UTR APA/Tandem Exon APA of select oncogenes.

(a) *CCND1* RNA Sequencing (RNA Seq.) data for exons 4 and 5 showing the read density and switch from using the dPAS after control siRNA (Con.) to using the pPAS after CFIm25 siRNA (CFIm25 KD) in HeLa cells. (b) A schematic showing the three PAS signals within *CCND1*′s 3′UTR. The *CCND1* full length 3′UTR is ~3,261 nt long with the ORF ~888 nt long. (Fig. 3a and 3b adapted from Masamha *et al*. [25]). (c) A schematic showing the known PAS, and sites and possible sequences of two potential unannotated PASs within *CCNE1*′s 3′UTR. The *CCNE1* ORF is ~with the full length 3′UTR ~535 nucleotides long. (Fig. 3c is adapted from Xue *et al*. [19]). Abbreviations: pPAS: proximal polyadenylation signal; dPAS: distal polyadenylation signal; ORF: open reading frame; PAS: polyadenylation signal.

around the PAS, the "first come, first served" model where the first transcribed PAS is used and the agonist/antagonist model is based on differential modulation by protein factors interacting with the pre-mRNA [7, 11, 14, 15]. Another model is based on observations that the dPAS frequently consists of the optimal canonical hexamer (AAUAAA) and the pPAS often consists of "weaker" variants of the sequence [16–18]. The implication of this trend is that the default choice is to utilize the canonical dPAS, giving rise to transcripts with longer 3′UTRs. While the model of having two PASs, a pPAS and a dPAS, has been used to tease out 3′UTR APA events, genes may have more than two PASs, resulting in complex 3′UTR length changes [19].

2.2. *The extensive occurrence of 3′UTR APA in animal biology*

Although 3′UTR APA has been documented for years for individual genes, it is only recently that it has gained stature as a widespread mechanism of gene regulation involving changes in the 3′UTR usage of multiple genes on a global scale. Across mammalian species there are more similarities in tissue specific 3′UTR lengths than there are within the same species. In general, the same PAS is used for a given gene in the same tissue type regardless of species. In contrast, different PASs are used for the same gene within tissues from the same species [12, 20]. Unique "APA signatures" have been reported during different developmental stages of *C. elegans* and zebrafish [16, 21]. There are global changes in the length of the 3′UTR during spermatogenesis in mice, in stem cell biology, as well as during rapid cellular proliferation [22–25]. In addition, recent studies show that changes in the 3′UTR length can be induced by environmental stress including hypoxia and cold/heat shock [26–30]. Hence, characteristic changes in the 3′UTR can be used as signatures for numerous cellular processes.

3. Potential functional consequences of 3′UTR APA

The size of the 3′UTR affects different aspects of mRNA metabolism including stability and translatability, as well as localization [31–34].

The 3′UTR acts as a loading dock for regulatory elements including RNA binding proteins (RBPs) and microRNAs (miRNAs) [35, 36]. Changes in the length and, hence, the sequence content of the 3′UTR due to 3′UTR APA may have different effects on different transcripts.

First, differences in 3′UTR length can affect subcellular localization and allow for spatial functionalization. In the brain, global APA results in the generation of isoforms with different subcellular localization [37]. Transcripts with longer 3′UTRs localized to neurites within the same cell at a higher rate than those with shorter 3′UTRs [38]. The spatial separation of distinct 3´UTR transcripts enables differential site-specific translation and, hence, protein synthesis [38]. A significant number of differentially localized mRNAs contain non-coding *cis* elements that facilitate localization of the transcript [37]. For some transmembrane proteins, RBPs bind to some of these *cis* elements found within the long 3′UTR transcripts mediating their translocation to the cell membrane, allowing for increased cell surface expression [32].

In addition to these localization elements, mRNAs have many other binding motifs that are targets for RBPs [39, 40]. RBPs bind to specific sequence elements within the 3′UTR and alter the stability of the bound mRNAs [41]. In addition to RBPs, the 3′UTR is also the binding site for miRNAs [42]. Binding by these miRNAs targets transcripts for degradation and/or translational inhibition [43, 44]. Interestingly, an estimated 75% of all the miRNA binding sites within the 3′UTR in one cell line were also bound by at least one RBP, suggesting that there is extensive interplay between miRNAs and RBPs and their regulation of 3′UTRs [45]. Hence, binding of the 3′UTR by miRNAs and different RBPs can affect transcript stability.

In addition to having RBP binding sites, the 3′UTR is also the binding site of other factors, including polysomes, thus affecting translation. In the brain, *BDNF* isoforms with long 3′UTRs are located in higher density polyribosome fractions compared to isoforms with shorter 3′UTRs. This result in higher translational efficiency of *BDNF* transcripts with longer 3′UTRs compared to those with shorter 3′UTRs [46]. A different study looking at multiple transcripts found that in six out of seven human cell lines, mRNAs with shorter 3′UTRs had higher polyribosome occupancies, resulting in increased translational efficiency. This may have been due to effects of miRNA regulation [47].

In conclusion, the actual impact of 3′UTR changes on individual transcripts may be transcript dependent. In the following section we will discuss what is known about the molecular impact of these effects in cancer.

3.1. *Molecular impact of 3′UTR APA changes on oncogenes*

An estimated 60% of human genes have predicted miRNA binding sites within their 3′UTR [36]. In the case of several oncogenes, shortening of the 3′UTRs results in increased mRNA stability and mRNA transcript half-life. Truncation of the 3′UTR eliminates miRNA binding sites located within the longer 3′UTR of these genes. This includes the oncogenes *CCND1* and *IMP-1* [48]. Shortening of the 3′UTR also resulted in increased protein levels of several oncogenes including *GLS* and *CCND1*, followed by increased cell proliferation [25]. Although these studies show that shortening of the 3′UTRs of select oncogenes results in increased mRNA stability and oncogene expression due to elimination of miRNA binding sites, more widespread studies have shown that 3′UTR shortening only mechanistically affects mRNA stability of a small subset of transcripts [49]. In contrast to *CCND1*, another cell cycle oncogene *CCNE1* undergoes 3′UTR lengthening in several tumor types [19]. The full length 3′UTR of *CCND1* is six times longer than the full length 3′UTR of *CCNE1* which is only ~535 nucleotides long (Fig. 3). Thus, there are major differences in the length and sequence content between the two 3′UTRs, and APA changes may affect the transcripts of these oncogenes very differently.

3.2. *Molecular impact of 3′UTR APA changes on tumor suppressors*

In contrast to oncogenes, shortening of the 3′UTR and loss of some miRNA target sites on anti-proliferative gene transcripts results in increased binding efficiency of miRNAs to the remaining miRNA binding sites located upstream of the pPAS. This follows the model that shortening

of the 3′UTR brings some miRNA binding sites which were located far from the 3′end in the longer 3′UTR closer to the end. The relocation of the miRNAs closer to the edge of the shorter transcript could potentially make them more functional and susceptible to miRNA binding. This is followed by decreased mRNA stability of the anti-proliferative gene transcripts including those involved in differentiation in cancer, ultimately facilitating increased cell proliferation [50]. Thus, the impact of 3′UTR shortening and loss of miRNA binding sites within the longer 3′UTR transcript may have different effects on the transcript depending on whether it is an oncogene or a tumor suppressor gene.

Another potential impact of 3′UTR shortening on tumor suppressor activity is more indirect and involves 3′UTRs of different genes that compete for the same miRNAs and, hence, act as competing endogenous RNAs (ceRNAs). In this case, shortening of the 3′UTR of a ceRNA transcript frees up miRNAs which then bind to the tumor suppressor in *trans*, resulting in mRNA degradation and repression of the tumor suppressor. Analysis of RNA Sequencing (RNA Seq.) data of ER+ breast cancer tumors identified 427 genes with recurrent 3′UTR shortening. One of those genes, *ESP15*, was identified as a ceRNA transcript and underwent 3′UTR shortening in about a third of tumors analyzed. Truncation of the *ESP15* 3′UTR freed up miRNAs that were then available to bind to and suppress the 3′UTR of the tumor suppressor *PTEN* [51]. Two other genes, *DNMT3B* and *TET3* have also been identified as ceRNAs for *PTEN* [52]. Other tumor suppressors that could also be potentially affected by this mechanism include *LARP1* and *PHF6* [51]. There are other ceRNAs whose 3′UTRs alter the availability of miRNAs that can bind the 3′UTR of target genes and may have prognostic potential in cancer. An example of a ceRNA is the FOXF1 Adjacent Noncoding Developmental Regulatory RNA (*FENDRR*) long-non-coding RNA (lncRNA) which is downregulated in patients with lung cancer and is associated with decreased overall survival of patients [53].

To counteract tumorigenicity, the traditional expectation is that shortening of the 3′UTR of anti-cancer genes and loss of miRNA binding sites will result in increased expression of tumor suppressors [54]. The gene *MGMT* is involved in chemotherapy induced DNA repair and antagonizes the efficacy of anti-cancer drugs. *MGMT* mRNAs with longer 3′UTRs are

subject to miRNA binding and regulation which results in decreased protein expression. This enhances the tumor's sensitivity to temazolamide treatment in patients with glioblastoma [55]. Thus, changes in the 3′UTR length can potentially affect a patient's response to cancer therapy.

4. Naturally occurring global 3′UTR APA patterns in cancer and their physiological implications

APA changes in tumors can potentially act as prognostic biomarkers. One of the earliest studies of genome wide 3′UTR changes in cancer was used to molecularly classify clinically indistinguishable tumor subtypes [56]. Several studies of naturally occurring 3′UTR APA events in cancer have involved the analysis of microarray data. A major limitation of microarray data for APA is the limited number of probes and number of genes that can be analyzed for APA events. RNA Seq., as well as targeted 3′UTR RNA Seq. data, is being increasingly used to identify global APA patterns in different types of cancer.

When the RNA Seq. data from seven different tumor types was analyzed, 1,346 genes were shown to change their 3′UTR length through APA. The majority of these events (91%) involved tumor-specific shortening of the 3′UTR through 3′UTR APA [57]. A different study analyzing tumor and normal samples from TCGA identified a total of 77 APA events across 13 different cancer types [19]. In that same cohort, 45 events were determined as being complex, with no clearly defined 3′UTR lengthening or shortening [19]. Specific changes in APA and their prognostic implications in specific tumor types are described in the following sections.

4.1. *Breast cancer*

A lot of work has been done to study 3′UTR APA in breast cancer. A training set using six breast cancer data samples identified a prognostic 3′UTR APA signature, which when applied to 251 primary breast tumors, was able to predict disease free survival [58]. The breast cancer subtype triple negative breast cancer (TNBC) which does not express the estrogen

receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor (HER2/neu), makes up 15–20 % of all breast cancers [59]. Analysis of microarray data from the NCBI Gene Expression Omnibus repository of 520 TNBC patient samples and 82 normal samples uncovered 113 mRNAs with significantly shortened 3′UTRs. There were 52 cases of 3′UTR lengthening. The higher incidence of shortened 3′UTRs of *SNX3*, *YME1L1*, and *USP9X* was validated in TNBC cell lines by 3′RACE and qRT-PCR. Furthermore, high short:long ratios for these three genes was associated with poor prognosis in patients with TNBC. High levels of truncated 3′UTRs for *TOP2A* and *SNX3* correlated with high tumor grade [60]. In another study, analysis of 164 TNBC microarray samples identified a set of genes whose shortened 3′UTRs could be used to predict lymph node metastasis with potential for use in tumor staging [61]. Furthermore, a different analysis of 327 TNBC microarray data samples identified a pattern of genes with truncated 3′UTRs which correlated with tumor-recurrence free survival [62].

A major issue with TNBC is its clinical and pathological heterogeneity [59]. This limits treatment options, and better classification and stratification of different subtypes of TNBC is needed for targeted therapies. This includes clinical subtyping at the molecular level. Analysis of mRNA from 165 TNBC patient samples with 33 normal matched pair samples by microarray identified 1,118 genes with truncated 3′UTRs and 513 genes with lengthened 3′UTRs. Furthermore, analysis of the 3′UTR APA events resulted in the identification of four TNBC subtypes based on the 3′UTR length. The subtypes included the luminal androgen subtype (LAR), which expresses the androgen receptor (AR) and is characterized by the activation of the AR hormone signaling pathway. Also identified using this method was a mesenchymal-like immune activated subtype, which included immune system associated genes as well as genes involved in epithelial to mesenchymal transition and differentiation. The third subtype identified based on 3′UTR length was the basal-like subtype, which had basal like signatures involving select enrichment in genes involved in the cell cycle and cell division. The final subtype, suppressed subtype, showed significant downregulation of several archetypal TNBC pathways including cell death, cell growth, and stem cell like genes. The suppressed TNBC subtype was associated with the worst disease-free survival of all

the other subtypes [63]. However, there was poor correlation between 3′UTR APA events from the TNBC patient microarray samples and those from RNA Seq. data from the TNBC cell line MDA MB231. This suggests that what is seen in cell lines may differ from what is happening in patient tumors [63].

Analysis of 3′UTR APA patterns can also be used to differentiate between TNBC and other types of breast cancer. A comparison of TNBC (520) patient cancer microarray data with ER+ (209) patient tumor microarrays showed that TBNC had higher short:long 3′UTR ratios for *SNX3*, *USP9X*, and *YME1L1* transcripts than ER+ breast cancers [60].

4.2. *Colorectal cancer*

An initial analysis of targeted 3′UTR sequencing (3′Seq.) data from 15 patients with colorectal carcinoma (CRC) that was validated in 50 patients (with five matched normal pairs) identified 3′UTR APA changes during tumor progression. Widespread 3′UTR shortening was observed as tumors progressed from normal mucosa to adenoma (286 genes), from normal mucosa to carcinoma (304 genes), and colorectal adenoma to carcinoma (322 genes). Validation of the 3′UTR shortening by qRT-PCR was done for *PPIE and DNMK* during transformation from normal to adenoma. Additional qRT-PCR validation of 3′UTR lengthening was done for *PDXK* transcripts during CRC progression from adenoma to carcinoma [64]. Hence, 3′UTR APA changes can potentially be used as biomarkers for cancer progression.

4.3. *Pancreatic cancer*

Venkat *et al.* analyzed RNA Seq. data from 148 pancreatic ductal carcinoma (PDAC) tumors and 184 normal pancreata. There were 266 significant 3′UTR shortening events which included several genes known to promote PDAC proliferation, survival, and migration. This was compared to 186 3′UTR lengthening events. The APA events were recurrent with over 90% of patient samples expressing 68 gene transcripts with truncated 3′UTRs while 28 genes had lengthened 3′UTRs. The authors were also able to link 3′UTR shortening with poor prognosis in a group of PDAC

patients that was independent of any currently known clinical subtypes of PDAC [54]. The RNA Seq. data was validated by 3′RACE of *ALDOA*, *ENO1*, *TRIP10*, *FLNA*, *PPP2R2D*, *SAT1*, *PAF1*, and casein kinase (*CSNK1A1*/CK1 alpha) in both PDAC cell lines and patient tumor samples [54].

4.4. *Lung cancer*

A study used three lung cancer training data sets to develop and test a prognostic 3′UTR signature in lung cancer. This was then used to predict prognosis in 442 lung adenocarcinoma patient samples and shortening of the 3′UTR of select genes was associated with decreased survival [58]. A different analysis of RNA Seq. data from 26 matched non-small cell lung cancer (NSCLC) and normal adjacent tissue, identified APA changes in 25 patient samples. A total of 1,855 genes had shortened 3′UTRs while 58 genes had longer 3′UTRs in NSCLC samples [65]. Hence in this case, 3′UTR APA changes were used to differentiate between tumor and non-tumor samples.

5. RNA-Seq data and APA

A lot of what is currently known about global APA changes is from short-read second generation sequencing data. Several bioinformatics tools have been developed to determine APA from standard RNA Seq. data [66, 67]. However, short-read RNA Seq. data often has lower sequencing read coverage of longer 3´UTRs, making accurate and extensive PAS mapping challenging. This has resulted in the development and use of several 3′end enriched RNA Seq. techniques, most of which involve oligo dT-priming to increase read coverage at the 3′end by selectively targeting polyadenylated transcripts [66]. More recently, third generation sequencing technology involving sequencing of long-reads is being used to detect APA. One method in particular, Iso Sequencing by Pacific Biosciences (PacBio Iso Seq.), allows the sequencing of full-length transcripts without the need for transcript reassembly as required for short-read data. Since the poly(A) tail is included in the full-length read, this enables direct identification of PASs [67]. There are several databases that have been developed to map

PAS and hence determine alternative polyadenylation. These include the poly(A)-DB3 and APAlyzer [68, 69] developed by the Tian lab, APADB [70], and APAatlas [71].

6. Drivers of 3′UTR APA in cancer

6.1. *Cleavage and polyadenylation factors as drivers of 3′UTR APA in cancer*

A number of proteins including 3′end processing factors, splicing factors, and other RBPs have been identified as regulators of 3′UTR APA [18, 72–76]. As a result, there has been a lot of interest in determining the role of these proteins, particularly 3′end processing factors as regulators of 3′UTR APA in cancer [48, 56, 57]. A study of 23 core cleavage and polyadenylation factors from a TBNC tumor microarray data set identified 12 factors that were more highly expressed in TNBC tissue compared to normal adjacent tissue. High levels of FIP1 (*CPSF4*) mRNA were associated with the highest number (18% or >500) of APA events [63]. In colorectal cancer, PCF11 and PABPN1 were downregulated in colon cancer tissue while CPSF 100 (*CPSF3*), CstF64 (*CSTF2*), and CFIm25 (*NUDT2)* were upregulated [77]. However, high levels of mRNAs do not necessarily translate to high protein levels. In addition, there been few systematic studies on the combined effects of different levels of these factors in cancer and their functional dominance in determining APA patterns. In neuroblastoma, PCF11 levels could be more dominant in determining APA than other 3′end processing factors [78]. Thus, to study the mechanistic impact of these proteins on 3′UTR APA, a common approach has been the knockdown of specific 3′end factors in cancer cell lines to demonstrate changes in APA patterns.

6.1.1. *Mammalian cleavage factors I and II (CFIm/CFIIm)*

NUDT21 codes for CFIm25, which binds as a homodimer to two UGUA sequence elements within the 3′UTR and forms a complex with the

CFIm59/CFIm68 subunits. Depletion of CFIm25 in HeLa cells (a cervical cancer cell line) followed by RNA. Seq. resulted in the 3′UTR shortening of 1,453 genes, including the oncogenes *CCND1* and *GLS*. This was companied by increased cell proliferation, increased cell migration, and colony formation. Analysis of glioblastoma RNA Seq. data of patients with glioblastoma whose tumors expressed low levels of CFIm25, identified 59 genes with truncated 3′UTRs, with 24 of those genes overlapping with the genes identified in HeLa cells. In a mouse xenograft model of glioblastoma, knockdown of CFIm25 in the glioblastoma cell line LN229 that overexpresses CFIm25 was associated with increased tumor volume, while overexpression of CFIm25 in U251 that has low levels of CFIm25 was associated with decreased tumor volume [25]. A subsequent study of TGGA RNA Seq. data showed that all high-grade glioma (glioblastoma) subtypes had low CFIm25 levels. Depletion of CFIm25 in LN229 cells resulted in 350 genes with truncated 3′UTRs and increased 3′UTR lengthening of 24 genes. *PAK2*, which has oncogenic roles in cancer, was the top gene candidate with the highest level of 3′UTR shortening [79]. A similar reduction in CFIm25 mRNA levels (TCGA dataset) and protein levels was detected in hepatocellular carcinoma (HCC). Knockdown of CFIm25 in HepG2 and Bel7402 HCC cell lines resulted in shortening of the 3′UTRs of select genes and increased tumor cell growth and tumorigenicity [80].

In contrast to CFIm25, depletion of PCF1, which belongs to the CFIIm complex, had the opposite effect, favoring lengthening of the 3′UTR of genes in neuroblastoma mouse cells as well as tissues from a transgenic PCF11 inducible knockout model. Furthermore, lower levels of PCF11 were associated with increased survival in patients with neuroblastoma [78].

6.1.2. *CPSFs*

Knockdown of CPSF160 (*CPSF1*) using shRNA in TNBC cell lines MDA MB 231 and MDA MB 468, resulted in decreased cell proliferation and increased apoptosis. RNA Seq. analysis of MDA MB 231 cells after *CPSF1* depletion resulted in 3′UTR shortening of 243 gene transcripts and increased 3′UTR lengthening of 336 genes [63].

6.1.3. *CstFs*

Transcriptome analysis identified CstF64 (*CSTF2*) as a 3′end processing factor with highly upregulated expression in 26 NSCLC tumor samples, and high CstF64 protein levels were detected in six NSCLC cell lines. Depletion of CstF64 by siRNA in the NSCLC cell line H460 followed by RNA Seq. identified several 3′UTR APA events and there was a 38 gene overlap when this was compared to a 100 paired TCGA NSCLC sample data set [65]. In a different study, treatment of a TNBC cell line MDA MB468 and an immortalized control MCF10A triple negative cell line with epidermal growth factor (EGF) resulted in increased levels of CstF64 mRNA and protein, as well as increased shortening of the 3′UTRs of *SNX3, USP9X*, *and YME1L1*. Direct silencing of CstF64 using siRNA resulted in decreased cell proliferation [60].

6.2. *Other factors*

Besides, 3′end processing factors, other proteins have been implicated in 3′UTR APA in cancer [81]. PABPN1 depletion in TNBC cell lines MDA MB 231 and MDA MB 468 resulted in decreased cell proliferation and enhanced apoptosis. RNA Seq. data analysis after shRNA knockdown of *PABPN1* in MDA MB 231 was accompanied by global 3′UTR shortening of 858 genes and 3′UTR lengthening of 321 genes [63].

7. Concluding remarks

3′UTR APA patterns, as well as subsets of genes that undergo recurrent changes in tumors, have the potential to be used as biomarkers for disease detection and may have prognostic potential in cancer. Determining the molecular mechanisms that drive 3′UTR APA changes may result in the identification of potential therapeutic targets.

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Frequently used abbreviations

APA	alternative polyadenylation
CFm	mammalian cleavage factor
CPSF	cleavage and polyadenylation specificity factor
CstF	cleavage stimulation factors
dPAS	distal polyadenylation signal
PAS	polyadenylation signal
pPAS	proximal polyadenylation signal
PABPN1	poly Adenylate-binding protein nuclear 1
RBPs	RNA binding proteins
RNA Seq.	RNA Sequencing
TCGA	The Cancer Genome Atlas
TNBC	Triple negative breast cancer
3′UTR	3′untranslated region

References

1. Mandel C, Bai Y and Tong L. (2008) Protein factors in pre-mRNA 3′-end processing. *Cell Molecular Life Science*, **65**(7–8), 1099–1122.
2. Rüegsegger U, Beyer K and Keller W. (1996) Purification and characterization of human cleavage factor I involved in the 3′ end processing of messenger RNA precursors. *Journal of Biological Chemistry*, **271**(11), 6107–6113.
3. Rüegsegger U, Blank D and Keller W. (1998) Human pre-mRNA cleavage factor Im is related to spliceosomal SR proteins and can be reconstituted in vitro from recombinant subunits. *Molecular Cell*, **1**(2), 243–253.
4. Kim H and Lee Y. (2001) Interaction of poly(A) polymerase with the 25-kDa subunit of cleavage factor I. *Biochemical and Biophysical Research Communications*, **289**(2), 513–518.
5. Clerici M, *et al.* (2018) Structural basis of AAUAAA polyadenylation signal recognition by the human CPSF complex. *Nature Structural & Molecular Biology*, **25**(2), 135–138.
6. Sun Y, *et al.* (2018) Molecular basis for the recognition of the human AAUAAA polyadenylation signal. *Proceedings of the National Academy of Sciences*, **115**(7), E1419.
7. Danckwardt S, Hentze M, and Kulozik A. (2008) 3′ end mRNA processing: Molecular mechanisms and implications for health and disease. *The EMBO Journal*, **27**(3), 482–498.
8. Millevoi S and Vagner S. Molecular mechanisms of eukaryotic pre-mRNA 3′ end processing regulation. *Nucleic Acids Research*, **38**(9), 2757–2774.

9. Shi Y, *et al.* (2009) Molecular architecture of the human pre-mRNA 3′ processing complex. *Molecular cell*, **33**(3), 365–376.
10. Tian B, *et al.* (2005) A large-scale analysis of mRNA polyadenylation of human and mouse genes. *Nucleic Acids Research*, **33**(1), 201–212.
11. Shi, Y. (2012) Alternative polyadenylation: New insights from global analyses. *RNA*, **18**(12), 2105–2117.
12. Derti A, *et al.* (2012) A quantitative atlas of polyadenylation in five mammals. *Genome Research*, **22**(6), 1173–1183.
13. Turner RE, Pattison AD and Beilharz TH. (2018) Alternative polyadenylation in the regulation and dysregulation of gene expression. *Semin Cell Developmental Biology*, **75**, 61–69.
14. Denome RM and Cole CN. (1988) Patterns of polyadenylation site selection in gene constructs containing multiple polyadenylation signals. *Molecular and Cellular Biology*, **8**(11), 4829–4839.
15. Wang R, *et al.* (2018) A compendium of conserved cleavage and polyadenylation events in mammalian genes. *Genome Research*, **28**(10), 1427–1441.
16. Mangone M, *et al.* The landscape of C. elegans 3′UTRs. *Science*, **329**(5990), 432–435.
17. Lackford B, *et al.* (2014) Fip1 regulates mRNA alternative polyadenylation to promote stem cell self-renewal. *The EMBO Journal*, **33**(8), 878–889.
18. Yao C, *et al.* (2012) Transcriptome-wide analyses of CstF64-RNA interactions in global regulation of mRNA alternative polyadenylation. *Proceedings of the National Academy of Sciences of USA*, **109**(46), 18773–18778.
19. Xue Z, *et al.* (2018) Recurrent tumor-specific regulation of alternative polyadenylation of cancer-related genes. *BMC Genomics*, **19**(1), 536.
20. Lianoglou S, *et al.* (2013) Ubiquitously transcribed genes use alternative polyadenylation to achieve tissue-specific expression. *Genes & Development*, **27**(21), 2380–2396.
21. Ulitsky I, *et al.* (2012) Extensive alternative polyadenylation during zebrafish development. *Genome Research*, **22**(10), 2054–2066.
22. Li W, *et al.* (2016) Alternative cleavage and polyadenylation in spermatogenesis connects chromatin regulation with post-transcriptional control. *BMC Biology*, **14**(1), 6.
23. Ji Z and Tian B. (2009) Reprogramming of 3′ untranslated regions of mRNAs by alternative polyadenylation in generation of pluripotent stem cells from different cell types. *PLOS ONE*, **4**(12), e8419.
24. Huang Y, *et al.* (2016) Specific Tandem 3′UTR patterns and gene expression profiles in mouse Thy1+ germline stem cells. *PLOS ONE*, **10**(12), e0145417.
25. Masamha CP, *et al.* (2014) CFIm25 links alternative polyadenylation to glioblastoma tumour suppression. *Nature*, **510**(7505), 412–416.
26. Yoon OK and Brem RB. (2010) Noncanonical transcript forms in yeast and their regulation during environmental stress. *RNA*, **16**(6), 1256–1267.

27. de Lorenzo L, *et al.* (2017) Noncanonical alternative polyadenylation contributes to gene regulation in response to hypoxia. *The Plant Cell*, **29**(6), 1262–1277.
28. Hollerer I, *et al.* (2016) The differential expression of alternatively polyadenylated transcripts is a common stress-induced response mechanism that modulates mammalian mRNA expression in a quantitative and qualitative fashion. *RNA*, **22**(9), 1441–1453.
29. Kraynik SM, *et al.* (2015) The stress-induced heat shock protein 70.3 expression is regulated by a dual-component mechanism involving alternative polyadenylation and HuR. *Biochimica et Biophysica Acta (BBA) — Gene Regulatory Mechanisms*, **1849**(6), 688–696.
30. Liu Y, *et al.* (2013) Cold-induced RNA-binding proteins regulate circadian gene expression by controlling alternative polyadenylation. *Scientific Reports*, **3**, 2054.
31. Chekulaeva M, Hentze MW and phrussi A. (2006) Bruno acts as a dual repressor of oskar translation, promoting mRNA oligomerization and formation of silencing particles. *Cell*, **124**(3), 521–533.
32. Berkovits BD and Mayr C. (2015) Alternative 3[prime] UTRs act as scaffolds to regulate membrane protein localization. *Nature*, **522**(7556), 363–367.
33. An JJ, *et al.* (2008) Distinct role of long 3′UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell*, **134**(1), 175–187.
34. Gruber AR, *et al.* (2014) Global 3′ UTR shortening has a limited effect on protein abundance in proliferating T cells. *Nature Communications*, **5**.
35. Mayr C. (2017) Regulation by 3′-untranslated regions. *Annual Review of Genetics*, **51**(1), 171–194.
36. Friedman RC, *et al.* (2009) Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research*, **19**(1), 92–105.
37. Corral-Debrinski M. (2007) mRNA specific subcellular localization represents a crucial step for fine-tuning of gene expression in mammalian cells. *Biochimica et Biophysica Acta (BBA) — Molecular Cell Research*, **1773**(4), 473–475.
38. Taliaferro JM, *et al.* (2016) Distal alternative last exons localize mRNAs to neural projections. *Molecular Cell*, **61**(6), 821–833.
39. Ray D, *et al.* (2013) A compendium of RNA-binding motifs for decoding gene regulation. *Nature*, **499**, 172.
40. Dominguez D, *et al.* (2018) Sequence, structure, and context preferences of human RNA binding proteins. *Molecular Cell*, **70**(5), 854–867.e9.
41. Barreau C, Paillard L and Osborne HB. (2006) AU-rich elements and associated factors: Are there unifying principles? *Nucleic Acids Research*, **33**(22), 7138–7150.
42. Bartel, DP. (2009) MicroRNAs: Target recognition and regulatory functions. *Cell*, **136**(2), 215–233.
43. Pushparaj PN, *et al.* (2008) siRNA, miRNA, and shRNA: In vivo applications. *Journal of Dental Research*, **87**(11), 992–1003.
44. Siomi H and Siomi M. Posttranscriptional regulation of microRNA biogenesis in animals. *Molecular Cell*. **38**(3), 323–332.

45. Plass M, Rasmussen SH and Krogh A. (2017) Highly accessible AU-rich regions in 3′ untranslated regions are hotspots for binding of regulatory factors. *PLoS Computational Biology*, **13**(4), e1005460–e1005460.
46. Lau AG, *et al.* (2010) Distinct 3′UTRs differentially regulate activity-dependent translation of brain-derived neurotrophic factor (BDNF). *Proceedings of the National Academy of Sciences*, **107**(36), 15945–15950.
47. Fu Y, *et al.* (2018) Crosstalk between alternative polyadenylation and miRNAs in the regulation of protein translational efficiency. *Genome Research*, **28**(11), 1656–1663.
48. Mayr C and Bartel DP. (2009) Widespread shortening of 3′UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. *Cell*, **138**(4), 673–684.
49. Nam J-W, *et al.* (2014) Global analyses of the effect of different cellular contexts on microRNA targeting. *Molecular Cell*, **53**(6), 1031–1043.
50. Hoffman Y, *et al.* (2016) 3′UTR shortening potentiates microRNA-based repression of pro-differentiation genes in proliferating human cells. *PLOS Genetics*, **12**(2), e1005879.
51. Park HJ, *et al.* (2018) 3′ UTR shortening represses tumor-suppressor genes in trans by disrupting ceRNA crosstalk. *Nature Genetics*, **50**(6), 783–789.
52. Roquid KAR, Alcantara KMM, and Garcia RL. (2020), Identification and validation of mRNA 3′untranslated regions of DNMT3B and TET3 as novel competing endogenous RNAs of the tumor suppressor PTEN. *International Journal of Oncology*, **56**(2), 544–558.
53. Liu Y, *et al.* (2019) Integrated analysis reveals five potential ceRNA biomarkers in human lung adenocarcinoma. *PeerJ*, **7**, e6694.
54. Venkat S, *et al.* (2020) Alternative polyadenylation drives oncogenic gene expression in pancreatic ductal adenocarcinoma. *Genome Research*, **30**(3), 347–360.
55. Kreth S, *et al.* (2013) In human glioblastomas transcript elongation by alternative polyadenylation and miRNA targeting is a potent mechanism of MGMT silencing. *Acta Neuropathologica*, **125**(5), 671–681.
56. Singh P, *et al.* (2009) Global changes in processing of mRNA 3′ untranslated regions characterize clinically distinct cancer subtypes. *Cancer Research*, **69**(24), 9422–9430.
57. Xia Z, *et al.* (2014) Dynamic analyses of alternative polyadenylation from RNA-seq reveal a 3′-UTR landscape across seven tumour types. *Nature Communication*, **5**, 5274.
58. Lembo A, Di Cunto F and Provero P. (2012) Shortening of 3′UTRs correlates with poor prognosis in breast and lung cancer. *PLOS ONE*, **7**(2), e31129.
59. Metzger-Filho O, *et al.* (2012) *Dissecting the heterogeneity of triple-negative breast cancer. Journal of Clinical Oncology*, **30**(15), 1879–1887.
60. Akman HB, *et al.* (2015) 3′UTR shortening and EGF signaling: Implications for breast cancer. *Human Molecular Genetics*, **24**(24), 6910–6920.

61. Wang, L, *et al.* (2019) Integrative 3′ untranslated region-based model to identify patients with low risk of axillary lymph node metastasis in operable triple-negative breast cancer. *The Oncologist*, **24**(1), 22–30.
62. Wang L, *et al.* (2016) The 3′UTR signature defines a highly metastatic subgroup of triple-negative breast cancer. *Oncotarget*, **7**(37).
63. Wang L, *et al.* (2020) Dissecting the heterogeneity of the alternative polyadenylation profiles in triple-negative breast cancers. *Theranostics*, **10**(23), 10531–10547.
64. Morris AR, *et al.* (2012) Alternative cleavage and polyadenylation during colorectal cancer development. *Clinical Cancer Research*, **18**(19), 5256–5266.
65. Zhang S, *et al.* (2019) Genome-wide profiling reveals alternative polyadenylation of mRNA in human non-small cell lung cancer. *Journal of Translational Medicine*, **17**(1), 257.
66. Chen W, *et al.* (2017) Alternative polyadenylation: Methods, findings, and impacts. *Genomics, Proteomics & Bioinformatics*, **15**(5), 287–300.
67. Shah A, *et al.* (2021) Benchmarking sequencing methods and tools that facilitate the study of alternative polyadenylation. *Genome Biology*, **22**(1), 291.
68. Wang R and Tian B. (2020) APAlyzer: A bioinformatics package for analysis of alternative polyadenylation isoforms. *Bioinformatics*, **36**(12), 3907–3909.
69. Wang R, *et al.* (2017) PolyA_DB 3 catalogs cleavage and polyadenylation sites identified by deep sequencing in multiple genomes. *Nucleic Acids Research*, **46**(D1), D315–D319.
70. Müller S, *et al.* (2014) APADB: A database for alternative polyadenylation and microRNA regulation events. *Database (Oxford)*, **2014**.
71. Hong W, *et al.* (2020) APAatlas: Decoding alternative polyadenylation across human tissues. *Nucleic Acids Research*, **48**(D1), D34–D39.
72. Gruber AR, *et al.* (2012) Cleavage factor Im is a key regulator of 3′ UTR length. *RNA Biology*, **9**(12), 1405–1412.
73. Li W, *et al.* (2015) Systematic profiling of poly(A)+ transcripts modulated by core 3′ end processing and splicing factors reveals regulatory rules of alternative cleavage and polyadenylation. *PLOS Genetics*, **11**(4), e1005166.
74. Berg MG, *et al.* (2012) U1 snRNP determines mRNA length and regulates isoform expression. *Cell*, **150**(1), 53–64.
75. Bava F-A, *et al.* (2013) CPEB1 coordinates alternative 3[prime]-UTR formation with translational regulation. *Nature*, **495**(7439), 121–125.
76. Jenal M, *et al.* (2012) The poly(A)-binding protein nuclear 1 suppresses alternative cleavage and polyadenylation sites. *Cell*, **149**(3), 538–553.
77. Mao Z, *et al.* (2020) Post-transcriptional dysregulation of microRNA and alternative polyadenylation in colorectal cancer. *Frontiers in Genetics*, **11**(64).
78. Ogorodnikov A, *et al.* (2018) Transcriptome 3′ end organization by PCF11 links alternative polyadenylation to formation and neuronal differentiation of neuroblastoma. *Nature Communications*, **9**(1), 5331.

79. Chu Y, *et al.* (2019) Nudt21 regulates the alternative polyadenylation of Pak1 and is predictive in the prognosis of glioblastoma patients. *Oncogene*, **38**(21), 4154–4168.
80. Sun M, *et al.* (2017) NUDT21 regulates 3′-UTR length and microRNA-mediated gene silencing in hepatocellular carcinoma. *Cancer Letters*, **410**, 158–168.
81. Masamha CP and Wagner EJ. (2018) The contribution of alternative polyadenylation to the cancer phenotype. *Carcinogenesis*, **39**(1), 2–10.

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Chapter 4

Tandem CCCH Zinc Finger RNA-Binding Proteins and Their Roles in Tumor Progression

Megan B. Stemberger*, Nina C. Lee*, and Gerald M. Wilson[#]

Department of Biochemistry and Molecular Biology and Marlene and Stewart Greenebaum NCI Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Abstract

The steady-state level of every mRNA expressed by a cell is governed by its rates of synthesis and degradation. Synthetic rates principally reflect the rate of transcription, but in select examples, are also influenced by the efficiency and specificity of pre-mRNA processing and/or nucleocytoplasmic transport. Cumulatively, these mechanisms function to deliver processed mRNA molecules to the cytoplasm where they may serve as templates for synthesis of nascent proteins. However, the longevity of mRNAs in the cytoplasm is also very tightly regulated. Some mRNAs are extremely stable, remaining in the cytoplasm for many

*both authors contributed equally

[#]Corresponding author's email: gwilson@som.umaryland.edu

hours or even days, while others are degraded within minutes, barely enough time for a few rounds of translation to occur. The degradation rate of each mRNA is regulated by specific sequences within each transcript. Among the plethora of *cis*-acting sequences that control mRNA decay kinetics, the AU-rich elements (AREs) are the best-known, and generally function to suppress expression of encoded genes by accelerating mRNA decay. AREs control mRNA decay by selectively recruiting *trans*-acting factors collectively termed ARE-binding proteins (ABPs), which can include proteins that accelerate or inhibit mRNA degradation. One of the best-characterized regulators of ARE-directed mRNA decay is the protein tristetraprolin (TTP) — the prototype of a small family of CCCH zinc finger-containing proteins that form high-affinity complexes with ARE sequences. In this chapter, we will describe the function and regulation of the TTP family of proteins in the control of mRNA degradation and the relationships between these proteins and the development and progression of neoplastic diseases.

1. The tristetraprolin family of CCCH zinc finger proteins

1.1. *Discovery and domain organization of TTP and its homologs*

The first full-length cDNA clone for TTP was reported by Perry Blackshear's group in 1990 from a screen for insulin-induced genes [1]. Several groups independently identified the same transcript, giving rise to its synonyms Nup475, TIS11, and G0S24 [2–4]. TTP is a 34 kDa protein encoded by the *ZFP36* gene that initially presented two notable sequence features. The first was its significant enrichment in proline (46 of 319 amino acid residues in the mouse protein; 53 of 326 residues in humans), including the three stretches of four contiguous proline residues (tetraproline) for which it was named [1]. The second was an internal domain consisting of two tandemly arrayed CCCH-class zinc fingers, prompting an early hypothesis that TTP might be a transcription factor [5]. Subsequent studies, however, established that TTP was an RNA-binding

Figure 1. Domain organization of TTP and its family members.
Numbers indicate the number of amino acid residues in each protein.

protein, whose recruitment to mRNA targets was associated with accelerated mRNA decay [6, 7].

In the early 1990's, cDNAs encoding proteins closely related to TTP were also isolated [8]. Butyrate response factor 1 (BRF1, also known as TIS11b, cMG1, and ERF1) is a 36 kDa protein encoded by the *ZFP36L1* gene; alternative pre-mRNA splicing gives rise to a 44 kDa variant with an extended N-terminal domain. Butyrate response factor 2 (BRF2, also known as TIS11d or ERF2) is a 51 kDa protein encoded by the *ZFP36L2* gene (Fig. 1). Like TTP, both of these homologs are widely expressed among most vertebrate species with the exception of birds [9]. A fourth family member encoded by the *ZFP36L3* gene appears to be expressed only in select rodents [10], and will not be discussed further in this chapter.

TTP, BRF1, and BRF2 all contain tandem zinc finger (TZF) domains that function as RNA-binding modules (Fig. 2). These domains are 64 amino acids in length and contain two CCCH zinc fingers, so designated because each contains three cysteines and one histidine residue responsible for coordinating a zinc ion [11]. The roles of metal ions in TZF domain folding and RNA-binding activity have been most rigorously studied for TTP. Here, zinc ion coordination by both zinc fingers is critical for high-affinity RNA binding, since: 1) mutating any of the metal ion-coordinating residues from either zinc finger motif abrogates RNA-binding activity [12], and 2) the TZF domain will not form ribonucleoprotein (RNP) complexes on RNA ligands in the absence of associated metal ions [13]. Metal ion coordination by the TTP TZF domain is not exclusive for Zn^{2+}; associated Co^{2+}, Cd^{2+}, Fe^{2+}, and Fe^{3+} all yield complexes capable of binding RNA substrates with varying affinities, although iron binding also renders the TZF domain sensitive to cysteine oxidation that diminishes

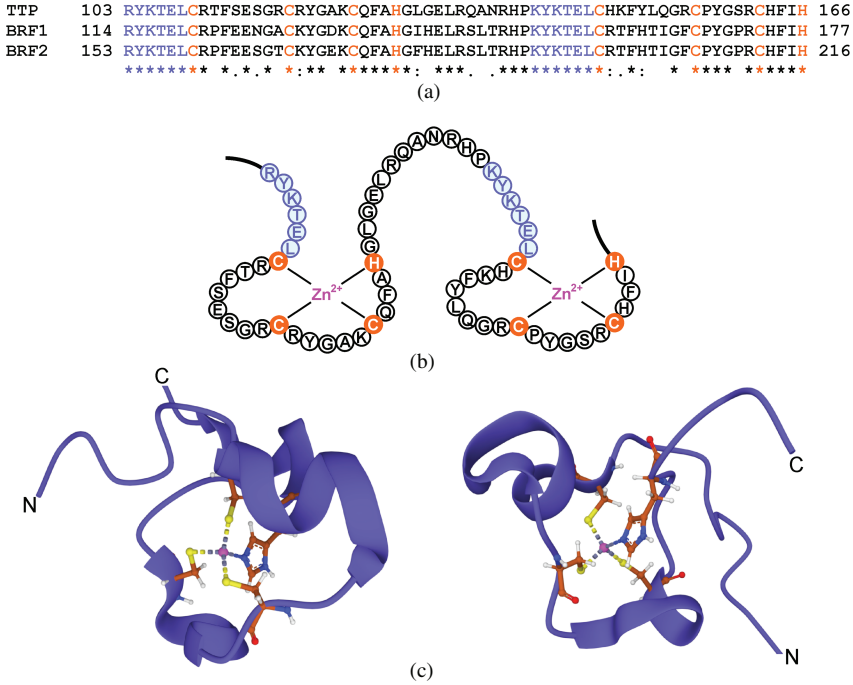

Figure 2. Tandem zinc finger (TZF) domains from TTP and its homologs.

(a) Sequence alignment between TZF domains of human TTP, BRF1, and BRF2. Numbers indicate amino acid residue positions. (R/K)YKTEL motifs are in blue and Zn^{2+}-coordinating residues are in orange. (b) Schematic showing residues coordinating Zn^{2+} from each zinc finger motif of human TTP. (c) Structure of the N-terminal (left) and C-terminal (right) zinc fingers from human BRF2 showing positions of zinc ions (magenta) and coordinating amino acid residues (adapted from PDB ID: 1GRO).

RNA-binding activity [14–16]. By contrast, TZF interactions with Cu^{+} or Au^{+} induce misfolding that abrogates RNA binding [17, 18].

The amino acid sequence (R/K)YTKEL is located immediately upstream of each zinc finger. Amino acid spacing between the metal ion-coordinating residues is also identical between TTP family members (Cys-X_8-Cys-X_5-Cys-X_3-His, where X can represent any amino acid), as is the 18-amino acid spacing between the individual zinc finger motifs [9]. Overall, amino acid sequence identity between the TZF domains of TTP,

BRF1, and BRF2 is approximately 70%, although key differences are observed. In particular, serine residues located at positions 113 and 160 within the TZF domain of TTP (residue numbers are for the human protein) are altered in the other family members; given the role of phosphorylation in regulating the activity of these proteins, these distinctions may be important for homolog-specific control of gene regulation.

Like many RNA-binding proteins, TTP can shuttle between the nuclear and cytoplasmic compartments of the cell [19, 20]. Nuclear import is mediated by a sequence within the TZF domain that is conserved among all mammalian TTP family members. A leucine-rich nuclear export sequence (NES) directs protein transport to the cytoplasm by binding the CRM1 nuclear export receptor but does not require RNA binding. The NES domain for TTP is positioned adjacent to the amino-terminus, while for BRF1 and BRF2, it is located near the C-terminus (Fig. 1). TTP and its family members also contain a conserved binding site for the carbon catabolite repression 4-negative on TATA-less (CCR4-NOT) transcription complex subunit 1 (CNOT1) scaffolding protein that plays a key role in TTP function, and extensive arrays of phosphorylation-competent serine and threonine residues throughout [21, 22]. Phosphorylation cascades are intricately involved in the regulation of TTP expression, localization, and function; these control mechanisms and their impact on gene expression and human disease are discussed in later sections.

1.2. *Physiological roles of TTP family proteins*

Although TTP was originally identified as an acutely inducible gene, the functions of its encoded protein remained elusive for several years. This changed in 1996 with the development of the TTP knockout (KO) mouse. TTP KO mice are born viable but develop symptoms associated with hyperinflammation within the first weeks of life including cachexia, decreased growth, arthritis, conjunctivitis, and dermatitis, as well as autoimmunity and elevated myeloid cell populations [23]. These symptoms prompted the hypothesis that TTP may modulate inflammatory reactions and hematopoiesis. The major pro-inflammatory cytokine tumor necrosis factor α (TNFα) mediates many of the pathological consequences of TTP

deletion, since injected TNFα antibodies potently attenuated hyperinflammatory phenotypes in TTP KO animals [23]. In macrophages from TTP KO mice, elevated TNFα production results from stabilization of TNFα mRNA [24]. TTP accelerates TNFα mRNA decay by binding to an AU-rich element (ARE) within the mRNA 3′-untranslated region (UTR) [25]. AREs direct rapid turnover of a variety of labile transcripts, including many that encode inflammatory factors and oncoproteins [26, 27]. These *cis*-acting sequences often consist of repeated or overlapping AUUUA motifs within U-rich contexts, but can exhibit significant variability between different mRNAs. Computational analysis suggests that approximately 7–10% of mammalian protein-coding genes contain AREs within their 3′UTRs [28, 29]. The mechanisms of ARE recognition by TTP and associated acceleration of mRNA turnover will be discussed in section 2.

While TTP and its family members BRF1 and BRF2 share many common features, including conserved TZF RNA-binding domains that recognize similar RNA substrates [6, 9] and docking sites for the CNOT1 scaffolding protein (Fig. 1), animal studies revealed significant differences in their physiological roles. Homozygous knockout of BRF1 in mice is embryonic lethal by day 11 of development, owing to failure of placental fusion and associated lack of circulation [30, 31]. BRF1 is normally highly expressed in the developing allantois and is required for its fusion with the chorion during placental formation [30]. By contrast, homozygous BRF2 KO mice do survive embryonic development but die soon after birth, usually within two weeks [32]. These animals exhibit a significant loss of hematopoietic progenitor cells relative to normal mice and correspondingly lower numbers of all associated hematopoietic cell lineages; death was attributed to thrombocytopenia and hemorrhage. Together, these observations prompted the hypothesis that BRF2 plays a critical role in the maintenance and/or function of hematopoietic stem cells. Paradoxically, mice lacking the first exon of BRF2, which still express low levels of an N-terminally truncated protein, do survive and appear normal, although females are infertile with embryos dying around the 2-cell stage [33].

Several non-exclusive models may explain the diversity of phenotypes observed between the TTP, BRF1, and BRF2 knockout mouse models.

Some differences could be due to variations in the tissue specificity of homolog expression, but a more comprehensive explanation will likely require the hypothesis that there are differences in the mRNA subpopulations targeted by each TTP family member, and/or that mRNA binding yields varying consequences for each protein. Other possibilities that could conceivably contribute to homolog-specific functions include sensitivity to local RNA secondary structure, co-expression of competing or cooperating RNA-binding proteins, tissue-specific expression of RNA substrates, subcellular localization of each protein and/or RNA substrate, and unique phosphorylation or other post-translational modifications that might modulate their interactions with RNA or other cellular factors [9, 22, 34].

2. Mechanisms of mRNA regulation by TTP

Mechanisms regulating mRNA stability by the TTP family of RNA-binding proteins have been most rigorously described for TTP itself. In this section, we explore the means by which TTP discriminates and associates with RNA substrates and the functional consequences that result. Comparable information regarding BRF1 and BRF2 are included where it is available.

2.1. *The deadenylation-dependent mRNA decay pathway*

The methods by which cells degrade mRNA molecules can be broadly classified into two groups. The first maintains the fidelity of gene expression by rapidly degrading mRNAs that contain premature termination codons (nonsense-mediated mRNA decay), lack termination codons (non-stop mRNA decay), or are associated with stalled ribosomes (no-go mRNA decay). By contrast, the second group of mechanisms controls the levels of functional mRNA molecules present in the cell [35, 36]. Among this latter group, the regulated turnover of the vast majority of mRNAs is mediated by the deadenylation-dependent mRNA decay pathway (Fig. 3). Its initial and rate-limiting catalytic step is 3′→5′ shortening of the mRNA poly(A) tail by any of several potential deadenylating exoribonucleases, where enzyme selection and activity are influenced by the composition of each mRNA ribonucleoprotein complex (mRNP) [37, 38]. Removal of the

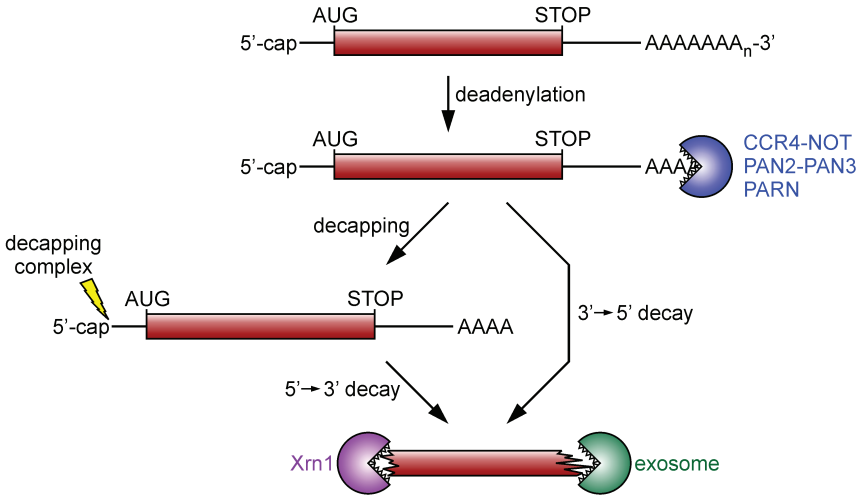

Figure 3. The deadenylation-dependent mRNA decay pathway.

The 3′-poly(A) tail is shortened in the 3′→5′ direction by deadenylating activities (blue) that can include the CCR4-NOT complex, a complex of poly(A) specific ribonuclease subunits PAN2 and PAN3, or a distinct poly(A)-specific ribonuclease (PARN). Conformational changes induced by poly(A) shortening recruit the decapping complex to excise the mRNA 5′-cap structure. The 5′- and 3′-ends of the mRNA body are then rapidly degraded by 5′-3′ exoribonuclease 1 (Xrn1; purple) and the exosome complex (green), respectively.

poly(A) tail and its array of associated poly(A)-binding proteins then provides access to a complex of nonspecific 3′→5′ exoribonucleases and associated factors called the exosome that rapidly degrades the body of the mRNA [39]. However, shortening of the poly(A) tail also induces a reconfiguration of mRNP structure that recruits a 5′-decapping complex, most frequently including the enzyme decapping mRNA 2 (DCP2) [40]. With its 5′-cap removed, the mRNA is then accessible to 5′→3′ exoribonuclease activities, particularly Xrn1 [41]. Using these coordinated mechanisms, shortening of an mRNA poly(A) tail can thus rapidly initiate degradation of the mRNA body from both ends simultaneously.

Although the vast majority of cellular mRNAs are degraded by the deadenylation-dependent decay pathway, the rates at which this occurs vary widely among different transcripts. Some are degraded within

minutes of synthesis while others can remain in the cell for many hours or even days. Cellular mRNA decay is generally well-approximated by first-order kinetics, permitting decay rates to be compared based on mRNA half-lives ($t_{1/2}$), the time necessary for 50% of a given mRNA species to degrade following cessation of synthesis. The diversity of half-lives exhibited by different mRNAs and their ability to be modulated in biological systems serves two critical roles in the regulation of gene expression. First, mRNAs with short half-lives will reach new steady-state levels more quickly than stable mRNAs following changes in their synthesis rates [42, 43], thereby reducing the time between a change in transcriptional activity and its biological response. Second, the half-lives of many mRNAs can be regulated by a variety of stimuli, making it possible to manipulate protein synthesis rates independent of or in coordination with alterations in transcription rates [35, 36, 44].

The decay rate of each mRNA is directed by specific *cis*-acting sequences contained within each transcript. Some of these sequences function constitutively while others can be regulated in response to cellular signaling pathways. ARE sequences are among the best-characterized *cis*-acting mRNA stability determinants, and function by recruiting *trans*-acting factors (Fig. 4). Over 20 different ARE-binding proteins (ABPs) and a putative ARE-targeting microRNA (miRNA) are known [26, 44, 45], but functional significance has been defined for only a subset of these interacting molecules. Some ABPs like TTP and KH-type splicing regulatory protein (KSRP) accelerate mRNA decay, others like ELAV-like RNA binding protein 1 (ELAVL1, also known as HuR) and the inducible 70 kDa heat shock protein Hsp70 function antagonistically to inhibit mRNA degradation [6, 46–48]. Additional ABPs including TIA1

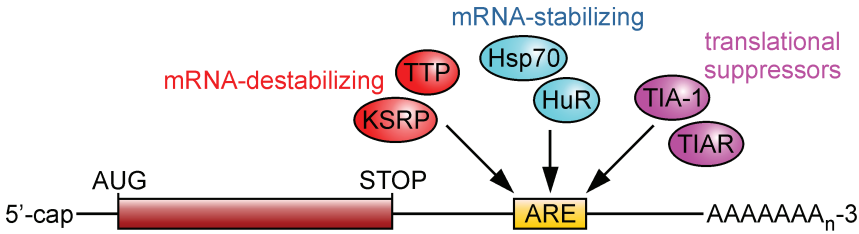

Figure 4. Select ARE-binding proteins and their principal functions on mRNA targets.

cytotoxic granule associated RNA binding protein (TIA-1) and TIA1 cytotoxic granule associated RNA binding protein like 1 (TIAR) suppress translation of mRNA substrates, while some like ARE/poly(U)-binding/degradation factor 1 (AUF1) can have positive or negative effects on mRNA decay and/or translation, depending on their mRNA and cellular contexts [49–51]. Together, this complex network of related but variable RNA target sites and numerous associating factors directs gene-specific control of mRNA decay, while at the same time being exquisitely responsive to cellular signals that can modulate the turnover rates of select transcripts. While much remains unknown about the mechanisms coordinating this degree of regulatory specificity and responsiveness, they are likely to take advantage of differences in the binding specificity, affinity, and dynamics of the various ABPs, their expression and subcellular localization in discrete cell types, combinatorial roles involving multiple ABPs, and the ability of cellular signaling mechanisms to control each of these characteristics.

2.2. *TTP recognition of mRNA substrates*

TNFα mRNA was the first confirmed substrate of TTP [24], with a 3′UTR-localized ARE sequence identified as the primary binding site [52]. Subsequent studies defining the biochemical basis for RNA recognition by TTP focused principally on the TZF domain alone. Using this model, quantitative fluorescence anisotropy-based methods identified the RNA sequence 5′-UUAUUUAUU-3′ as the optimal (K_d = 3 nM) binding site [13]. This motif is present in the ARE from TNFα mRNA, as well as many other transcripts encoding cytokines and other factors controlling cell growth and inflammation. However, unlike ABPs AUF1 and Hsp70, which require only a U-rich RNA tract for high-affinity binding [53, 54], the TTP TZF domain shows remarkable sequence specificity *in vitro*, particularly involving adenosine residues within the binding site. Substitution of a G for either of the two A residues within the canonical RNA binding site decreased TZF-binding affinity by approximately 8-fold, while this energetic penalty approached 20-fold if an A was changed to a U or a C [13]. However, the TZF domain does accommodate some flexibility in the positioning of the A residues, as spacers of between

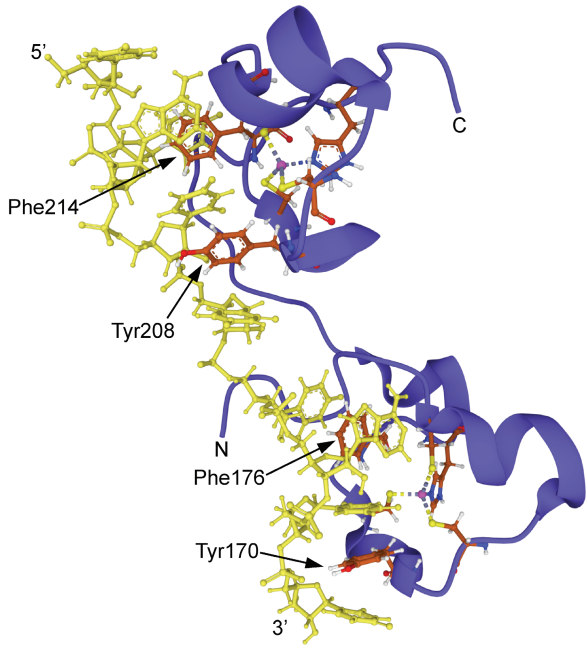

Figure 5. RNA recognition by the TZF domain of BRF2.

A ribbon schematic of the BRF2 TZF backbone is in blue with Zn^{2+} ions in magenta. The RNA substrate (yellow) does not directly bind the Zn^{2+}-coordination complexes but makes extensive contacts with amino acid residues flanking these sites. In particular, the labeled aromatic residue side chains make critical stacking interactions with nucleobases within each UAUU half-site. (adapted from PDB ID: 1GRO).

2 and 5 uridines permitted binding with K_d < 20 nM. The model established by thermodynamic analyses of the TTP TZF domain binding to RNA substrates is consistent with an NMR structure of the BRF2 TZF domain complexed with the TTP-optimized nonameric RNA ligand [55], which revealed that RNA association is mediated by interactions between each zinc finger and a tandemly arrayed UAUU half-site (Fig. 5). Highly conserved aromatic residues in the spacer regions between Zn^{2+}-coordinating residues are also critical for RNA-binding activity by either integrating between RNA bases or participating in stacking interactions with the zinc ion-coordinating histidine residues [11]. The stability and

specificity of TZF:RNA complex formation is further augmented by hydrogen bonds and ionic interactions [13, 55].

Recently, several studies have identified cellular mRNA subpopulations that associate with TTP family proteins using variations of the crosslinking-immunoprecipitation (CLIP) technique [56]. Across multiple cell types, TTP-targeted mRNAs were enriched in AU-rich sequences, although only a minority included the canonical UUAUUUAUU target site [57–59]. Similar results were observed among the BRF1-associated mRNA population in activated T cells, which overlapped broadly with the TTP-targeted transcript pool [59]. Among the remaining TTP- and BRF1-targeted mRNA sequences, most included a UAUU half-site, suggesting that RNA ligands that are thermodynamically unfavorable *in vitro* may represent functional regulatory elements in cells. However, binding by TTP was not always sufficient to induce rapid turnover of mRNA targets, since many mRNAs identified as TTP ligands degrade very slowly in cells [58]. Together, these data indicate that TTP/BRF recruitment to mRNA substrates and downstream consequences on mRNA decay rates are likely to be context-dependent. One hypothesis that could explain some of these contextual effects is that RNA binding and recruitment of mRNA-destabilizing activities may be impacted by allosteric relationships between TTP family members and other RNA-binding proteins and/or noncoding RNAs. For example, one isoform of the ARE-binding protein AUF1 can bind the TZF domain of TTP and enhance its recruitment to RNA substrates [60]. Similarly, heterogeneous nuclear ribonucleoprotein F (hnRNP F) binding to both TTP and BRF1 enhances decay of select mRNA targets [61]. Reciprocally, TTP binding to components of the RNA-induced silencing complex (RISC) enhances the ability of miR-16 to accelerate decay of some ARE-containing RNAs [45]. Finally, the RNA-binding specificity of TTP and potentially its family members might be a regulated event. In a CLIP experiment using a TTP mutant that could not be phosphorylated via p38 mitogen-activated protein kinase ($p38^{MAPK}$) signaling, a much higher proportion of recovered RNA target sites included the canonical UUAUUUAUU sequence [62], indicating that this signaling pathway could modify the selectivity of TTP for RNA substrates.

2.3. *Acceleration of mRNA decay by TTP*

Early evidence for the mechanism by which TTP accelerates mRNA decay emerged by comparing the expression and decay of granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNA in bone marrow stromal cells from wild-type versus TTP knockout mice [63]. Cells from wild-type mice expressed two rapidly-degrading forms of GM-CSF mRNA: a full-length form and a truncated version that lacks its 3′ poly(A) tail. By contrast, the deadenylated form of the mRNA was largely absent in cells from TTP knockout mice, and GM-CSF mRNA half-life is significantly longer in these cells. Given that deadenylation is the initial step in the turnover of most cellular mRNAs (section 2.1., above), these data suggested that TTP accelerates 3′→5′ degradation of the poly(A) tail, since in the absence of TTP, GM-CSF mRNA is dramatically stabilized and a deadenylated intermediate does not accumulate.

In mammals, at least three distinct mRNA-deadenylating activities are known: the CCR4-NOT complex, the PAN2-PAN3 complex, and the poly(A)-specific ribonuclease PARN [37, 64, 65]. While an early study suggested that TTP might enhance PARN activity on mRNA substrates in cell-free assays [66], more recent work has established that TTP accelerates mRNA decay by recruiting CCR4-NOT [67]. This complex is assembled about the scaffolding protein CNOT1, whose binding partners include two 3′→5′ exoribonucleases, CCR4, and CCR4-NOT transcription complex subunit 8 (CNOT8, also known as CAF1), which show marked specificity for poly(A) sequences [68, 69]. However, CNOT1 also contains an internal domain that can bind to a sequence at the C-terminus of TTP [70]. Both BRF1 and BRF2 contain highly conserved CNOT1-binding domains at homologous locations (Fig. 1), suggesting that their mRNA-destabilizing functions are similarly mediated by CNOT1-tethered nucleases. Recent work also indicates that the stability of complex binding to TTP may be enhanced by interactions with CNOT9, a CCR4-NOT substituent with affinity for several conserved tryptophan residues located throughout TTP [71]. By binding to an ARE sequence, TTP can thus directly recruit the CCR4-NOT complex to an mRNA substrate (Fig. 6), bringing active deadenylating enzymes into close proximity to the mRNA poly(A) tail to execute the first step of its degradation. TTP has also been

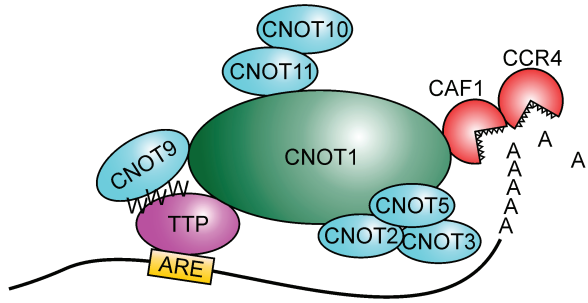

Figure 6. TTP-directed recruitment of the CCR4-NOT complex to mRNA substrates.

TTP bound to an mRNA substrate via an ARE sequence serves as a docking site for the CCR4-NOT complex by interactions with a central CNOT1 domain and CNOT9 binding to select tryptophan (W) residues of TTP. Recruitment of this complex facilitates access of the deadenylating exoribonucleases CAF1 and CCR4 to the mRNA 3′-poly(A) tail.

shown to interact with some factors required for subsequent mRNA degradation steps including decapping proteins, the 5′→3′ exoribonuclease Xrn1, and components of the exosome [72, 73].

2.4. *Additional gene regulatory functions of TTP*

While acceleration of mRNA deadenylation remains the best-described function of TTP, emerging evidence has revealed additional gene regulatory roles, particularly at the levels of translation and transcription. ARE sequences within mRNA 3′UTRs can inhibit translation via a number of different ARE-binding proteins, including TTP [74, 75]. For example, in tumor-associated macrophages, TTP dramatically suppressed production of several inflammatory cytokines but had limited effect on levels of their encoded mRNAs owing to constitutive $p38^{MAPK}$ signaling [76], which inhibits TTP's mRNA-destabilizing activity (section 3.2., below). In almost all cases, initiation is the regulated and rate-limiting step of protein synthesis [77]. One proposed mechanism by which TTP might suppress translation initiation is by the selective recruitment of eukaryotic initiation factor 4E2 (eIF4E2), a homolog of the canonical cytoplasmic cap-binding protein eIF4E [78]. eIF4E2 can bind the mRNA 5′-cap but does not interact with the scaffolding factor eIF4G required to recruit 40S ribosomal

subunits, thus slowing initiation frequency [79, 80]. A second but non-exclusive mechanism involves cooperative functions between TTP and the DEAD-box helicase RCK/p54, a general translational suppressor that is not known to interact directly with TTP but may be activated by it in the context of a larger macromolecular complex [81].

TTP can also suppress gene expression at the transcriptional level in multiple ways. One example is by inhibiting the function of the transcription factor nuclear factor-κB (NF-κB), a key regulator of immune and inflammatory responses. Inactive NF-κB is normally retained in the cytoplasm by associated inhibitory factors. In the canonical activation pathway, proteasomal degradation of the inhibitory protein IκBα releases the active transcription factor complex which is then translocated to the nucleus where it can bind and activate target gene promoters [82]. TTP can suppress this pathway, in part by binding to and preventing nuclear import of one of the transcriptionally active subunits (p65), thus abrogating its ability to induce transcription of NF-κB-responsive genes [83, 84]. A second mechanism by which TTP can suppress transcription is as a corepressor associated with nuclear steroid receptors [85]. This has been best characterized for estrogen receptor α (ERα), where TTP binding to promoter-bound ERα blocks association of the key coactivator SRC-1 while concomitantly recruiting histone deacetylases, particularly HDAC1, to silence targeted promoters [86].

Beyond regulating select genes at the levels of mRNA decay, transcription, and translation, evidence supporting additional roles for TTP has been reported. For example, stress granules (SGs) and processing bodies (P bodies) are granular, membrane-less organelles containing assemblies of translationally stalled RNP complexes and RNA degradative factors, respectively [87, 88]. TTP may be involved in shuttling ARE-containing mRNA targets between SGs and P bodies [27, 89], thus directing the fates of associated mRNAs between storage, translation, or degradation. A second example concerns TTP binding to AU-rich regulatory sequences within the RNA genome of the human immunodeficiency virus HIV-1. This interaction appears to inhibit viral replication by enhancing the production of non-functional splice variants of HIV-1 RNA [90]. Together, these examples highlight the diverse mechanisms by which TTP can regulate gene expression, and suggest that additional roles may yet be discovered.

3. Mechanisms of TTP regulation

3.1. *Regulation of TTP expression*

Expression of TTP is highly variable among different tissues and cell types. It is expressed constitutively in many tissues including those of the respiratory and female reproductive systems, bone marrow, and in many mature leukocyte lineages [91–93]. BRF1 and BRF2 show different patterns of expression across many cell types but include some common themes with TTP, including enriched expression in respiratory and female reproductive tissues [91]. However, in many other cells, particularly when quiescent or serum-starved, TTP is expressed at very low levels but can be rapidly and transiently induced by a variety of mitogenic stimuli including insulin, serum, select cytokines, growth factors, and phorbol esters [1, 94].

Mitogenic induction of TTP expression occurs principally at the transcriptional level, involving the coordinated activity of several transcription factor binding sites in the proximal *ZFP36* gene promoter, including early growth response 1 (EGR1) and activating protein 2 (AP-2) [95]. By contrast, TTP induction by the pro-inflammatory cytokine TNFα or bacterial lipopolysaccharide (LPS) is mediated by NF-κB signaling, targeting a distinct upstream site in the *ZFP36* promoter [96]. Epidermal growth factor (EGF)-directed stimulation of *ZFP36* transcription is dependent on the extracellular-related kinase (ERK)1/2 MAP kinase pathway, and involves recruitment of the transcription factor ELK1 to a downstream promoter element augmented by concomitant induction of EGR1, which in turn binds its cognate element in the proximal *ZFP36* promoter [97].

TTP expression is also regulated via post-transcriptional mechanisms. In monocyte and macrophage cell models, LPS rapidly induces *ZFP36* gene transcription (described above) but also stabilizes TTP mRNA through a process involving ARE sequences located within the mRNA 3′UTR [98]. In uninduced cells, TTP can also bind and accelerate degradation of its own mRNA via this ARE sequence [98, 99], which likely serves as a negative feedback loop to limit TTP expression levels. TTP mRNA is also subject to suppression by microRNAs including miR-29a and -29c [100, 101].

Finally, cellular TTP levels are regulated at the level of protein turnover. TTP protein normally degrades rapidly by a proteasome-dependent mechanism, albeit non-canonically in that degradation appears to be triggered by recognition of intrinsically disordered domains at the N- and C-termini and does not require ubiquitination [102]. This pathway is inhibited by $p38^{MAPK}$ signaling via TTP phosphorylation by the activated downstream kinase MK2. While over 30 distinct phosphorylation sites have been identified on TTP [22], MK2-directed modifications at Ser52 and Ser178 (numbering is for the mouse protein sequence; they correspond to Ser60 and Ser186 in the human homolog) appear to play a dominant role in maintaining the cytoplasmic localization and stability of TTP protein in activated macrophages [103]. The mechanism(s) by which these phosphorylation events stabilize TTP remain unclear, although an intriguing hypothesis is that phosphorylation-dependent association of cellular factors, particularly members of the 14-3-3 family of proteins, might protect TTP from proteasome-dependent turnover by directing or stabilizing local protein folding [22, 104]. By contrast, some other kinase pathways are associated with accelerated degradation of TTP. For example, mitogen-activated protein kinase kinase kinase 1 (MAP3K1, also known as MEKK1), a key upstream regulator of the c-Jun N-terminal kinase (JNK) pathway, phosphorylates TTP and induces ubiquitin-dependent proteasomal turnover by recruiting an E3 ubiquitin ligase complex [105]. Proteasome-dependent degradation of TTP is also accelerated in melanoma cells by sustained ERK activation [106], and in human breast cancer cells by pyruvate kinase M2 (PKM2) involving phosphorylation at Ser60 [107].

While phosphorylation-based mechanisms play major roles in the regulation of TTP expression (above) and function (below), much remains unknown in this area. The plethora of phosphorylation targets on TTP raises the potential for myriad cell type-specific and/or combinatorial influences on the consequences of TTP phosphorylation. The complexity of this system is compounded by the extremely poor similarity (<25%) between the peptide sequences of TTP and its family members BRF1 and BRF2 outside of their conserved TZF domains, including the locations and sequence contexts of phosphorylated residues [22]. Some phosphorylation-mediated signaling events do appear

to be conserved. For example, phosphorylation of BRF1 at Ser92 and Ser203 stabilizes the protein via interactions with 14-3-3 proteins [108], analogous to phosphorylation of TTP at Ser52 and Ser178. However, even in this case, variations in sequence context likely account for differences in kinase recognition, since BRF1 can be phosphorylated by either MK2 [109] or protein kinase B [108] at these sites. It is thus likely that, while some aspects of RNA recognition and substrate catabolism are conserved between members of the TTP protein family, their physiological and potentially pathological impacts will be distinguished by unique factors that independently regulate their expression and activity, including overlapping but distinct interfaces with cellular signaling pathways.

3.2. *Regulation of TTP function*

Similar to the mechanisms controlling TTP protein turnover, our emerging understanding of mechanisms by which TTP activity is regulated have largely been derived from observations linking MAP kinase signaling to mRNA decay kinetics. Early studies demonstrated that activation of the $p38^{MAPK}$ pathway stabilized ARE-containing mRNAs [110, 111] by slowing their deadenylation rates [112]. Subsequently, phosphorylation of TTP by the $p38^{MAPK}$-effector kinase MK2 was found to inhibit Caf1 deadenylase recruitment to TTP [113]. In 2013, a mechanistic rationale explaining these data was provided by a co-crystal structure of the internal TTP-binding domain of CNOT1 with a peptide corresponding to the C-terminal 15 amino acids of TTP [70]. A serine residue known to be phosphorylated by MK2 (Ser316 in the mouse protein; Ser323 in human) [114] is contained within this CNOT1-binding interface, and *in vitro* experiments demonstrated that phosphorylation at this site inhibited CNOT1 binding affinity by several hundred-fold [70]. Subsequent findings revealed that phosphorylation at Ser316 can be directed by both MK2 and the ERK-activated kinase RSK1 [115], explaining how the mRNA-destabilizing activity of TTP could be inhibited by both the $p38^{MAPK}$ and ERK pathways [116]. BRF1 and BRF2 both have serine residues homologous to Ser316 of TTP within their conserved CNOT1-binding domains, suggesting a

common mechanism of phosphorylation-inhibited recruitment of the deadenylase complex, but to date, the sole kinase shown to phosphorylate these residues in the BRF proteins is RSK1 [117].

4. Many mRNAs regulated by TTP family members encode pro-tumorigenic factors

Beyond their well-characterized suppression of inflammatory transcripts, TTP and its family members also accelerate the decay of many mRNAs that encode pro-tumorigenic factors. Among the first to be discovered was vascular endothelial growth factor (VEGF) mRNA, which is destabilized by both TTP and BRF1 [118, 119]. VEGF is a key activator of angiogenesis, which promotes cancer by facilitating the formation of new blood vessels in tumors that allow nutrient delivery and enhance tumor cell migration [120]. By destabilizing VEGF mRNA, TTP and BRF1 suppress production of VEGF protein, thus limiting tumor vascularization. A second well-characterized TTP substrate mRNA encodes cyclooxygenase 2 (COX-2, also known as prostaglandin-endoperoxide synthase 2) [121, 122]. COX-2 is an enzyme that catalyzes the first two steps in the prostaglandin biosynthetic pathway and is a major target of non-steroidal anti-inflammatory therapeutics [123]. COX-2 expression is normally very low in healthy tissue but sustained expression can contribute to the pro-oncogenic effects of tumor-associated inflammation [124–126].

The discovery that TTP family proteins target and destabilize select cancer-associated mRNAs prompted the hypothesis that suppression or inhibition of these proteins might contribute to cancer. This model has been supported by clinical correlation and transgenic animal experiments that are described below (section 5), while further studies have identified a plethora of mRNAs regulated by TTP and/or BRF proteins that encode factors contributing to a diverse array of tumorigenic phenotypes. In Table 1, select examples are classified into their relevant “Hallmarks of Cancer” as defined by Hanahan and Weinberg [127]. For a subset of these mRNA targets, mechanisms that link their deregulation due to loss of TTP or BRF proteins with enhancement of oncogenic functions are described in section 6.

Table 1. mRNA substrates of TTP family proteins associated with cancer[a].

Oncogenic phenotype	TTP-regulated mRNAs	BRF1-regulated mRNAs	BRF2-regulated mRNAs
Evading growth suppressors	DUSP1, LATS2		
Enabling replicative immortality	Myc, Cyclin D1, E2F1, MIP-2, p21, PLK3, PIM1	Cyclin D1, E2F1	Cyclin D1
Tumor-promoting inflammation	TNFα, COX-2, CCL2, CXCL8, CCL3, CD86, GM-CSF, IL-2, 3, 6, 8, 10, 12, and 23	TNFα, COX-2, GM-CSF, M-CSF, CXCL1, IL-8	
Activating invasion and metastasis	MMP1, uPA, uPAR, MUC4, TWIST, SNAIL, c-*fos*	uPA, uPAR	HDAC1
Resisting cell death	cIAP2, PLK3, BCL2, BNIP2	BCL2, BNIP2, cIAP2	BCL10, BNIP2, BIRC3
Deregulating cellular energetics	OPA1	HIF1A, PDK1, OPA1	OPA1

[a]listed RNA substrates were compiled from [128–136].

5. Suppression of TTP and BRF1 occurs frequently in cancer and is associated with poor patient outcomes

Comparisons of TTP mRNA levels between patient-matched tumor and non-transformed tissues revealed that TTP was downregulated by 50% or more in the majority of patient tumors tested, with particularly strong repression in tumors of the thyroid, lung, ovary, uterus, and breast [137]. These findings were corroborated by analyses of gene expression datasets across several thousand patient samples [137, 138]. Measuring TTP mRNA in cell models also showed significantly lower TTP expression in lung and colon cancer cell lines relative to healthy tissue [91].

While TTP expression is consistently lower in most tumors relative to healthy tissue across a wide range of cancer types, additional data link

suppression of TTP to tumor aggressiveness. For example, among primary tumors of the breast, pancreas, and prostate, low TTP expression is closely correlated with advanced grade tumors [133, 137, 139, 140], those comprised of poorly differentiated and actively dividing cells with atypical morphological features. Furthermore, among prostate cancer patients, TTP expression is significantly lower in metastases relative to primary tumors [137, 140]. An extensive survey of similar studies spanning a variety of tumor types was recently published by Saini *et al*. [141], emphasizing the pervasiveness of TTP suppression in cancer, particularly in advanced cases. Finally, low TTP expression is associated with poor patient prognosis in many cancers. In a cohort of 251 breast cancer patients, those whose excised tumors had low levels of TTP mRNA faced an over 2-fold increased risk of death from recurrent breast cancer than patients with high tumor TTP expression [137]. In a study of 90 pancreatic cancer patients, low tumor TTP expression was associated with an 80% decrease in median survival [133]. Similar results were observed in cohorts of glioblastomas as well as lung and prostate cancer patients [142–144]. Cumulatively, these data indicate that low TTP expression is associated with more aggressive cancers and poorer patient prognosis.

Most patient studies to date have focused on TTP, but emerging data suggest that altered expression of BRF1 and BRF2 is also associated with some cancers. In an intriguing mouse model, the *ZFP36L1* and *ZFP36L2* genes were both deleted in lymphocytes prior to development of the thymus [145]. These mice uniformly developed a T cell acute lymphoblastic leukemia involving upregulation of the pro-oncogenic transcription factor Notch1. However, deletion of either *ZFP36L1* or *ZFP36L2* in isolation yielded no pathogenic phenotype, suggesting that their encoded proteins may function redundantly as tumor suppressors in this context. Subsequent analyses of BRF1 expression in clinical samples have also supported a tumor suppressive role for this protein. For example, a large genome-wide association study (GWAS) identified mutations in the *ZFP36L1* gene that are associated with an increased risk of epithelial ovarian cancer [146], while BRF1 expression is also strongly downregulated in breast and bladder cancers relative to normal tissues [135].

Cell line data are less conclusive for BRF1 and BRF2, which show inconsistent decreases at the mRNA level in cancer cell lines relative to

normal tissues and in select cell models are even overexpressed [91]. Regarding the impact of BRF2 on tumor progression, very limited data are currently available, but what has been reported suggests that it may play pro-oncogenic roles in some contexts. For example, BRF2 is upregulated and associated with more aggressive disease in pancreatic ductal adenocarcinoma [147]. Furthermore, computational studies suggest that gene expression signatures including elevated BRF2 are associated with formation of bone metastases from breast cancer [148] and recurrence in bladder cancer [149]. This apparent dichotomy between the cancer-related roles of the TTP family members suggests the possibility of consistent, redundant, or even antagonistic functional relationships in different cellular contexts. Whether these diverse outcomes are the result of cell or tissue type-specific differences in the expression or relative distribution of TTP family members, variations in RNA substrate selectivity, or unique responses to cellular signaling inputs remains to be seen.

6. Consequences of altered TTP expression or function in cancer

In section 4, we introduced the concept that diminution of the expression and/or function of TTP family members in cancer could lead to stabilization and accumulation of pro-tumorigenic mRNAs, culminating in poor patient outcomes. This sequela is a result of alterations in tumor cell phenotypes including cell proliferation, senescence, invasiveness, metastasis, metabolism, and tumor-associated inflammation. The following subsections describe examples linking suppression of TTP family members to exacerbation of oncogenic pathways, and how each contributes to global changes associated with tumor development and progression.

6.1. *Cell proliferation*

For tumors to develop, cancer cells must enhance their proliferative rates relative to their non-transformed progenitors. While evidence from several systems supports an anti-proliferative function for TTP, the underlying mechanisms vary with cellular context. For example, in breast cancer,

decreased TTP mRNA levels are associated with more advanced tumors [137]. Restoring TTP expression in the aggressive breast cancer cell line MDA-MB-231 significantly slowed proliferation, both *in vitro* and in murine xenografts [150]. Cell cycle analysis revealed an accumulation of cells in S phase when TTP was expressed, consistent with restricted transition into the G2 phase. An independent study reported similar results in lung adenocarcinoma cells [151].

In the breast cancer cell model, the underlying mechanism for inhibiting proliferation appears to be independent of TTP's mRNA-destabilizing function, but rather involves its ability to inhibit NF-κB from inducing expression of the transcription factor c-Jun [150]. In the absence of TTP, enhanced c-Jun levels suppress expression of the G2 checkpoint kinase Wee1, thus abrogating its ability to restrict progress through the cell cycle (Fig. 7). Wee1 phosphorylates cyclin-dependent kinases 2 and 1 (CDK2 and CDK1) to prevent progression from S phase into G2 and G2 into mitosis, respectively [152], and normally prevents mitosis in response to DNA damage [153]. However, when TTP is overexpressed in these cells, inhibition of NF-κB prevents c-Jun production, resulting in Wee1 accumulation. Elevated Wee1 levels slow the cell cycle transition into G2, which accounts for the enriched population of cells retained in S phase when TTP is present [150]. Curiously, in ovarian cancer, c-Jun appears to directly activate rather than inhibit Wee1 transcription [154], suggesting yet another example where downstream consequences of these signaling pathways may be context-dependent.

TTP also slowed proliferation and tumor-forming potential of prostate cancer cell lines, but in this case, accompanied by a significant accumulation of cells in G1 phase, consistent with inhibition of the G1/S checkpoint [140]. Comparable results were observed when expressing TTP in B cell lymphomas [155]. In prostate cell models, an RNA-Seq screen

Figure 7. Control of the NF-κB/Jun/Wee1 axis by TTP in MDA-MB-231 cells.

identified over 1,700 mRNAs that were differentially expressed in TTP-expressing versus control cells, with virtually all showing lower expression when TTP was present. Over half of these were known substrates of TTP and/or contained canonical TTP-binding sites, making accelerated mRNA decay the most likely consequence of TTP in these cells. Among the TTP-suppressed mRNA population were several encoding cell cycle regulators like cyclin C and CDK1, but the mRNAs most dramatically impacted by TTP encoded metabolic factors [140], particularly those involved in mitochondrial respiration and maintenance of ATP levels. At present, mechanism(s) linking TTP-induced metabolic reprogramming to cell cycle arrest remain unknown. An independent study, however, demonstrated that TTP can also target and destabilize the mRNA encoding the G1/S regulatory transcription factor E2F1 in prostate cancer cell lines [156], suggesting a more direct means by which TTP might inhibit proliferation in this cell type.

Beyond directly modulating expression of cell cycle regulatory proteins or transcription factors, TTP also controls expression of some signaling molecules that activate proliferative mechanisms. An example is PIM1, an oncogenic serine/threonine kinase that accelerates the cell cycle by phosphorylating and activating the phosphatases cell division cycle 25A (CDC25A) and CDC25C, which are required for progression through G1/S and G2/M, respectively, and for blocking the activity of select cell cycle inhibitors [157]. While PIM1 is upregulated in a host of solid tumors and hematopoietic cancers [158], its mRNA can be directly targeted and suppressed by TTP in diverse cancer cell types [133, 159, 160].

Current data indicate that other TTP family members also inhibit cell proliferation in some cell models. For example, BRF1 inhibits proliferation of breast and bladder cancer lines while both BRF1 and BRF2 slowed growth in colon cancer cells [135, 136]. Cell cycle analyses in human embryonic kidney (HEK) 293 and colon cancer cell models revealed that BRF1 and BRF2 block progression at G1/S [136] and promote quiescence in developing B lymphocytes [161]. In the bladder cancer cell line J82, BRF1 expression also significantly reduced colony formation *in vitro* and tumor growth in a murine xenograft model [135]. The mechanism(s) by which BRF1 and BRF2 suppress proliferation also mirror examples described by TTP, notably via the targeted degradation

of select ARE-containing mRNAs including some that encode cell cycle regulatory proteins. RNA-Seq and transcriptome-wide protein pulldown approaches in J82 cells identified over 1,410 mRNAs likely to be direct functional targets of BRF1 including those encoding well-known G1/S cell cycle regulators cyclin D1 and E2F1 (examples in Table 1), and BRF1 and BRF2 both suppressed cyclin D1 expression in HEK293 and select colon cancer cell lines [136]. Together, these data indicate that all TTP family members can suppress proliferation of diverse cancer cell types, in most cases involving targeted degradation of similar mRNA substrates.

6.2. *Senescence*

Senescence is a phenomenon by which cells limit the number of replication cycles they can complete to prevent uncontrolled growth and accumulation of DNA damage. This mechanism, termed "replicative senescence," is based on progressive shortening of the telomere sequences at the ends of chromosomes following each round of DNA replication, and occurs naturally during various stages of embryonic development and tissue repair where senescent cells are ultimately cleared by the immune system [162, 163]. However, senescence can also be induced independent of replicative capacity by a host of cellular stresses, including DNA and oxidative damage, chronic mitogen signaling, and oncogene activation [163, 164]. These mechanisms normally serve as protection against tumorigenesis, although senescent cells can foster pro-tumorigenic microenvironments in xenograft models, likely owing to secretion of inflammatory cytokines but also potential reversal of the senescence phenotype and re-activation of the cell cycle [165]. In general, however, pre-cancerous cells must evade senescence in order to develop into malignant tumors, and often do so by suppressing or inactivating key mediators of senescence including the tumor suppressor p53 and the cyclin-dependent kinase inhibitor CDKN2A (also known as INK4A) as well as re-activation of telomerase or alternative mechanisms to extend telomeres [166–168].

To date, a number of studies have indicated that TTP promotes cellular senescence [128, 169]. An elegant example in a cancer cell model interrogated the role of TTP in human papilloma virus (HPV)-infected HeLa cervical adenocarcinoma cells [170]. Cellular transformation by

oncogenic HPV strains is mediated by viral proteins E6 and E7. While TTP did not impact expression of these viral-encoded factors, it did target and destabilize the mRNA encoding the cellular E6-associated protein (E6-AP), a ubiquitin ligase. Consequences of E6-AP binding to E6 include accelerated degradation of the tumor suppressor p53 and enhanced transcription of the human telomerase gene *hTert* [171], well-established mechanisms by which tumor cells evade senescence. However, when TTP was expressed in HeLa cells, targeted degradation of E6-AP mRNA resulted in decreased levels of E6-AP protein, enhanced p53 levels, and suppressed production of telomerase, accompanied by morphological and molecular markers of senescence [170]. This finding suggests that introduction or re-activation of TTP might inhibit aggressiveness of some tumor cells by re-activating senescence pathways. While it remains unknown whether BRF1 or BRF2 proteins are involved in the regulation of senescence, recent data indicate that both proteins can enhance p53 expression in select colon cancer cell lines [136].

6.3. *Cell migration, invasiveness, and metastasis*

Additional consequences of TTP suppression in some cancers are enhanced cell migration and invasiveness, key phenotypes associated with metastasis. Metastasis is a complex process that involves the epithelial-mesenchymal transition (EMT), a pathway by which altered expression of select genes cause epithelial cells to lose apical-basal polarity and adopt characteristics of mesenchymal cells including increased motility and the ability to degrade the extra-cellular matrix. EMT plays key roles in several normal physiological processes including development and wound healing, but is often utilized by cancer cells to facilitate migration and invasion while also evading apoptosis [172, 173]. In several colorectal cancer cell models, TTP suppressed mRNAs encoding three transcription factors that activate EMT pathways: SRY-box transcription factor 9 (SOX9), zinc finger E-box binding homeobox 1 (ZEB1), and metastasis-associated in colon cancer 1 (MACC1) [174]. As a result, TTP-expressing cells showed increased production of epithelial cell markers like E-cadherin, tighter cell junctions, and decreased levels of

mesenchymal markers like N-cadherin and vimentin. TTP also targeted and suppressed mRNAs encoding EMT-promoting transcription factors snail family transcriptional repressor 1 (SNAI1) and twist family bHLH transcription factor 1 (TWIST1) in ovarian cancer cells, concomitant with repression of mesenchymal cell features [132]. Together, these data support a role for TTP in preventing EMT.

However, TTP can also inhibit cancer cell migration and invasive potential independent of EMT. Suppressing TTP in head and neck squamous cell carcinoma lines enhanced invasiveness by increasing penetration through basement membranes [175]. This was achieved by upregulating mRNAs encoding interleukin (IL)-6 and matrix metalloproteinases 2 and 9, invasion-promoting factors whose expression is normally limited by TTP-directed mRNA degradation. A complementary model was reported in glioma cells, where TTP-induced decay of IL-13 mRNA prevented cell migration and invasion by suppressing the IL-13-activated PI3K/Akt/mTOR signaling pathway [143]. Migration of bladder cancer cells was inhibited by expression of BRF1 [135], although it is unclear at this point whether the mechanisms responsible are linked to EMT. Roles for BRF2 in the regulation of tumor cell metastatic functions remain unknown.

6.4. *Cell metabolism*

Cellular energy stores are largely replenished by production of ATP resulting from the catabolism of glucose. In healthy cells, this is initiated by glycolysis, which does not require molecular oxygen but achieves optimal energy yields under aerobic conditions where carbon atoms of pyruvate generated by glycolysis can be further oxidized to CO_2 by the citric acid cycle and associated oxidative phosphorylation in the mitochondria. However, in many cancers, glucose catabolism is primarily driven by glycolysis regardless of oxygen availability. This condition, known as the Warburg effect, requires dramatic increases in glucose consumption to meet tumor cells' energy needs and is associated with increased lactate production [176, 177]. Enhanced glycolytic activity in cancer cells can be facilitated by increased expression of hexokinase

2 (HK2), which catalyzes the first committed step of this pathway [178]. HK2 mRNA contains an ARE in its 3′UTR that is targeted by TTP. In breast and lung cancer cell models, expression of TTP inhibited production of HK2 protein by accelerating degradation of its mRNA, which in turn suppressed cellular glycolytic capacity [179]. Analyses of TTP-regulated mRNAs in prostate cancer cells also identified targets that encode proteins involved in cellular respiration, but interestingly these were enriched among factors that direct the aerobic citric acid cycle and electron transport chain pathways [140], further evidence of the context-dependent roles of TTP across different cancer types.

6.5. *Tumor-associated inflammation*

Among the first physiological functions of TTP to be discovered was its role as a suppressor of inflammatory signaling (section 1.2.), mediated by its recognition and destabilization of mRNAs encoding a host of pro-inflammatory cytokines and chemokines including TNFα, GM-CSF, and many others (Table 1). In cancer, local production of key cytokines contributes to tumor-promoting inflammation [127], which can be exacerbated by suppression of TTP expression. Early evidence linking TTP to inhibition of tumor-associated inflammation was observed in a v-H-*ras*-dependent mast cell model. These cells require constitutive expression of IL-3 to develop into tumors, as IL-3 generated and secreted by these cells then binds cell surface receptors and activates proliferative signaling in an autocrine loop [180]. IL-3 mRNA is a direct target of TTP, so expression of TTP in these cells was sufficient to disrupt this loop by degrading IL-3 mRNA; the resulting inhibition of IL-3 synthesis slowed cell proliferation *in vitro* and tumor growth in murine xenografts [181].

TTP can also use similar mechanisms to suppress tumor-associated inflammation in a paracrine fashion. In the breast cancer cell model T47D, downregulation of TTP resulted in stabilization of IL-16 mRNA and increased production and secretion of its encoded protein [182]. When cultured in spheroids with monocytes, enhanced IL-16 production in cells lacking TTP significantly increased monocyte infiltration, a finding mirrored by histological analysis of xenografted tumors. While these examples illustrate TTP suppression of localized inflammation by directly

targeting mRNAs encoding specific cytokines, these consequences may be amplified in some contexts owing to TTP's role as an inhibitor of NF-κB, a master regulator of pro-inflammatory gene transcription (section 2.4.). This association has been observed in aggressive prostate cancer, where TTP expression is inversely correlated with transcript levels for multiple NF-κB-responsive genes [183]. Collectively, these data support a role for TTP in antagonizing tumor-promoting inflammation.

7. Mechanisms suppressing TTP family members in cancer

Analogous to the varying context-dependent consequences of TTP and its homologs on recognition and regulation of mRNA targets, diverse mechanisms have been identified that inhibit the expression and/or function of these proteins in cancer.

7.1. *Gene mutations*

Genome analyses across >70,000 tumor samples identified a variety of truncating mutations (e.g., frameshift, nonsense) among genes encoding all three TTP family members, but also revealed that these were significantly enriched at the *ZFP36L1* gene locus, particularly among breast and bladder cancers [135]. An A→G substitution within the proximal promoter of the *ZFP36* gene was also associated with significant decreases in TTP expression and poor prognosis in a cohort of breast cancer patients [130]; this mutation occurs at a site predicted to interact with several transcription factors.

7.2. *Transcriptional suppression*

In humans, development of most tumors includes upregulation of the transcription factor Myc, which modulates expression of a host of genes associated with tumorigenesis including many that control the cell cycle, protein biosynthesis, stemness, cellular metabolic pathways, angiogenesis, and immune signaling [184, 185]. Among the genes regulated by Myc

are *ZFP36* and *ZFP36L1*. Myc binds near the transcriptional initiation site of each gene, suppressing RNA polymerase II recruitment and RNA synthesis [155]. Consistent with this model, experiments in breast, colorectal, and metastatic prostate cancers found TTP expression to be very low in tumors where Myc levels are elevated. A second oncogenic transcriptional mechanism that suppresses TTP is the Wnt/β-catenin system. This pathway is a central regulator of embryonic development and tissue stem cell function but is constitutively active in many cancers where it is a principal driver of EMT [186, 187]. In colorectal cancer cell lines, Wnt/β-catenin activity repressed TTP expression [174]. While it remains unknown whether this pathway suppresses *ZFP36* gene transcription directly or via a secondary regulator, support for the former hypothesis is given by putative binding sites for the β-catenin effector protein TCF7L2 within the *ZFP36* promoter and ENCODE project data, indicating that TZF7L2 binds to at least one of these sequences [188]. By contrast, TTP suppression by β-catenin signaling in squamous cell carcinoma lines may require or be augmented by Wnt/β-catenin-induced expression of transcriptional repressor proteins SNAI1 and SLUG, which also bind the *ZFP36* gene promoter [189]. A third mechanism by which the *ZFP36* gene may be transcriptionally suppressed is epigenetic. In some liver carcinoma cell lines and patient tissues, methylation of a single CpG site in the *ZFP36* promoter is sufficient to recruit a transcriptional repressor complex that inhibits TTP expression [190], while in bladder carcinoma tissues, extensive DNA methylation at the *ZFP36L1* promoter correlates with decreased mRNA levels [135].

7.3. *mRNA-targeted suppression*

While several mechanisms regulate TTP expression at post-transcriptional levels (section 3.1.), the sole example associated with TTP suppression in cancer to date is via miR-29a. MiRNAs are 20 to 22-nucleotide noncoding RNA molecules that function as *trans*-acting regulators of gene expression. When associated with the RNA-Induced Silencing Complex (RISC), miRNA recognition of mRNA targets by complementary base pairing suppresses protein production by inhibiting translation and/or accelerating mRNA degradation [191, 192]. Flexibility in mRNA substrate recognition

allows individual miRNAs to regulate hundreds of different mRNAs. Accordingly, many examples have been reported where dysregulation of individual miRNAs can reprogram extensive gene expression networks associated with cancer [193, 194]. One example is miR-29a, which exhibits tumor suppressive functions in some cancer types [195] but enhances EMT and metastasis in a Ras-driven murine breast cancer model [100]. miR-29a promoted these phenotypes by directly targeting and suppressing TTP mRNA, since silencing TTP alone was sufficient to promote EMT and growth of lung metastases in mice. Furthermore, surveys of clinical samples revealed that TTP expression is inversely correlated with miR-29a levels in metastatic human breast cancers and tumors of the pancreas [100, 196], suggesting that inhibition of this miRNA could conceivably have therapeutic benefit. Consistent with this hypothesis, a cell-permeable miR-29a peptide nucleic acid (PNA)-based inhibitor restored TTP expression in an aggressive breast cancer cell line concomitant with suppression of cell invasiveness [197].

7.4. *Protein inactivation and/or degradation*

In a growing number of cancer models, TTP function is suppressed by inactivation and/or targeted degradation of the protein, mostly mediated by phosphorylation-based mechanisms described previously (section 3). While many regulatory phosphorylation sites are conserved among all TTP family members [22], their roles in dysregulating BRF1 and BRF2 in cancer have not been well defined to date.

In several oncogenic settings, the $p38^{MAPK}$ pathway is a key mechanism for TTP suppression, including the pro-inflammatory environment that is a poor prognostic indicator in head and neck cancers [198]. When select head and neck squamous carcinoma cell lines were treated with IL-1β, activation of the RAS-like small GTP-binding protein rap1B induced signaling through the $p38^{MAPK}$ pathway, leading to phosphorylation and inactivation of TTP [175]. Since TTP normally suppresses expression of many cytokines, inhibition of TTP function in this model would enhance cellular production and secretion of pro-inflammatory factors, conceivably triggering a feed-forward loop to further amplify localized inflammatory signaling. The $p38^{MAPK}$ pathway is also implicated in

the accumulation of inactive, hyperphosphorylated TTP in malignant glioma tissues and cell lines [199]. The significance of TTP phosphorylation in this system was cleverly demonstrated by engineering a mutant form of TTP where eight serine residues known to be phosphorylated were changed to alanines; expressing this phospho-null mutant in a glioma cell model restored rapid degradation of TTP substrate mRNAs while also inhibiting cell proliferation and colony formation [200].

TTP suppression can also be mediated by ERK signaling, a mechanism clearly observed in melanoma. Approximately half of all melanomas are driven by the activating V600E mutation in the serine/threonine-protein kinase B-Raf (BRAF), resulting in constitutively high activity of the downstream mitogen-activated protein kinase kinase (MEK)-ERK signaling pathway [201]. TTP expression is very low in melanoma cell lines harboring the BRAF V600E mutation, but can be restored by treatment with MEK inhibitors [202]. TTP also accumulated in these cells following treatment with proteasome inhibitors, but as a phosphoprotein, consistent with the model that TTP phosphorylation via ERK signaling directs the protein for degradation by the proteasome. Finally, kinases PIM2 and pyruvate kinase M2 (PKM2) both bind and accelerate proteasome-directed turnover of TTP in breast cancer models but do so independently of their kinase functions [107, 203].

8. Potential therapeutic opportunities

Currently, there are no FDA-approved therapeutics that specifically restore expression or function of TTP or its family members. However, emerging pre-clinical data have revealed several approaches that show promise for reactivating TTP activity. The first is to induce TTP expression by enhancing transcription from the *ZFP36* gene. One method by which this has been achieved in cancer cell models is through histone deacetylase (HDAC) inhibitors. Histone deacetylases are a group of enzymes that remove acetyl groups from accessible lysine residues, which generally suppresses transcription by stabilizing condensed chromatin structures via the restored positive charges of these amino acid side chains and by preventing recruitment of transcription coactivator complexes that require acetyl-lysine docking sites [204]. Several different HDAC

inhibitors increased TTP mRNA and protein expression in colorectal cancer cell lines as well as the cervical cancer cell line HeLa [205]. Using the HDAC inhibitor trichostatin A (TSA) as a model, the mechanism of TTP re-activation was shown to be indirect, as TSA did not impact acetylation of the *ZFP36* gene promoter but instead induced expression of EGR1, a transcription factor that binds and enhances transcription of the *ZFP36* gene [97]. In an independent study, TTP expression was transcriptionally activated by the HDAC inhibitor Givinostat in fibroblast-like synoviocytes isolated from rheumatoid arthritis patients [206]. In both examples, the enhanced TTP produced was active, destabilizing known TTP substrate mRNAs, and in a colon cancer cell line, inhibiting cell proliferation. Transcription of the *ZFP36* gene is also increased in gastric and breast cancer cell models by thymoquinone, a bioactive compound isolated from seeds of the *Nigella sativa* plant reported to exhibit a variety of pharmacological properties, including analgesic, anti-microbial, and anti-cancer functions [207]. While the molecular mechanism(s) by which thymoquinone enhances *ZFP36* transcription remain unknown, the induced TTP protein was active, destabilizing several known mRNA substrates in these cells [131].

A second potential strategy to restore TTP activity in cancer cells is by inhibiting specific kinases or other signaling pathways. For example, phosphorylation and proteasome-dependent degradation of TTP in melanoma cells can be abrogated by sorafenib, an inhibitor of the rapidly accelerated fibrosarcoma (RAF) serine/threonine kinase [106]. Conceivably, targeted disruption of the RAF/MEK/ERK pathway at other points, possibly including FDA-approved MEK inhibitors like trametinib and cobimetinib [208], may also restore TTP function as a component of their anti-tumor activities.

Finally, it may be possible to restore function of TTP or other family members in cancer cells by direct protein delivery. A phospho-null TTP mutant (described in section 7.4.) accelerated decay of TTP target RNAs, slowed proliferation, and enhanced apoptosis in glioma cells [200], although this required expression within transfected cells. Recent studies involving BRF1, however, raise the possibility of direct protein entry into target cells. For example, cell-penetrating variants of BRF1 generated by adding the TAT domain from HIV or short (seven or nine residues)

polyarginine tracts to the N-terminus were readily internalized by cultured cells and tumor xenografts, suppressing expression of multiple angiogenic and inflammatory factors as well as slowing tumor growth and vascularization [209]. A similar experiment used a polyarginine-tagged BRF1 mutant that lacked the N-terminal domain but included a serine to aspartate mutation (S334D) in the C-terminal domain [210]. This protein was also efficiently delivered to breast cancer cells, where it inhibited several tumorigenic phenotypes including proliferation and invasion *in cellulo* while also suppressing growth, hypoxia, and EMT characteristics when injected into tumors in mice. While systemic delivery of such peptide therapeutics is unlikely to be viable in human subjects owing to immunological or other complicating factors, it is conceivable that such reagents might be deliverable via intratumoral injection, analogous to the BRF1 animal studies reported to date and emerging concepts in the field of immunotherapy [211]. While extensive work is still required to develop optimal mechanisms for re-activating or restoring function of TTP or its homologs in cancer, data reported over the past decade provide promising evidence that the overall objective is sound since TTP and BRF1, in particular, show strong antineoplastic activities across a variety of cancer types.

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References

1. Lai WS, Stumpo DJ and Blackshear PJ. (1990) Rapid insulin-stimulated accumulation of an mRNA encoding a proline-rich protein. *Journal of Biological Chemistry*, **265**, 16556–16563.
2. DuBois RN, McLane MW, Ryder K, Lau LF and Nathans D. (1990) A growth factor-inducible nuclear protein with a novel cysteine/histidine repetitive sequence. *Journal of Biological Chemistry*, **265**, 19185–19191.

3. Ma Q and Herschman HR. (1991) A corrected sequence for the predicted protein from the mitogen-inducible TIS11 primary response gene. *Oncogene*, **6**, 1277–1278.
4. Heximer SP and Forsdyke DR. (1993) A human putative lymphocyte G0/G1 switch gene homologous to a rodent gene encoding a zinc-binding potential transcription factor. *DNA and Cell Biology*, **12**, 73–88.
5. Taylor GA, Lai WS, Oakey RJ, Seldin MF, Shows TB, Eddy RL and Blackshear PJ. (1991) The human TTP protein: Sequence, alignment with related proteins, and chromosomal localization of the mouse and human genes. *Nucleic Acids Research*, **19**, 3454.
6. Brooks SA and Blackshear PJ. (2013) Tristetraprolin (TTP): Interactions with mRNA and proteins, and current thoughts on mechanisms of action. *Biochimica et Biophysica Acta*, **1829**, 666–679.
7. Sanduja S, Blanco FF and Dixon DA. (2011) The roles of TTP and BRF proteins in regulated mRNA decay. *Wiley Interdisciplinary Reviews: RNA*, **2**, 42–57.
8. Varnum BC, Ma QF, Chi TH, Fletcher B and Herschman HR. (1991) The TIS11 primary response gene is a member of a gene family that encodes proteins with a highly conserved sequence containing an unusual Cys-His repeat. *Molecular and Cellular Biology*, **11**, 1754–1758.
9. Wells ML, Perera L and Blackshear PJ. (2017) An ancient family of RNA-binding proteins: Still important! *Trends in Biochemical Sciences*, **42**, 285–296.
10. Blackshear PJ, Phillips RS, Ghosh S, Ramos SBV, Richfield EK and Lai WS. (2005) Zfp36l3, a rodent X chromosome gene encoding a placenta-specific member of the tristetraprolin family of CCCH tandem zinc finger proteins. *Biology of Reproduction*, **73**, 297–307.
11. Lai WS, Wells ML, Perera L and Blackshear PJ. (2019) The tandem zinc finger RNA binding domain of members of the tristetraprolin protein family. *Wiley Interdisciplinary Reviews: RNA,* **10**, e1531.
12. Lai WS, Kennington EA and Blackshear PJ. (2002) Interactions of CCCH zinc finger proteins with mRNA: Non-binding tristetraprolin mutants exert an inhibitory effect on degradation of AU-rich element-containing mRNAs. *Journal of Biological Chemistry*, **277**, 9606–9613.
13. Brewer BY, Malicka J, Blackshear PJ and Wilson GM. (2004) RNA sequence elements required for high affinity binding by the zinc finger domain of tristetraprolin: Conformational changes coupled to the bipartite nature of AU-rich mRNA-destabilizing motifs. *Journal of Biological Chemistry*, **279**, 27870–27877.
14. Michalek JL, Lee SJ and Michel SLJ. (2012) Cadmium coordination to the zinc binding domains of the non-classical zinc finger protein tristetraprolin affects RNA binding selectivity. *Journal of Inorganic Biochemistry*, **112**, 32–38.
15. diTargiani RC, Lee SJ, Wassink S and Michel SLJ. (2006) Functional characterization of iron-substituted tristetraprolin-2D (TTP-2D, NUP475-2D): RNA binding affinity and selectivity. *Biochemistry*, **45**, 13641–13649.

16. Lee SJ and Michel SLJ. (2010) Cysteine oxidation enhanced by iron in tristetraprolin, a zinc finger peptide. *Inorganic Chemistry*, **49**, 1211–1219.
17. Shimberg GD, Ok K, Neu HM, Splan KE and Michel SLJ. (2017) Cu(I) disrupts the structure and function of the nonclassical zinc finger protein tristetraprolin (TTP). *Inorganic Chemistry*, **56**, 6838–6848.
18. Ok K, Li W, Neu HM, Batelu S, Stemmler TL, Kane MA and Michel SLJ. (2020) The role of gold in inflammation and tristetraprolin activity. *Chemistry*, **26**, 1535–1547.
19. Murata T, Yoshino Y, Morita N and Kaneda N. (2002) Identification of nuclear import and export signals within the structure of the zinc finger protein TIS11. *Biochemical and Biophysical Research Communications*, **293**, 1242–1247.
20. Phillips RS, Ramos SBV and Blackshear PJ. (2002) Members of the tristetraprolin family of tandem CCCH zinc finger proteins exhibit CRM1-dependent nucleocytoplasmic shuttling. *Journal of Biological Chemistry*, **277**, 11606–11613.
21. Blackshear PJ and Perera L. (2014) Phylogenetic distribution and evolution of the linked RNA-binding and NOT1-binding domains in the tristetraprolin family of tandem CCCH zinc finger proteins. *Journal of Interferon & Cytokine Research*, **34**, 297–306.
22. Clark AR and Dean JLE. (2016) The control of inflammation via the phosphorylation and dephosphorylation of tristetraprolin: A tale of two phosphatases. *Biochemical Society Transactions*, **44**, 1321–1337.
23. Taylor GA, Carballo E, Lee DM, Lai WS, Thompson MJ, Patel DD, Schenkman DI, Gilkeson GS, Broxmeyer HE, Haynes BF, *et al.* (1996) A pathogenic role for TNFa in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. *Immunity*, **4**, 445–454.
24. Carballo E, Lai WS and Blackshear PJ. (1998) Feedback inhibition of macrophage tumor necrosis factor-a production by tristetraprolin. *Science*, **281**, 1001–1005.
25. Lai WS, Carballo E, Strum JR, Kennington EA, Phillips RS and Blackshear PJ. (1999) Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. *Molecular and Cellular Biology*, **19**, 4311–4323.
26. Barreau C, Paillard L and Osborne HB. (2005) AU-rich elements and associated factors: Are there unifying principles? *Nucleic Acids Research*, **33**, 7138–7150.
27. von Roretz C, Marco SD, Mazroui R and Gallouzi IE. (2011) Turnover of AU-rich-containing mRNAs during stress: A matter of survival. *Wiley Interdisciplinary Reviews: RNA,* **2**, 336–347.
28. Halees AS, El-Badrawi R and Khabar KSA. (2008) ARED Organism: Expansion of ARED reveals AU-rich element cluster variations between human and mouse. *Nucleic Acids Research*, **36**, D137–D140.
29. Gruber AR, Fallmann J, Kratochvill F, Kovarik P and Hofacker IL. (2011) AREsite: A database for the comprehensive investigation of AU-rich elements. *Nucleic Acids Research*, **39**, D66–D69.

30. Stumpo DJ, Byrd NA, Phillips RS, Ghosh S, Maronpot RR, Castranio T, Meyers EN, Mishina Y and Blackshear PJ. (2004) Chorioallantoic fusion defects and embryonic lethality resulting from disruption of Zfp36L1, a gene encoding a CCCH tandem zinc finger protein of the tristetraprolin family. *Molecular and Cellular Biology*, **24**, 6445–6455.
31. Bell SE, Sanchez MJ, Spasic-Boskovic O, Santalucia T, Gambardella L, Burton GJ, Murphy JJ, Norton JD, Clark AR and Turner M. (2006) The RNA binding protein Zfp36l1 is required for normal vascularisation and post-transcriptionally regulates VEGF expression. *Developmental Dynamics*, **235**, 3144–3155.
32. Stumpo DJ, Broxmeyer HE, Ward T, Cooper S, Hangoc G, Chung YJ, Shelley WC, Richfield EK, Ray MK, Yoder MC, *et al.* (2009) Targeted disruption of Zfp36l2, encoding a CCCH tandem zinc finger RNA-binding protein, results in defective hematopoiesis. *Blood*, **114**, 2401–2410.
33. Ramos SBV, Stumpo DJ, Kennington EA, Phillips RS, Bock CB, Ribeiro-Neto F and Blackshear PJ. (2004) The CCCH tandem zinc-finger protein Zfp36l2 is crucial for female fertility and early embryonic development. *Development*, **131**, 4883–4893.
34. Sandler H and Stoecklin G. (2008) Control of mRNA decay by phosphorylation of tristetraprolin. *Biochemical Society Transactions*, **36**, 491–496.
35. Wu X and Brewer G. (2012) The regulation of mRNA stability in mammalian cells: 2.0. *Gene*, **500**, 10–21.
36. Schoenberg DR and Maquat LE. (2012) Regulation of cytoplasmic mRNA decay. *Nature Reviews Genetics*, **13**, 246–259.
37. Chen C-YA and Shyu A-B. (2011) Mechanisms of deadenylation-dependent decay. *Wiley Interdisciplinary Reviews: RNA,* **2**, 167–183.
38. Goldstrohm AC and Wickens M. (2008) Multifunctional deadenylase complexes diversify mRNA control. *Nature Reviews Molecular and Cellular Biology*, **9**, 337–344.
39. Schmid M and Jensen TH. (2008) The exosome: A multipurpose RNA-decay machine. *Trends in Biochemical Sciences*, **33**, 501–510.
40. Arribas-Layton M, Wu D, Lykke-Andersen J and Song H. (2013) Structural and functional control of the eukaryotic mRNA decapping machinery. *Biochimica et Biophysica Acta*, **1829**, 580–589.
41. Jones CI, Zabolotskaya MV and Newbury SF. (2012) The 5′ -> 3′ exoribonuclease XRN1/Pacman and its functions in cellular processes and development. *Wiley Interdisciplinary Reviews: RNA,* **3**, 455–468.
42. Hargrove JL and Schmidt FH. (1989) The role of mRNA and protein stability in gene expression. *FASEB Journal*, **3**, 2360–2370.
43. White EJF, Brewer G and Wilson GM. (2013) Post-transcriptional control of gene expression by AUF1: Mechanisms, physiological targets, and regulation. *Biochimica et Biophysica Acta*, **1829**, 680–688.

44. Garneau NL, Wilusz J and Wilusz CJ. (2007) The highways and byways of mRNA decay. *Nature Reviews Molecular and Cellular Biology*, **8**, 113–126.
45. Jing Q, Huang S, Guth S, Zarubin T, Motoyama A, Chen J, Di Padova F, Lin S-C, Gram H and Han J. (2005) Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell*, **120**, 623–634.
46. Briata P, Chen CY, Ramos A and Gherzi R. (2013) Functional and molecular insights into KSRP function in mRNA decay. *Biochimica et Biophysica Acta*, **1829**, 689–694.
47. Schultz CW, Preet R, Dhir T, Dixon DA and Brody JR. (2020) Understanding and targeting the disease-related RNA binding protein human antigen R (HuR). *Wiley Interdisciplinary Reviews: RNA,* **11**, e1581.
48. Kishor A, Tandukar B, Ly YV, Toth EA, Suarez Y, Brewer G and Wilson GM. (2013) Hsp70 is a novel posttranscriptional regulator of gene expression that binds and stabilizes selected mRNAs containing AU-rich elements. *Molecular and Cellular Biology*, **33**, 71–84.
49. White EJF, Matsangos AE and Wilson GM. (2017) AUF1 regulation of coding and noncoding RNA. *Wiley Interdisciplinary Reviews: RNA*, **8**, e1393.
50. Gratacós FM and Brewer G. (2010) The role of AUF1 in regulated mRNA decay. *Wiley Interdisciplinary Reviews: RNA,* **1**, 457–473.
51. Waris S, Wilce MCJ and Wilce JA. (2014) RNA recognition and stress granule formation by TIA proteins. *International Journal of Molecular Sciences*, **15**, 23377–23388.
52. Lai WS, Carballo E, Thorn JM, Kennington EA and Blackshear PJ. (2000) Interactions of CCCH zinc finger proteins with mRNA: Binding of tristetraprolin-related zinc-finger proteins to AU-rich elements and destabilization of mRNA. *Journal of Biological Chemistry*, **275**, 17827–17837.
53. Wilson GM, Sutphen K, Chuang K and Brewer G. (2001) Folding of A+U-rich RNA elements modulates AUF1 binding: Potential roles in regulation of mRNA turnover. *Journal of Biological Chemistry*, **276**, 8695–8704.
54. Wilson GM, Sutphen K, Bolikal S, Chuang K and Brewer G. (2001) Thermodynamics and kinetics of Hsp70 association with A+U-rich mRNA-destabilizing sequences. *Journal of Biological Chemistry*, **276**, 44450–44456.
55. Hudson BD, Martinez-Yamout MA, Dyson HJ and Wright PE. (2004) Recognition of the mRNA AU-rich element by the zinc finger domain of TIS11d. *Nature Structural & Molecular Biology*, **11**, 257–264.
56. Ascano M, Hafner M, Cekan P, Gerstberger S and Tuschl T. (2012) Identification of RNA-protein interaction networks using PAR-CLIP. *Wiley Interdisciplinary Reviews: RNA,* **3**, 159–177.
57. Mukherjee N, Jacobs NC, Hafner M, Kennington EA, Nusbaum JD, Tuschl T, Blackshear PJ and Ohler U. (2014) Global target mRNA specification and regulation by the RNA-binding protein ZFP36. *Genome Biology*, **15**, R12.
58. Sedlyarov V, Fallmann J, Ebner F, Huemer J, Sneezum L, Ivin M, Kreiner K, Tanzer A, Vogl C, Hofacker I, *et al.* (2016) Tristetraprolin binding site atlas in the

macrophage transcriptome reveals a switch for inflammation resolution. *Molecular Systems Biology*, **12**, 868.

59. Moore MJ, Blachere NE, Fak JJ, Park CY, Sawicka K, Parveen S, Zucker-Scharff I, Moltedo B, Rudensky AY and Darnell RB. (2018) ZFP36 RNA-binding proteins restrain T cell activation and anti-viral immunity. *eLife Sciences*, **7**, e33057.
60. Kedar VP, Zucconi BE, Wilson GM and Blackshear PJ. (2012) Direct binding of specific AUF1 isoforms to the tandem zinc finger domains of tristetraprolin (TTP) family proteins. *Journal of Biological Chemistry*, **287**, 5459–5471.
61. Reznik B, Clement SL and Lykke-Andersen J. (2014) hnRNP F complexes with tristetraprolin and stimulates ARE-mRNA decay. *PLoS ONE*, **9**, e100992.
62. Tiedje C, Diaz-Muñoz MD, Trulley P, Ahlfors H, Laaß K, Blackshear PJ, Turner M and Gaestel M. (2016) The RNA-binding protein TTP is a global post-transcriptional regulator of feedback control in inflammation. *Nucleic Acids Research*, **44**, 7418–7440.
63. Carballo E, Lai WS and Blackshear PJ. (2000) Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. *Blood*, **95**, 1891–1899.
64. Collart MA. (2016) The Ccr4-Not complex is a key regulator of eukaryotic gene expression. *Wiley Interdisciplinary Reviews: RNA,* **7**, 438–454.
65. Cheng Y and Li G. (2012) Role of the ubiquitin ligase Fbw7 in cancer progression. *Cancer and Metastasis Reviews*, **31**, 75–87.
66. Lai WS, Kennington EA and Blackshear PJ. (2003) Tristetraprolin and its family members can promote the cell-free deadenylation of AU-rich element-containing mRNAs by poly(A) ribonuclease. *Molecular and Cellular Biology*, **23**, 3798–3812.
67. Sandler H, Kreth J, Timmers HTh and Stoecklin G. (2011) Not1 mediates recruitment of the deadenylase Caf1 to mRNAs targeted for degradation by tristetraprolin. *Nucleic Acids Research*, **39**, 4373–4386.
68. Raisch T, Chang CT, Levdansky Y, Muthukumar S, Raunser S and Valkov E. (2019) Reconstitution of recombinant human CCR4-NOT reveals molecular insights into regulated deadenylation. *Nature Communications*, **10**, 3173.
69. Tang TTL, Stowell JAW, Hill CH and Passmore LA. (2019) The intrinsic structure of poly(A) RNA determines the specificity of Pan2 and Caf1 deadenylases. *Nature Structural & Molecular Biology*, **26**, 433–442.
70. Fabian MR, Frank F, Rouya C, Siddiqui N, Lai WS, Karetnikov A, Blackshear PJ, Nagar B and Sonenberg N. (2013) Structural basis for the recruitment of the human CCR4-NOT deadenylase complex by tristetraprolin. *Nature Structural & Molecular Biology*, **20**, 735–739.
71. Bulbrook D, Brazier H, Mahajan P, Kliszczak M, Fedorov O, Marchese FP, Aubareda A, Chalk R, Picaud S, Strain-Damerell C, *et al.* (2018) Tryptophan-mediated interactions between tristetraprolin and the CNOT9 subunit are required for CCR4-NOT deadenylase complex recruitment. *Journal of Molecular Biology*, **430**, 722–736.

72. Lykke-Andersen J and Wagner E. (2005) Recruitment and activation of mRNA decay enzymes by two ARE-mediated decay activation domains in the proteins TTP and BRF-1. *Genes & Development*, **19**, 351–361.
73. Hau HH, Walsh RJ, Ogilvie RL, Williams DA, Reilly CS and Bohjanen PR. (2007) Tristetraprolin recruits functional mRNA decay complexes to ARE sequences. *Journal of Cellular Biochemistry*, **100**, 1477–1492.
74. Zhang T, Kruys V, Huez G and Gueydan C. (2002) AU-rich element-mediated translational control: Complexity and multiple activities of trans-acting factors. *Biochemical Society Transactions*, **30**, 952–958.
75. Otsuka H, Fukao A, Funakami Y, Duncan KE and Fujiwara T. (2019) Emerging evidence of translational control by AU-rich element-binding proteins. *Frontiers in Genetics*, **10**, 332.
76. Kratochvill F, Gratz N, Qualls JE, Van De Velde L, Chi H, Kovarik P and Murray PJ. (2015) Tristetraprolin limits inflammatory cytokine production in tumor-associated macrophages in an mRNA decay-independent manner. *Cancer Research*, **75**, 3054–3064.
77. Hershey JWB, Sonenberg N and Mathews MB. (2012) Principles of translational control: An overview. *Cold Spring Harbor Perspectives in Biology*, **4**, a011528.
78. Tao X and Gao G. (2015) Tristetraprolin recruits eukaryotic initiation factor 4E2 to repress translation of AU-rich element-containing mRNAs. *Molecular and Cellular Biology*, **35**, 3921–3932.
79. Rom E, Kim HC, Gingras A-C, Marcotrigiano J, Favre D, Olsen H, Burley SK and Sonenberg N. (1998) Cloning and characterization of 4EHP, a novel mammalian eIF4E-related cap-binding protein. *Journal of Biological Chemistry*, **273**, 13104–13109.
80. Ptushkina M, Berthelot K, von der Haar T, Geffers L, Warwicker J and McCarthy JEG. (2001) A second eIF4E protein in Schizosaccharomyces pombe has distinct eIF4G-binding properties. *Nucleic Acids Research*, **29**, 4561–4569.
81. Qi M, Wang Z, Zhang Z, Shao Q, Zeng A, Li X, Li W, Wang C, Tian F, Li Q, *et al.* (2012) AU-rich-element-dependent translation repression requires the cooperation of tristetraprolin and RCK/p54. *Molecular and Cellular Biology*, **32**, 913–928.
82. Liu T, Zhang L, Joo D and Sun SC. (2017) NF-kB signaling in inflammation. *Signal Transduction and Targeted Therapy*, **2**, 17023.
83. Liang J, Lei T, Song Y, Yanes N, Qi Y and Fu M. (2009) RNA-destabilizing factor tristetraprolin negatively regulates NF-kB signaling. *Journal of Biological Chemistry*, **284**, 29383–29390.
84. Schichl YM, Resch U, Hofer-Warbinek R and de Martin R. (2009) Tristetraprolin impairs NF-kB/p65 nuclear translocation. *Journal of Biological Chemistry*, **284**, 29571–29581.
85. Barrios-García T, Gómez-Romero V, Tecalco-Cruz Á, Valadéz-Graham V and León-Del-Río A. (2016) Nuclear tristetraprolin acts as a corepressor of multiple steroid nuclear receptors in breast cancer cells. *Molecular Genetics and Metabolism Reports*, **7**, 20–26.

86. Barrios-Garcia T, Tecalco-Cruz A, Gomez-Romero V, Reyes-Carmona S, Meneses-Morales I and Leon-Del-Rio A. (2014) Tristetraprolin represses estrogen receptor a transactivation in breast cancer cells. *Journal of Biological Chemistry*, **289**, 15554–15565.
87. Protter DSW and Parker R. (2016) Principles and properties of stress granules. *Trends in Cell Biology*, **26**, 668–679.
88. Luo Y, Na Z and Slavoff SA. (2018) P-bodies: Composition, properties, and functions. *Biochemistry*, **57**, 2424–2431.
89. Franks TM and Lykke-Andersen J. (2007) TTP and BRF proteins nucleate processing body formation to silence mRNAs with AU-rich elements. *Genes & Development*, **21**, 719–735.
90. Maeda M, Sawa H, Tobiume M, Tokunaga K, Hasegawa H, Ichinohe T, Sata T, Moriyama M, Hall WW, Kurata T, *et al.* (2006) Tristetraprolin inhibits HIV-1 production by binding to genomic RNA. *Microbes and Infection*, **8**, 2647–2656.
91. Carrick DM and Blackshear PJ. (2008) Comparative expression of tristetraprolin (TTP) family member transcripts in normal human tissues and cancer cell lines. *Archives of Biochemistry and Biophysics*, **462**, 278–285.
92. Karlsson M, Zhang C, Méar L, Zhong W, Digre A, Katona B, Sjöstedt E, Butler L, Odeberg J, Dusart P, *et al.* (2021) A single-cell type transcriptomics map of human tissues. *Science Advances*, **7**, eabh2169.
93. Uhlen M, Karlsson MJ, Zhong W, Tebani A, Pou C, Mikes J, Lakshmikanth T, Forsström B, Edfors F, Odeberg J, *et al.* (2019) A genome-wide transcriptomic analysis of protein-coding genes in human blood cells. *Science*, **366**, eaax9198.
94. Taylor GA, Thompson MJ, Lai WS and Blackshear PJ. (1996) Mitogens stimulate the rapid nuclear to cytoplasmic translocation of tristetraprolin, a potential zinc-finger transcription factor. *Journal of Molecular Endocrinology*, **10**, 140–146.
95. Lai WS, Thompson MJ, Taylor GA, Liu Y and Blackshear PJ. (1995) Promoter analysis of *Zfp-36*, the mitogen-inducible gene encoding the zinc finger protein tristetraprolin. *Journal of Biological Chemistry*, **270**, 25266–25272.
96. Chen YL, Jiang YW, Su YL, Lee SC, Chang MS and Chang CJ. (2013) Transcriptional regulation of tristetraprolin by NF-kB signaling in LPS-stimulated macrophages. *Molecular Biology Reports*, **40**, 2867–2877.
97. Florkowska M, Tymoszuk P, Balwierz A, Skucha A, Kochan J, Wawro M, Stalinska K and Kasza A. (2012) EGF activates TTP expression by activation of ELK-1 and EGR-1 transcription factors. *BMC Molecular Biology*, **13**, 8.
98. Tchen CR, Brook M, Saklatvala J and Clark AR. (2004) The stability of tristetraprolin mRNA is regulated by mitogen-activated protein kinase p38 and by tristetraprolin itself. *Journal of Biological Chemistry*, **279**, 32393–32400.
99. Brooks SA, Connolly JE and Rigby WFC. (2004) The role of mRNA turnover in the regulation of tristetraprolin expression: Evidence for an extracellular signal-regulated kinase-specific, AU-rich element-dependent, autoregulatory pathway. *The Journal of Immunology*, **172**, 7263.

100. Gebeshuber CA, Zatloukal K and Martinez J. (2009) miR-29a suppresses tristetraprolin, which is a regulator of epithelial polarity and metastasis. *EMBO Reports*, **10**, 400–405.
101. Guo J, Li J, Zhao J, Yang S, Wang L, Cheng G, Liu D, Xiao J, Liu Z and Zhao Z. (2017) MiRNA-29c regulates the expression of inflammatory cytokines in diabetic nephropathy by targeting tristetraprolin. *Scientific Reports*, **7**, 2314.
102. Ngoc LV, Wauquier C, Soin R, Bousbata S, Twyffels L, Kruys V and Gueydan C. (2014) Rapid proteasomal degradation of posttranscriptional regulators of the TIS11/tristetraprolin family is induced by an intrinsically unstructured region independently of ubiquitination. *Molecular and Cellular Biology*, **34**, 4315–4328.
103. Brook M, Tchen CR, Santalucia T, McIlrath J, Arthur JS, Saklatvala J and Clark AR. (2006) Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. *Molecular and Cellular Biology*, **26**, 2408–2418.
104. Bustos DM. (2012) The role of protein disorder in the 14-3-3 interaction network. *Molecular BioSystems*, **8**, 178–184.
105. Schichl YM, Resch U, Lemberger CE, Stichlberger D and de Martin R. (2011) Novel phosphorylation-dependent ubiquitination of tristetraprolin by mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase 1 (MEKK1) and tumor necrosis factor receptor-associated factor 2 (TRAF2). *Journal of Biological Chemistry*, **286**, 38466–38477.
106. Bourcier C, Griseri P, Grépin R, Bertolotto C, Mazure N and Pagès G. (2011) Constitutive ERK activity induces downregulation of tristetraprolin, a major protein controlling interleukin8/CXCL8 mRNA stability in melanoma cells. *American Journal of Physiology-Cell Physiology*, **301**, C609.
107. Huang L, Yu Z, Zhang Z, Ma W, Song S and Huang G. (2016) Interaction with pyruvate kinase M2 destabilizes tristetraprolin by proteasome degradation and regulates cell proliferation in breast cancer. *Scientific Reports*, **6**, 22449.
108. Benjamin D, Schmidlin M, Min L, Gross B and Moroni C. (2006) BRF1 protein turnover and mRNA decay activity are regulated by protein kinase B at the same phosphorylation sites. *Molecular and Cellular Biology*, **26**, 9497–9507.
109. Maitra S, Chou CF, Luber CA, Lee KY, Mann M and Chen CY. (2008) The AU-rich element mRNA decay-promoting activity of BRF1 is regulated by mitogen-activated protein kinase-activated protein kinase 2. *RNA*, **14**, 950–959.
110. Winzen R, Kracht M, Ritter B, Wilhem A, Chen C-YA, Shyu A-B, Muller M, Gaestel M, Resch K and Holtmann H. (1999) The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. *EMBO Journal*, **18**, 4969–4980.
111. Lasa M, Mahtani KR, Finch A, Brewer G, Saklatvala J and Clark AR. (2000) Regulation of cyclooxygenase 2 mRNA stability by the mitogen-activated protein kinase p38 signaling cascade. *Molecular and Cellular Biology*, **20**, 4265–4274.

112. Dean JLE, Sarsfield SJ, Tsounakou E and Saklatvala J. (2003) p38 mitogen-activated protein kinase stabilizes mRNAs that contain cyclooxygenase-2 and tumor necrosis factor AU-rich elements by inhibiting deadenylation. *Journal of Biological Chemistry*, **278**, 39470–39476.
113. Marchese FP, Aubareda A, Tudor C, Saklatvala J, Clark AR and Dean JLE. (2010) MAPKAP kinase 2 blocks tristetraprolin-directed mRNA decay by inhibiting CAF1 deadenylase recruitment. *Journal of Biological Chemistry*, **285**, 27590–27600.
114. Chrestensen CA, Schroeder MJ, Shabanowitz J, Hunt DF, Pelo JW, Worthington MT and Sturgill TW. (2004) MAPKAP kinase 2 phosphorylates tristetraprolin on *in vivo* sites including Ser^{178}, a site required for 14-3-3 binding. *Journal of Biological Chemistry*, **279**, 10176–10184.
115. Hsieh HH, Chen YA, Chang YJ, Wang HH, Yu YH, Lin SW, Huang YJ, Lin S and Chang CJ. (2021) The functional characterization of phosphorylation of tristetraprolin at C-terminal NOT1-binding domain. *Journal of Inflammation*, **18**, 22.
116. Deleault KM, Skinner SJ and Brooks SA. (2008) Tristetraprolin regulates TNF-a mRNA stability via a proteasome dependent mechanism involving the combined action of the ERK and p38 pathways. *Molecular Immunology*, **45**, 13–24.
117. Adachi S, Homoto M, Tanaka R, Hioki Y, Murakami H, Suga H, Matsumoto M, Nakayama KI, Hatta T, Iemura S, *et al.* (2014) ZFP36L1 and ZFP36L2 control LDLR mRNA stability via the ERK-RSK pathway. *Nucleic Acids Research*, **42**, 10037–10049.
118. Ciais D, Cherradi N, Bailly S, Grenier E, Berra E, Pouyssegur J, LaMarre J and Feige J-J. (2004) Destabilization of vascular endothelial growth factor mRNA by the zinc-finger protein TIS11b. *Oncogene*, **23**, 8673–8680.
119. Essafi-Benkhadir K, Onesto C, Stebe E, Moroni C and Pages G. (2007) Tristetraprolin inhibits Ras-dependent tumor vascularization by inducing vascular endothelial growth factor mRNA degradation. *Molecular Biology of the Cell*, **18**, 4648–4658.
120. Nishida N, Yano H, Nishida T, Kamura T and Kojiro M. (2006) Angiogenesis in cancer. *Vascular Health and Risk Management*, **2**, 213–219.
121. Boutand O, Dixon DA, Oates JA and Sawaoka H. (2003) Tristetrapolin binds to the COX-2 mRNA 3′ untranslated region in cancer cells. In *Advances in Prostaglandin, Leukotriene, and Other Bioactive Lipid Research: Basic Science and Clinical Applications*, Yazici Z, Folco GC, Drazen JM, Nigam S & Shimizu T (eds.), Springer, Boston, MA, USA, pp. 157–160.
122. Phillips K, Kedersha N, Shen L, Blackshear PJ and Anderson P. (2004) Arthritis suppressor genes TIA-1 and TTP dampen the expression of tumor necrosis factor a, cyclooxygenase 2, and inflammatory arthritis. *Proceedings of the National Academy of Sciences of the United States*, **101**, 2011–2016.
123. Smith WL, DeWitt DL and Garavito RM. (2000) Cyclooxygenases: Structural, cellular, and molecular biology. *Annual Review of Biochemistry*, **69**, 145–182.
124. Williams CS, Mann M and DuBois RN. (1999) The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene*, **18**, 7908–7916.

125. Gómez-Valenzuela F, Escobar E, Pérez-Tomás R and Montecinos VP. (2021) The inflammatory orofile of the tumor microenvironment, orchestrated by cyclooxygenase-2, promotes epithelial-mesenchymal transition. *Frontiers in Oncology*, **11**, 686792.
126. Chimal-Ramírez GK, Espinoza-Sánchez NA and Fuentes-Pananá EM. (2015) A role for the inflammatory mediators Cox-2 and metalloproteinases in cancer stemness. *Anti-Cancer Agents in Medicinal Chemistry*, **15**, 837–855.
127. Hanahan D and Weinberg RA. (2011) Hallmarks of cancer: The next generation. *Cell*, **144**, 646–674.
128. Ross CR, Brennan-Laun SE and Wilson GM. (2012) Tristetraprolin: Roles in cancer and senescence. *Ageing Research Reviews*, **11**, 473–484.
129. Sanduja S, Blanco FF, Young LE, Kaza V and Dixon DA. (2012) The role of tristetraprolin in cancer and inflammation. *Frontiers in Bioscience*, **17**, 174–188.
130. Upadhyay R, Sanduja S, Kaza V and Dixon DA. (2013) Genetic polymorphisms in RNA binding proteins contribute to breast cancer survival. *International Journal of Cancer*, **132**, E128–E138.
131. Lee SR, Mun JY, Jeong MS, Lee HH, Roh YG, Kim WT, Kim MH, Heo J, Choi YH, Kim SJ, *et al.* (2019) Thymoquinone-induced tristetraprolin inhibits tumor growth and metastasis through destabilization of MUC4 mRNA. *International Journal of Molecular Sciences*, **20**, 2614.
132. Yoon NA, Jo HG, Lee UH, Park JH, Yoon JE, Ryu J, Kang SS, Min YJ, Ju SA, Seo EH, *et al.* (2016) Tristetraprolin suppresses the EMT through the down-regulation of Twist1 and Snail1 in cancer cells. *Oncotarget*, **7**, 8931–8943.
133. Wei ZR, Liang C, Feng D, Cheng YJ, Wang WM, Yang DJ, Wang YX and Cai QP. (2016) Low tristetraprolin expression promotes cell proliferation and predicts poor patients outcome in pancreatic cancer. *Oncotarget*, **7**, 17737–17750.
134. Zekavati A, Nasir A, Alcaraz A, Aldrovandi M, Marsh P, Norton JD and Murphy JJ. (2014) Post-transcriptional regulation of BCL2 mRNA by the RNA-binding protein ZFP36L1 in malignant B cells. *PLoS ONE*, **9**, e102625.
135. Loh XY, Sun QY, Ding LW, Mayakonda A, Venkatachalam N, Yeo MS, Silva TC, Xiao JF, Doan NB, Said JW, *et al.* (2020) RNA-binding protein ZFP36L1 suppresses hypoxia and cell-cycle signaling. *Cancer Research*, **80**, 219–233.
136. Suk FM, Chang CC, Lin RJ, Lin SY, Liu SC, Jau CF and Liang YC. (2018) ZFP36L1 and ZFP36L2 inhibit cell proliferation in a cyclin D-dependent and p53-independent manner. *Scientific Reports*, **8**, 2742.
137. Brennan SE, Kuwano Y, Alkharouf N, Blackshear PJ, Gorospe M and Wilson GM. (2009) The mRNA-destabilizing protein tristetraprolin is suppressed in many cancers, altering tumorigenic phenotypes and patient prognosis. *Cancer Research*, **69**, 5168–5176.
138. Hitti E, Bakheet T, Al-Souhibani N, Moghrabi W, Al-Yahya S, Al-Ghamdi M, Al-Saif M, Shoukri MM, Lánczky A, Grépin R, *et al.* (2016) Systematic analysis of

AU-rich element expression in cancer reveals common functional clusters regulated by key RNA-binding proteins. *Cancer Research*, **76**, 4068.

139. Goddio MV, Gattelli A, Slomiansky V, Lacunza E, Gingerich T, Tocci JM, Facchinetti MM, Curino AC, LaMarre J, Abba MC, *et al.* (2012) Mammary differentiation induces expression of tristetraprolin, a tumor suppressor AU-rich mRNA-binding protein. *Breast Cancer Research and Treatment*, **135**, 749–758.
140. Berglund AE, Scott KEN Li W, Yang C, Fernandez MR, Schaub FX, Cleveland JL and Rounbehler RJ (2016) Tristetraprolin disables prostate cancer maintenance by impairing proliferation and metabolic function. *Oncotarget*, **7**, 83462–83475.
141. Saini Y, Chen J and Patial S. (2020) The tristetraprolin family of RNA-binding proteins in cancer: Progress and future prospects. *Cancers*, **12**, 1539.
142. Rounbehler RJ, Berglund AE, Gerke T, Takhar MM, Awasthi S, Li W, Davicioni E, Erho NG, Ross AE, Schaeffer EM, *et al.* (2018) Tristetraprolin is a prognostic biomarker for poor outcomes among patients with low-grade prostate cancer. *Cancer Epidemiology, Biomarkers & Prevention*, **27**, 1376–1383.
143. Zeng B, Zhu D, Su Z, Li Z and Yu Z. (2016) Tristetraprolin exerts tumor suppressive functions on the tumorigenesis of glioma by targeting IL-13. *International Immunopharmacology*, **39**, 63–70.
144. Fallahi M, Amelio AL, Cleveland JL and Rounbehler RJ. (2014) CREB targets define the gene expression signature of malignancies having reduced levels of the tumor suppressor tristetraprolin. *PLoS One*, **9**, e115517.
145. Hodson DJ, Janas ML, Galloway A, Bell SE, Andrews S, Li CM, Pannell R, Siebel CW, MacDonald HR, De Keersmaecker K, *et al.* (2010) Deletion of the RNA-binding proteins ZFP36L1 and ZFP36L2 leads to perturbed thymic development and T lymphoblastic leukemia. *Nature Immunology*, **11**, 717–724.
146. Earp MA, Kelemen LE, Magliocco AM, *et al.* (2014) Genome-wide association study of subtype-specific epithelial ovarian cancer risk alleles using pooled DNA. *Human Genetics*, **133**, 481–497.
147. Yonemori K, Seki N, Kurahara H, Osako Y, Idichi T, Arai T, Koshizuka K, Kita Y, Maemura K and Natsugoe S. (2017) ZFP36L2 promotes cancer cell aggressiveness and is regulated by antitumor microRNA-375 in pancreatic ductal adenocarcinoma. *Cancer Science*, **108**, 124–135.
148. Park SB, Chung CK, Gonzalez E and Yoo C. (2018) Causal inference network of genes related with bone metastasis of breast cancer and osteoblasts using causal Bayesian networks. *Journal of Bone Metabolism*, **25**, 251–266.
149. Han Y, Zheng Q, Tian Y, Ji Z and Ye H. (2019) Identification of a nine-gene panel as a prognostic indicator for recurrence with muscle-invasive bladder cancer. *Journal of Surgical Oncology*, **119**, 1145–1154.
150. Xu L, Ning H, Gu L, Wang Q, Lu W, Peng H, Cui W, Ying B, Ross CR, Wilson GM, *et al.* (2015) Tristetraprolin induces cell cycle arrest in breast tumor cells through targeting AP-1/c-Jun and NF-kB pathway. *Oncotarget*, **6**, 41679–41691.

151. Dong F, Li C, Wang P, Deng X, Luo Q, Tang X and Xu L. (2018) The RNA binding protein tristetraprolin down-regulates autophagy in lung adenocarcinoma cells. *Experimental Cell Research*, **367**, 89–96.
152. Ghelli Luserna di Rorà A, Cerchione C, Martinelli G and Simonetti G. (2020) A WEE1 family business: Regulation of mitosis, cancer progression, and therapeutic target. *Journal of Hematology & Oncology*, **13**, 126.
153. Do K, Doroshow JH and Kummar S. (2013) Wee1 kinase as a target for cancer therapy. *Cell Cycle*, **12**, 3348–3353.
154. Li J, Pan C, Boese AC, Kang J, Umano AD, Magliocca KR, Yang W, Zhang Y, Lonial S, Jin L, *et al.* (2020) DGKA provides platinum resistance in ovarian cancer through activation of c-JUN-WEE1 signaling. *Clinical Cancer Research*, **26**, 3843–3855.
155. Rounbehler RJ, Fallahi M, Yang C, Steeves MA, Li W, Doherty JR, Schaub FX, Sanduja S, Dixon DA, Blackshear PJ, *et al.* (2012) Tristetraprolin impairs Myc-induced lymphoma and abolishes the malignant state. *Cell*, **150**, 563–574.
156. Lee HH, Lee SR and LeemSH. (2014) Tristetraprolin regulates prostate cancer cell growth through suppression of E2F1. *Journal of Microbiology and Biotechnology*, **24**, 287–294.
157. Zhao W, Qiu R, Li P and Yang J. (2017) PIM1: a promising target in patients with triple-negative breast cancer. *Medical Oncology*, **34**, 142.
158. Ouhtit A, Muzumdar S, Gupta I, Shanmuganathan S and Tamimi Y. (2015) Understanding the functional discrepancy of Pim-1 in cancer. *Frontiers in Bioscience*, **7**, 239–247.
159. Mahat DB, Brennan-Laun SE, Fialcowitz-White EJ, Kishor A, Ross CR, Pozharskaya T, Rawn JD, Blackshear PJ, Hassel BA and Wilson GM. (2012) Coordinated expression of tristetraprolin post-transcriptionally attenuates mitogenic induction of the oncogenic Ser/Thr kinase Pim-1. *PLoS One*, **7**, e33194.
160. Selmi T, Martello A, Vignudelli T, Ferrari E, Grande A, Gemelli C, Salomoni P, Ferrari S and Zanocco-Marani T. (2012) ZFP36 expression impairs glioblastoma cell lines viability and invasiveness by targeting multiple signal transduction pathways. *Cell Cycle*, **11**, 1977–1987.
161. Galloway A, Saveliev A, Lukasiak S, Hodson DJ, Bolland D, Balmanno K, Ahlfors H, Monzón-Casanova E, Mannurita SC, Bell LS, *et al.* (2016) RNA-binding proteins ZFP36L1 and ZFP36L2 promote cell quiescence. *Science*, **352**, 453–459.
162. Muñoz-Espín D and Serrano M. (2014) Cellular senescence: From physiology to pathology. *Nature Reviews Molecular and Cellular Biology*, **15**, 482–496.
163. van Deursen JM. (2014) The role of senescent cells in ageing. *Nature*, **509**, 439–446.
164. Victorelli S and Passos JF. (2017) Telomeres and cell senescence — Size matters not. *eBioMedicine*, **21**, 14–20.
165. Wyld L, Bellantuono I, Tchkonia T, Morgan J, Turner O, Foss F, George J, Danson S and Kirkland JL. (2020) Senescence and cancer: A review of clinical implications of senescence and senotherapies. *Cancers*, **12**, 2134.

166. Collado M and Serrano M. (2010) Senescence in tumours: Evidence from mice and humans. *Nature Reviews Cancer*, **10**, 51–57.
167. Akincilar SC, Unal B and Tergaonkar V. (2016) Reactivation of telomerase in cancer. *Cellular and Molecular Life Sciences*, **73**, 1659–1670.
168. Zhang JM and Zou L. (2020) Alternative lengthening of telomeres: From molecular mechanisms to therapeutic outlooks. *Cell & Bioscience*, **10**, 30.
169. Abdelmohsen K, Kuwano Y, Kim HH and Gorospe M. (2008) Posttranscriptional gene regulation by RNA-binding proteins during oxidative stress: Implications for cellular senescence. *Biological Chemistry*, **389**, 243–255.
170. Sanduja S, Kaza V and Dixon DA. (2009) The mRNA decay factor tristetraprolin (TTP) induces senescence in human papillomavirus-transformed cervical cancer cells by targeting E6-AP ubiquitin ligase. *Aging*, **1**, 803–817.
171. Mantovani F and Banks L. (2001) The human papillomavirus E6 protein and its contribution to malignant progression. *Oncogene*, **20**, 7874–7887.
172. Kalluri R and Weinberg RA. (2009) The basics of epithelial-mesenchymal transition. *Journal of Clinical Investigation*, **119**, 1420–1428.
173. Campbell K. (2018) Contribution of epithelial-mesenchymal transitions to organogenesis and cancer metastasis. *Current Opinion in Cell Biology*, **55**, 30–35.
174. Montorsi L, Guizzetti F, Alecci C, Caporali A, Martello A, Giacinto Atene C, Parenti S, Pizzini S, Zanovello P, Bortoluzzi S, *et al.* (2016) Loss of ZFP36 expression in colorectal cancer correlates to wnt/b-catenin activity and enhances epithelial-to-mesenchymal transition through upregulation of ZEB1, SOX9 and MACC1. *Oncotarget*, **7**, 59144–59157.
175. Van Tubergen EA, Banerjee R, Liu M, Broek RV, Light E, Kuo S, Feinberg SE, Willis AL, Wolf G, Carey T, *et al.* (2013) Inactivation or loss of TTP promotes invasion in head and neck cancer via transcript stabilization and secretion of MMP9, MMP2, and IL-6. *Clinical Cancer Research*, **19**, 1169–1179.
176. Liberti MV and Locasale JW. (2016) The Warburg effect: How does it benefit cancer cells? *Trends in Biochemical Sciences*, **41**, 211–218.
177. Vander Heiden MG, Cantley LC and Thompson CB. (2009) Understanding the Warburg effect: The metabolic requirements of cell proliferation. *Science*, **324**, 1029–1033.
178. Patra KC, Wang Q, Bhaskar PT, Miller L, Wang Z, Wheaton W, Chandel N, Laakso M, Muller WJ, Allen EL, *et al.* (2013) Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. *Cancer Cell*, **24**, 213–228.
179. Kim DJ, Vo MT, Choi SH, Lee JH, Jeong SY, Hong CH, Kim JS, Lee UH, Chung HM, Lee BJ, *et al.* (2019) Tristetraprolin-mediated hexokinase 2 expression regulation contributes to glycolysis in cancer cells. *Molecular Biology of the Cell*, **30**, 542–553.
180. Nair AP, Diamantis ID, Conscience JF, Kindler V, Hofer P and Moroni C. (1989) A v-H-ras-dependent hemopoietic tumor model involving progression from a clonal stage of transformation competence to autocrine interleukin 3 production. *Molecular and Cellular Biology*, **9**, 1183–1190.

181. Stoecklin G, Gross B, Ming X-F and Moroni C. (2003) A novel mechanism of tumor suppression by destabilizing AU-rich growth factor mRNA. *Oncogene*, **22**, 3554–3561.
182. Milke L, Schulz K, Weigert A, Sha W, Schmid T and Brüne B. (2013) Depletion of tristetraprolin in breast cancer cells increases interleukin-16 expression and promotes tumor infiltration with monocytes/macrophages. *Carcinogenesis*, **34**, 850–857.
183. Gerke T, Beltran H, Wang X, Lee GM, Sboner A, Karnes RJ, Klein EA, Davicioni E, Yousefi K, Ross AE, *et al.* (2019) Low tristetraprolin expression is associated with lethal prostate cancer. *Cancer Epidemiology, Biomarkers & Prevention*, **28**, 584–590.
184. Gabay M, Li Y and Felsher DW. (2014) MYC activation is a hallmark of cancer initiation and maintenance. *Cold Spring Harbor Perspectives in Medicine*, **4**, a014241.
185. Dang CV. (2012) *MYC* on the path to cancer. *Cell*, **149**, 22–35.
186. Nusse R and Clevers H. (2017) Wnt/b-catenin signaling, disease, and emerging therapeutic modalities. *Cell*, **169**, 985–999.
187. Valenta T, Hausmann G and Basler K. (2012) The many faces and functions of b-catenin. *EMBO Journal*, **31**, 2714–2736.
188. Rosenbloom KR, Dreszer TR, Pheasant M, Barber GP, Meyer LR, Pohl A, Raney BJ, Wang T, Hinrichs AS, Zweig AS, *et al.* (2010) ENCODE whole-genome data in the UCSC Genome Browser. *Nucleic Acids Research*, **38**, D620–D625.
189. Zanfi ED, Fantini S, Lotti R, Bertesi M, Marconi A, Grande A, Manfredini R, Pincelli C and Zanocco-Marani T. (2020) Wnt/CTNNB1 signal transduction pathway inhibits the expression of ZFP36 in squamous cell carcinoma, by Inducing transcriptional repressors SNAI1, SLUG and TWIST. *International Journal of Molecular Sciences*, **21**, 5692.
190. Sohn BH, Park IY, Lee JJ, Yang SJ, Jang YJ, Park KC, Kim DJ, Lee DC, Sohn HA, Kim TW, *et al.* (2010) Functional switching of TGF-b1 signaling in liver cancer via epigenetic modulation of a single CpG site in TTP Promoter. *Gastroenterology*, **138**, 1898–1908.
191. Saliminejad K, Khorram KHR, Soleymani FS and Ghaffari SH. (2019) An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. *Journal of Cellular Physiology*, **234**, 5451–5465.
192. Bartel DP. (2009) MicroRNAs: Target recognition and regulatory functions. *Cell*, **136**, 215–233.
193. Acunzo M, Romano G, Wernicke D and Croce CM. (2015) MicroRNA and cancer — A brief overview. *Advances in Biological Regulation*, **57**, 1–9.
194. Hata A and Lieberman J. (2015) Dysregulation of microRNA biogenesis and gene silencing in cancer. *Science Signaling*, **8**, re3.
195. Wang Y, Zhang X, Li H, Yu J and Ren X. (2013) The role of miRNA-29 family in cancer. *European Journal of Cell Biology*, **92**, 123–128.

196. Sun XJ, Liu BY, Yan S, Jiang TH, Cheng HQ, Jiang HS, Cao Y and Mao AW. (2015) MicroRNA-29a promotes pancreatic cancer growth by inhibiting tristetraprolin. *Cellular Physiology and Biochemistry*, **37**, 707–718.
197. Al-Ahmadi W, Al-Ghamdi M, Al-Souhibani N and Khabar KSA. (2013) miR-29a inhibition normalizes HuR over-expression and aberrant AU-rich mRNA stability in invasive cancer. *The Journal of Pathology*, **230**, 28–38.
198. Charles KA, Harris BDW, Haddad CR, Clarke SJ, Guminski A, Stevens M, Dodds T, Gill AJ, Back M, Veivers D, *et al.* (2016) Systemic inflammation is an independent predictive marker of clinical outcomes in mucosal squamous cell carcinoma of the head and neck in oropharyngeal and non-oropharyngeal patients. *BMC Cancer*, **16**, 124.
199. Suswam E, Li Y, Zhang X, Gillespie GY, Li X, Shacka JJ, Lu L, Zheng L and King PH. (2008) Tristetraprolin down-regulates interleukin-8 and vascular endothelial growth factor in malignant glioma cells. *Cancer Research*, **68**, 674–682.
200. Suswam EA, Shacka JJ, Walker K, Lu L, Li X, Si Y, Zhang X, Zheng L, Nabors LB, Cao H, *et al.* (2013) Mutant tristetraprolin: A potent inhibitor of malignant glioma cell growth. *Journal of Neuro-Oncology*, **113**, 195–205.
201. Ascierto PA, Kirkwood JM, Grob JJ, Simeone E, Grimaldi AM, Maio M, Palmieri G, Testori A, Marincola FM and Mozzillo N. (2012) The role of BRAF V600 mutation in melanoma. *Journal of Translational Medicine*, **10**, 85.
202. Grechishnikova D and Poptsova M. (2016) Conserved 3′UTR stem-loop structure in L1 and Alu transposons in human genome: Possible role in retrotransposition. *BMC Genomics*, **17**, 992.
203. Ren C, Yang T, Qiao P, Wang L, Han X, Lv S, Sun Y, Liu Z, Du Y and Yu Z. (2018) PIM2 interacts with tristetraprolin and promotes breast cancer tumorigenesis. *Molecular Oncology*, **12**, 690–704.
204. Haberland M, Montgomery RL and Olson EN. (2009) The many roles of histone deacetylases in development and physiology: Implications for disease and therapy. *Nature Reviews Genetics*, **10**, 32–42.
205. Sobolewski C, Sanduja S, Blanco FF, Hu L and Dixon DA. (2015) Histone deacetylase inhibitors activate tristetraprolin expression through induction of early growth response protein 1 (EGR1) in colorectal cancer cells. *Biomolecules*, **5**, 2035–2055.
206. Angiolilli C, Kabala PA, Grabiec AM, Rossato M, Lai WS, Fossati G, Mascagni P, Steinkühler C, Blackshear PJ, Reedquist KA, *et al.* (2018) Control of cytokine mRNA degradation by the histone deacetylase inhibitor ITF2357 in rheumatoid arthritis fibroblast-like synoviocytes: Beyond transcriptional regulation. *Arthritis Research & Therapy*, **20**, 148.
207. Imran M, Rauf A, Khan IA, Shahbaz M, Qaisrani TB, Fatmawati S, Abu-Izneid T, Imran A, Rahman KU and Gondal TA. (2018) Thymoquinone: A novel strategy to combat cancer: A review. *Biomedicine & Pharmacotherapy*, **106**, 390–402.
208. Cheng Y and Tian H. (2017) Current development status of MEK inhibitors. *Molecules*, **22**, 1551.

209. Planel S, Salomon A, Jalinot P, Feige JJ and Cherradi N. (2010) A novel concept in antiangiogenic and antitumoral therapy: Multitarget destabilization of short-lived mRNAs by the zinc finger protein ZFP36L1. *Oncogene*, **29**, 5989–6003.
210. Rataj F, Planel S, Denis J, Roelants C, Filhol O, Guyon L, Feige JJ and Cherradi N. (2019) Targeting AU-rich element-mediated mRNA decay with a truncated active form of the zinc-finger protein TIS11b/BRF1 impairs major hallmarks of mammary tumorigenesis. *Oncogene*, **38**, 5174–5190.
211. Melero I, Castanon E, Alvarez M, Champiat S and Marabelle A. (2021) Intratumoural administration and tumour tissue targeting of cancer immunotherapies. *Nature Reviews Clinical Oncology*, **18**, 558–576.

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Chapter 5

Roles of the Hu/ELAV Family of mRNA-Stabilizing Proteins in Tumor Development

Cyril Sobolewski[1], Sahida Afroz[2], Samantha Z. Brown[3], Jonathan R. Brody[3,*], and Dan A. Dixon[2,**]

[1]*University of Lille, Inserm, CHU Lille, U1286-INFINITE — Institute for Translational Research in Inflammation, F-59000, Lille, France*
[2]*Department of Molecular Biosciences, University of Kansas, and University of Kansas Cancer Center, Lawrence, KS 66045, United States*
[3]*Department of Surgery, Brenden-Colson Center for Pancreatic Care, Oregon Health & Sciences University, Portland, OR 97201, United States*

Abstract

The Hu/ELAV family of RNA-binding proteins consists of the members HuR, HuB, HuC, and HuD (*ELAVL1, ELAVL2, ELAVL3, ELAVL4,*

* Corresponding author's email: brodyj@ohsu.edu
** Corresponding author's email: dan_dixon@ku.edu

respectively). These factors contribute to a variety of physiological and pathological processes, primarily dependent on the cell/tissue type they are expressed. HuB, HuC, and HuD are mainly expressed in neuronal tissues and play a role in neuronal development and differentiation, whereas HuR is ubiquitously expressed and has various functions in multiple organ systems. Through their ability to recognize and bind many target mRNAs, Hu/ELAV proteins modulate gene expression through post-transcriptional regulation impacting mRNA stability and translation. In the context of cancer, the role of HuR as a tumor-promoting factor has been demonstrated in several organ types with higher expression observed in most human cancers. In this chapter, we focus on the role Hu/ELAV proteins in human cancer with an emphasis on the role of HuR in promoting altered gene expression contributing to cancer hallmarks.

1. Introduction

Post-transcriptional regulation gathers several mechanisms necessary for precise control of gene expression. These regulatory events include mRNA processing events involving splicing and polyadenylation. In addition, they also dictate the fate of mRNAs through mechanisms controlling mRNA degradation and/or translation. Through these actions, post-transcriptional regulation serves as a key regulatory point to modulate protein production of a particular gene in response to changing cellular environment and conditions [1].

The necessity of post-transcriptional regulation is apparent, as 40–50% of global gene expression changes in response to cellular signals occur at the level of mRNA stability [2, 3]. This regulation requires several *trans*-acting factors, including RNA-binding proteins, microRNAs (miRNAs), and long-non-coding RNAs (lncRNAs), which recognize specific sequences within the mRNAs [3]. A notable 3′UTR element present in many inflammatory cytokines, growth factors, and proto-oncogenes is the AU-rich element or ARE [4, 5]. The functional ARE is most often composed of adjacent AUUUA sequence motifs, but several ARE classes and clusters have been defined based on the number and context of the AUUUA pentamers [5]. The importance of this particular RNA element is evident, since estimates ranging from 5–8% of coding genes contain a 3′UTR ARE sequence [6]. Through its presence, the ARE promotes target

mRNA degradation and/or translational suppression within the cytoplasm [5, 6]. AREs mediate this through association with adenylate and uridylate-rich element-binding proteins (AUBPs) and miRNAs that bind with high affinity [4, 7–9]. AUBPs, independently or in close association with non-coding RNAs, are potent regulators of mRNA stability and/or translation and these effects are mediated by directing their target transcripts into small cytoplasmic compartments such as processing-bodies (P-bodies) or stress granules, where mRNAs are respectively degraded or stored translationally silent [10–12]. To date, more than 20 different AUBPs regulating mRNA stability and/or translation have been identified and their expression level and function may strongly differ depending on the cellular context (e.g., inflammation) or cell type [13, 14]. Furthermore, AUBPs exhibit pleiotropic effects on gene expression, since a single AUBP can bind to multiple mRNAs and binding can occur among different classes of AREs [13, 15].

The ability of AUBPs to regulate expression of multiple genes involved in pathological processes indicates that a phenotype observed with the alteration of one given AUBP may result from a concerted deregulation of several mRNA transcripts. This complexity is further enhanced by various post-translational modifications (e.g., phosphorylation), which can regulate an AUBP subcellular localization and activity [16, 17]. Moreover, these proteins form a complex regulatory network, due to the competition or the coordination between AUBPs on the same transcripts or to modulate miRNAs/lncRNA-dependent regulation of gene expression, but also through their ability to regulate their own expression [14, 18].

AUBPs play an important role in various physiological processes and accordingly, their abnormal expression/activity have been associated with various pathologies including cancers, along with inflammatory and metabolic disorders preceding carcinogenesis (e.g., inflammatory bowel disease, pancreatitis, fatty liver disease) [19–27]. These changes in AUBP activity indicate that alteration of these proteins may create a favorable landscape fostering tumor development and progression. The Hu/ELAV (Embryonic Lethal Abnormal Vision) family of AUBPs represents one of the earliest groups of RNA-binding proteins identified as being associated with tumorigenesis [20, 22, 28, 29], and is the primary focus of this chapter.

2. Hu/ELAV family of RNA-binding proteins

The Hu/ELAV family of proteins were originally identified as specific antigens in a paraneoplastic neurological syndrome (the Hu syndrome) from patients with small-cell lung cancer [30, 31]. The presence of anti-Hu antibodies in serum recognizes a nuclear antigen present in neurons, along with identifying specific proteins of 35–40 kDa in brain and tumor extracts [30, 32]. Subsequently, cDNAs encoding the Hu proteins HuD (*ELAVL4*), HuC (*ELAVL3*), HuB (*ELAVL2*; Hel-N1), and HuR (*ELAVL1*; HuA) were cloned and characterized [28, 33–35].

The Hu/ELAV family shares homology with the *Drosophila elav* gene primarily in three RNA recognition motifs (RRMs) present in all Hu/ELAV members, with a high degree of sequence homology (70–85%) among the vertebrate ELAV proteins (Fig. 1) [29–31]. Physical and biochemical studies have shown that the tandem RRMs 1 and 2 participate in ARE binding, where RRM3 interacts with poly(A) tails and supports protein-protein interactions [29, 36–42]. Positioned between RRMs 2 and 3, the Hu/ELAV proteins contain a less conserved basic hinge region

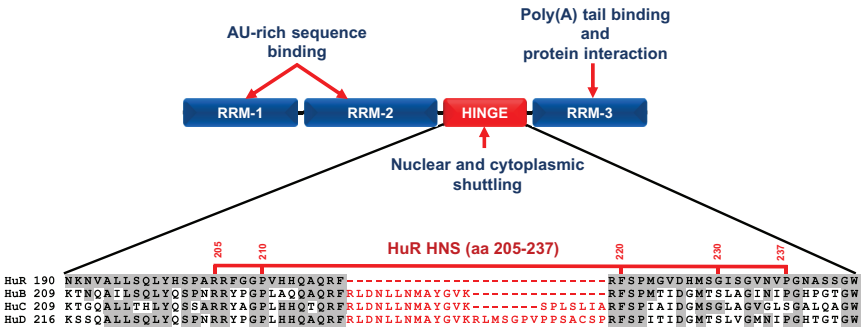

Figure 1. Comparison of human Hu/ELAV proteins.

HuR, HuB, HuC, and HuD (*ELAVL1*, *ELAVL2*, *ELAVL3*, and *ELAVL4*, respectively) consist of RNA recognition sequences (RRM) RRM-1, RRM-2, and RRM-3 involved with AU-rich element binding, poly(A) tail binding, and protein-protein interactions. A conserved basic hinge region between RRMs 2 and 3 is involved with nucleocytoplasmic shuttling; conserved residues with HuR are shown in gray. The region responsible for HuR nucleocytoplasmic shuttling (HNS) within the hinge region is shown. Adapted from [43].

(Fig. 1). Similar to other RNA-binding proteins, the hinge region contains *cis*-acting elements responsible for Hu/ELAV protein nucleocytoplasmic shuttling. Contained within the HuR hinge region is the HuR Nucleocytoplasmic Shuttling sequence or HNS that serves as an integrated nuclear localization signal and a nuclear export signal for HuR [43, 44]. Interestingly, this HNS region of HuR is divergent with HuB, HuC, and HuD, which may in part explain distinct nuclear localization and nuclear export signals as observed with HuD [45].

The human Hu/ELAV family genes *ELAVL1*, *ELAVL2*, *ELAVL3*, and *ELAVL4* encode the proteins HuR, HuB, HuC, and HuD, respectively. Based on their tissue expression pattern, the Hu/ELAV proteins can be grouped into ubiquitous (HuR) or neural-specific (HuB, HuC, HuD) (Fig. 2). HuR is ubiquitously expressed in human tissues [29, 46]. The neural-specific Hu/ELAVs HuB, HuC, and HuD largely show heightened levels of expression in the brain, pituitary, and testes [31, 47]. This tissue specificity indicates that neuronal Hu/ELAV proteins play an important role in neuronal development and neurological diseases as supported through numerous experimental studies [22, 29, 48]. However, the ubiquitously expressed HuR has been shown to be involved in a variety of biological functions, with an emphasis on cancer development [49, 50].

Insights into the physiological function of Hu/ELAV proteins have been accomplished through the creation of respective genetically engineered mouse models. With regard to cancer, most insights have come from *Elavl1* (HuR) modified mouse models. Initial findings using whole-body *Elavl1* deletion demonstrated HuR to be necessary for viability, as knockout mice die within 10 days due to defects in embryonic development with a loss of progenitor cells of myeloid and intestinal lineage being observed [51, 52]. The use of tissue-specific conditional knockout models has allowed for identifying cancer-associated phenotypes where HuR is a major contributing factor [50]. In myeloid-specific HuR knockout mice, HuR was shown to be critical for macrophage-derived VEGFA expression [53]. This loss of myeloid HuR led to impaired angiogenesis and tumor growth, indicating the role of HuR in promoting macrophage-dependent tumor vascularization. In a similar myeloid HuR knockout model, inflammatory cytokine levels were increased, and these mice had exacerbated chemical-induced ulcerative colitis and were prone

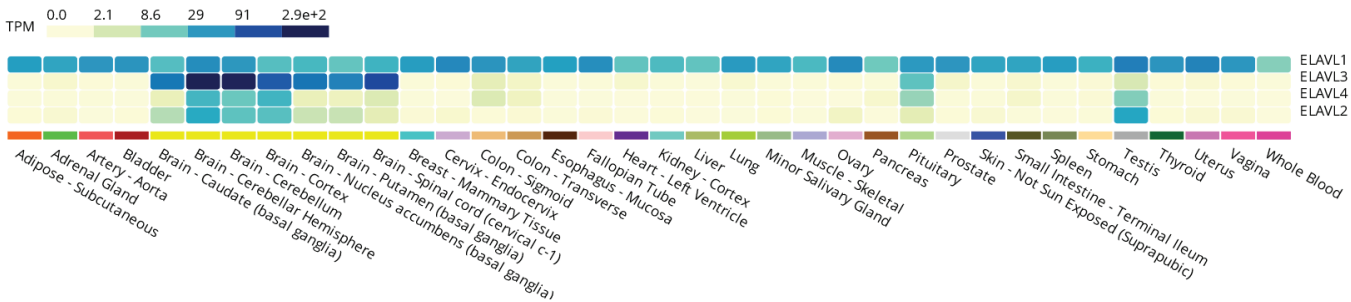

Figure 2. Hu/ELAV expression in human tissues.

Expression level of HuR, HuC, HuD, and HuB (*ELAVL1*, *ELAVL3*, *ELAVL4,* and *ELAVL2*, respectively) transcripts in human tissues. Data was obtained using the GTEx portal (https://gtexportal.org/home/index.html) and represented as a heatmap showing transcripts per million (TPM).

to colitis-associated cancer [54]. In the intestine, loss of HuR led to intestinal mucosal atrophy through alteration of Wnt signaling activity [55]. This loss of intestinal HuR also impacted tumorigenesis, as these mice displayed a 3-fold decrease in tumor burden, with reduced proliferation and increased apoptosis observed in the intestinal epithelium [56]. Deletion of *Elavl1* in microglia/macrophages has been recently shown to inhibit glioblastoma. Knockout of HuR in these cells led to reduced tumor growth and proliferation that was associated with extended survival in a murine model of glioblastoma [57]. Finally, using an inducible HuR transgenic mouse model, we demonstrated that HuR overexpression in the pancreas caused chronic pancreatitis [27]. In this model, elevated HuR cooperated with oncogenic K-Ras resulting in a >3-fold increase in pancreatic ductal adenocarcinoma (PDAC) incidence.

With regard to the neuronal Hu/ELAV members, the links to cancer development are not as directly apparent as with HuR. *Elavl2* (HuB) knockout mice display primarily reproductive phenotypes, as HuB-deficient female mice are infertile due to defective primordial follicle formation with small ovaries and defective oocytes development. Lethality was observed at a high rate (80%) by weaning due to growth retardation [58]. *Elavl3* (HuC) knockout mice are viable, however display spontaneous epileptic seizure, demonstrating the role of HuC in the regulation of neurotransmitter glutamate levels [59]. Similarly, *Elavl4* (HuD) knockout mice are viable without showing any apparent adult brain morphological defects. These mice do display altered neuronal development, indicating a role for HuD in regulating the proliferative activity and neuronal cell-fate acquisition of neural stem/progenitor cells [60]. Interestingly, current findings have shown a role for HuD in the pancreas as a regulator of insulin translation in pancreatic β cells. In HuD knockout mice, insulin production was elevated, whereas HuD-overexpressing mice exhibited lower insulin levels in islets and in plasma [61].

3. Hu/ELAV expression in cancer development

Based on its ubiquitous expression pattern, elevated level of HuR is observed to occur in a number of tumor types (Fig. 3, top panel) indicating its putative role as a tumor promoter through mRNA stabilization of

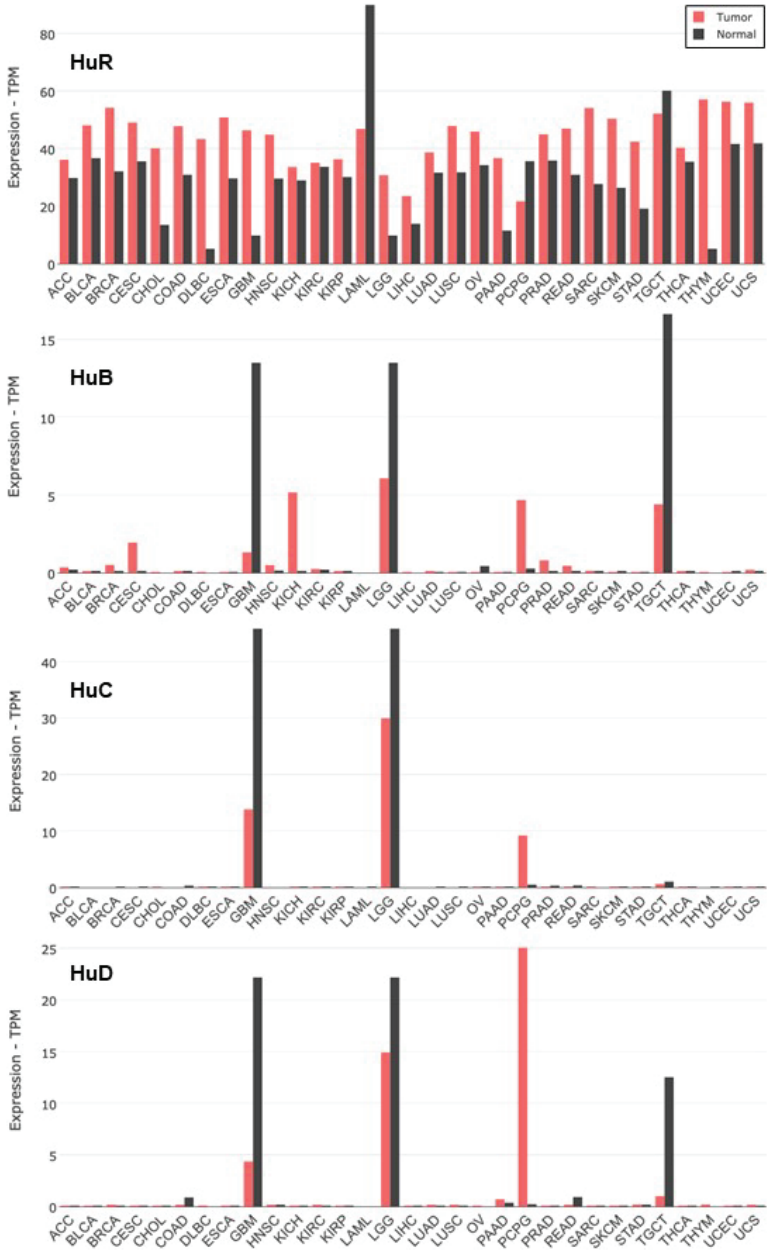
Tumor
Normal
HuR
HuB
HuC
HuD
Expression - TPM
ACC BLCA BRCA CESC CHOL COAD DLBC ESCA GBM HNSC KICH KIRC KIRP LAML LGG LIHC LUAD LUSC OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA THYM UCEC UCS

Figure 3. (Continued on Facing Page) Hu/ELAV expression in human cancers.

Expression level of HuR, HuB, HuC, and HuD transcripts in paired tumor and normal tissues originating from different cancers. Data were retrieved from the Gepia database (http://gepia2.cancer-pku.cn). The height of bar represents the median expression in transcripts per million (TPM) of indicated tumor type or normal tissue.

Abbreviation	Complete name
ACC	Adrenocortical carcinoma
BLCA	Bladder urothelial carcinoma
BRCA	Breast carcinoma
CESC	Cervical squamous cell carcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
DLBC	Diffuse lymphoid B-cell lymphoma
ESCA	Esophageal carcinoma
GBM	Glioblastoma multiforme
HNSC	Head and neck squamous cell carcinoma
KICH	Kidney chromophobe
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute myeloid leukemia
LGG	Brain low grade glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma
OV	Ovarian serous carcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
SKCM	Skin cutaneous melanoma
STAD	Stomach adenocarcinoma
TGCT	Testicular germ cell tumors
THYM	Thymoma
UCEC	Uterine corpus endometrial carcinoma
UCS	Uterine carcinosarcoma

various cancer-associated transcripts (see below). In contrast, the expression of neural-specific HuB, HuC, and HuD appears mainly downregulated in tumors of neural and testes origin. Shown in Figure 3, brain glioblastoma and low-grade gliomas, along with testicular germ cell tumors, have decreased tumor expression, suggesting that neural-specific Hu/ELAV genes may have tumor suppressor capabilities. Interestingly, HuB, HuC, and HuD are upregulated in pheochromocytoma and paragangliomas, which are tumors that are neuronal in nature, producing adrenaline and noradrenaline, whereas HuR is downregulated in this tumor type.

As HuR is highly expressed in several cancers (Fig. 3), a number of studies have been conducted to demonstrate that tumor aggressiveness and poor outcomes are associated with increased HuR levels in numerous tumor types [49, 50, 62]. For instance, in bladder, breast, cervical, colorectal, esophageal, kidney, lung, and pancreatic cancers, high HuR levels and cytoplasmic HuR accumulation has been shown to correlate with worse histological grading and increased incidence of lymphatic spread and distant metastasis, leading to worse clinical outcomes [63–72]. A heat map illustrating overall survival for multiple cancer types based on respective Hu/ELAV mRNA expression level is shown in Figure 4(a), with similar trends observed with HuR expression as seen in published observations of individual tumor types.

In contrast to HuR, elevated expression of the neuronal Hu/ELAV members appears to exert a protective effect and associates with lower risk (Fig. 4a). An example of this is observed with brain low grade glioma (labeled LGG), where high HuR expression associates with worse survival and high HuB, HuC, and HuD associates with better outcomes (Fig. 4b). However, these observations are challenged by studies indicating that some neuronal Hu/ELAV members are also deregulated in non-neuronal cancerous tissues. Early studies evaluating neuroendocrine markers for small-cell lung carcinoma (SCLC) identified HuB to be present in a SCLC cell line and tumors [73]. More recently, elevated HuB was observed in various cancer cell lines where it plays a role in proto-oncogene activation [74]. Similarly, using genome wide CRISPR transcriptional activation screening, HuB was identified to be a contributing factor in cisplatin resistance in esophageal squamous cell carcinoma [75]. In oral squamous

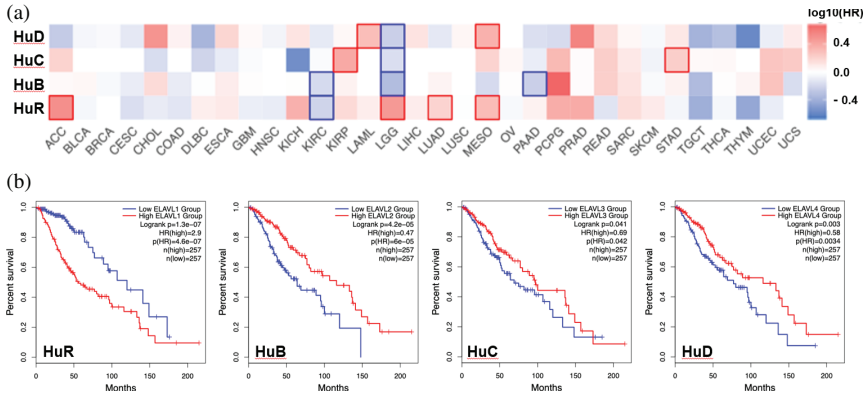

Figure 4. Clinical relevance of Hu/ELAV members.

(a) Heat map showing the overall survival for multiple cancer types based on the expression level of HuD, HuC, HuB or HuR. Overall survival is represented in a HR (hazard ratio) in logarithmic scale (log10) for different genes, with the red and blue blocks denote higher and lower risks, respectively, with an increase in Hu/ELAV expression. Significant results are indicated with an outlined box. (b) Kaplan-Meier survival curves as a function of low (blue) versus high (red) expression of HuR, HuB, HuC or HuD in brain lower grade glioma (LGG) derived from the Gepia database (http://gepia2.cancer-pku.cn).

cell carcinoma, expression of HuD was observed to be associated with histological differentiation, nodal metastasis, and mode of invasion, through regulation of VEGF, MMP-2, and MMP-9 [76]. While HuD mRNA did not appear to be expressed or associated with survival in head and neck squamous cell carcinoma (labeled HNSC; Figs. 3, 4), these differences could be attributed to HuD mRNA versus protein expression in tumor tissue or a reflection of the sample population examined. HuD expression was also shown to be induced by ionizing irradiation in lung cancer cells, promoting cell viability post-radiation [77]. In contrast, loss of HuD was observed in pancreatic neuroendocrine tumors, which correlated with low $p27^{Kip1}$ levels and poor prognosis, indicating a protective effect in this tumor type [78]. Similarly, recent studies have shown that HuB and HuD, but not HuC, can repress telomerase activity in human neuroblastoma cells by binding the telomerase noncoding RNA *TERC* and preventing HuR binding, inhibiting the function of HuR in promoting telomerase activity and in delaying cellular senescence [79].

4. Role of HuR as a post-transcriptional tumor promoter

through its ability to bind and promote the expression of various cancer-associated mRNAs in various models of cancer, HuR has been recognized as a critical mediator of cancer progression (Fig. 5a) [20, 49, 80]. Through this pleiotropic effect on cancer gene expression, HuR has been found to contribute to the various hallmarks of cancer [81] such as: sustaining proliferative signaling [82–85], evading growth suppressors [51, 86], resisting cell death [87–90], enabling replicative immortality [91, 92], inducing angiogenesis [93–95], activating invasion and metastasis [96, 97], promoting tumor-associated inflammation [23, 27, 98], reprogramming energy metabolism [99–101], and evading immune destruction [102].

HuR target mRNAs have primarily been identified using transcriptomic approaches in cancer models that have altered expression levels of HuR and in assays detecting HuR-bound mRNA complexes (e.g., CLIP-seq, RNP-IP). These mRNA targets are currently available in several databases (e.g., POSTAR3 database: http://111.198.139.65/, Starbase: https://starbase.sysu.edu.cn/) [103]. Interestingly, approximately half of the top 500 HuR-bound mRNAs were identified to contain a 3′UTR ARE motif using the ARED-Plus database (https://brp.kfshrc.edu.sa/ared/#pagetop) [104] (Fig. 5b), suggesting that HuR-mediated gene regulation can extend beyond ARE-containing transcripts. Although these targets may differ between cancer types, it is likely that the tumor-promoting function of HuR results from the alteration of a network of cancer-associated genes rather than a single target [13]. In support of this, gene ontology analysis of HuR targets shows enrichment in pathways involved with various cancer processes (e.g., DNA damage and repair, Wnt signaling) (Fig. 5c), similar to those observed in colorectal cancer and hepatocellular carcinoma [13, 14].

Ample evidence exists dating back to the early 2000s, demonstrating HuR's tumor promoting ability *in vitro* in cancer cells [80]. More recently, genetic approaches used to inhibit HuR (e.g., siRNA or gene deletion) have recently been used to demonstrate the role of HuR in promoting

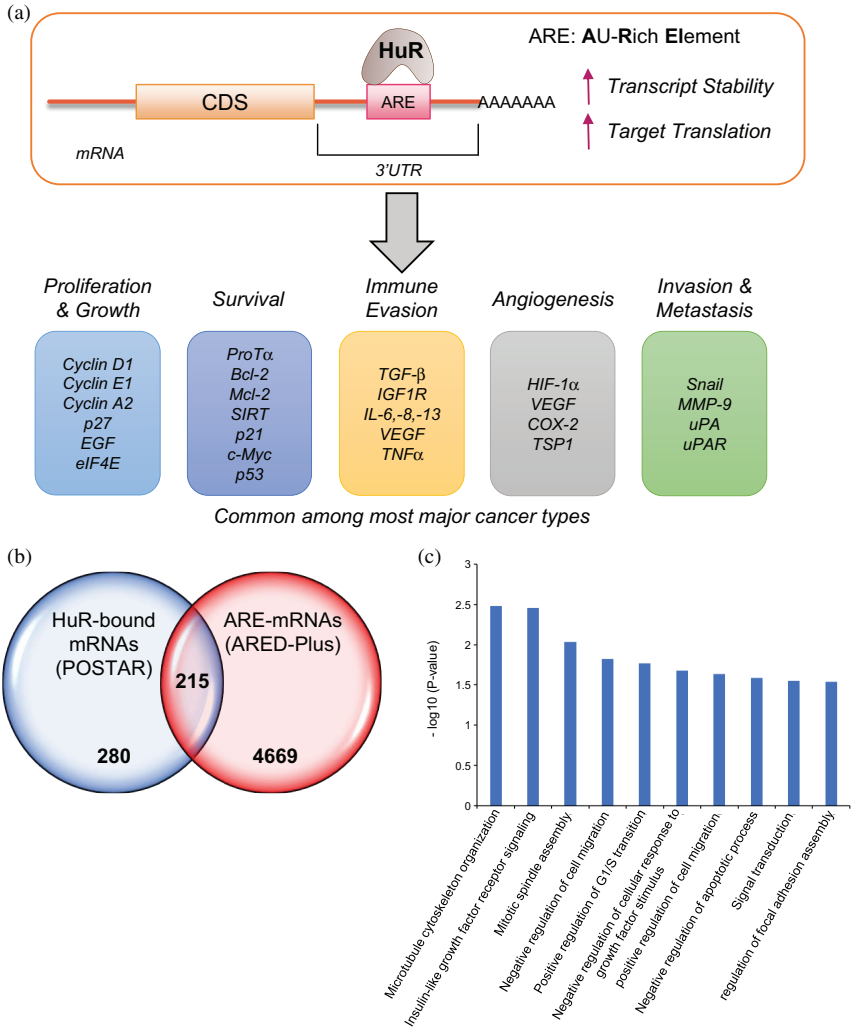

Figure 5. HuR regulation of cancer-associated gene expression.

(a) HuR-mediated regulation of the stability and/or translation of target mRNAs associated with cancer traits. Listed are a subset of HuR-regulated mRNAs common among major cancer types involved in promoting various hallmarks of tumorigenesis. (b) Cross comparison between HuR-bound mRNA targets (Top 500, retrieved from the POSTAR2 database: POSTAR3: http://111.198.139.65/) and mRNA transcripts bearing an ARE motif within the 3′UTR (ARED-Plus: https://brp.kfshrc.edu.sa/ared/#page-top). (c) Gene ontology enrichment analysis for biological processes with the top 500 HuR-bound targets (POSTAR3 database). Enrichment is represented with a -log10 *P* value.

tumor growth *in vivo* [56, 69, 92, 101, 105–108]. Interestingly, deletion of *ELAVL1* using CRISPR-Cas9 methodology in both pancreatic and colorectal cancer cell lines demonstrated that lack of HuR severely attenuated *in vivo* tumor growth as compared to isogenic cells expressing HuR [109]. The ability of these HuR knockout lines to form tumors *in vivo* was rescued by re-expressing HuR or by expressing HuR targets such as IDH1, which are frequently lost concomitantly with HuR [101, 109]. While these preclinical models are useful in demonstrating the noteworthy role HuR plays in tumorigenesis, targeting HuR alone may prove to be a challenge in the clinical setting. Thus, inhibiting HuR is likely to be most effective when done in combination with other therapeutic strategies, consistent with other targeted approaches used in many cancer therapies.

5. Concluding remarks

Based on the large body of research gathered on the Hu/ELAV family of RNA-binding proteins, a clearer picture of their contribution in physiological and pathological processes has emerged. In the context of cancer, HuR is generally accepted as a key post-transcriptional regulator promoting tumorigenesis in most tumor types. Conversely, the neuronal Hu/ELAV members appear to act in a tumor suppressor capacity. Further efforts in characterizing their role and identifying their target mRNAs involved in tumor development, will aid in defining specific neuronal Hu/ELAV members as tumor promoters and potential drug targets, or alternatively determine if they can serve as tumor suppressors where their expression could potentially antagonize HuR-mediated mRNA stabilization.

As various reports have shown that downregulation or loss of HuR can alter cancer-associated gene expression and impede tumor cell growth, identifying methods to selectively target HuR have been an area of active current investigation. These include drug discovery campaigns identifying small molecules that inhibit the ability of HuR to bind to target mRNA [80, 106, 110–114] and inhibit the cytoplasmic translocation of HuR [80, 87, 115, 116]. The latest efforts in this area have identified a new class of molecules targeting HuR protein dimerization and shown favorable results in glioma disease progression [117, 118]. Based on recent

advances in the field in understanding the molecular mechanisms that regulate RBP-RNA interactions [119], a future direction would be in the development of RBP-site blockers. These RNA molecules would work similar to miRNA target site blockers [120] and may increase specificity by inhibiting the interaction between HuR and a target mRNA. To move forward in identifying HuR as a cancer therapeutic target, it will become important to demonstrate drug specificity, as off-targeting effects on the neuronal Hu/ELAV members may be likely with the potential for neurological implications and/or inhibition of neuronal Hu/ELAV tumor suppressor activity. Ultimately, these findings will prove helpful in demonstrating RNA-binding proteins as a cancer-relevant class of gene regulatory proteins that are not only therapeutically important but also amendable for pharmaceutical targeting.

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References

1. Moore AE, Young LE and Dixon DA. (2011) MicroRNA and AU-rich element regulation of prostaglandin synthesis. *Cancer and Metastasis Reviews*, **30**, 419–435.
2. Cheadle C, Fan J, Cho-Chung YS, Werner T, Ray J, Do L, Gorospe M and Becker KG. (2005) Control of gene expression during T cell activation: Alternate regulation of mRNA transcription and mRNA stability. *BMC Genomics*, **6**, 75.

3. Garneau NL, Wilusz J and Wilusz CJ. (2007) The highways and byways of mRNA decay. *Nature Reviews Molecular Cell Biology*, **8**, 113–126.
4. Beisang D and Bohjanen PR. (2012) Perspectives on the ARE as it turns 25 years old. *Wiley Interdisciplinary Reviews: RNA*, **3**, 719–731.
5. Barreau C, Paillard L and Osborne HB. (2005) AU-rich elements and associated factors: Are there unifying principles? *Nucleic Acids Research*, **33**, 7138–7150.
6. Bakheet T, Williams BR and Khabar KS. (2006) ARED 3.0: The large and diverse AU-rich transcriptome. *Nucleic Acids Research*, **34**, D111–114.
7. Stumpo DJ, Lai WS and Blackshear PJ. (2010) Inflammation: Cytokines and RNA-based regulation. *Wiley Interdisciplinary Reviews: RNA*, **1**, 60–80.
8. Young LE, Moore AE, Sokol L, Meisner-Kober N and Dixon DA. (2012) The mRNA stability factor HuR inhibits microRNA-16 targeting of COX-2. *Molecular Cancer Research*, **10**, 167–180.
9. Jing Q, Huang S, Guth S, Zarubin T, Motoyama A, Chen J, Di Padova F, Lin SC, Gram H and Han J. (2005) Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell*, **120**, 623–634.
10. Anderson P, Kedersha N and Ivanov P. (2015) Stress granules, P-bodies and cancer. *Biochimica et Biophysica Acta*, **1849**, 861–870.
11. Blanco FF, Sanduja S, Deane NG, Blackshear PJ and Dixon DA. (2014) Transforming growth factor beta regulates P-body formation through induction of the mRNA decay factor tristetraprolin. *Molecular and Cellular Biology*, **34**, 180–195.
12. Legrand N, Dixon DA and Sobolewski C. (2020) Stress granules in colorectal cancer: Current knowledge and potential therapeutic applications. *World Journal of Gastroenterology*, **26**, 5223–5247.
13. Legrand N, Dixon DA and Sobolewski C. (2019) AU-rich element-binding proteins in colorectal cancer. *World Journal of Gastrointestinal Oncology*, **11**, 71–90.
14. Dolicka D, Sobolewski C, Correia de Sousa M, Gjorgjieva M and Foti M. (2020) mRNA post-transcriptional regulation by AU-rich element-binding proteins in liver inflammation and cancer. *International Journal of Molecular Sciences*, **21**.
15. Lopez de Silanes I, Quesada MP and Esteller M. (2007) Aberrant regulation of messenger RNA 3′-untranslated region in human cancer. *Cellular Oncology*, **29**, 1–17.
16. Grammatikakis I, Abdelmohsen K and Gorospe M. (2017) Posttranslational control of HuR function. *Wiley Interdisciplinary Reviews: RNA*, **8**.
17. Brauer U, Zaharieva E and Soller M. (2014) Regulation of ELAV/Hu RNA-binding proteins by phosphorylation. *Biochemical Society Transactions*, **42**, 1147–1151.
18. Simone LE and Keene JD. (2013) Mechanisms coordinating ELAV/Hu mRNA regulons. *Current Opinion in Genetics & Development*, **23**, 35–43.
19. Srikantan S and Gorospe M. (2012) HuR function in disease. *Frontiers in Bioscience*, **17**, 189–205.
20. Abdelmohsen K and Gorospe M. (2010) Posttranscriptional regulation of cancer traits by HuR. *Wiley Interdisciplinary Reviews: RNA*, **1**, 214–229.

21. Zhang Z, Zong C, Jiang M, Hu H, Cheng X, Ni J, Yi X, Jiang B, Tian F, Chang MW, Su W, Zhu L, Li J, Xiang X, Miao C, Gorospe M, de Cabo R, Dou Y, Ju Z, Yang J, Jiang C, Yang Z and Wang W. (2020) Hepatic HuR modulates lipid homeostasis in response to high-fat diet. *Nature Communications*, **11**, 3067.
22. Pascale A and Govoni S. (2012) The complex world of post-transcriptional mechanisms: Is their deregulation a common link for diseases? Focus on ELAV-like RNA-binding proteins. *Cellular and Molecular Life Sciences*, **69**, 501–517.
23. Young LE, Sanduja S, Bemis-Standoli K, Pena EA, Price RL and Dixon DA. (2009) The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. *Gastroenterology*, **136**, 1669–1679.
24. Dixon DA, Balch GC, Kedersha N, Anderson P, Zimmerman GA, Beauchamp RD and Prescott SM. (2003) Regulation of cyclooxygenase-2 expression by the translational silencer TIA-1. *Journal of Experimental Medicine*, **198**, 475–481.
25. Dixon DA, Tolley ND, Bemis-Standoli K, Martinez ML, Weyrich AS, Morrow JD, Prescott SM and Zimmerman GA. (2006) Expression of COX-2 in platelet-monocyte interactions occurs via combinatorial regulation involving adhesion and cytokine signaling. *Journal of Clinical Investigation*, **116**, 2727–2738.
26. Brody JR and Dixon DA. (2018) Complex HuR function in pancreatic cancer cells. *Wiley Interdisciplinary Reviews: RNA*, **9**, e1469.
27. Peng W, Furuuchi N, Aslanukova L, Huang YH, Brown SZ, Jiang W, Addya S, Vishwakarma V, Peters E, Brody JR, Dixon DA and Sawicki JA. (2018) Elevated HuR in pancreas promotes a pancreatitis-like inflammatory microenvironment that facilitates tumor development. *Molecular and Cellular Biology*, **38**.
28. Szabo A, Dalmau J, Manley G, Rosenfeld M, Wong E, Henson J, Posner JB and Furneaux HM. (1991) HuD, a paraneoplastic encephalomyelitis antigen, contains RNA-binding domains and is homologous to Elav and Sex-lethal. *Cell*, **67**, 325–333.
29. Hinman MN and Lou H. (2008) Diverse molecular functions of Hu proteins. *Cellular and Molecular Life Sciences*, **65**, 3168–3181.
30. Okano HJ and Darnell RB. (1997) A hierarchy of Hu RNA binding proteins in developing and adult neurons. *Journal of Neuroscience*, **17**, 3024–3037.
31. Antic D and Keene JD. (1997) Embryonic lethal abnormal visual RNA-binding proteins involved in growth, differentiation, and posttranscriptional gene expression. *The American Journal of Human Genetics*, **61**, 273–278.
32. Dalmau J, Furneaux HM, Cordon-Cardo C and Posner JB. (1992) The expression of the Hu (paraneoplastic encephalomyelitis/sensory neuronopathy) antigen in human normal and tumor tissues. *The American Journal of Pathology*, **141**, 881–886.
33. Sakai K, Gofuku M, Kitagawa Y, Ogasawara T, Hirose G, Yamazaki M, Koh CS, Yanagisawa N and Steinman L. (1994) A hippocampal protein associated with paraneoplastic neurologic syndrome and small cell lung carcinoma. *Biochemical and Biophysical Research Communications*, **199**, 1200–1208.

34. Levine TD, Gao F, King PH, Andrews LG and Keene JD. (1993) Hel-N1: An autoimmune RNA-binding protein with specificity for 3′ uridylate-rich untranslated regions of growth factor mRNAs. *Molecular and Cellular Biology*, **13**, 3494–3504.
35. Ma WJ, Cheng S, Campbell C, Wright A and Furneaux H. (1996) Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein. *Journal of Biological Chemistry*, **271**, 8144–8151.
36. Pabis M, Popowicz GM, Stehle R, Fernandez-Ramos D, Asami S, Warner L, Garcia-Maurino SM, Schlundt A, Martinez-Chantar ML, Diaz-Moreno I and Sattler M. (2019) HuR biological function involves RRM3-mediated dimerization and RNA binding by all three RRMs. *Nucleic Acids Research*, **47**, 1011–1029.
37. Ripin N, Boudet J, Duszczyk MM, Hinniger A, Faller M, Krepl M, Gadi A, Schneider RJ, Sponer J, Meisner-Kober NC and Allain FH. (2019) Molecular basis for AU-rich element recognition and dimerization by the HuR C-terminal RRM. *Proceedings of the National Academy of Sciences of the United States*, **116**, 2935–2944.
38. Wang X and Tanaka Hall TM. (2001) Structural basis for recognition of AU-rich element RNA by the HuD protein. *Nature Structural & Molecular Biology*, **8**, 141–145.
39. Inoue M, Muto Y, Sakamoto H and Yokoyama S. (2000) NMR studies on functional structures of the AU-rich element-binding domains of Hu antigen C. *Nucleic Acids Research*, **28**, 1743–1750.
40. Ma WJ, Chung S and Furneaux H. (1997) The Elav-like proteins bind to AU-rich elements and to the poly(A) tail of mRNA. *Nucleic Acids Research*, **25**, 3564–3569.
41. Kasashima K, Sakashita E, Saito K and Sakamoto H. (2002) Complex formation of the neuron-specific ELAV-like Hu RNA-binding proteins. *Nucleic Acids Research*, **30**, 4519–4526.
42. Fialcowitz-White EJ, Brewer BY, Ballin JD, Willis CD, Toth EA and Wilson GM. (2007) Specific protein domains mediate cooperative assembly of HuR oligomers on AU-rich mRNA-destabilizing sequences. *Journal of Biological Chemistry*, **282**, 20948–20959.
43. Fan XC and Steitz JA. (1998) HNS, a nuclear-cytoplasmic shuttling sequence in HuR. *Proceedings of the National Academy of Sciences of the United States*, **95**, 15293–15298.
44. Keene JD. (1999) Why is Hu where? Shuttling of early-response-gene messenger RNA subsets. *Proceedings of the National Academy of Sciences of the United States*, **96**, 5–7.
45. Kasashima K, Terashima K, Yamamoto K, Sakashita E and Sakamoto H. (1999) Cytoplasmic localization is required for the mammalian ELAV-like protein HuD to induce neuronal differentiation. *Genes Cells*, **4**, 667–683.

46. Good PJ. (1997) The role of elav-like genes, a conserved family encoding RNA-binding proteins, in growth and development. *Seminars in Cell and Developmental Biology*, **8**, 577–584.
47. Good PJ. (1995) A conserved family of elav-like genes in vertebrates. *Proceedings of the National Academy of Sciences of the United States*, **92**, 4557–4561.
48. Zhou HR, He K, Landgraf J, Pan X and Pestka JJ. (2014) Direct activation of ribosome-associated double-stranded RNA-dependent protein kinase (PKR) by deoxynivalenol, anisomycin and ricin: A new model for ribotoxic stress response induction. *Toxins*, **6**, 3406–3425.
49. Wang J, Guo Y, Chu H, Guan Y, Bi J and Wang B. (2013) Multiple functions of the RNA-binding protein HuR in cancer progression, treatment responses and prognosis. *International Journal of Molecular Sciences*, **14**, 10015–10041.
50. Zucal C, D'Agostino V, Loffredo R, Mantelli B, NatthakanThongon, Lal P, Latorre E and Provenzani A. (2015) Targeting the multifaceted HuR protein, benefits and caveats. *Current Drug Targets*, **16**, 499–515.
51. Ghosh M, Aguila HL, Michaud J, Ai Y, Wu MT, Hemmes A, Ristimaki A, Guo C, Furneaux H and Hla T. (2009) Essential role of the RNA-binding protein HuR in progenitor cell survival in mice. *Journal of Clinical Investigation*, **119**, 3530–3543.
52. Katsanou V, Milatos S, Yiakouvaki A, Sgantzis N, Kotsoni A, Alexiou M, Harokopos V, Aidinis V, Hemberger M and Kontoyiannis DL. (2009) The RNA-binding protein Elavl1/HuR is essential for placental branching morphogenesis and embryonic development. *Molecular and Cellular Biology*, **29**, 2762–2776.
53. Chang SH, Lu YC, Li X, Hsieh WY, Xiong Y, Ghosh M, Evans T, Elemento O and Hla T. (2013) Antagonistic function of the RNA-binding protein HuR and miR-200b in post-transcriptional regulation of vascular endothelial growth factor-A expression and angiogenesis. *Journal of Biological Chemistry*, **288**, 4908–4921.
54. Yiakouvaki A, Dimitriou M, Karakasiliotis I, Eftychi C, Theocharis S and Kontoyiannis DL. (2012) Myeloid cell expression of the RNA-binding protein HuR protects mice from pathologic inflammation and colorectal carcinogenesis. *Journal of Clinical Investigation*, **122**, 48–61.
55. Liu L, Christodoulou-Vafeiadou E, Rao JN, Zou T, Xiao L, Chung HK, Yang H, Gorospe M, Kontoyiannis D and Wang JY. (2014) RNA-binding protein HuR promotes growth of small intestinal mucosa by activating the Wnt signaling pathway. *Mol Biol Cell*, **25**, 3308–3318.
56. Giammanco A, Blanc V, Montenegro G, Klos C, Xie Y, Kennedy S, Luo J, Chang SH, Hla T, Nalbantoglu I, Dharmarajan S and Davidson NO. (2014) Intestinal epithelial HuR modulates distinct pathways of proliferation and apoptosis and attenuates small intestinal and colonic tumor development. *Cancer Research*, **74**, 5322–5335.

57. Wang J, Leavenworth JW, Hjelmeland AB, Smith R, Patel N, Borg B, Si Y and King PH. (2019) Deletion of the RNA regulator HuR in tumor-associated microglia and macrophages stimulates anti-tumor immunity and attenuates glioma growth. *Glia*, **67**, 2424–2439.
58. Kato Y, Iwamori T, Ninomiya Y, Kohda T, Miyashita J, Sato M and Saga Y. (2019) ELAVL2-directed RNA regulatory network drives the formation of quiescent primordial follicles. *EMBO Reports*, **20**, e48251.
59. Ince-Dunn G, Okano HJ, Jensen KB, Park WY, Zhong R, Ule J, Mele A, Fak JJ, Yang C, Zhang C, Yoo J, Herre M, Okano H, Noebels JL and Darnell RB. (2012) Neuronal Elav-like (Hu) proteins regulate RNA splicing and abundance to control glutamate levels and neuronal excitability. *Neuron*, **75**, 1067–1080.
60. Akamatsu W, Fujihara H, Mitsuhashi T, Yano M, Shibata S, Hayakawa Y, Okano HJ, Sakakibara S, Takano H, Takano T, Takahashi T, Noda T and Okano H. (2005) The RNA-binding protein HuD regulates neuronal cell identity and maturation. *Proceedings of the National Academy of Sciences of the United States*, **102**, 4625–4630.
61. Lee EK, Kim W, Tominaga K, Martindale JL, Yang X, Subaran SS, Carlson OD, Mercken EM, Kulkarni RN, Akamatsu W, Okano H, Perrone-Bizzozero NI, de Cabo R, Egan JM and Gorospe M. (2012) RNA-binding protein HuD controls insulin translation. *Molecular Cell*, **45**, 826–835.
62. Kotta-Loizou I, Giaginis C and Theocharis S. (2014) Clinical significance of HuR expression in human malignancy. *Medical Oncology*, **31**, 161.
63. Miyata Y, Watanabe S, Sagara Y, Mitsunari K, Matsuo T, Ohba K and Sakai H. (2013) High expression of HuR in cytoplasm, but not nuclei, is associated with malignant aggressiveness and prognosis in bladder cancer. *PLoS One*, **8**, e59095.
64. Denkert C, Weichert W, Winzer KJ, Muller BM, Noske A, Niesporek S, Kristiansen G, Guski H, Dietel M and Hauptmann S. (2004) Expression of the ELAV-like protein HuR is associated with higher tumor grade and increased cyclooxygenase-2 expression in human breast carcinoma. *Clinical Cancer Research*, **10**, 5580–5586.
65. Lim SJ, Kim HJ, Kim JY, Park K and Lee CM. (2007) Expression of HuR is associated with increased cyclooxygenase-2 expression in uterine cervical carcinoma. *International Journal of Gynecological Pathology*, **26**, 229–234.
66. Denkert C, Koch I, von Keyserlingk N, Noske A, Niesporek S, Dietel M and Weichert W. (2006) Expression of the ELAV-like protein HuR in human colon cancer: Association with tumor stage and cyclooxygenase-2. *Modern Pathology*, **19**, 1261–1269.
67. Lim SJ, Lee SH, Joo SH, Song JY and Choi SI. (2009) Cytoplasmic expression of HuR is related to cyclooxygenase-2 expression in colon cancer. *Cancer Research Treatment*, **41**, 87–92.
68. Zhang C, Xue G, Bi J, Geng M, Chu H, Guan Y, Wang J and Wang B. (2014) Cytoplasmic expression of the ELAV-like protein HuR as a potential prognostic marker in esophageal squamous cell carcinoma. *Tumour Biology*, **35**, 73–80.

69. Danilin S, Sourbier C, Thomas L, Lindner V, Rothhut S, Dormoy V, Helwig JJ, Jacqmin D, Lang H and Massfelder T. (2010) Role of the RNA-binding protein HuR in human renal cell carcinoma. *Carcinogenesis*, **31**, 1018–1026.
70. Wang J, Wang B, Bi J and Zhang C. (2011) Cytoplasmic HuR expression correlates with angiogenesis, lymphangiogenesis, and poor outcome in lung cancer. *Medical Oncology*, **28**(Suppl 1), S577–585.
71. Kim GY, Lim SJ and Kim YW. (2011) Expression of HuR, COX-2, and survivin in lung cancers; cytoplasmic HuR stabilizes cyclooxygenase-2 in squamous cell carcinomas. *Modern Pathology*, **24**, 1336–1347.
72. Richards NG, Rittenhouse DW, Freydin B, Cozzitorto JA, Grenda D, Rui H, Gonye G, Kennedy EP, Yeo CJ, Brody JR and Witkiewicz AK. (2010) HuR status is a powerful marker for prognosis and response to gemcitabine-based chemotherapy for resected pancreatic ductal adenocarcinoma patients. *Annals of Surgery*, **252**, 499–505; discussion 505–496.
73. King PH. (1997) Differential expression of the neuroendocrine genes Hel-N1 and HuD in small-cell lung carcinoma: Evidence for down-regulation of HuD in the variant phenotype. *International Journal of Cancer*, **74**, 378–382.
74. Hatanaka T, Higashino F, Tei K and Yasuda M. (2019) The neural ELAVL protein HuB enhances endogenous proto-oncogene activation. *Biochemical and Biophysical Research Communications*, **517**, 330–337.
75. Zhao WS, Yan WP, Chen DB, Dai L, Yang YB, Kang XZ, Fu H, Chen P, Deng KJ, Wang XY, Xie XW, Chen HS and Chen KN. (2019) Genome-scale CRISPR activation screening identifies a role of ELAVL2-CDKN1A axis in paclitaxel resistance in esophageal squamous cell carcinoma. *American Journal of Cancer Research*, **9**, 1183–1200.
76. Sasahira T, Kurihara M, Yamamoto K, Ueda N, Nakashima C, Matsushima S, Bhawal UK, Kirita T and Kuniyasu H. (2014) HuD promotes progression of oral squamous cell carcinoma. *Pathobiology*, **81**, 206–214.
77. Choi KJ, Lee JH, Kim KS, Kang S, Lee YS and Bae S. (2011) Identification of ELAVL4 as a modulator of radiation sensitivity in A549 non-small cell lung cancer cells. *Oncology Reports*, **26**, 55–63.
78. Kim C, Jeong DE, Heo S, Ji E, Rho JG, Jung M, Ahn S, Kim YJ, Kim YS, Nam SW, Kulkarni RN, Lee KB, Lee EK and Kim W. (2018) Reduced expression of the RNA-binding protein HuD in pancreatic neuroendocrine tumors correlates with low p27(Kip1) levels and poor prognosis. *The Journal of Pathology*, **246**, 231–243.
79. Cheng X, Gu X, Xia T, Ma Z, Yang Z, Feng HL, Zhao Y, Ma W, Ju Z, Gorospe M, Yi X, Tang H and Wang W. (2021) HuB and HuD repress telomerase activity by dissociating HuR from TERC. *Nucleic Acids Research*, **49**, 2848–2858.
80. Schultz CW, Preet R, Dhir T, Dixon DA and Brody JR. (2020) Understanding and targeting the disease-related RNA binding protein human antigen R (HuR). *Wiley Interdisciplinary Reviews: RNA*, **11**, e1581.

81. Hanahan D and Weinberg RA. (2011) Hallmarks of cancer: The next generation. *Cell*, **144**, 646–674.
82. Holmes B, Benavides-Serrato A, Freeman RS, Landon KA, Bashir T, Nishimura RN and Gera J. (2018) mTORC2/AKT/HSF1/HuR constitute a feed-forward loop regulating Rictor expression and tumor growth in glioblastoma. *Oncogene*, **37**, 732–743.
83. Wang W, Caldwell MC, Lin S, Furneaux H and Gorospe M. (2000) HuR regulates cyclin A and cyclin B1 mRNA stability during cell proliferation. *EMBO Journal*, **19**, 2340–2350.
84. Yuan Z, Sanders AJ, Ye L, Wang Y and Jiang WG. (2011) Knockdown of human antigen R reduces the growth and invasion of breast cancer cells in vitro and affects expression of cyclin D1 and MMP-9. *Oncology Reports*, **26**, 237–245.
85. Zhang Z, Huang A, Zhang A and Zhou C. (2017) HuR promotes breast cancer cell proliferation and survival via binding to CDK3 mRNA. *Biomedicine & Pharmacotherapy*, **91**, 788–795.
86. Balkhi MY, Iwenofu OH, Bakkar N, Ladner KJ, Chandler DS, Houghton PJ, London CA, Kraybill W, Perrotti D, Croce CM, Keller C and Guttridge DC. (2013) miR-29 acts as a decoy in sarcomas to protect the tumor suppressor A20 mRNA from degradation by HuR. *Science Signaling*, **6**, ra63.
87. Blanco FF, Jimbo M, Wulfkuhle J, Gallagher I, Deng J, Enyenihi L, Meisner-Kober N, Londin E, Rigoutsos I, Sawicki JA, Risbud MV, Witkiewicz AK, McCue PA, Jiang W, Rui H, Yeo CJ, Petricoin E, Winter JM and Brody JR. (2016) The mRNA-binding protein HuR promotes hypoxia-induced chemoresistance through posttranscriptional regulation of the proto-oncogene PIM1 in pancreatic cancer cells. *Oncogene*, **35**, 2529–2541.
88. Guo J, Lv J, Chang S, Chen Z, Lu W, Xu C, Liu M and Pang X. (2016) Inhibiting cytoplasmic accumulation of HuR synergizes genotoxic agents in urothelial carcinoma of the bladder. *Oncotarget*, **7**, 45249–45262.
89. Lin GL, Ting HJ, Tseng TC, Juang V and Lo YL. (2017) Modulation of the mRNA-binding protein HuR as a novel reversal mechanism of epirubicin-triggered multidrug resistance in colorectal cancer cells. *PLoS One*, **12**, e0185625.
90. Zhu H, Berkova Z, Mathur R, Sehgal L, Khashab T, Tao RH, Ao X, Feng L, Sabichi AL, Blechacz B, Rashid A and Samaniego F. (2015) HuR suppresses fas expression and correlates with patient outcome in liver cancer. *Molecular Cancer Research*, **13**, 809–818.
91. Tang H, Wang H, Cheng X, Fan X, Yang F, Zhang M, Chen Y, Tian Y, Liu C, Shao D, Jiang B, Dou Y, Cong Y, Xing J, Zhang X, Yi X, Songyang Z, Ma W, Zhao Y, Wang X, Ma J, Gorospe M, Ju Z and Wang W. (2018) HuR regulates telomerase activity through TERC methylation. *Nature Communications*, **9**, 2213.
92. Chand SN, Zarei M, Schiewer MJ, Kamath AR, Romeo C, Lal S, Cozzitorto JA, Nevler A, Scolaro L, Londin E, Jiang W, Meisner-Kober N, Pishvaian MJ, Knudsen

KE, Yeo CJ, Pascal JM, Winter JM and Brody JR. (2017) Posttranscriptional regulation of PARG mRNA by HuR facilitates DNA repair and resistance to PARP inhibitors. *Cancer Research*, **77**, 5011–5025.

93. Goldberg-Cohen I, Furneauxb H and Levy AP. (2002) A 40-bp RNA element that mediates stabilization of vascular endothelial growth factor mRNA by HuR. *Journal of Biological Chemistry*, **277**, 13635–13640.
94. Levy NS, Chung S, Furneaux H and Levy AP. (1998) Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR. *Journal of Biological Chemistry*, **273**, 6417–6423.
95. Osera C, Martindale JL, Amadio M, Kim J, Yang X, Moad CA, Indig FE, Govoni S, Abdelmohsen K, Gorospe M and Pascale A. (2015) Induction of VEGFA mRNA translation by CoCl2 mediated by HuR. *RNA Biology*, **12**, 1121–1130.
96. Li Z, Wang Y, Hu R, Xu R and Xu W. (2018) LncRNA B4GALT1-AS1 recruits HuR to promote osteosarcoma cells stemness and migration via enhancing YAP transcriptional activity. *Cell Proliferation*, **51**, e12504.
97. Xu W, Chen C, Xu R, Li Y, Hu R, Li Z and Zhu X. (2018) Knockdown of HuR represses osteosarcoma cells migration, invasion and stemness through inhibition of YAP activation and increases susceptibility to chemotherapeutic agents. *Biomedicine & Pharmacotherapy*, **102**, 587–593.
98. Nabors LB, Gillespie GY, Harkins L and King PH. (2001) HuR, a RNA stability factor, is expressed in malignant brain tumors and binds to adenine- and uridine-rich elements within the 3′ untranslated regions of cytokine and angiogenic factor mRNAs. *Cancer Research*, **61**, 2154–2161.
99. Cascajo MV, Abdelmohsen K, Noh JH, Fernandez-Ayala DJ, Willers IM, Brea G, Lopez-Lluch G, Valenzuela-Villatoro M, Cuezva JM, Gorospe M, Siendones E and Navas P. (2016) RNA-binding proteins regulate cell respiration and coenzyme Q biosynthesis by post-transcriptional regulation of COQ7. *RNA Biology*, **13**, 622–634.
100. Diaz-Munoz MD, Bell SE, Fairfax K, Monzon-Casanova E, Cunningham AF, Gonzalez-Porta M, Andrews SR, Bunik VI, Zarnack K, Curk T, Heggermont WA, Heymans S, Gibson GE, Kontoyiannis DL, Ule J and Turner M. (2015) The RNA-binding protein HuR is essential for the B cell antibody response. *Nature Immunology*, **16**, 415–425.
101. Zarei M, Lal S, Parker SJ, Nevler A, Vaziri-Gohar A, Dukleska K, Mambelli-Lisboa NC, Moffat C, Blanco FF, Chand SN, Jimbo M, Cozzitorto JA, Jiang W, Yeo CJ, Londin ER, Seifert EL, Metallo CM, Brody JR and Winter JM. (2017) Posttranscriptional upregulation of IDH1 by HuR establishes a powerful survival phenotype in pancreatic cancer cells. *Cancer Research*, **77**, 4460–4471.
102. Brauss TF, Winslow S, Lampe S, Scholz A, Weigert A, Dehne N, von Stedingk K, Schmid T and Brune B. (2017) The RNA-binding protein HuR inhibits expression of CCL5 and limits recruitment of macrophages into tumors. *Molecular Carcinogenesis*, **56**, 2620–2629.

103. Zhu Y, Xu G, Yang YT, Xu Z, Chen X, Shi B, Xie D, Lu ZJ and Wang P. (2019) POSTAR2: Deciphering the post-transcriptional regulatory logics. *Nucleic Acids Research*, **47**, D203–D211.
104. Bakheet T, Hitti E and Khabar KSA. (2018) ARED-Plus: An updated and expanded database of AU-rich element-containing mRNAs and pre-mRNAs. *Nucleic Acids Research*, **46**, D218–D220.
105. Filippova N, Yang X, Wang Y, Gillespie GY, Langford C, King PH, Wheeler C and Nabors LB. (2011) The RNA-binding protein HuR promotes glioma growth and treatment resistance. *Molecular Cancer Research*, **9**, 648–659.
106. Lal P, Cerofolini L, D'Agostino VG, Zucal C, Fuccio C, Bonomo I, Dassi E, Giuntini S, Di Maio D, Vishwakarma V, Preet R, Williams SN, Fairlamb MS, Munk R, Lehrmann E, Abdelmohsen K, Elezgarai SR, Luchinat C, Novellino E, Quattrone A, Biasini E, Manzoni L, Gorospe M, Dixon DA, Seneci P, Marinelli L, Fragai M and Provenzani A. (2017) Regulation of HuR structure and function by dihydrotanshinone-I. *Nucleic Acids Research*, **45**, 9514–9527.
107. Lopez de Silanes I, Fan J, Yang X, Zonderman AB, Potapova O, Pizer ES and Gorospe M. (2003) Role of the RNA-binding protein HuR in colon carcinogenesis. *Oncogene*, **22**, 7146–7154.
108. Wang H, Chen Y, Guo J, Shan T, Deng K, Chen J, Cai L, Zhou H, Zhao Q, Jin S and Xia J. (2018) Dysregulation of tristetraprolin and human antigen R promotes gastric cancer progressions partly by upregulation of the high-mobility group box 1. *Scientific Reports*, **8**, 7080.
109. Lal S, Cheung EC, Zarei M, Preet R, Chand SN, Mambelli-Lisboa NC, Romeo C, Stout MC, Londin E, Goetz A, Lowder CY, Nevler A, Yeo CJ, Campbell PM, Winter JM, Dixon DA and Brody JR. (2017) CRISPR knockout of the HuR gene causes a xenograft lethal phenotype. *Molecular Cancer Research*, **15**, 696–707.
110. Muralidharan R, Mehta M, Ahmed R, Roy S, Xu L, Aube J, Chen A, Zhao YD, Herman T, Ramesh R and Munshi A. (2017) HuR-targeted small molecule inhibitor exhibits cytotoxicity towards human lung cancer cells. *Scientific Reports*, **7**, 9694.
111. D'Agostino VG, Lal P, Mantelli B, Tiedje C, Zucal C, Thongon N, Gaestel M, Latorre E, Marinelli L, Seneci P, Amadio M and Provenzani A. (2015) Dihydrotanshinone-I interferes with the RNA-binding activity of HuR affecting its post-transcriptional function. *Scientific Reports*, **5**, 16478.
112. Wu X, Lan L, Wilson DM, Marquez RT, Tsao WC, Gao P, Roy A, Turner BA, McDonald P, Tunge JA, Rogers SA, Dixon DA, Aube J and Xu L. (2015) Identification and validation of novel small molecule disruptors of HuR-mRNA interaction. *ACS Chemical Biology*, **10**, 1476–1484.
113. Dong R, Chen P, Polireddy K, Wu X, Wang T, Ramesh R, Dixon DA, Xu L, Aube J and Chen Q. (2020) An RNA-binding protein, Hu-antigen R, in pancreatic cancer epithelial to mesenchymal transition, metastasis, and cancer stem cells. *Molecular Cancer Therapeutics*.

114. Wu X, Gardashova G, Lan L, Han S, Zhong C, Marquez RT, Wei L, Wood S, Roy S, Gowthaman R, Karanicolas J, Gao FP, Dixon DA, Welch DR, Li L, Ji M, Aube J and Xu L. (2020) Targeting the interaction between RNA-binding protein HuR and FOXQ1 suppresses breast cancer invasion and metastasis. *Communications Biology*, **3**, 193.
115. Blanco FF, Preet R, Aguado A, Vishwakarma V, Stevens LE, Vyas A, Padhye S, Xu L, Weir SJ, Anant S, Meisner-Kober N, Brody JR and Dixon DA. (2016) Impact of HuR inhibition by the small molecule MS-444 on colorectal cancer cell tumorigenesis. *Oncotarget*, **7**, 74043–74058.
116. Meisner NC, Hintersteiner M, Mueller K, Bauer R, Seifert JM, Naegeli HU, Ottl J, Oberer L, Guenat C, Moss S, Harrer N, Woisetschlaeger M, Buehler C, Uhl V and Auer M. (2007) Identification and mechanistic characterization of low-molecular-weight inhibitors for HuR. *Nature Chemical Biology*, **3**, 508–515.
117. Filippova N, Yang X, Ananthan S, Sorochinsky A, Hackney JR, Gentry Z, Bae S, King P and Nabors LB. (2017) Hu antigen R (HuR) multimerization contributes to glioma disease progression. *Journal of Biological Chemistry*, **292**, 16999–17010.
118. Filippova N, Yang X, Ananthan S, Calano J, Pathak V, Bratton L, Vekariya RH, Zhang S, Ofori E, Hayward EN, Namkoong D, Crossman DK, Crowley MR, King PH, Mobley J and Nabors LB. (2021) Targeting the HuR oncogenic role with a new class of cytoplasmic dimerization inhibitors. *Cancer Research*, **81**, 2220–2233.
119. Corley M, Burns MC, and Yeo GW. (2020) How RNA-binding proteins interact with RNA: molecules and mechanisms. *Molecular Cell*, **78**, 9–29.
120. Ebert MS and Sharp PA. (2010) MicroRNA sponges: Progress and possibilities. *RNA*, **16**, 2043–2050.

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Chapter 6

Pro- and Anti-Tumorigenic Activities of the RNA-binding Protein AUF1

Nina C. Lee and Gerald M. Wilson*

Department of Biochemistry and Molecular Biology and Marlene and Stewart Greenebaum NCI Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Abstract

ARE/poly(U)-binding/degradation factor 1 (AUF1) is a small family of RNA-binding proteins with diverse roles in the regulation of gene expression. Its best-characterized functions are in the posttranscriptional control of mRNAs containing AU-rich elements (AREs), including many that encode proteins associated with inflammation and cancer. However, the consequences of AUF1 binding to RNA substrates are diverse and can be highly variable across different cellular contexts. Transcript recruitment can be associated with RNA degradation, RNA stabilization, or modulation of translation efficiency, and can be regulated by a plethora of factors. AUF1 can also bind select microRNAs

*Corresponding author's email: gwilson@som.umaryland.edu

(miRNAs) and long noncoding RNAs (lncRNAs), further expanding the repertoire of its substrate pool and potential to modulate cellular gene expression networks. In this chapter, we first provide an overview of AUF1 and current knowledge of its mechanisms of action and regulation. Subsequently, we discuss roles of AUF1 in the development and progression of cancer. AUF1 expression is frequently dysregulated in neoplastic diseases, impacting production of factors associated with a variety of cancer cell phenotypes. However, the relationships between AUF1 levels and tumor progression are complex, as its diverse functional roles and context-dependent regulatory controls enable both pro- and anti-tumorigenic functions for this protein family.

1. Post-transcriptional control of gene expression by AU-rich elements (AREs)

Gene expression can be controlled at many steps, including transcription, pre-mRNA processing, nucleocytoplasmic transport, mRNA turnover, translation, and at post-translational levels. A subset of these mechanisms regulates the abundance and/or function of mature cytoplasmic mRNAs by modulating their degradation kinetics or translational efficiency which, by extension, controls the levels of their encoded protein products. The turnover and translational efficiency of individual mRNAs are generally regulated by specific *cis*-acting sequences in each transcript, frequently located within their 3′-untranslated regions (3′UTRs) [1–3].

Among the most rigorously defined of these mRNA regulatory sequences is a family termed the AU-rich elements (AREs) which may be present in as many as 10–15% of all human mRNAs [4, 5]. AREs show remarkable sequence diversity but are generally U-rich stretches that often contain one or more AUUUA motifs, frequently in an overlapping pattern. AREs control the stability and translation of many mRNAs. These sequences are particularly enriched in mRNAs encoding inflammatory and pro-tumorigenic factors, including cytokines, chemokines, growth factors, and oncoproteins [6, 7]. This makes them intriguing potential therapeutic targets in cancer or chronic inflammation [8–10]. AREs are recognized by ARE-binding proteins (ARE-BPs), a growing collection of over 20 proteins that bind these *cis*-elements and induce

diverse fates for targeted transcripts [6, 11–16]. For example, binding by some ARE-BPs promotes rapid transcript degradation while others inhibit mRNA decay rates. By contrast, recruitment of a subset of ARE-BPs regulates mRNA translation, in some cases by shuttling targeted mRNAs to membrane-less organelles called stress granules — subcellular assemblies that are enriched in translationally stalled ribonucleoprotein (RNP) complexes [17].

While the roles of AREs in mRNA metabolism have been well characterized over the past three decades, recent evidence also supports functional relationships between AREs and long noncoding RNAs (lncRNAs). lncRNAs are defined as RNA molecules longer than 200 nt that are unlikely to encode functional protein products but generally resemble mRNAs in many other respects including transcription by RNA polymerase II and post-transcriptional processing via splicing, 5′-capping, and 3′-polyadenylation [18]. Recent transcriptome-wide surveys indicate that the total number of lncRNAs rivals that of the mRNA transcriptome (~20,000) but may be several-fold higher [19]. lncRNAs exert a wide variety of regulatory functions based on their ability to interact with specific DNA, RNA, or protein targets. In the nucleus, lncRNAs can regulate transcription, subnuclear architecture, and splicing, while cytoplasmic functions include modulating mRNA decay kinetics and translational efficiency [20]. Three mechanisms employed by lncRNAs to regulate mRNA targets are: 1) by recruiting specific *trans*-acting factors to lncRNA-targeted mRNAs, 2) by sequestering RNA-binding proteins and microRNAs (miRNAs) away from mRNA substrates through a process known as "sponging", and 3) by recruiting RNA-modifying enzymes to direct local base methylation on mRNA targets [21–23]. The first two of these mechanisms are used by a subset of lncRNAs to regulate ARE functions [24–26]. However, ARE-BP binding can also directly regulate the decay kinetics of some lncRNAs [27, 28], analogous to the role of these *trans*-acting proteins in the control of mRNA turnover.

Since individual ARE-BPs and lncRNAs modulate expression of many different mRNAs, changes in the expression and/or function of these factors can have severe pathophysiological consequences based on their ability to impact multiple gene networks. This has perhaps been best described in cancer, where dysregulation of a post-transcriptional factor

can alter expression of multiple oncogenes and tumor suppressor genes which can drive a host of cancer-related phenotypes including proliferation, protection from apoptosis, angiogenesis, invasion, and metastasis [8, 28–32]. These oncogenic and tumor suppressive roles of ARE-targeted gene regulatory mechanisms make them attractive targets for therapeutic development.

2. The ARE-binding protein AUF1

In 1991, Gary Brewer reported the purification of an RNA-binding factor that accelerated degradation of c-*myc* mRNA in a cell-free mRNA decay system [33]. Two years later, his group cloned a cDNA encoding this factor, which they named ARE/poly(U)-binding/degradation factor 1 (AUF1) [34]. In 1995, a similar cDNA was independently isolated by Fuyuki Ishikawa's group as heterogeneous nuclear ribonucleoprotein D (hnRNP D) [35]. Subsequent work in the Brewer lab revealed that AUF1 is a family of four protein isoforms generated by alternative splicing of a single pre-mRNA, and designated each member based on its apparent molecular weight as $p37^{AUF1}$, $p40^{AUF1}$, $p42^{AUF1}$, and $p45^{AUF1}$ [36, 37].

The *AUF1/hnRNPD* gene is located on chromosome 4q21 and consists of 10 exons [37–39]. Each isoform contains two tandemly-arranged RNA Recognition Motifs (RRMs), similar to the RNA-binding domains of many other RNA-binding proteins [40], as well as a glutamine (Q)-rich domain C-terminal to the RRMs (Fig. 1). Differences between AUF1 isoforms are based on selective inclusion or exclusion of protein sequences

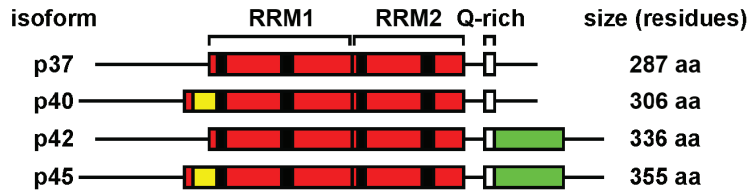

Figure 1. Isoforms of AUF1.

All isoforms have two non-identical RNA recognition motifs (red) characterized by internal RNP-1 and RNP-2 sequence motifs (black bars), while $p40^{AUF1}$ and $p45^{AUF1}$ contain additional protein sequence encoded by alternatively spliced exon 2 (yellow), and $p42^{AUF1}$ and $p45^{AUF1}$ from alternatively spliced exon 7 (green).

encoded by exons 2 and 7. While early biophysical analyses suggested that AUF1 proteins were dimeric [41–43], more recent studies with very highly purified recombinant AUF1 proteins indicate that they may instead exist as monomers in solution [44].

2.1. *RNA binding by AUF1*

All AUF1 isoforms retain some functional similarities including the ability to bind AREs and modify local RNA structure [45]. However, each isoform also exhibits unique characteristics derived from their structural differences, which likely contribute to the isoform-dependent variations in function described in later sections of this chapter. First, subcellular localization of AUF1 proteins is heavily influenced by sequences encoded by exon 7. Isoforms containing this sequence ($p42^{AUF1}$ and $p45^{AUF1}$) are almost exclusively nuclear in most cell types while $p37^{AUF1}$ and $p40^{AUF1}$ shuttle between the cytoplasmic and nuclear compartments [34, 46–49]. Second, the presence of exon 2- or 7-encoded domains modulates binding affinity for ARE substrates, albeit in two different ways (Table 1). On extended RNA substrates, multiple rounds of AUF1 binding generate oligomeric RNP structures. Inclusion of exon 2 sequences inhibits initial binding of AUF1 to RNA ligands, so $p40^{AUF1}$ and $p45^{AUF1}$ recognize these substrates with significantly poorer affinity than do $p37^{AUF1}$ and $p42^{AUF1}$ [37, 43]. Conversely, exon 7 sequences only modestly impact the initial AUF1 binding step on an RNA ligand, but instead dramatically enhance subsequent recruitment of AUF1 proteins to generate multimeric RNP complexes. Finally, the local structure of the RNA ligand in AUF1 RNP complexes is modulated by the exon 7-encoded domain (Table 1). Initial ARE binding events involving any AUF1 isoform induce a condensation of local RNA structure that is retained in higher-order AUF1 complexes involving $p37^{AUF1}$ and $p40^{AUF1}$, which lack the exon 7 domain. By contrast, oligomeric $p42^{AUF1}$ and $p45^{AUF1}$ complexes retain bound ARE substrates in extended conformations [43]. Together, these mechanisms permit isoform-dependent control of AUF1 access to and affinity for RNA substrates, as well as the conformational consequences of RNP assembly.

While AREs were the first substrates reported to bind AUF1, subsequent work has greatly expanded the range of RNA targets for this protein.

Table 1. Isoform-dependence of AUF1 RNA-binding and -remodeling activities[a].

	ARE-binding affinity		Conformation of ARE ligands	
Isoform	**First protein bound[b]**	**Second protein bound[b]**	**First protein bound**	**Second protein bound**
$p37^{AUF1}$	++++	++	condensed	condensed
$p40^{AUF1}$	++	+	condensed	condensed
$p42^{AUF1}$	+++	++++	condensed	open
$p45^{AUF1}$	++	++	condensed	open

[a]data compiled from [43, 50].

[b]relative ranking of RNA-protein binding affinities, from weakest (+) to strongest (++++).

A comprehensive survey of cellular RNA binding sites for AUF1 performed using photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) revealed extensive binding to U- and GU-rich motifs in addition to canonical ARE sequences [51]. Biochemical studies indicated a relatively large RNA footprint for high-affinity AUF1 binding (>30 nt), yet only 15 nt of ARE-like sequence were required to nucleate RNP formation [52]. These data indicate that non-base-specific interactions make substantial contributions to overall binding free energy, and potentially explain the relatively promiscuous binding of AUF1 to RNA targets. A surprising result of the PAR-CLIP study was the discovery that AUF1 proteins directly bound a subset of miRNAs [51]. Despite their small size (20–22 nt), some miRNAs bind AUF1 with affinities rivalling much longer ARE sequences [44, 53], suggesting that AUF1 might employ distinct mechanisms to recognize these RNA substrates. To date, no high-resolution structural studies on AUF1-RNA complexes have been reported that might resolve this paradox.

2.2. *Functional consequences of AUF1 binding to RNA*

On mRNA targets, AUF1 binding can induce a variety of fates including mRNA degradation, stabilization, and modulation of translation efficiency [12, 45, 54]. AUF1 was initially discovered based on its ability to accelerate decay of c-*myc* mRNA in cell-free mRNA decay assays [33]

and, for over a decade, this remained its principal known function. Additional data supported a primary role for the p37 isoform in accelerating ARE-directed mRNA decay, since: 1) $p37^{AUF1}$ shows the highest affinity for ARE ligands among all AUF1 isoforms [37, 43], 2) the affinity of $p37^{AUF1}$ for ARE substrates *in vitro* closely correlates with the mRNA-destabilizing activity of these RNA sequences *in cellulo* [55], and 3) cellular decay of ARE-reporter mRNAs was accelerated more robustly by ectopic overexpression of $p37^{AUF1}$ than by any other AUF1 isoform [56]. In select systems, however, other AUF1 isoforms have also been associated with accelerating decay of mRNA targets, particularly $p42^{AUF1}$. For example, AUF1-mediated degradation of fibroblast growth factor 9 (FGF9) mRNA in human embryonic kidney HEK293 cells was selective for the p42 isoform [57], while rapid decay of c-*fos* mRNA in hemin-treated K562 erythroleukemia cells was most efficiently achieved by overexpression of either $p37^{AUF1}$ or $p42^{AUF1}$ [58]. In some other cases, however, AUF1 proteins inhibit rather than enhance the decay of target transcripts. Overexpression of $p37^{AUF1}$ or $p42^{AUF1}$ stabilized an ARE reporter mRNA in NIH 3T3 fibroblasts [59] while $p37^{AUF1}$ also slowed the decay of mRNAs encoding the transcription factors snail family transcriptional repressor 1 (SNAI1) and twist family bHLH transcription factor 1 (TWIST1) in MCF-7 breast cancer cells [60]. Finally, comparing ribosome profiles between AUF1-expressing and AUF1-depleted cells identified over 100 AUF1-bound mRNAs that were translationally enhanced or repressed by AUF1 [51], indicating that AUF1 proteins can regulate production of select proteins without altering mRNA decay kinetics. A validated example of this mechanism is transforming growth factor-β-activated kinase (TAK1). In the pro-monocytic leukemia cell line THP-1, AUF1 induces expression of TAK1 protein but has no effects on the stability or steady-state level of TAK1 mRNA [61].

The precise molecular mechanisms by which AUF1 regulates mRNA decay and translation remain unknown. The proteins have no intrinsic nuclease activity but can form complexes with several cellular factors implicated in mRNA turnover and translation including eukaryotic translation initiation factor 4G (eIF4G), poly(A)-binding protein (PABP), and heat shock proteins Hsp70, Hsc70, and Hsp27 [62–64]. In cells, the vast majority of mRNAs are degraded via the deadenylation-dependent mRNA

decay pathway, initiated by recruitment or activation of deadenylating enzymes that shorten the mRNA 3′-poly(A) tail [2, 65]. Subsequently, the mRNA 5′-cap is excised by decapping factors, enabling processive degradation of the mRNA body by 5′ → 3′ and 3′ → 5′ exoribonucleases. One possibility is that AUF1 functions by nucleating the assembly of a multi-subunit *trans*-acting complex on ARE-containing mRNAs that recruits and/or activates deadenylating nucleases [12, 66]. A second potential mechanism is that this complex, via its engagement with PABP, might alter the interaction between PABP and the mRNA poly(A) tail, thereby enhancing or restricting deadenylase access.

Current data indicate that AUF1 directs a variety of metabolic consequences on different mRNA substrates, and examples in later sections will highlight how these effects can vary by cell type and in response to signaling pathways. These features prompt a third, non-exclusive hypothesis to explain how AUF1 might mediate such a diversity of context-specific regulatory functions: by acting as an RNA chaperone — modulating local RNA conformational landscapes to enhance or obstruct *trans*-factor binding to overlapping or proximal sites [45]. Evidence for such a model includes observations that AUF1 can enhance translation of c-*myc* mRNA by displacing the translation inhibitory factor TIA1 cytotoxic granule associated RNA binding protein like 1 (TIAR) [67] and that AUF1 binding to RNA polymerase II subunit D (POLR2D) mRNA enhances miRNA let-7b access to an adjacent binding site [44]. AUF1 can similarly modulate accessibility of mRNA targets for miRNA-loaded RNA-induced silencing complexes (RISC). For example, AUF1 inhibits recruitment of the catalytic RISC component Argonaute 2 (AGO2) to c-*myc* mRNA but enhances AGO2 binding to the HOXB8 transcript [68]. AUF1 also enhances loading of the mRNA-destabilizing protein tristetraprolin on ARE substrates [69] and integration of the miRNA let-7b into RISC [53]. Finally, recent data have revealed important roles for AUF1 in the replication and gene expression of select RNA viruses, in some cases clearly requiring AUF1's RNA chaperone-like functions. Replication of some flaviviruses including West Nile, dengue, and Zika are enhanced by the RNA remodeling activity of $p45^{AUF1}$ [70, 71], involving an RGG/RG-rich motif located near the protein C-terminus [72]. By contrast, AUF1 binding to RNA from enterovirus and human rhinovirus inhibits infections [73],

which in the case of enterovirus 71 involves suppressing translation initiated by an internal ribosome entry site in the viral RNA [74].

Cumulatively, these data support a model whereby the gene regulatory consequences of AUF1 binding to an RNA substrate could be largely mediated by its RNA chaperone activity, since it would explain: 1) the RNA substrate-dependence of AUF1 function, which would depend on gene-specific sequences near the AUF1 docking site, 2) the isoform-dependence of AUF1 activity, as each isoform shows distinct combinations of binding affinities, oligomerization potentials, and RNA conformational consequences on RNA substrates (Table 1), and 3) how signaling events that alter AUF1's RNA chaperone activities might impact post-transcriptional gene regulation, a concept explored further under section 3.

2.3. *Potential DNA-mediated consequences of AUF1 binding*

While AUF1 proteins are best known for RNA-targeted effects on gene expression, they also have important functions involving DNA substrates. Potential roles for these proteins in DNA repair processes were first suggested by the AUF1 PAR-CLIP study where AUF1 was found to target and regulate mRNAs encoding several DNA repair proteins [51]. However, more recent work indicates that AUF1 can also take a direct role in DNA repair, since chromatin-bound AUF1 can re-localize to DNA double-strand breaks following induction of DNA damage, while silencing AUF1 inhibited DNA end resection [75]. Furthermore, an independent screen for AUF1-interacting proteins in oral cancer cell lines identified several that were associated with base excision and double-strand break repair pathways [76]. Direct roles for AUF1 proteins in transcription are supported by observations that $p40^{AUF1}$ binds and activates the promoter for the complement receptor 2 gene and is a component of the lipopolysaccharide-responsive transcription factor LR1 in B cells [77, 78]. Similarly, multiple isoforms of AUF1 can regulate transcription of genes encoding enkephalin during brain development and the onco-developmental protein α-fetoprotein [79, 80]. Finally, all AUF1 isoforms bind the G-rich stand of the telomere repeat sequence [81], while the C-terminal RRM domain of AUF1

destabilizes guanosine quadruplex structures associated with this DNA substrate [82]. Together, these data prompted speculation that AUF1 might be involved in maintaining telomeres by enhancing access for the telomerase enzyme. Subsequent data showing direct activation of the telomerase reverse transcriptase (TERT) gene by AUF1 [83, 84] and telomere erosion and premature aging in AUF1-deficient mice [83] are highly supportive of this model, which AUF1 mediates by both local substrate remodeling and direct transcriptional activity.

3. Regulation of AUF1

A growing body of evidence has identified mechanisms that influence the expression, isoform distribution, localization, and activity of AUF1. Cumulatively, these diverse and frequently cell type-specific regulatory systems may make major contributions to the broad spectrum of physiological roles played by AUF1 in different tissue types and disease states.

3.1. *Regulation of AUF1 expression and isoform distribution*

While expression of AUF1 is altered in a variety of cancer types (section 5), specific mechanisms responsible for its regulation have only been described in a small number of cases. At the transcriptional level, the *AUF1* gene is induced by elevated nuclear factor-κB (NF-κB) activity in an oral squamous cell carcinoma line [85] and by the cell cycle regulator E2F transcription factor 1 (E2F1) in select cell models [86]. Interleukin (IL)-6 signaling induces *AUF1* gene transcription in breast cancer-associated fibroblasts by activating the transcription factor signal transducer and activator of transcription 3 (STAT3) [87].

AUF1 mRNA levels can also be regulated by post-transcriptional mechanisms. For example, estradiol treatment stabilizes AUF1 mRNAs in the endometrial and myometrial tissues of ovariectomized sheep [88], while in an osteosarcoma cell model, AUF1 expression is suppressed by miRNAs miR-141 and miR-146b-5p [89]. Furthermore, select AUF1 mRNA splice variants are targeted for degradation by the

nonsense-mediated mRNA decay pathway [90], while others may be suppressed by AUF1 itself through a negative feedback loop [91]. In mice, the distribution of AUF1 isoforms varies between different cell types or tissues and may be altered in response to select stimuli [92, 93], yet it remains unknown whether relative isoform ratios are principally due to variations in splicing efficiency, mRNA turnover, translation, or protein degradation kinetics. Some evidence for isoform-specific control of AUF1 protein levels was given by observations in the cervical carcinoma cell line HeLa that $p37^{AUF1}$ and $p40^{AUF1}$ are subject to ubiquitin-proteasome-directed turnover, while the larger isoforms are not and hence are more stable [94]. In THP-1 cells, however, all AUF1 protein isoforms exhibit very slow turnover rates but are destabilized by interactions with select phosphorylated forms of Hsp27 [95] — a stress-inducible factor that promotes ubiquitination and proteasomal degradation of multiple protein targets [96, 97].

3.2. *Regulation of AUF1 localization and function*

Beyond modulating AUF1 expression, diverse mechanisms have been identified that can control the RNA-binding activity of AUF1 and/or the regulatory consequences of AUF1 engagement. First, AUF1 binding is highly sensitive to RNA structural environments since local RNA folding at the substrate binding site inhibits AUF1 recruitment [98, 99]. Second, cytoplasmic localization of $p37^{AUF1}$, and to some extent $p40^{AUF1}$, can be enhanced by association with the protein 14-3-3σ concomitant with accelerated decay of ARE reporter mRNAs [100]. Third, some molecular binding partners can sequester AUF1 away from mRNA substrates. For example, the circular RNA *circPCNX* competes for AUF1 binding with other substrates including that encoding the cell cycle inhibitor p21. Overexpression of *circPCNX* in HeLa cells decreases AUF1 binding to p21 mRNA, resulting in stabilization and accumulation of the p21 transcript and enhancing production of its encoded protein [101].

AUF1 proteins are also subject to a variety of post-translational modifications. To date, the best-characterized of these is phosphorylation, which occurs on most if not all AUF1 isoforms [34, 66, 102] but is clearly

cell type-specific. For example, polysome-associated $p40^{AUF1}$ is phosphorylated at two sites within the exon 2-encoded domain in THP-1 cells. Removal of these phosphate groups following stimulation of protein kinase C (PKC) signaling with phorbol esters is associated with stabilization of tumor necrosis factor-α (TNFα) and IL-1β mRNAs [47]. Phosphorylation of $p40^{AUF1}$ at these sites modestly inhibited binding to an ARE substrate *in vitro* but precluded its ability to structurally remodel the RNA ligand [103]. Conversely, AUF1 phosphorylation by the chimeric tyrosine kinase nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) was associated with stabilization of c-*myc* and cyclin D1 mRNAs [104]. By contrast, most or all AUF1 isoforms display basal levels of serine phosphorylation in cardiomyocytes, but increased phosphorylation on threonine following PKC stimulation concomitant with enhanced binding of $p45^{AUF1}$ to sarco(endo)plasmic reticulum calcium ATPase 2a (SERCA2a) mRNA accelerated degradation of the transcript [105]. Beyond phosphorylation, methylation has been detected on multiple arginine residues in the C-terminal domains of $p37^{AUF1}$ and $p45^{AUF1}$ [106, 107]. In the case of $p45^{AUF1}$, methylation requires protein arginine methyltransferase 1 (PRMT1) and enhances RNA-binding affinity and the RNA chaperone activity of $p45^{AUF1}$ on West Nile Virus RNA [107]. Finally, all AUF1 isoforms can interact with peptidylprolyl *cis/trans* isomerase NIMA-interacting 1 (Pin1) in eosinophils, where Pin1 activity suppresses AUF1 binding to granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNA, thereby stabilizing the transcript [108].

4. Cancer-related roles of AUF1 targets

AUF1 binding to RNA substrates is highly dynamic and exhibits relatively loose substrate specificity, favoring uridine-rich sequences even without the AUUUA motifs common in AREs [42, 51]. These properties allow AUF1 to rapidly sample cellular transcriptomes to recognize a broad spectrum of RNA targets. The potential for AUF1 to impact cancer-related processes was recognized soon after its discovery when high affinity binding was observed to ARE sequences from c-*fos* and c-*myc* mRNAs, which both encode oncogenic transcription factors [34, 55]. Subsequent

transcriptome-wide screens for AUF1-targeted RNAs identified a plethora of substrates that encode factors associated with virtually every hallmark of cancer including regulators of DNA repair, cell proliferation, apoptosis, angiogenesis, inflammation, and metastasis [51, 109–111]. Expression of these proteins is tightly controlled since their overexpression or depletion can deregulate cellular pathways that contribute to tumor development or progression.

Some effects of AUF1 on target transcripts have clear and direct anti-tumorigenic consequences. One example is by accelerating the degradation of B cell lymphoma 2 (*bcl*-2) mRNA [112], since suppressing expression of its encoded protein limits the apoptosis resistance phenotype common to many cancer cells [113]. AUF1 may also inhibit cancer development indirectly by reducing inflammation. Elevated and sustained inflammation is linked to the development and progression of cancer by producing pro-tumorigenic microenvironments [114, 115]. A key role for AUF1 in suppressing inflammation was rigorously demonstrated in an AUF1 knockout mouse model, where overproduction of TNFα and IL-1β led to chronic dermatitis and extreme hypersensitivity to endotoxic shock [116, 117]. However, AUF1 activity on some other RNA substrates indicates pro-tumorigenic roles such as the stabilization of mRNAs encoding the transcription regulatory proteins SNAI1 and TWIST1 [60]. Increased levels of these proteins activate gene expression pathways that induce epithelial-mesenchymal transition (EMT), a key mechanism promoting tumor cell invasion and metastasis in many cell types [118]. Together, these data indicate that AUF1 does not play universal pro- or anti-tumorigenic roles, rather, its consequences on tumor growth and development are highly context dependent. This is perhaps most obviously demonstrated by its regulation of c-*myc* mRNA, which is dominated by distinct mechanisms in different cell types. In bladder cancer cells, AUF1 accelerates degradation of *c-myc* mRNA, limiting the pro-tumorigenic potential of its encoded transcription factor [119]. By contrast, in a leukemia model, AUF1 enhances translation of c-*myc* mRNA, increasing Myc protein levels and cellular proliferation rates [67]. Specific examples highlighting the diverse mechanisms by which AUF1 regulates target transcripts associated with cancer and the pathophysiological consequences of these events

are the foci of sections 5 and 6. Potential explanations for the plurality of outcomes associated with AUF1 binding to RNA substrates and their dependence on cellular context will be discussed under section 7.

5. Pro-tumorigenic functions of AUF1

Surveys of gene expression datasets reveal that AUF1 mRNA levels are significantly enhanced in many tumors relative to normal tissue, including cancers of the bladder, colon, testis, and acute myeloid leukemia (Fig. 2a). A focused study of surgical resections from thyroid cancers found that levels of AUF1 proteins were highly elevated in thyroid carcinomas and that overexpression of AUF1 and Hu protein R (HuR), another ARE-binding protein, could discriminate malignant follicular thyroid carcinoma from goiter tissue or follicular adenoma [120]. Although overall AUF1 expression is elevated in a wide variety of tumor types, the consequences of AUF1 on cancer patient outcomes are not uniform. In this section we describe evidence supporting pro-tumorigenic roles for AUF1, including associated RNA targets and functional consequences of AUF1 binding. Key examples are summarized in Table 2.

Surveys of patient data reveal a subset of cancer types where elevated AUF1 expression is associated with poor patient outcomes, including liver cancer and sarcomas (Figs. 2b and 2c). Data from mouse models support pro-oncogenic effects when the p37 isoform was systemically overexpressed, although effects were limited to specific tissue types. For example, $p37^{AUF1}$-overexpressing mice had enhanced levels of mRNAs encoding several cancer-relevant proteins including the transcription factors Myc, Jun, and Fos as well as the cell cycle regulator cyclin D1 in tissues that normally have low physiological levels of AUF1, such as skeletal muscle and liver [121]. Mice expressing the highest levels of $p37^{AUF1}$ spontaneously developed sarcomas that were rich in AUF1 and cyclin D1, though dysplasia was observed in every $p37^{AUF1}$-overexpressing mouse line and other tumors were occasionally observed in various locations including the esophagus, gastrointestinal tract, pancreas, and bladder. All identified tumors shared similar morphological features, were highly mitotic and vascularized, and contained significant necrotic regions although metastases were only observed in a single case [121].

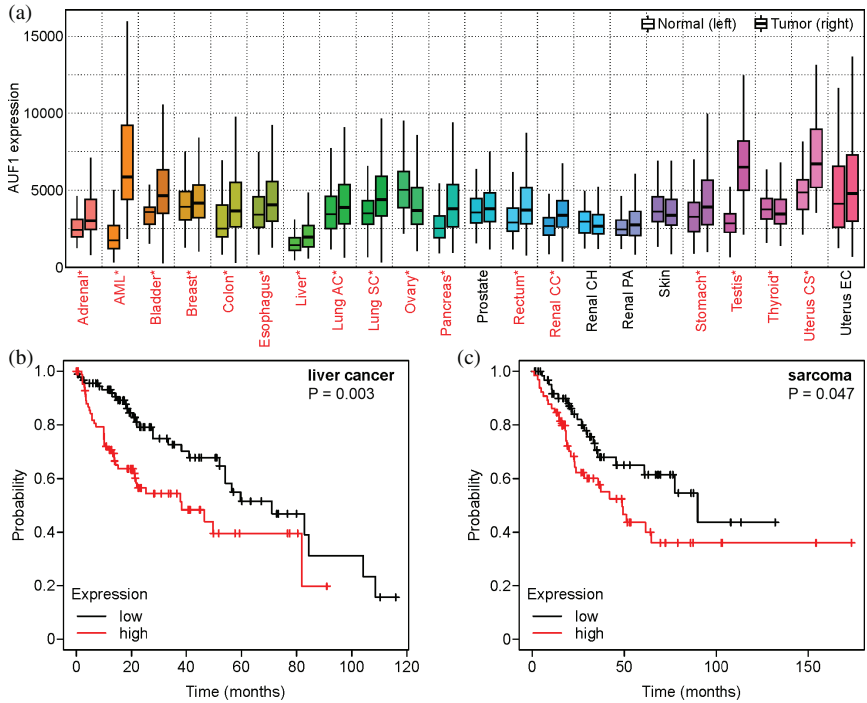

Figure 2. High expression of AUF1 is common in cancer and associated with poor survival in some tumor types.

(a) Comparisons of AUF1 mRNA levels in tumors relative to normal tissues across a wide array of cancer types performed using data reported in [122]. Tissues where AUF1 expression is significantly different between tumors and normal tissues are labeled in red. Abbreviations: AML: acute myelogenous leukemia; AC: adenocarcinoma; SC: squamous cell carcinoma; CC: clear cell carcinoma; CH: chromophobe; PA: papillary cell carcinoma; CS: carcinosarcoma; EC: endometrial carcinoma. Kaplan-Meier plots of (b) liver cancer and (c) sarcoma cases showing that high tumor expression of AUF1 (red line, highest expressing quartile) correlates with poorer survival relative to low tumor AUF1 expression (black line, lowest expressing quartile). mRNA levels were compared from RNA-Seq datasets reported in [123].

5.1. *Pro-tumorigenic AUF1-directed mRNA degradation events*

In some cancers, AUF1 promotes tumor development by accelerating decay of select RNA targets. One example of this phenomenon occurs in thyroid cancer. Two studies have demonstrated that malignant thyroid

tissues contain higher cytoplasmic and overall concentrations of AUF1 proteins than non-transformed tissues [120, 124]. Since AUF1 mRNA is not systematically upregulated in thyroid cancers (Fig. 2a), these data suggest that AUF1 levels might be induced at translational or post-translational stages of gene regulation in some cancers. In papillary thyroid cancer, one of the most common endocrine cancer types, part of AUF1's role in promoting proliferation, migration, and invasion phenotypes functions by binding and destabilizing the mRNA encoding the transcription factor zinc finger and BTB domain containing 2 (ZBTB2) [125]. The consequent decrease in ZBTB2 protein levels suppresses transcription from its target genes, including that encoding tripartite motif containing 58 (TRIM58), an E3 ubiquitin ligase reported to inhibit tumor development [126, 127]. By accelerating decay of ZBTB2 mRNA, AUF1 indirectly inhibits production of the tumor suppressor TRIM58, thus driving tumor development by impeding an antitumor pathway.

A second example where mRNA degradation by AUF1 elicits pro-tumorigenic consequences involves selective targeting of Dicer1 mRNA, which encodes the cytoplasmic ribonuclease III-type endoribonuclease required for cleaving pre-miRNA into mature miRNA molecules [128]. AUF1 directly targets and destabilizes Dicer1 mRNA, decreasing the concentration of its encoded protein and production of mature miRNAs [129]. Consistent with this model, AUF1 and Dicer1 protein levels are negatively correlated across a diverse panel of cancer types [129]. An example demonstrating the pro-tumorigenic consequences of AUF1-directed suppression of Dicer1 occurs in liver cancer, where downstream inhibition of miRNA maturation represses production of the liver-specific miRNA, miRNA-122 [130]. miRNA-122 has been characterized as a tumor suppressor since it promotes apoptosis by inhibiting expression of the anti-apoptotic factor BCL like 2 (BCL2L2), but this miRNA is frequently downregulated in hepatocellular carcinoma [131]. When AUF1 is overexpressed in these cells, levels of pre-miRNA-122 increase, while Dicer1 and mature miRNA-122 populations both decrease, which protects the cells from apoptosis and enhances the cancer phenotype [130].

5.2. *Pro-tumorigenic AUF1-directed mRNA stabilization events*

Tumor cells in breast and select other cancer types are normally intermingled with stromal cells including leukocytes and fibroblasts. Paracrine signaling between tumor and stroma can prompt development of tissue microenvironments that enhance tumor growth and invasiveness [132]. An example is the transition of breast fibroblasts to myofibroblasts. One factor that promotes myofibroblast formation is IL-6 secreted by breast cancer cells, which inhibits production of tumor suppressor proteins such as tumor protein p53 and the 16-kDa variant of cyclin dependent kinase inhibitor 2A ($p16^{INK4A}$) while activating expression of myofibroblast markers [87]. These activities are mediated by the transcription factor STAT3, which induces transcription from many gene targets including *AUF1*. In turn, the expressed AUF1 proteins bind and stabilize select mRNA targets, including C-X-C motif chemokine ligand 12 (CXCL12, also known as SDF-1), IL-6, and transforming growth factor β1 (TGFB1), enhancing production of these paracrine factors and establishing a feed-forward loop to further activate the myofibroblast. Overexpression of the p37 isoform alone is sufficient to sensitize fibroblasts to IL-6 and increase paracrine factor expression [87].

Another set of AUF1-regulated RNAs contribute to EMT and the cancer stem cell phenotype in breast epithelial cells. Increased levels of AUF1, specifically $p37^{AUF1}$, bound and stabilized mRNAs encoding the EMT transcription factors SNAI1 and TWIST1, increasing levels of their encoded proteins [60]. In turn, these transcription factors activated expression of stem cell markers CD44 and aldehyde dehydrogenase 1 family member A1 (ALDH1A1), and induced production of several mesenchymal marker proteins including N-cadherin and vimentin. Accumulation of these proteins enhanced tumorsphere formation and EMT, leading to stronger invasion and migration phenotypes *in cellulo*, and accelerated the formation of tumors in mice supporting a pro-tumorigenic role for AUF1 in this cancer type [60].

Stabilization of mRNA targets by AUF1 also contributes to tumor aggressiveness in osteosarcoma models. Decay of mRNAs encoding the

mesenchymal transcription factor zinc finger E-box binding homeobox 1 (ZEB1) and the AKT-activating kinase phosphoinositide-dependent kinase-1 (PDK1) are both inhibited when AUF1 is overexpressed [89]. In this model, $p37^{AUF1}$-dependent stabilization of ZEB1 mRNA increased the expression of mesenchymal proteins and contributed to EMT, while proliferation, migration, and invasion phenotypes were also enhanced by PDK1 activation of the AKT kinase pathway. Kinase activation by AUF1 was also observed in colorectal cancer cell lines, including both the AKT1 and extracellular signal-related kinase (ERK) pathways [133], although specific mRNA targets linking AUF1 to ERK signaling in these cells remain unknown. These consequences of AUF1 are pro-tumorigenic, however, since silencing AUF1 enhanced production of epithelial markers but inhibited expression of mesenchymal factors associated with EMT, while also slowing cell motility, invasiveness, and tumor growth in xenograft models. Finally, upregulation of AUF1 protein, especially the p37, p40, and p42 isoforms, is strongly associated with poor prognosis, reduced survival, and advanced colorectal cancer stage in human subjects [133].

Some similar pro-tumorigenic properties of AUF1 were also noted in esophageal squamous cell carcinoma where AUF1 protein levels were enhanced in tumors versus non-transformed tumor-adjacent tissues and correlated with tumor stage [134]. In the human esophageal carcinoma cell line Eca-109, depletion of AUF1 slowed cell proliferation and increased apoptosis, suggesting that AUF1 protects tumors from programmed cell death even as it accelerates their growth. Enhanced levels of AUF1 altered expression of multiple genes in esophageal cancer cells, including some associated with mitogen-activated protein kinase (MAPK) signaling and general transcription. Among the transcripts upregulated by AUF1 was a novel target, GTP cyclohydrolase I (GCH1) mRNA, whose encoded protein catalyzes the first and rate-limiting step in the synthesis of tetrahydrobiopterin (BH4), an essential cofactor required for nitric oxide formation [135]. Silencing GCH1 alone suppressed proliferation and colony formation in these cells, indicating that it is an important factor in esophageal cancer and that its induction by AUF1 may contribute to cancer progression [134].

5.3. *Pro-tumorigenic AUF1-directed enhancement of translation*

Among the first identified targets of AUF1 was c-*myc* mRNA [34]. It encodes the transcription factor Myc, which regulates expression of 15% of all known genes and contributes to multiple cancerous phenotypes [136–138]. Dysregulation of Myc is vital for the nucleation of many cancers and can disrupt both normal proliferation and cell death checkpoints, allowing rapid progression into a neoplastic phenotype. In the leukemia cell models K562 and THP-1 as well as two cell lines derived from solid tumors, silencing AUF1 expression did not alter c-*myc* mRNA levels, but rather reduced levels of Myc protein concomitant with decreased ribosome density on c-*myc* mRNA, indicating that AUF1 enhances the translational efficiency of this transcript [67]. Consistent with this model, overexpression of any AUF1 isoform increased Myc protein levels independent of changes in c-*myc* mRNA abundance. AUF1 proteins enhance Myc translation in these models by competing with the translational inhibitory protein TIAR, leading to Myc-dependent acceleration of cell proliferation [67].

Table 2. Pro-tumorigenic activities of AUF1.

Type of cancer	AUF1 isoform	Function	Phenotype	Source
Leukemia	All	Enhanced translation of c-*myc* mRNA	Increases cell proliferation	[67]
Breast	p37	Stabilization of TWIST1 and SNAI1 mRNAs	Enhances stemness	[60]
Breast	p37	Stabilization of IL-6 and myofibroblast marker mRNAs	Sensitizes activation of cancer-associated myofibroblasts	[87, 139]
Various (in mice)	p37	Enhanced levels of c-*myc*, c-*jun*, c-*fos*, and cyclin D1 mRNAs	Increases in tumor formation	[121]

(*Continued*)

Table 2. (*Continued*)

Type of cancer	AUF1 isoform	Function	Phenotype	Source
Osteosarcoma	p37	Stabilization of PDK1 and ZEB1 mRNAs	Enhances AKT signaling and EMT	[89]
Papillary thyroid cancer	Unknown	Degradation of ZBTB2 mRNA	Suppresses TRIM58 to enhance proliferation	[125]
Colorectal	p37, p40, p42	Unknown	Enhances AKT pathway and EMT transition	[133]
Esophageal	Unknown	Stabilization of GCH1 mRNA	Enhances proliferation and colony formation	[134]
Hepatocellular	Unknown	Degradation of Dicer1 mRNA	Protects against apoptosis	[130]

6. Anti-tumorigenic functions of AUF1

In contrast to the previous section, there have also been numerous cases reported where AUF1 exhibits anti-tumorigenic properties. In some cancer types, high expression of AUF1 can be protective and improve prognosis. For example, in lung and gastric cancers, elevated AUF1 expression is associated with better patient outcomes (Fig. 3). All anti-tumorigenic functions of AUF1 reported to date involve accelerating decay of select RNA targets, implying a potential separation between the mechanisms directing pro- versus anti-tumorigenic functions of AUF1 proteins. The best-characterized examples of AUF1's anti-tumorigenic activities are summarized in Table 3.

As described above (section 5.3.), c-*myc* mRNA is a well-known AUF1 substrate. In the invasive bladder cancer cell line UMUC3, elevated AUF1 levels target the ARE within the c-*myc* mRNA 3′UTR, accelerating its degradation and thereby decreasing the potential of the encoded protein to drive tumor development [119]. However, this example also highlights a key paradox regarding AUF1 regulation and function in cancer. While AUF1 is upregulated in many tumors (Fig. 2a), its effects on mRNA substrates can dramatically vary depending on cell type. For example, in

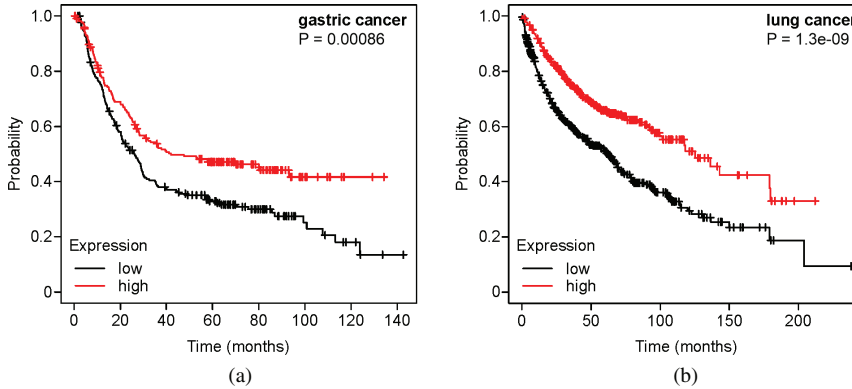

Figure 3. High expression of AUF1 is associated with improved survival in some tumor types.

Kaplan-Meier plots of (a) gastric and (b) lung cancer cases showing that high tumor expression of AUF1 (red line, highest expressing quartile) correlates with improved survival relative to low tumor AUF1 expression (black line, lowest expressing quartile). mRNA levels were compared from RNA-Seq datasets reported in [123].

bladder cancer cells, AUF1 exhibits a potent mRNA-destabilizing activity rather than the mRNA-stabilizing functions described previously in the osteosarcoma line [89] or the translational enhancement function observed in several other cancer cell models [67]. Potential hypotheses that may explain these discrepancies are discussed in section 7.

Beyond c-*myc*, AUF1-enhanced degradation of several other mRNAs also promotes anti-tumorigenic phenotypes. For example, AUF1 binds ARE sequences within the 3′UTR of *bcl*-2 mRNA, which encodes a vital anti-apoptotic factor, and causes its degradation in Jurkat T cell leukemia and mouse 3T3 fibroblast cells [140]. Activation of apoptosis in Jurkat cells by UVC irradiation increased AUF1 protein levels, especially $p45^{AUF1}$. This normally nuclear protein isoform also accumulated in the cytoplasm following UVC treatment, suggesting potential alterations in nuclear import/export cycling. While all AUF1 isoforms can bind *bcl*-2 mRNA, elevated cytoplasmic $p45^{AUF1}$ levels were associated with its destabilization [140]. Suppression of Bcl-2 protein expression or function potently enhances apoptotic sensitivity in many cancer cell types, making it an attractive target for novel anti-neoplastic therapeutic strategies [135, 141].

A distinct pro-apoptotic role of AUF1 functions by accelerating degradation of follistatin (FST) mRNA, which encodes a protein that protects cells from apoptosis in response to stress [142]. One of the key stressors that induces FST expression is glucose deprivation, where increased FST protein levels accumulate in the nucleolus and delay apoptotic signaling through a process that involves inhibition of ribosomal RNA synthesis [143]. FST induction is mediated by relocalization of all AUF1 isoforms into the nucleus by a mechanism which remains unknown but requires activity of the AMP-dependent protein kinase (AMPK) pathway. The resulting decrease in cytoplasmic AUF1 concentrations prevents these proteins from binding and degrading the cytoplasmic FST mRNA [142]. For cells deep within tumors that are nutrient-poor, interventions aimed at overexpressing AUF1 or relocalizing it to the cytoplasm could thus hinder cancer cell survival by destabilizing the anti-apoptotic FST transcript and increasing cellular susceptibility to apoptosis.

AUF1 also has a role in preventing metastatic properties of colon cancer and melanoma cells by binding and destabilizing autotaxin (ATX) mRNA [144]. ATX is an enzyme that catalyzes the formation of lysophosphatidic acid and is one of the most upregulated genes in metastatic cancer [145]. The ATX product regulates several cancer-related cellular activities including proliferation and migration. As such, AUF1-dependent degradation of ATX mRNA inhibits cancer cell migration. While all AUF1 isoforms interact with the ATX mRNA 3′UTR, it is unknown which are responsible for accelerating ATX1 mRNA decay [144].

In non-small cell lung cancer, AUF1 can enhance antitumor immunity by binding and enhancing degradation of programmed death ligand 1 (PD-L1, also known as CD274) mRNA [146]. The PD-L1 protein is overexpressed in a wide range of tumor types and inactivates infiltrating T cells by binding via their programmed cell death 1 (PD-1, also called PDCD1) receptors [147]. However, an oncogenic lncRNA, SChLAP1, competes with PD-L1 mRNA for binding to AUF1 and sequesters the RNA-binding protein. Disengagement of AUF1 stabilizes PD-L1 mRNA and increases expression of its encoded protein, promoting immune evasion. Overexpression of SChLAP1 occurs in multiple cancer types, suggesting that impairment of AUF1's anti-tumorigenic function by this

lncRNA might be a common method for immune system resistance by enhancing PD-1/PD-L1 interactions [146, 148–150].

Finally, while SChLAP1 functions as a lncRNA inhibitor of AUF1 function, AUF1 can also degrade select oncogenic lncRNAs directly such as ANRIL and NEAT1 [25, 51]. However, these tumor suppressive roles for AUF1 can also be inhibited by a distinct oncogenic lncRNA called P14AS, which is frequently upregulated in colorectal cancers and promotes proliferation and tumor formation. P14AS specifically binds $p37^{AUF1}$ and $p40^{AUF1}$, competing them away from ANRIL and other RNA substrates, thus preventing AUF1 from suppressing expression of these oncogenic RNAs [25].

7. Conclusions and questions in the field

AUF1 proteins regulate expression of myriad genes that encode pro- and anti-oncogenic products, and while AUF1 is frequently upregulated in human cancers, its relationship to tumor phenotypes and patient outcomes

Table 3. Anti-tumorigenic activities of AUF1.

Type of cancer	AUF1 isoform	Function	Phenotype	Source
Bladder	Unknown	Degradation of c-*myc* mRNA	Slows cell cycle and anchorage-independent growth	[119]
Leukemia Cell line/fibroblasts	p45	Degradation of *bcl*-2 mRNA	Enhances apoptosis	[140]
Colon	p37, p40 (potentially all)	Degradation of ANRIL and other oncogenic lncRNAs	Slows cell proliferation and tumor formation	[25]
Colon/Melanoma	Unknown	Degradation of ATX mRNA	Slows metastasis and proliferation	[144]
Cervical adenocarcinoma	All	Degradation of follistatin mRNA	Enhances apoptosis	[142]
Non-small cell lung carcinoma	Unknown	Degradation of PD-L1 mRNA	Prevents immune evasion	[146]

is complex. Based on the biochemical and cell biological studies reported to date, the disparate effects of AUF1 on different tumor types may be rationalized via three major factors:

1) AUF1 function varies depending on the mRNA substrate: Data from paired PAR-CLIP and RNA substrate expression studies provide the most robust demonstration of this principle, since altering AUF1 levels increased or decreased levels of different RNA targets, while a separate subpopulation showed changes in ribosome loading independent of effects on mRNA steady-states [51]. Based on the RNA chaperone model of AUF1 function (section 2.2.), regulatory consequences of AUF1 binding could be dependent on: 1) the structural remodeling activity of AUF1 on specific RNA targets, 2) the presence of proximal *cis*-acting elements within the mRNA that are exposed or occluded following AUF1 recruitment, and 3) the expression and/or activity of *trans*-acting factors that might interact with these flanking RNA sequences.
2) AUF1 function varies depending on the protein isoform: Specific isoforms of AUF1 have distinct effects on some RNA targets. For example, in THP-1 monocytes, $p40^{AUF1}$ stabilizes IL-10 mRNA, while $p37^{AUF1}$ does not [151]. Other examples where mRNA regulation is selective for individual AUF1 isoforms are described above. In most cases, it is not clear whether this phenomenon results from selective binding to RNA targets or distinct functional outcomes mediated by specific isoforms. Since the PAR-CLIP data also revealed that each AUF1 isoform binds distinct but overlapping subpopulations of cellular RNA [51], both hypotheses remain possible at this point. Accordingly, individual AUF1 isoforms might contribute to the diversity of functional consequences on RNA substrates because of differences in: 1) preferences/tolerances for specific RNA sequence and/or RNA structural elements, 2) subcellular localization, 3) AUF1 oligomerization potential and local RNA structural remodeling (Table 1), and by extension, accessibility for protein and/or RNA *trans*-acting factors binding proximal sites, 4) susceptibility to post-translational modifications, and 5) interactions with ancillary factors. An example

of the last point is AUF1's role in modulating tristetraprolin loading onto RNA substrates, which is aided by $p45^{AUF1}$ but not by the p37 isoform [69].

3) AUF1 function varies depending on cellular context: Examples described in sections 5 and 6 demonstrate that AUF1 can play pro- or anti-tumorigenic roles in different tissues. Furthermore, its pro- or anti-tumorigenic properties are facilitated by inducing or inhibiting distinct oncogenic phenotypes depending on the cell model tested. Specific cell types can impact AUF1 functions and pathophysiological relevance based on variations in: 1) the populations of AUF1 substrate RNAs expressed, since AUF1 can only act upon the RNA targets with which it is presented, 2) AUF1 abundance and isoform distribution, 3) subcellular localization of AUF1 isoforms expressed, 4) co-expression of ancillary factors including other RNA-binding proteins, miRNAs, and lncRNAs that contribute to or antagonize AUF1 binding or function, and 5) signaling mechanisms that can modify AUF1 expression and/or function.

This elaborate context-dependence of AUF1 function and its relationship to the cancer phenotype mandates that potential AUF1-targeted anti-tumor therapies must also be designed and applied in a context-dependent manner. AUF1 has many pro-tumorigenic activities but also plays anti-tumorigenic roles in some tissues. As such, an AUF1 inhibitor strategy is anticipated to be beneficial only in cancers where elevated AUF1 concentrations are promoting tumor aggressiveness. The survey of current literature reported in sections 5 and 6 indicate that the mRNA degradation activity of AUF1 is principally anti-tumorigenic, while most examples where AUF1 induces gene expression by stabilizing or enhancing translation of mRNA substrates are pro-tumorigenic. These properties suggest several means by which AUF1 function might be therapeutically manipulated (Fig. 4). First, compounds that selectively inhibit the mRNA stabilization and/or translation enhancement functions of AUF1 could have utility against a wide variety of tumor types where AUF1 contributes to the oncogenic phenotype. Rational design of such compounds would require that AUF1 use distinct mechanisms to stabilize and/or enhance

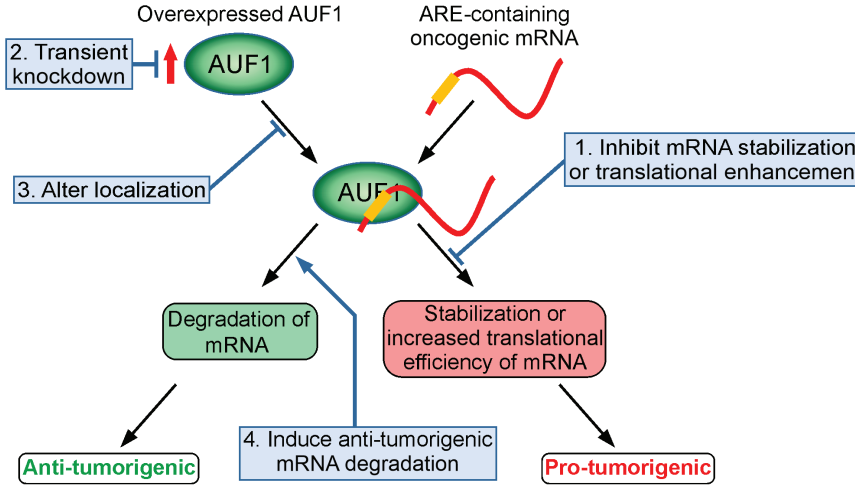

Figure 4. Potential therapeutic strategies to target AUF1.

In many cancers, overexpression of AUF1 increases potential for binding ARE substrate mRNAs, including many that encode pro-oncogenic factors. Stabilization or translational enhancement of these mRNA targets are generally associated with pro-tumorigenic outcomes, while mRNA degradative functions of AUF1 are more commonly linked to anti-oncogenic phenotypes. Four possible anti-tumor therapeutic strategies targeting AUF1 are highlighted in blue boxes and described in the text.

translation of target mRNAs versus those employed in accelerating decay of mRNA substrates. Second, transient suppression of AUF1 may be useful, particularly in cases where elevated AUF1 contributes to chemoresistance by suppressing apoptotic signaling. Support for this approach was observed in breast cancer cell models, where AUF1 knockdown sensitized cells to cisplatin treatment [60]. A third option could be to develop compounds that manipulate the subcellular localization of AUF1. Since cytoplasmic AUF1 is generally associated with regulation of mRNA targets, inducing nuclear accumulation may be sufficient to suppress the pro-tumorigenic mRNA stabilization and translational enhancement activities. Finally, in cancers where AUF1 plays anti-tumorigenic roles, activation of AUF1 expression or its mRNA-destabilizing functions may prove beneficial. Evidence for this approach has been observed in lung cancer cells, where prostaglandin A_2 treatment induced expression of

$p45^{AUF1}$ concomitant with accelerated decay of cyclin D1 mRNA and arrest of the cell cycle [152]. However, given the extreme tissue variability of AUF1 function, the utility of any therapeutics that manipulate AUF1 expression, localization, or activity would dramatically benefit from delivery methods that direct AUF1-inhibitory (or AUF1-enhancing) compounds to specific tissues [153, 154] or by administering as pro-drugs activatable only in select cell types [155].

Before therapeutic targeting of AUF1 can realistically be considered, some key questions need resolution. First, what is the biochemical basis by which AUF1 recognizes and remodels RNA substrates? Atomic-level structures of AUF1:RNA complexes would be ideal for this purpose, but AUF1 has thus far been refractory to crystallization or structural analysis by nuclear magnetic resonance (NMR) beyond individual RRM domains [156, 157], suggesting that the protein may include extensively disordered regions beyond the minimal RNA-binding interface. Second, what mechanism(s) determine whether AUF1 binding stabilizes, destabilizes, or modulates translation of individual mRNA substrates? Functional discrimination is likely mediated by the mRNA target itself but could involve selective recruitment of ancillary *trans*-factors to the mRNA or possibly even distinct modes of AUF1 binding to different substrates. Third, what cellular mechanisms are responsible for inducing AUF1 expression in many cancers? Inhibition of these pathways may be appealing methods for suppressing AUF1 production in some aggressive tumor types. Finally, what methods do cells employ to regulate AUF1 function? Identifying the signaling pathways that modify AUF1 proteins and the regulatory consequences of these modifications is likely to reveal additional targets for therapeutic intervention.

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References

1. Mayr C. (2017) Regulation by 3′-untranslated regions. *Annual Review of Genetics*, **51**, 171–194.
2. Wu X and Brewer G. (2012) The regulation of mRNA stability in mammalian cells: 2.0. *Gene*, **500**, 10–21.
3. Truitt ML and Ruggero D. (2016) New frontiers in translational control of the cancer genome. *Nature Reviews Cancer*, **16**, 288–304.
4. Gruber AR, Fallmann J, Kratochvill F, Kovarik P and Hofacker IL. (2011) AREsite: A database for the comprehensive investigation of AU-rich elements. *Nucleic Acids Research*, **39**, D66–D69.
5. Bakheet T, Williams BRG and Khabar KSA. (2006) ARED 3.0: The large and diverse AU-rich transcriptome. *Nucleic Acids Research*, **34**, D111–D114.
6. Barreau C, Paillard L and Osborne HB. (2005) AU-rich elements and associated factors: Are there unifying principles? *Nucleic Acids Research*, **33**, 7138–7150.
7. Beisang D and Bohjanen PR. (2012) Perspectives on the ARE as it turns 25 years old. *Wiley Interdisciplinary Reviews: RNA,* **3**, 719–731.
8. Griseri P and Pagès G. (2014) Control of pro-angiogenic cytokine mRNA half-life in cancer: The role of AU-rich elements and associated proteins. *Journal of Interferon & Cytokine Research*, **34**, 242–254.
9. Khabar KSA. (2017) Hallmarks of cancer and AU-rich elements. *Wiley Interdisciplinary Reviews: RNA*, **8**, e1368.
10. Vlasova-St.Louis I and Bohjanen PR. (2017) Post-transcriptional regulation of cytokine and growth factor signaling in cancer. *Cytokine & Growth Factor Reviews*, **33**, 83–93.
11. Otsuka H, Fukao A, Funakami Y, Duncan KE and Fujiwara T. (2019) Emerging evidence of translational control by AU-rich element-binding proteins. *Frontiers in Genetics*, **10**, 332.
12. Gratacós FM and Brewer G. (2010) The role of AUF1 in regulated mRNA decay. *Wiley Interdisciplinary Reviews: RNA,* **1**, 457–473.
13. Briata P, Chen CY, Ramos A and Gherzi R. (2013) Functional and molecular insights into KSRP function in mRNA decay. *Biochimica et Biophysica Acta*, **1829**, 689–694.
14. Brooks SA and Blackshear PJ. (2013) Tristetraprolin (TTP): Interactions with mRNA and proteins, and current thoughts on mechanisms of action. *Biochimica et Biophysica Acta*, **1829**, 666–679.
15. Schultz CW, Preet R, Dhir T, Dixon DA and Brody JR. (2020) Understanding and targeting the disease-related RNA binding protein human antigen R (HuR). *Wiley Interdisciplinary Reviews: RNA,* **11**, e1581.
16. Waris S, Wilce MCJ and Wilce JA. (2014) RNA recognition and stress granule formation by TIA proteins. *International Journal of Molecular Sciences*, **15**, 23377–23388.

17. Protter DSW and Parker R. (2016) Principles and properties of stress granules. *Trends in Cell Biology*, **26**, 668–679.
18. Ulitsky I and Bartel DP. (2013) lincRNAs: Genomics, evolution, and mechanisms. *Cell*, **154**, 26–46.
19. Uszczynska-Ratajczak B, Lagarde J, Frankish A, Guigó R and Johnson R. (2018) Towards a complete map of the human long non-coding RNA transcriptome. *Nature Reviews Genetics*, **19**, 535–548.
20. Statello L, Guo CJ, Chen LL and Huarte M. (2021) Gene regulation by long non-coding RNAs and its biological functions. *Nature Reviews Molecular Cell Biology*, **22**, 96–118.
21. Rashid F, Shah A and Shan G. (2016) Long non-coding RNAs in the cytoplasm. *Genomics Proteomics Bioinformatics*, **14**, 73–80.
22. Sebastian-delaCruz M, Gonzalez-Moro I, Olazagoitia-Garmendia A, Castellanos-Rubio A and Santin I. (2021) The role of lncRNAs in gene expression regulation through mRNA stabilization. *Noncoding RNA*, **7**, 3.
23. Noh JH, Kim KM, McClusky WG, Abdelmohsen K and Gorospe M. (2018) Cytoplasmic functions of long noncoding RNAs. *Wiley Interdisciplinary Reviews: RNA,* **9**, e1471.
24. Peng WX, Koirala P, Zhang W, Ni C, Wang Z, Yang L and Mo YY. (2020) lncRNA RMST enhances DNMT3 expression through interaction with HuR. *Molecular Therapy*, **28**, 9–18.
25. Ma W, Qiao J, Zhou J, Gu L and Deng D. (2020) Characterization of novel LncRNA P14AS as a protector of ANRIL through AUF1 binding in human cells. *Molecular Cancer*, **19**, 42.
26. Huang J, Zhang A, Ho TT, Zhang Z, Zhou N, Ding X, Zhang X, Xu M and Mo YY. (2016) Linc-RoR promotes c-Myc expression through hnRNP I and AUF1. *Nucleic Acids Research*, **44**, 3059–3069.
27. Yoon JH, Kim J and Gorospe M. (2015) Long noncoding RNA turnover. *Biochimie*, **117**, 15–21.
28. Jonas K, Calin GA and Pichler M. (2020) RNA-binding proteins as important regulators of long non-coding RNAs in cancer. *International Journal of Molecular Sciences*, **21**, 2969.
29. Lukong KE, Chang K, Khandjian EW and Richard S. (2008) RNA-binding proteins in human genetic disease. *Trend in Genetics*, **24**, 416–425.
30. Pereira B, Billaud M and Almeida R. (2017) RNA-binding proteins in cancer: Old players and new actors. *Trends in Cancer*, **3**, 506–528.
31. Wang H, Ding N, Guo J, Xia J and Ruan Y. (2016) Dysregulation of TTP and HuR plays an important role in cancers. *Tumor Biology*, **37**, 14451–14461.
32. Abdelmohsen K and Gorospe M. (2010) Posttranscriptional regulation of cancer traits by HuR. *Wiley Interdisciplinary Reviews: RNA,* **1**, 214–229.
33. Brewer G. (1991) An A+U-rich element RNA-binding factor regulates c-*myc* mRNA stability in vitro. *Molecular and Cellular Biology*, **11**, 2460–2466.

34. Zhang W, Wagner BJ, Ehrenman K, Schaefer AW, DeMaria CT, Crater D, DeHaven K, Long L and Brewer G. (1993) Purification, characterization, and cDNA cloning of an AU-rich element RNA-binding protein, AUF1. *Molecular and Cellular Biology*, **13**, 7652–7665.
35. Kajita Y, Nakayama J, Aizawa M and Ishikawa F. (1995) The UUAG-specific RNA-binding protein, heterogeneous nuclear ribonucleoprotein D0. *Journal of Biological Chemistry*, **270**, 22167–22175.
36. Ehrenman K, Long L, Wagner BJ and Brewer G. (1994) Characterization of cDNAs encoding the murine A+U-rich RNA-binding protein AUF1. *Gene*, **149**, 315–319.
37. Wagner BJ, DeMaria CT, Sun Y, Wilson GM and Brewer G. (1998) Structure and genomic organization of the human AUF1 gene: Alternative pre-RNA splicing generates four protein isoforms. *Genomics*, **48**, 195–202.
38. Wagner BJ, Long L, Rao PN, Pettenati MJ and Brewer G. (1996) Localization and physical mapping of genes encoding the A+U-rich element RNA-binding protein AUF1 to human chromosomes 4 and X. *Genomics*, **34**, 219–222.
39. Dempsey LA, Li M, DePace A, Bray-Ward P and Maizels N. (1998) The human *HNRPD* locus maps to 4q21 and encodes a highly conserved protein. *Genomics*, **49**, 378–384.
40. Maris C, Dominguez C and Allain FHT. (2005) The RNA recognition motif, a plastic RNA-binding platform to regulate post-transcriptional gene expression. *FEBS Journal*, **272**, 2118–2131.
41. DeMaria CT, Sun Y, Long L, Wagner BJ and Brewer G. (1997) Structural determinants in AUF1 required for high affinity binding to A+U-rich elements. *Journal of Biological Chemistry*, **272**, 27635–27643.
42. Wilson GM, Sun Y, Lu H and Brewer G. (1999) Assembly of AUF1 oligomers on U-rich RNA targets by sequential dimer association. *Journal of Biological Chemistry*, **274**, 33374–33381.
43. Zucconi BE, Ballin JD, Brewer BY, Ross CR, Huang J, Toth EA and Wilson GM. (2010) Alternatively expressed domains of AU-rich element RNA-binding protein 1 (AUF1) regulate RNA-binding affinity, RNA-induced protein oligomerization, and the local conformation of bound RNA ligands. *Journal of Biological Chemistry*, **285**, 39127–39139.
44. Sänger L, Bender J, Rostowski K, Golbik R, Lilie H, Schmidt C, Behrens S and Friedrich S. (2021) Alternatively spliced isoforms of AUF1 regulate a miRNA-mRNA interaction differentially through their YGG motif. *RNA Biology*, **18**, 843–853.
45. White EJF, Matsangos AE and Wilson GM. (2017) AUF1 regulation of coding and noncoding RNA. *Wiley Interdisciplinary Reviews: RNA*, **8**, e1393.
46. Moore AE, Chenette DM, Larkin LC and Schneider RJ. (2014) Physiological networks and disease functions of RNA-binding protein AUF1. *Wiley Interdisciplinary Reviews: RNA,* **5**, 549–564.

47. Wilson GM, Lu J, Sutphen K, Sun Y, Huynh Y and Brewer G. (2003) Regulation of A+U-rich element-directed mRNA turnover involving reversible phosphorylation of AUF1. *Journal of Biological Chemistry*, **278**, 33029–33038.
48. Arao Y, Kuriyama R, Kayama F and Kato S. (2000) A nuclear matrix-associated factor, SAF-B, interacts with specific isoforms of AUF1/hnRNP D. *Archives of Biochemistry and Biophysics*, **380**, 228–236.
49. Inoue A, Arao Y, Omori A, Ichinose S, Nishio K, YamamotoN, Kinoshita Y and Mita S. (2003) Identification of S1 proteins B2, C1 and D1 as AUF1 isoforms and their major role as heterogeneous nuclear ribonucleoprotein proteins. *Biochemical Journal*, **372**, 775–785.
50. Wilson GM, Sutphen K, Moutafis M, Sinha S and Brewer G. (2001) Structural remodeling of an A+U-rich RNA element by cation or AUF1 binding. *Journal of Biological Chemistry*, **276**, 38400–38409.
51. Yoon JH, De S, Srikantan S, Abdelmohsen K, Grammatikakis I, Kim J, Kim KM, Noh JH, White EJF, Martindale JL, *et al.* (2014) PAR-CLIP analysis uncovers AUF1 impact on target RNA fate and genome integrity. *Nature Communications*, **5**, 5248.
52. Zucconi BE and Wilson GM. (2013) Assembly of functional ribonucleoprotein complexes by AU-rich element RNA-binding protein 1 (AUF1) requires base-dependent and -independent RNA contacts. *Journal of Biological Chemistry*, **288**, 28034–28048.
53. Yoon JH, Jo MH, White EJF, De S, Hafner M, Zucconi BE, Abdelmohsen K, Martindale JL, Yang X, Wood WH, *et al.* (2015) AUF1 promotes let-7b loading on Argonaute 2. *Genes & Development*, **29**, 1599–1604.
54. von Roretz C, Marco SD, Mazroui R and Gallouzi IE. (2011) Turnover of AU-rich-containing mRNAs during stress: A matter of survival. *Wiley Interdisciplinary Reviews: RNA,* **2**, 336–347.
55. DeMaria CT and Brewer G. (1996) AUF1 binding affinity to A+U-rich elements correlates with rapid mRNA degradation. *Journal of Biological Chemistry*, **271**, 12179–12184.
56. Sarkar B, Xi Q, He C and Schneider RJ. (2003) Selective degradation of AU-rich mRNAs promoted by the p37 AUF1 protein isoform. *Molecular and Cellular Biology*, **23**, 6685–6693.
57. Chen T-M, Hsu CH, Tsai SJ and Sun HS. (2010) AUF1 p42 isoform selectively controls both steady-state and PGE2-induced FGF9 mRNA decay. *Nucleic Acids Research*, **38**, 8061–8071.
58. Loflin P, Chen C-YA and Shyu A-B. (1999) Unraveling a cytoplasmic role for hnRNP D in the *in vivo* mRNA destablization directed by the AU-rich element. *Genes & Development*, **13**, 1884–1897.
59. Paschoud S, Dogar AM, Kuntz C, Grisoni-Neupert B, Richman L and Kuhn LC. (2006) Destabilization of interleukin-6 mRNA requires a putative RNA stem-loop structure, an AU-rich element, and the RNA-binding protein AUF1. *Molecular and Cellular Biology*, **26**, 8228–8241.

60. AlAhmari MM, Al-Khalaf HH, Al-Mohanna FH, Ghebeh H and Aboussekhra A. (2020) AUF1 promotes stemness in human mammary epithelial cells through stabilization of the EMT transcription factors TWIST1 and SNAIL1. *Oncogenesis*, **9**, 70.
61. Sarkar S, Han J, Sinsimer KS, Liao B, Foster RL, Brewer G and Pestka S. (2011) RNA-binding protein AUF1 regulates lipopolysaccharide-induced IL10 expression by activating IkB kinase complex in monocytes. *Molecular and Cellular Biology*, **31**, 602–615.
62. Laroia G, Cuesta R, Brewer G and Schneider RJ. (1999) Control of mRNA decay by heat shock-ubiquitin-proteosome pathway. *Science*, **284**, 499–502.
63. Lu JY, Bergman N, Sadri N and Schneider RJ. (2006) Assembly of AUF1 with eIF4G-poly(A) binding protein complex suggests a translation function in AU-rich mRNA decay. *RNA*, **12**, 883–893.
64. Sinsimer KS, Gratacos FM, Knapinska AM, Lu J, Krause CD, Wierzbowski AV, Maher LR, Scrudato S, Rivera YM, Gupta S, *et al.* (2008) Chaperone Hsp27, a novel subunit of AUF1 protein complexes, functions in AU-rich element-mediated mRNA decay. *Molecular and Cellular Biology*, **28**, 5223–5237.
65. Schoenberg DR and Maquat LE. (2012) Regulation of cytoplasmic mRNA decay. *Nature Reviews Genetics*, **13**, 246–259.
66. White EJF, Brewer G and Wilson GM. (2013) Post-transcriptional control of gene expression by AUF1: Mechanisms, physiological targets, and regulation. *Biochimica et Biophysica Acta*, **1829**, 680–688.
67. Liao B, Hu Y and Brewer G. (2007) Competitive binding of AUF1 and TIAR to *MYC* mRNA controls its translation. *Nature Structural & Molecular Biology*, **14**, 511–518.
68. Wu X, Chesoni S, Rondeau G, Tempesta C, Patel R, Charles S, Daginawala N, Zucconi BE, Kishor A, Xu G, *et al.* (2013) Combinatorial mRNA binding by AUF1 and Argonaute 2 controls decay of selected target mRNAs. *Nucleic Acids Research*, **41**, 2644–2658.
69. Kedar VP, Zucconi BE, Wilson GM and Blackshear PJ. (2012) Direct binding of specific AUF1 isoforms to the tandem zinc finger domains of tristetraprolin (TTP) family proteins. *Journal of Biological Chemistry*, **287**, 5459–5471.
70. Friedrich S, Engelmann S, Schmidt T, Szczepankiewicz G, Bergs S, Liebert UG, Kümmerer BM, Golbik RP, Behrens S and Diamond MS. (2018) The host factor AUF1 p45 supports flavivirus propagation by triggering the RNA switch required for viral genome cyclization. *Journal of Virology*, **92**, e01647-17.
71. Friedrich S, Schmidt T, Geissler R, Lilie H, Chabierski S, Ulbert S, Liebert UG, Golbik RP and Behrens S. (2014) AUF1 p45 promotes West Nile Virus replication by an RNA chaperone activity that supports cyclization of the viral genome. *Journal of Virology*, **88**, 11586–11599.
72. Meyer A, Golbik RP, Sänger L, Schmidt T, Behrens S and Friedrich S. (2019) The RGG/RG motif of AUF1 isoform p45 is a key modulator of the protein's RNA chaperone and RNA annealing activities. *RNA Biology*, **16**, 960–971.

73. Cathcart AL, Rozovics JM and Semler BL. (2013) Cellular mRNA decay protein AUF1 negatively regulates enterovirus and human rhinovirus infections. *Journal of Virology*, **87**, 10423–10434.
74. Lin JY, Li ML and Brewer G. (2014) mRNA decay factor AUF1 binds the internal ribosomal entry site of enterovirus 71 and inhibits virus replication. *PLoS One*, **9**, e103827.
75. Alfano L, Caporaso A, Altieri A, Dell'Aquila M, Landi C, Bini L, Pentimalli F and Giordano A. (2019) Depletion of the RNA binding protein HNRNPD impairs homologous recombination by inhibiting DNA-end resection and inducing R-loop accumulation. *Nucleic Acids Research*, **47**, 4068–4085.
76. Kumar M, Matta A, Masui O, Srivastava G, Kaur J, Thakar A, Shukla NK, RoyChoudhury A, Sharma M, Walfish PG, *et al.* (2015) Nuclear heterogeneous nuclear ribonucleoprotein D is associated with poor prognosis and interactome analysis reveals its novel binding partners in oral cancer. *Journal of Translational Medicine*, **13**, 285.
77. Tolnay M, Lambris JD and Tsokos GC. (1997) Transcriptional regulation of the complement receptor 2 gene: Role of a heterogeneous nuclear ribonucleoprotein. *Journal of Immunology*, **159**, 5492–5501.
78. Dempsey LA, Hanakahi LA and Maizels N. (1998) A specific isoform of hnRNP D interacts with DNA in the LR1 heterodimer: Canonical RNA binding motifs in a sequence-specific duplex DNA binding protein. *Journal of Biological Chemistry*, **273**, 29224–29229.
79. Dobi A, Szemes M, Lee C, Palkovits M, Lim F, Gyorgy A, Mahan MA and Agoston DV. (2006) AUF1 Is expressed in the developing brain, binds to AT-rich double-stranded DNA, and regulates enkephalin gene expression. *Journal of Biological Chemistry*, **281**, 28889–28900.
80. Jiao R, He QY, Chen H, Hua Z, Jiao Q and Chiu J. (2006) AUF1-like protein binds specifically to DAS *cis*-acting element that regulates mouse a-fetoprotein gene expression. *Journal of Cellular Biochemistry*, **98**, 1257–1270.
81. Eversole A and Maizels N. (2000) In vitro properties of the conserved mammalian protein hnRNP D suggest a role in telomere maintenance. *Molecular and Cellular Biology*, **20**, 5425–5432.
82. Enokizono Y, Konishi Y, Nagata K, Ouhashi K, Uesugi S, Ishikawa F and Katahira M. (2005) Structure of hnRNP D complexed with single-stranded telomere DNA and unfolding of the quadruplex by heterogenous nuclear ribonucleoprotein D. *Journal of Biological Chemistry*, **280**, 18862–18870.
83. Pont AR, Sadri N, Hsiao SJ, Smith S and Schneider RJ. (2012) mRNA decay factor AUF1 maintains normal aging, telomere maintenance, and suppression of senescence by activation of telomerase transcription. *Molecular Cell*, **47**, 5–15.
84. Kang X, Chen W, Kim RH, Kang MK and Park NH. (2008) Regulation of the hTERT promoter activity by MSH2, the hnRNPs K and D, and GRHL2 in human oral squamous cell carcinoma cells. *Oncogene*, **28**, 565–574.

85. Kumar V, Kumar A, Kumar M, Lone MR, Mishra D and Chauhan SS. (2022) NFkB (RelA) mediates transactivation of hnRNPD in oral cancer cells. *Scientific Reports*, **12**, 5944.
86. Zhang T, Guan G, Zhang J, Zheng H, Li D, Wang W, Lu F and Chen X. (2022) E2F1-mediated AUF1 upregulation promotes HCC development and enhances drug resistance via stabilization of AKR1B10. *Cancer Science*, **113**, 1154–1167.
87. Hendrayani S, Al-Khalaf HH and Aboussekhra A. (2014) The cytokine IL-6 reactivates breast stromal fibroblasts through transcription factor STAT3-dependent up-regulation of the RNA-binding protein AUF1. *Journal of Biological Chemistry*, **289**, 30962–30976.
88. Ing NH. (2010) Estradiol up-regulates expression of the A+U-rich binding factor 1 (AUF1) gene in the sheep uterus. *Journal of Steroid Biochemistry and Molecular Biology*, **122**, 172–179.
89. Al-Khalaf HH and Aboussekhra A. (2014) MicroRNA-141 and microRNA-146b-5p inhibit the prometastatic mesenchymal characteristics through the RNA-binding protein AUF1 targeting the transcription factor ZEB1 and the protein kinase AKT. *Journal of Biological Chemistry*, **289**, 31433–31447.
90. Banihashemi L, Wilson GM, Das N and Brewer G. (2006) Upf1/Upf2 regulation of 3′ untranslated region splice variants of AUF1 links nonsense-mediated and A+U-rich element-mediated mRNA decay. *Molecular and Cellular Biology*, **26**, 8743–8754.
91. Wilson GM, Sun Y, Sellers J, Lu H, Penkar N, Dillard G and Brewer G. (1999) Regulation of AUF1 expression via conserved alternatively spliced elements in the 3′ untranslated region. *Molecular and Cellular Biology*, **19**, 4056–4064.
92. Sheflin LG and Spaulding SW. (2000) Testosterone and dihydrotestosterone regulate AUF1 isoforms in a tissue-specific fashion in the mouse. *American Journal of Physiology-Endocrinology and Metabolism*, **278**, E50–E57.
93. Lu J-Y and Schneider RJ. (2004) Tissue distribution of AU-rich mRNA-binding proteins involved in regulation of mRNA decay. *Journal of Biological Chemistry*, **279**, 12974–12979.
94. Laroia G and Schneider RJ. (2002) Alternate exon insertion controls selective ubiquitination and degradation of different AUF1 protein isoforms. *Nucleic Acids Research*, **30**, 3052–3058.
95. Knapinska AM, Gratacos FM, Krause CD, Hernandez K, Jensen AG, Bradley JJ, Wu X, Pestka S and Brewer G. (2011) Chaperone Hsp27 modulates AUF1 proteolysis and AU-rich element-mediated mRNA degradation. *Molecular and Cellular Biology*, **31**, 1419–1431.
96. Vahid S, Thaper D, Gibson KF, Bishop JL and Zoubeidi A. (2016) Molecular chaperone Hsp27 regulates the Hippo tumor suppressor pathway in cancer. *Scientific Reports*, **6**, 31842.
97. Qi Z, Shen L, Zhou H, Jiang Y, Lan L, Luo L and Yin Z. (2014) Phosphorylation of heat shock protein 27 antagonizes TNF-a induced HeLa cell apoptosis via regulating

TAK1 ubiquitination and activation of p38 and ERK signaling. *Cell Signaling*, **26**, 1616–1625.

98. Wilson GM, Sutphen K, Chuang K and Brewer G. (2001) Folding of A+U-rich RNA elements modulates AUF1 binding: Potential roles in regulation of mRNA turnover. *Journal of Biological Chemistry*, **276**, 8695–8704.
99. Fialcowitz EJ, Brewer BY, Keenan BP and Wilson GM. (2005) A hairpin-like structure within an AU-rich mRNA-destabilizing element regulates *trans*-factor binding selectivity and mRNA decay kinetics. *Journal of Biological Chemistry*, **280**, 22406–22417.
100. He C and Schneider RJ. (2006) 14-3-3s is a p37 AUF1-binding protein that facilitates AUF1 transport and AU-rich mRNA decay. *EMBO Journal*, **25**, 3823–3831.
101. Tsitsipatis D, Grammatikakis I, Driscoll RK, Yang X, Abdelmohsen K, Harris SC, Yang JH, Herman AB, Chang MW, Munk R, *et al.* (2021) AUF1 ligand circPCNX reduces cell proliferation by competing with p21 mRNA to increase p21 production. *Nucleic Acids Research*, **49**, 1631–1646.
102. Bell O, Gaberman E, Kilav R, Levi R, Cox KB, Molkentin JD, Silver J and Naveh-Many T. (2005) The protein phosphatase calcineurin determines basal parathyroid hormone gene expression. *Molecular Endocrinology*, **19**, 516–526.
103. Wilson GM, Lu J, Sutphen K, Suarez Y, Sinha S, Brewer B, Villanueva-Feliciano EC, Ylsa RM, Charles S and Brewer G. (2003) Phosphorylation of $p40^{AUF1}$ regulates binding to A+U-rich mRNA-destabilizing elements and protein-induced changes in ribonucleoprotein structure. *Journal of Biological Chemistry*, **278**, 33039–33048.
104. Fawal M, Armstrong F, Ollier S, Dupont H, Touriol C, Monsarrat B, Delsol G, Payrastre B and Morello D. (2006) A "liaison dangereuse" between AUF1/hnRNPD and the oncogenic tyrosine kinase NPM-ALK. *Blood*, **108**, 2780–2788.
105. Blum JL, Samarel AM and Mestril R. (2005) Phosphorylation and binding of AUF1 to the 3′-untranslated region of cardiomyocyte SERCA2a mRNA. *American Journal of Physiology-Heart and Circulatory Physiology*, **289**, H2543–H2550.
106. Fellows A, Deng B, Mierke DF, Robey RB and Nichols RC. (2013) Peptides modeled on the RGG domain of AUF1/hnRNP-D regulate 3′UTR-dependent gene expression. *International Immunopharmacology*, **17**, 132–141.
107. Friedrich S, Schmidt T, Schierhorn A, Lilie H, Szczepankiewicz G, Bergs S, Liebert UG, Golbik RP and Behrens S. (2016) Arginine methylation enhances the RNA chaperone activity of the West Nile virus host factor AUF1 p45. *RNA*, **22**, 1574–1591.
108. Esnault S, Shen ZJ, Whitesel E and Malter JS. (2006) The peptidyl-prolyl isomerase Pin1 regulates granulocyte-macrophage colony-stimulating factor mRNA stability in T lymphocytes. *Journal of Immunology*, **177**, 6999–7006.
109. Bhattacharya S, Giordano T, Brewer G and Malter JS. (1999) Identification of AUF-1 ligands reveals vast diversity of early response gene mRNAs. *Nucleic Acids Research*, **27**, 1464–1472.
110. Mazan-Mamczarz K, Hagner PR, Dai B, Wood WH, Zhang Y, Becker KG, Liu Z and Gartenhaus RB. (2008) Identification of transformation-related pathways in a

breast epithelial cell model using a ribonomics approach. *Cancer Research*, **68**, 7730–7735.

111. Lal A, Mazan-Mamczarz K, Kawai T, Yang X, Martindale JL and Gorospe M. (2004) Concurrent versus individual binding of HuR and AUF1 to common labile target mRNAs. *EMBO Journal*, **23**, 3092–3102.
112. Ishimaru D, Zuraw L, Ramalingam S, Sengupta TK, Bandyopadhyay S, Reuben A, Fernandes DJ and Spicer EK. (2010) Mechanism of regulation of bcl-2 mRNA by nucleolin and A+U-rich element-binding factor 1 (AUF1). *Journal of Biological Chemistry*, **285**, 27182–27191.
113. Radha G and Raghavan SC. (2017) BCL2: A promising cancer therapeutic target. *Biochimica et Biophysica Acta — Reviews on Cancer*, **1868**, 309–314.
114. Marx J. (2004) Inflammation and cancer: The link grows stronger. *Science*, **306**, 966–968.
115. Aggarwal BB, Shishodia S, Sandur SK, Pandey MK and Sethi G. (2006) Inflammation and cancer: How hot is the link? *Biochemical Pharmacology*, **72**, 1605–1621.
116. Lu J-Y, Sadri N and Schneider RJ. (2006) Endotoxic shock in AUF1 knockout mice mediated by failure to degrade proinflammatory cytokine mRNAs. *Genes & Development*, **20**, 3174–3184.
117. Sadri N and Schneider RJ. (2009) Auf1/*Hnrnpd*-deficient mice develop pruritic inflammatory skin disease. *Journal of Investigative Dermatology*, **129**, 657–670.
118. Kalluri R and Weinberg RA. (2009) The basics of epithelial-mesenchymal transition. *Journal of Clinical Investigation*, **119**, 1420–1428.
119. Li X, Tian Z, Jin H, Xu J, Hua X, Yan H, Liufu H, Wang J, Li J, Zhu J, *et al.* (2018) Decreased c-Myc mRNA stability via the microRNA 141-3p/AUF1 axis is crucial for p63a inhibition of cyclin D1 gene transcription and bladder cancer cell tumorigenicity. *Molecular and Cellular Biology*, **38**, e00273-18.
120. Trojanowicz B, Sekulla C, Dralle H and Hoang-Vu C. (2016) Expression of ARE-binding proteins AUF1 and HuR in follicular adenoma and carcinoma of thyroid gland. *Neoplasma*, **63**, 371–377.
121. Gouble A, Grazide S, Meggetto F, Mercier P, Delsol G and Morello D. (2002) A new player in oncogenesis: AUF1/hnRNPD overexpression leads to tumorigenesis in transgenic mice. *Cancer Research*, **62**, 1489–1495.
122. Bartha Á and Győrffy B. (2021) TNMplot.com: A web tool for the comparison of gene expression in normal, tumor and metastatic tissues. *International Journal of Molecular Sciences*, **22**, 2622.
123. Győrffy B. (2021) Survival analysis across the entire transcriptome identifies biomarkers with the highest prognostic power in breast cancer. *Computational and Structural Biotechnology Journal*, **19**, 4101–4109.
124. Trojanowicz B, Brodauf L, Sekulla C, Lorenz K, Finke R, Dralle H and Hoang-Vu C. (2009) The role of AUF1 in thyroid carcinoma progression. *Endocrine-Related Cancer*, **16**, 857–871.

125. Du X, Wang JM, Zhang DL, Wu T, Zeng XY, Jiang JY and Du ZX. (2021) AUF1 promotes proliferation and invasion of thyroid cancer via downregulation of ZBTB2 and subsequent TRIM58. *Frontiers in Oncology*, **11**, 681736.
126. Liu X, Long Z, Cai H, Yu S and Wu J. (2020) TRIM58 suppresses the tumor growth in gastric cancer by inactivation of b-catenin signaling via ubiquitination. *Cancer Biology & Therapy*, **21**, 203–212.
127. Yuan P, Zhou Y, Wang R, Chen S, Wang Q, Xu Z, Liu Y and Yang H. (2020) TRIM58 interacts with pyruvate kinase M2 to inhibit tumorigenicity in human osteosarcoma cells. *BioMed Research International*, **2020**, 8450606.
128. Bartel DP. (2004) MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell*, **116**, 281–297.
129. Abdelmohsen K, Tominaga-Yamanaka K, Srikantan S, Yoon JH, Kang MJ and Gorospe M. (2012) RNA-binding protein AUF1 represses Dicer expression. *Nucleic Acids Research*, **40**, 11531–11544.
130. Wu X, Yang Y, Huang Y, Chen Y, Wang T, Wu S, Tong L, Wang Y, Lin L, Hao M, *et al.* (2018) RNA-binding protein AUF1 suppresses miR-122 biogenesis by down-regulating Dicer1 in hepatocellular carcinoma. *Oncotarget*, **9**, 14815–14827.
131. Lin CJ, Gong HY, Tseng HC, Wang WL and Wu JL. (2008) miR-122 targets an anti-apoptotic gene, Bcl-w, in human hepatocellular carcinoma cell lines. *Biochemical and Biophysical Research Communications*, **375**, 315–320.
132. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL and Weinberg RA. (2005) Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell*, **121**, 335–348.
133. Tian XY, Li J, Liu TH, Li DN, Wang JJ, Zhang H, Deng ZL, Chen FJ and Cai JP. (2020) The overexpression of AUF1 in colorectal cancer predicts a poor prognosis and promotes cancer progression by activating ERK and AKT pathways. *Cancer Medicine*, **9**, 8612–8623.
134. Gao Y, Wang W, Cao J, Wang F, Geng Y, Cao J, Xu X, Zhou J, Liu P and Zhang S. (2017) Upregulation of AUF1 is involved in the proliferation of esophageal squamous cell carcinoma through GCH1. *International Journal of Oncology*, **49**, 2001–2010.
135. Werner ER, Blau N and Thöny B. (2011) Tetrahydrobiopterin: Bbiochemistry and pathophysiology. *Biochemical Journal*, **438**, 397–414.
136. Kim J, Lee J and Iyer VR. (2008) Global identification of Myc target genes reveals its direct role in mitochondrial biogenesis and its E-box usage in vivo. *PLoS One*, **3**, e1798.
137. Bretones G, Delgado MD and León J. (2015) Myc and cell cycle control. *Biochimica et Biophysica Acta*, **1849**, 506–516.
138. Gabay M, Li Y and Felsher DW. (2014) MYC activation is a hallmark of cancer initiation and maintenance. *Cold Spring Harbor Perspectives in Medicine*, **4**, a014241.

139. Hendrayani S, Al-Harbi B, Al-Ansari MM, Silva G and Aboussekhra A. (2016) The inflammatory/cancer-related IL-6/STAT3/NF-kB positive feedback loop includes AUF1 and maintains the active state of breast myofibroblasts. *Oncotarget*, **7**, 41974–41985.
140. Lapucci A, Donnini M, Papucci L, Witort E, Tempestini A, Bevilacqua A, Niolin A, Brewer G, Schiavone N and Capaccioli S. (2002) AUF1 is a *bcl-2* A+U-rich element-binding protein involved in *bcl-2* mRNA destabilization during apoptosis. *Journal of Biological Chemistry*, **277**, 16139–16146.
141. Bodur C and Basaga H. (2012) Bcl-2 inhibitors: Emerging drugs in cancer therapy. *Current Medicinal Chemistry*, **19**, 1804–1820.
142. Gao X, Dong H, Lin C, Sheng J, Zhang F, Su J and Xu Z. (2014) Reduction of AUF1-mediated follistatin mRNA decay during glucose starvation protects cells from apoptosis. *Nucleic Acids Research*, **42**, 10720–10730.
143. Gao X, Wei S, Lai K, Sheng J, Su J, Zhu J, Dong H, Hu H and Xu Z. (2010) Nucleolar follistatin promotes cancer cell survival under glucose-deprived conditions through inhibiting cellular rRNA synthesis. *Journal of Biological Chemistry*, **285**, 36857–36864.
144. Sun S, Zhang X, Lyu L, Li X, Yao S and Zhang J. (2016) Autotaxin expression is regulated at the post-transcriptional level by the RNA-binding proteins HuR and AUF1. *Journal of Biological Chemistry*, **291**, 25823–25836.
145. Boutin JA and Ferry G. (2009) Autotaxin. *Cellular and Molecular Life Sciences*, **66**, 3009.
146. Du Z, Niu S, Wang J, Wu J, Li S and Yi X. (2021) SChLAP1 contributes to non-small cell lung cancer cell progression and immune evasion through regulating the AUF1/PD-L1 axis. *Autoimmunity*, **54**, 225–233.
147. Ostrand-Rosenberg S, Horn LA and Haile ST. (2014) The programmed death-1 immune-suppressive pathway: Barrier to antitumor immunity. *Journal of Immunology*, **193**, 3835–3841.
148. Prensner JR, Zhao S, Erho N, Schipper M, Iyer MK, Dhanasekaran SM, Magi-Galluzzi C, Mehra R, Sahu A, Siddiqui J, *et al.* (2014) RNA biomarkers associated with metastatic progression in prostate cancer: A multi-institutional high-throughput analysis of *SChLAP1*. *Lancet Oncology*, **15**, 1469–1480.
149. Ji J, Xu R, Ding K, Bao G, Zhang X, Huang B, Wang X, Martinez A, Wang X, Li G, *et al.* (2019) Long noncoding RNA SChLAP1 forms a growth-promoting complex with HNRNPL in human glioblastoma through stabilization of ACTN4 and activation of NF-kB Signaling. *Clinical Cancer Research*, **25**, 6868–6881.
150. Bai X, Zhang S, Qiao J, Xing X, Li W, Zhang H and Xie J. (2021) Long non-coding RNA SChLAP1 regulates the proliferation of triple negative breast cancer cells via the miR-524-5p/HMGA2 axis. *Molecular Medicine Reports*, **23**, 446.
151. Sarkar S, Sinsimer KS, Foster RL, Brewer G and Pestka S. (2008) AUF1 isoform-specific regulation of anti-inflammatory IL10 expression in monocytes. *Journal of Interferon & Cytokine Research*, **28**, 679–691.

152. Lin S, Wang W, Wilson GM, Yang X, Brewer G, Holbrook NJ and Gorospe M. (2000) Down-regulation of cyclin D1 expression by prostaglandin A_2 is mediated by enhanced cyclin D1 mRNA turnover. *Molecular and Cellular Biology*, **20**, 7903–7913.
153. Attia MF, Anton N, Wallyn J, Omran Z and Vandamme TF. (2019) An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. *Journal of Pharmacy and Pharmacology*, **71**, 1185–1198.
154. Zhao Z, Ukidve A, Kim J and Mitragotri S. (2020) Targeting strategies for tissue-specific drug delivery. *Cell*, **181**, 151–167.
155. Rautio J, Meanwell NA, Di L and Hageman MJ. (2018) The expanding role of prodrugs in contemporary drug design and development. *Nature Reviews Drug Discovery*, **17**, 559–587.
156. Katahira M, Miyanoiri Y, Enokizono Y, Matsuda G, Nagata T, Ishikawa F and Uesugi S (2001) Structure of the C-terminal RNA-binding domain of hnRNP D0 (AUF1), its interactions with RNA and DNA, and change in backbone dynamics upon complex formation with DNA. *Journal of Molecular Biology*, **311**, 973–988.
157. Choi YJ, Yoon JH and Chang JH. (2016) Crystal structure of the N-terminal RNA recognition motif of mRNA decay regulator AUF1. *BioMed Research International*, **2016**, 3286191.

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Chapter 7

Oncogenic Consequences of Dysregulated Translation Initiation

Forum Kayastha[1,2,*], Bandish Kapadia[1,2,**], and Ronald Gartenhaus[1,2]

[1]*Division of Hematology and Oncology, Department of Internal Medicine, Virginia Commonwealth University School of Medicine, and Massey Cancer Center, Richmond, VA 23298, USA*
[2]*Richmond VA Medical Center, Richmond, VA 23249, USA*

Abstract

As a convergent mechanism downstream of many oncogenic signals, rewired translational output is rapidly emerging as a primary focus in cancer research. The perturbed regulation of the translational machinery in cancer has protean effects, including dysregulation of both translation factor(s) expression and activity, which are distinctive to types of different stages of a given cancer. This temporospatial fluctuation in tumor cell gene expression confers advantages to the malignant cell,

all authors contributed equally
* Corresponding author's email: forum.kayastha@vcuhealth.org
** Corresponding author's email: bandish.kapadia@vcuhealth.org

as it responds to local signaling and chemotactic stimuli in its microenvironment, enabling its progression from localized to metastatic tumor growth. The impact of altered translational output is manifest multifold including, enhanced ribosome synthesis, augmented translation of select mRNAs such as oncogenic transcription factors, and silencing/inhibition of translation regulatory components, resulting in more robust *de novo* protein biosynthesis. Eukaryotic translational regulation is principally achieved at the initiation stage for rapid, reversible, and spatial control of gene expression. This chapter will highlight the functional relevance of key translation initiation factors in cellular homeostasis and their dependency on tumor development and progression. This emerging paradigm broadens the spectrum of potential targets and offers significant opportunities for innovative cancer therapies. Indeed, as discussed below, there is expanding scientific interest in exploiting these new insights in protein synthesis by targeting specific cellular translational apparatus components for cancer therapeutics. Although clinical trial data are scant to date, promising early results in several tumor types strongly support pursuing translational control as an exciting new area for therapeutic development in human cancer.

1. Introduction

Protein biosynthesis is a key modulator in defining cellular phenotype, and its output is efficiently controlled during growth and development. Global analysis of mammalian gene expression demonstrated that mRNA levels could explain ~40% of the variability in protein content and further revealed that the translation rate plays a pivotal role in synchronizing cellular protein levels [1]. Although overall protein biosynthesis rates are generally higher in cancer cells, it is now recognized that subsets of oncogenic mRNAs are specifically regulated at the translation level [2].

Over the past two decades, molecular and biochemical studies have provided remarkable insights into the process of translation and the factors that assist the ribosome in producing proteins (Table 1). Elevated protein synthesis is an important feature of many tumors which often arises due to enhanced upstream signaling events converging on eukaryotic initiation factor (eIF) 4F, the key regulator of the mRNA-ribosome recruitment phase of translation initiation [3]. eIF4F is a heteromeric complex that is comprised of the cap-binding protein, eIF4E (Eukaryotic

Table 1. Functions of eIFs and subunits in translation initiation.

Initiation factor	Sub-units	Interacting eIFs/ subunits	Functions	References
eIF1	eIF1	eIF2, eIF3, eIF5, 40S ribosome	Necessary for scanning and involved in initiation site selection. Prepares 40S ribosome for mRNA loading and promotes scanning with eIF1A, promotes binding between eIF2 ternary complex and 40S ribosomal subunit, and prevents untimely hydrolysis of eIF2.GTP to eIF2.GDP by eIF5 GAP to stop downstream events.	[138]
	eIF1A	eIF5B, 40S ribosome		[139, 140]
eIF2	eIF2	eIF1, eIF2B, eIF3, eIF5, initiator tRNA, 40S ribosome	Recruits initiator tRNA to 40S ribosome, constructs the eIF2.TC complex and delivers into 40S ribosomal subunit. Promotes binding of the initiator tRNA to 40S ribosomal subunits and phosphorylation state of eIF2α modulates the rate of formation of the ternary complex.	[141, 142]
	eIF2B	eIF2	Activates eIF2 factor by stimulating the release of GDP, helps in regeneration of eIF2.	[143]
eIF3	eIF3a-eIF3m	eIF1, eIF1A, eIF4G, eIF5, 40S ribosome	The eIF-3 complex stimulates mRNA recruitment to the 43S PIC and scanning of the mRNA for AUG recognition. Play a role in disassembly and recycling of post-termination ribosomal complexes and subsequently prevents premature joining of the 40S and 60S ribosomal subunits prior to initiation.	[15, 144, 145]
eIF4	eIF4G	eIF4E, eIF4A, eIF3, RNA, PABP, MNK1	Member of eIF4F cap-binding complex, supports binding of eIF3, eIF4a, eIF4e, PABP, and mRNA, and stimulates helicase and ATPase activity of eIF4A.	[146, 147]

(*Continued*)

Table 1. (*Continued*)

Initiation factor	Sub-units	Interacting eIFs/ subunits	Functions	References
	eIF4A	eIF4G, eIF4B, RNA	ATP-dependent RNA helicase, member of eIF4F cap-binding complex, and unwinds secondary structure in 5′ UTR of the mRNA.	[146]
	eIF4E	eIF4G, 5′m7G cap	Binds to mRNA at 5′ m7G cap, member of eIF4F cap-binding complex, and stimulates eIF4A helicase activity with eIF4G.	[131, 146, 148]
	eIF4B	eIF4A, eIF3, RNA	Required for the binding of mRNA to ribosomes. Binds near the 5′-terminal cap of mRNA in the presence of eIF4F and ATP. Promotes the ATPase activity and the ATP-dependent RNA unwinding activity of eIF4A.	[146, 149]
	eIF4H	eIF4A, RNA	Enhances RNA helicase activity of eIF4a (eIF4F), RNA-binding protein, and homologous to N-terminus activity of eIF4A.	[130]
eIF5	eIF5	eIF1, eIFA1, eIF2, eIF3, 40S ribosome	Catalyzes the hydrolysis of GTP bound to the 40S ribosomal initiation complex with the subsequent joining of a 60S ribosomal subunit, resulting in the release of eIF-2 and the guanine nucleotide.	[150, 151]
	eIF5B	eIF1A, 80S ribosome	Ribosome-dependent GTPase, responsible for connection between ribosomal subunits.	[139]
eIF6	eIF6	60S ribosome	Prevents premature binding between 40S and 60S subunits, and regulates ribosome biogenesis.	[152]

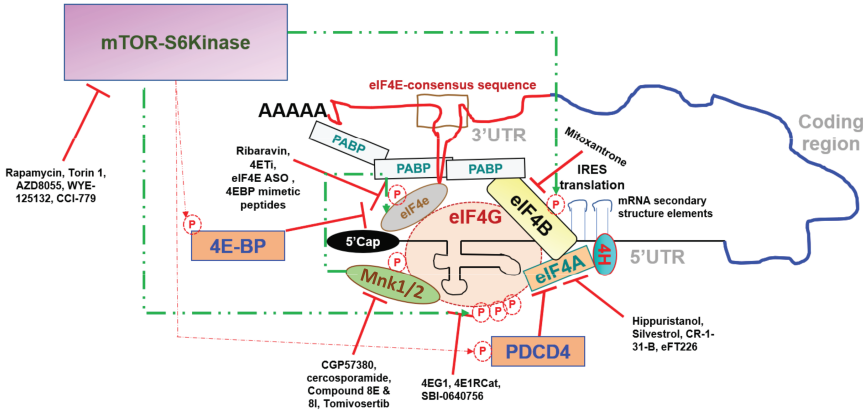

Figure 1. Schematic illustration of the major protein involved in the regulation of translation initiation along with their potent inhibitors.

Since eIF4F unperturbed activity is central to the sustained output of key oncogenes essential for tumor cell development, progression, and metastasis, altering this activity can dramatically impact cell fitness. Successful formation of eIF4F complex on the mRNA cap further promotes the pre-initiation complex (PIC) recruitment, followed by 5′ UTR scanning to reach the start codon AUG and joining of 60S ribosomal subunit. This event marks the completion of translation initiation. The activity of the complex is regulated by mTORC1 (via eIF4B and eIF4G mediated phospho-modifications (green arrow) along with 4E-BP1 and PDCD4 dependent repression (red arrow)). Various inhibitors of translation initiation factors have been identified targeting eIF4E, eIF4G, eIF4A, and eIF4B either directly or indirectly, curtailing their activities.

Eukaryotic Initiation Factor 4E (eIF4E), the helicase Eukaryotic Initiation Factor 4A (eIF4A), and the large scaffolding protein Eukaryotic Initiation Factor 4G (eIF4G) (Fig. 1). This complex promotes many of the malignant cell characteristics, such as sustained oncogenic signaling, proliferation, evading apoptosis, replicative immortality, angiogenesis, invasion, and metastasis. Expression levels of the eIF4F complex proteins, their intracellular repressor(s), and their phospho-modification(s), are associated with the aggressiveness of histological subtypes of cancers, response to treatment, as well as poor disease outcome and survival. Below, we will focus on the eIF4F complex, how it contributes to chemoresistance, as well as its potential therapeutic targeting employing small-molecule inhibitors.

2. eIF4G (Eukaryotic Initiation Factor 4G)

eIF4G was discovered, purified, and characterized by Browning and colleagues from wheat germ [4]. Functional evaluation of eIF4G demonstrated that it cross-links to the 5′ terminal cap of oxidized mRNA in the presence of eIF-4A, eIF-4E, and ATP, thus stimulating ATP-dependent RNA unwinding [5]. A decade later, Hentze proposed a model that positioned eIF4G as a multipurpose ribosome adapter [6]. The seminal importance of eIF4G in cap-dependent translation was underscored while studying proteases encoded by the picornaviruses, poliovirus, and foot-and-mouth-disease virus that specifically cleaved eIF4G on the N-terminal [7].

Mammals have three related eIF4G proteins: 1) eIF4G1, widely referred to in the literature as eIF4GI, 2) eIF4G3 referred to as eIF4GII, and 3) eIF4G2 referred to as death-associated protein (DAP5)/p97/novel APOBEC-1 target no. 1 (NAT1). eIF4GI and eIF4GII act as a scaffold for binding poly(A)-binding protein (PABP), eIF4E, eIF4A, eIF3, and MAP kinase-interacting kinase (MNK), although DAP5 encoded from 5′ distal initiation codon lacks the N-terminal PABP and eIF4E binding site. eIF4GI contains two RNA-binding domains (RBDs), one positioned amino-terminal to HEAT-1(Huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1), and the other overlapping HEAT-1. The cap-binding protein, eIF4E, can directly insert into the NH2-terminal domain of eIF4G, while the central region associates with eIF3, a multimeric complex that is bound to the 40S subunit [8]. The binding of eIF4A to HEAT-1 stimulates ATP hydrolysis and RNA binding [9]. These RBDs dramatically enhance the interaction between eIF4E and the cap. MNK1, which phosphorylates eIF4E, binds to the eIF4G at its C-terminal HEAT domain [10], and interaction is regulated by Protein Kinase Cα (PKCα). PKCα phosphorylates eIF4GI at Ser^{1186}, thereby eliciting tethering of MNK1 to eIF4G protein with subsequent enhancement of global translation [11].

The importance of eIF4G in translational regulation was further bolstered by *in vitro* biochemical examination of Borman and colleagues.

Using truncated proteins, they reported that the central domain (which interacts with eIF4A) was sufficient to stimulate cap-independent mRNA loading [12]. Further, the central domain of eIF4GI binds strongly and specifically to a structural element within the encephalomyocarditis virus IRES (internal ribosome entry site) upstream of the initiation codon [6], signifying that the protein is also relevant for cap-independent translation. Another important aspect of eIF4G-mediated translational regulation was reported by Yanagiya. The N-terminal region of yeast eIF4G promotes eIF4F–PABP association on mRNAs [13], thus circularizing the active translational complex for sustained multi-ribosomal units mediated translation as well as MCTS1-DENR-mediated re-initiation of translation [14]. The association between the central domain of eIF4G and eIF3 is mediated by the eIF3c, d, and e subunits, which are unaffected by eIF4A's association; an interaction proposed to be the underlying mechanism by which the 43S PIC is recruited mRNA [15]. Therefore, it is postulated that eIF4G acts as a channel between the active translational complexes and the 40S ribosomal subunit. Collectively, eIF4G plays a critical role in recruiting the translational machinery to mRNA.

Consistent with eIF4GI's central role in translational regulation, the protein undergoes several post-translational modifications from upstream kinases. Brian and colleagues identified serum-stimulated phosphorylation sites (Ser^{1108}, Ser^{1148}, and Ser^{1192}) within its C-terminal. More importantly, these phosphosites were sensitive to the oncogenic PI3K-mTOR (Phosphoinositide 3 kinase- mammalian target of Rapamycin) pathway [16]. Additionally, eIF4GI plays a crucial role downstream of mTOR signaling. mTOR-S6Kinase mediated phosphorylation of eIF4E-binding proteins (4E-BPs) diminishes its interaction with eIF4E. This released eIF4E tethers with eIF4G1 to drive cap-dependent translation [17]. Likewise, programmed cell death 4 (PDCD4), an S6Kinase sensitive gene, disrupts the interaction between eIF4A and eIF4G1, leading to translational inhibition [18]. However, the impact of mTOR-signaling driven phosphomodification of eIF4G on the above-mentioned interactions is still unknown. These observations outline the impact of any extracellular stimulus or cellular stress mediated modification of eIF4GI and provide

the basis for further studies to assess how phosphorylation may modulate eIF4GI activity/interaction with other proteins in regulating the energy-driven translational apparatus.

Given its central role in augmenting translational machinery, eIF4GI governs *de novo* protein biosynthesis and its quality, thereby impacting cell phenotype and function. Aberrant overexpression of eIF4GI is tightly linked with the prognosis of several cancers, such as lung squamous cell carcinoma, inflammatory breast cancer, cervical cancers, nasopharyngeal carcinoma, and others [19–22] (Table 2). A high variation frequency of eIF4GI levels has been observed in pancreatic cancer, ovarian cancer, and head and neck squamous cell carcinomas (HNSCC) [22, 23]. Mechanistic studies in breast and nasopharyngeal cancers indicate that the overexpression of eIF4G1 promotes malignant transformation and invasion, while the depletion of eIF4G1 leads to the significant inhibition of cell cycle progression, invasion, and colony formation *in vitro* and *in vivo* [20, 21, 24]. Polysomal evaluation of eIF4GI depleted breast cancer cells revealed

Table 2. Aberrant expression of eIF4F factors in human cancer.

Protein	Cancer types	Differential expression	Model	Reference
eIF4A	Hepatocellular carcinoma	Overexpression	Cell-derived xenograft (CDX) and patient-derived xenograft (PDX) models in colorectal cancer	[153, 154]
	Non-small cell lung carcinoma (NSCLC)			[155]
	Endometrial cancer			
	Cervical cancer			[156]
	Breast cancer			[157]
	Melanoma			[158]
eIF4B	Ovarian granulosa		Knockdown decreases translation and proliferation	[100, 159]
	B-cell lymphoma			[104]
	Leukemia			[160]
eIF4E	Breast cancer		Overexpression leads to variety of cancers in mouse model	[161–165]
	Bladder cancer			[166]
	Brain cancer			[167]

Table 2. (*Continued*)

Protein	Cancer types	Differential expression	Model	Reference
	Cervix cancer			[168]
	Colon cancer			[169]
	Liver cancer			[170]
	Lung cancer			[160, 171]
	Head and neck cancer			[172, 173]
	Lymphoma and ovary cancer			[160]
	Esophagus cancer			[174]
	Prostate cancer			[67, 160]
	Gastric cancer			[154]
	Skin cancer			[160, 175]
eIF4E	Breast cancer and colon cancer	Hyper phosphorylation	Knock-in mouse replacing Serine 209 with alanine	[176, 177]
	Lung cancer			[178]
	Lymphoma			[66]
	Ovary cancer			[179]
	Prostate cancer			[67, 177]
	Gastric cancer			[176]
eIF4G	Breast cancer	Overexpression	Knock-down Inhibited *in vivo* Xenograft Tumor Growth	[20]
	Cervix cancer			[180]
	Squamous cell lung carcinoma			[19, 181]
	Nasopharyngeal carcinoma			[182]
eIF4H	Colorectal and esophagus cancer	Overexpression	Overexpression: increased cell transformation Knockdown: inhibited tumor growth migration and increased sensitization to chemotherapy	[125, 132]

that it also regulates mRNA translation of key DNA damage repair pathway effectors including p53-binding protein 1 (53BP1), Growth Arrest And DNA Damage Inducible Alpha (GADD45a), surviving, and X-Linked Inhibitor Of Apoptosis (XIAP) [25]. Apart from transcriptional amplification and post-translational modification, the gene is already regulated by a post-transcriptional mechanism. Downregulation of eIF4GII by miR-520c-3p represses diffuse large B cell lymphoma development [26]. In contrast to eIF4GI and eIF4GII, the precise role of Dap5 in translational regulation is still emerging. Initial experiments by Imataka *et al*. [27], as well as Yamanaka *et al*. [28], revealed that Dap5 inhibits cap-dependent as well as IRES-dependent translation. Conversely, Henis-Korenbilt *et al*. noted that Dap5 amplified cap-dependent translation and could promote IRES-driven expression [29]. A recent report supports the observation that Dap5 promotes Eukaryotic Translation Initiation Factor 3 Subunit D (eIF3d)-driven, but not eIF4E-independent, cap-dependent mRNA translation [30]. Other labs have found that depletion of Dap5 impedes global translation and cell proliferative genes, including S-Phase Kinase Associated Protein 2 (Skp2), Cyclin-Dependent Kinase 2 (CDK2), and Human antigen R (HuR) in human glioblastoma cells [30–32].

In light of the fact that eIF4G is crucial for cell proliferation and noted to be overexpressed in both solid as well as hematological malignancies, several groups have tried to target eIF4G using small molecule inhibitors that would interfere with its interacting partners (Table 3). Three inhibitors that target eIF4E-eIF4G association, 4EGI-1, 4E1RCat, and 4E2RCat, were identified by high-throughput screening of chemical libraries.

Table 3. Agents targeting eIF4G at different levels.

Small molecules	**Mechanism**	***In vitro* IC_{50}**	***In vivo* models**	**Clinical development**	**References**
4EG1	Inhibits eIF4E-eIF4G association	6μM	U87 xenograft mouse model	Pre-Clinical	[33, 183]
4E1RCat		3.2μM	Eμ-Myc model	Pre-Clinical	[184]
SBI-0640756		1μM	Melanoma tumor mouse model	Pre-Clinical	[35]

Structural studies of the eIF4E and 4EGI-1 complex showed that 4EGI-1 allosterically modifies eIF4E by binding to its hydrophobic pocket, which is distal from the eIF4G binding site, leading to localized conformational changes and subsequent suppression of the eIF4E-eIF4G association [33]. 4EGI-1 induces apoptosis and enhances radiotherapy sensitivity in cancer cells by augmenting Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis through cellular FADD-like IL-1β-converting enzyme (FLICE)-inhibitory protein (cFLIP) downregulation and death receptor 5 (DR5) induction upon 4E-BP1 dephosphorylation [34]. SBI-0640756 (SBI-756), a first-in-class inhibitor that targets eIF4G1 and disrupts the eIF4F complex, attenuates the growth of clinically unresponsive melanomas by disrupting the eIF4F translation initiation complex [35]. Recently, SBI-756, in combination with Venetoclax, has shown a synergistic effect in pre-clinical models of DLBCL and Mantle Cell Lymphoma (MCL) [36]. Furthermore, SBI-0640756 and RNA aptamers have been designed to directly target eIF4GI in melanoma models [35, 37]. Overall, despite the limited clinical success to date, inhibitors of eIF4G and/or eIF4G1/eIF4E interactions represent an attractive strategy to potentiate the effectiveness of existing cancer therapies and to overcome chemoresistance to drugs.

3. eIF4E

eIF4E is a protein that plays a key role in translational initiation. Indeed, eIF4E, a 25 kDa protein, binds the m^7G cap structure at the 5′ end of most eukaryotic mRNAs along with other initiation factors, eIF4G, eIF4A, and eIF4B, which mediates the recruitment of the mRNA on the 40S ribosomal subunit to initiate translation. The eIF4E family consists of three members [38]: 1) eIF4E1 (the most studied and hereafter referred as eIF4E), 2) eIF4E-2 (4E-HP), and 3) eIF4E3 (emerging data suggest it may act as a tumor suppressor [39]). eIF4E remains one of the best-characterized proteins in different organisms, including yeast, wheat, mouse, and humans. The major binding sites are the cap-binding site and the dorsal surface, which serves as a binding surface for both eIF4G and 4E-BP proteins [40].

Even though all eukaryotic mRNAs have a cap structure, not all cellular transcribed mRNAs are equally sensitive to eIF4E activity [41]. mRNAs harboring long structured 5′-UTRs (including proto-oncogenes and genes encoding growth regulatory proteins), known as "weak" mRNAs, are more sensitive to eIF4E levels. The translation of these "eIF4E-sensitive mRNAs" is preferentially stimulated by increased eIF4E activity. For example, housekeeping mRNAs such as GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) and β-actin are less sensitive to eIF4E as compared to mRNAs involved in cell growth, proliferation, immune responses, and angiogenesis, e.g., c-MYC, cyclins, B-cell lymphoma 2 (BCL-2), Myeloid Cell Leukemia Sequence 1 (MCL1), osteopontin, survivin, vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), and matrix metalloproteinase 9 (MMP-9) [39, 42, 43]. eIF4E is also reported to be involved in the translational control of another class of mRNAs (mRNAs with 5′ terminal oligopyrimidine tract), referred to as TOP mRNAs [44, 45]. These mRNAs encode elongation factors, ribosomal proteins, and fused proteins [46, 47]. Importantly, it has been demonstrated that eIF4E enhances the intrinsic RNA helicase activity of eIF4A. eIF4E relieves the repression of the eIF4A through conformational changes of eIF4G. These ternary changes in the complex structure stimulate the eIF4A helicase activity on the mRNA 5′-UTR in the presence of the accessory protein eIF4B to promote cap-independent translation [48].

Apart from mammalian translational regulation, eIF4E is important for mRNA export. The protein is observed to be localized in the nucleus but absent in Cajal bodies, nucleolus, and nuclear speckles [50], suggesting that the protein is involved in the export of specific mRNAs for efficient translation. The export function is mediated through the transcript carrying the 5′ cap as well as the ~50nt eIF4E-sensitive element in the 3′UTR. These elements are required for efficient cap formation and eIF4E binding for export into the cytoplasm. Here, the protein in conjunction with leucine-rich pentatricopeptide repeat protein (LRPPRC), UAP56 (a DExD-box helicase 39B), and heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) form a complex referred to as a ribonucleoparticle (RNP) [51]. Interestingly, the mRNA that is highly dependent on eIF4E expression for efficient translation is also exported from the nucleus in an eIF4E-dependent manner [40]. It was also recently established that eIF4E could

bind with trimethyl guanosine (TMG) cap, however, the affinity of human eIF4E towards TMG cap was less than that of m^7g cap [52]. eIF4E is also known to regulate histone H4 mRNA translation in a cap-independent manner. The histone H4 mRNA encodes a paired-stem loop element within its open reading frame, which mimics the eIF4E-sensitive element of many mRNAs. Intriguingly, the interaction between eIF4E was noted on the amino-terminal part and not on the eIF4E-sensitive element, which could mediate histone H4 mRNA translation by tethering the *de novo* protein biosynthetic machinery [49]. More detailed studies are needed to determine the biological relevance of this interaction.

eIF4E expression and activity are tightly regulated by multiple cellular processes. These include: 1) gene amplification of eIF4E, 2) transcriptional activation, 3) intracellular eIF4E inhibitor (eIF4E-binding protein, 4E-BP), and 4) post-translational modifications. Gene amplification has been observed in a subset of head and neck, and breast cancers [53, 54]. There have been several reports of promoter-mediated self-regulation of eIF4E expression. The eIF4E promoter encodes an E-box element and is a direct transcriptional target of c-Myc [55]. Interestingly, c-Myc is an mRNA export and translation target of eIF4E as well, suggesting a potential positive feedback mechanism [56]. The hnRNPK (Heterogeneous Nuclear Ribonucleoprotein K) protein also binds to the core polypyrimidine element of the eIF4E promoter, and its regulation of eIF4E contributes to neoplastic transformation [57]. Recent studies demonstrate that eIF4E is also a C/EBP target [58]. Finally, the stability of eIF4E mRNA is increased by both HuR and TIAR proteins. Of clinical relevance, increased HuR levels and activated NFκB are associated with increased eIF4E expression in primary M4 and M5 subtype AML (adult acute myeloid leukemia). While eIF4E is ubiquitously expressed, it appears that eIF4E functions in a tissue-specific manner [59]. Various tissue-specific regulators of eIF4E have been determined to play a key role in modulating the activity of eIF4E. Promyelocytic leukemia protein (PML) nuclear bodies have been seen associated with the eIF4E nuclear bodies resulting in the inhibition of the mRNA nuclear export role of eIF4E [50]. Additionally, PRH-eIF4E interaction (proline-rich homeodomain, expression of PRH is limited in cell types) is shown to disrupt eIF4E nuclear bodies acting as a key mechanism of balancing cellular proliferation and differentiation [60].

Apart from intracellular regulators of nuclear export, the translational activity of eIF4E is also impeded by interaction with eIF4E-binding proteins (4E-BP). 4E-BP competes with eIF4E in the interaction with eIF4G for the formation of active translational complexes, thus acting as an intracellular translational repressor [61]. The family consists of three isoforms — 4E-BP1, 4E-BP2, and 4E-BP3, which have similar functions with differential tissue expression. The interaction of 4E-BP to eIF4E heavily relies on the 4E-BP phosphorylation state. Upon phosphorylation by mTORC1, the affinity of 4E-BP to eIF4E is reduced, leading to its dissociation from eIF4E and allowing the formation of active eIF4F complexes [44]. In addition to the regulation of mTORC1/4E-BP, the activity of eIF4E is also regulated by Mnk1/2(Mitogen-activated protein kinase (MAPK)-interacting protein kinases 1 and 2)-driven phosphorylation. MNK1/2, which are downstream of the extracellular-signal-regulated kinase (ERK) [62] and the p38 MAPK signaling cascades [39], phosphorylates eIF4E at its sole phosphorylation site, Ser^{209} [10]. The phospho-modification of eIF4E results in altered translation for a subset of mRNAs, although the mechanisms underlying the effect of this phosphorylation event on modified translational output and transcript-specificity remain unresolved [39]. In addition to phosphorylation, eIF4E is also known to be ubiquitinated, however, the corresponding E3 ligase(s) and deubiquitinase(s) have not been identified [63].

eIF4E is a potent oncogene, elevated in up to 30% of human malignancies, including carcinomas of the breast, prostate, lung, head, and neck as well as in many leukemias and lymphomas (Table 2). Increased eIF4E expression is associated with poor prognosis in certain malignancies. Consistent with this observation is that a relatively modest level of ectopic expression (~2.5-fold, a range observed in several human cancers) of eIF4E in NIH 3T3 was sufficient to cause transformation [61]. The assessment from the cell-based observations was further bolstered by subsequent *in vivo* work in mice, which demonstrated that enhanced expression of eIF4E enhanced Eμ-Myc-driven lymphomas and caused cancers in a multitude of organs when expressed from the β-actin promoter [64]. Elevated eIF4E protein levels impact transformation, tumorigenesis, metastasis, and drug resistance in both experimental cancer models and certain human cancers. Unsurprisingly, the knockdown of eIF4E by small interfering RNA (siRNA) can suppress oncogenic transformation in many

tumor models [40]. Strikingly, ectopic expression of the eIF4ES209A phospho-null mutant protein failed to cause neoplastic transformation in NIH 3T3 cells and the Eμ-Myc lymphoma mouse model, positioning Mnk driven phosphorylation as an ideal therapeutic target in curtailing eIF4E-driven transformation [65, 66].

In light of the fact that eIF4E is a convergence node for the numerous cancers driven by perturbed upstream signaling cascades, there has been considerable effort to target eIF4E directly or indirectly for cancer therapy (Table 4). Graff *et al*., using antisense oligonucleotide (ASO) against eIF4E, reported a robust reduction in eIF4E-sensitive genes in human xenograft models. eIF4E ASO also reduced eIF4E levels in the mouse (80% in the liver), but importantly, it had no effect on body weight, organ weight, or liver transaminase levels [67], suggesting a broad therapeutic window. Another class of promising inhibitors targets eIF4E-eIF4G interactions, as

Table 4. Small molecules hampering eIF4E activity.

Agent	Mechanism	*In vitro* IC_{50}	*In vivo* models	Clinical development	References
Ribavirin	eIF4E–cap interaction	12 μM	ALL xenograft mice	Phase II	[63, 185]
Bn7GMP		16 μM	Lung cancer xenograft mouse model	Pre-Clinical	[186]
eIF4E ASO LY2275796	eIF4E synthesis	10 nM	Lung cancer xenograft mouse model	Phase I	[187]
4EBP mimetic peptides		—	Breast carcinoma allografts in BALB/C mice model	Pre-Clinical	[63]
Rapamycin	m-TOR activity	2 nM	Human anal xenograft mouse models	FDA approved	[188]
CCI-779		1.7 μM	Human pancreatic cancer mouse model	FDA approved	[189]
RAD001		2.4 nM	Ovarian cancer model	Phase II	[190]

(*Continued*)

Table 4. (*Continued*)

Agent	Mechanism	*In vitro* IC_{50}	*In vivo* models	Clinical development	References
AP23573		0.2 nM	Lung cancer xenograft mouse model	Phase II	[191]
Torin 1		2 nM	Human colon cancer stem cell like cell xenograft mouse model	Pre-Clinical	[192]
AZD8055		0.8 nM	Papillary cell carcinoma xenograft mouse model	Phase I	[193]
WYE-125132		0.2 nM	Lung cancer xenograft mouse model	Pre-Clinical	[194]

discussed above. Clinical and pre-clinical studies demonstrated that ribavirin, a broad-spectrum antiviral drug, inhibits the activities of eIF4E. Ribavirin monotherapy led to objective clinical benefit in many of these patients. Many tissue-specific factors will likely profoundly influence the utility of ribavirin as a more broadly used anti-cancer drug [68]. 4Ei-1, a 5′ cap mimetic, inhibited cap-dependent translation and exhibited anti-neoplastic activities with minimal toxic effects. High throughput screening efforts for more effective 5′-cap mimetics are ongoing [61]. Another strategy to diminish eIF4E activity has been to target its kinase, MNK. These small inhibitory molecules include CGP57380 [39], cercosporamide and Compounds 8E and 8I (5-(2-(phenylamino) pyrimidine-4-yl) thiazol-2(3H)-one derivatives [69]). Another promising inhibitor is Tomivosertib which inhibits both MNK1/2 and is currently under clinical evaluation [63].

4. eIF4A/DDX2

The isolation of eIF4A/DDX2 (Dead-box helicase 2) was accomplished by Jamie Grifo and colleagues [70]. Ray *et al*. noted that eIF4A is an

ATP-dependent RNA helicase, although eIF4F was much more efficient than eIF4A on a molar basis [71]. Since the protein was the first reported RNA helicase, Rogers and the group proposed eIF4A/DDX2 as the gold standard and founding member, thus dubbed as "godfather" of the DEAD-box family [72]. There are two mammalian isoforms involved in translation, eIF4A1 and eIF4A2, while eIF4A3, another isoform, is reported to be associated with non-sense mediated decay via the exon junction complex. The proteins are found to be highly conserved, sharing more than 90–95% sequence identity and ~90% homology with their murine homologs. Although eIF4A1 and eIF4A2 show ~90% sequence identity, they are not functionally redundant *in vivo*. The expression levels of eIF4A1 and eIF4A2 vary in a tissue-dependent manner. eIF4A1 is expressed more in proliferating cells in contrast to eIF4A2, which is up-regulated in growth-arrested differentiated cells, suggesting differential regulation of cell fate. eIF4A is a minimal dead-box protein that encompasses only the conserved helicase domain with a short N-terminal and almost absent C-terminal [73]. Biochemical characterization demonstrated that it has weak intrinsic ATP-dependent RNA helicase activity, which was reported to be modulated by its interacting partners, eIF4B, eIF4H, eIF4G, eIF4E, and PDCD4, the latter postulated to be an endogenous eIF4A inhibitor [74].

Before the formation of an active ribosome complex, eIF4A is recruited on an m7G cap structure in conjunction with eIF4E and eIF4G for the formation of the ternary eIF4F complex. This results in the unwinding of 5′-UTR of mRNA's secondary structure to allow ribosome scanning. Not surprisingly, eIF4A1 knockdown leads to a profound decrease of [^{35}S]-methionine incorporation of nascent polypeptide synthesis and global mRNA distribution in polysome profiles [73]. Interestingly, depletion of eIF4A1 results in eIF4A2 enhanced expression, however, upregulation of eIF4A2 does not compensate for the reduction in mRNA translation efficacy upon eIF4A1 depletion [75]. In addition, eIF4A1 and eIF4A2 have distinct binding partners, eIF4A1 predominantly binds to eIF4G [76] while eIF4A2 preferentially binds to CCR4-NOT Transcription Complex Subunit 7 (cNOT7) [77]. The activity of eIF4A1 is highest when it is incorporated into the eIF4F complex and is potentiated by its interacting partners eIF4B and eIF4H [78]. General translation initiation is

impaired after eIF4A inhibition; the requirement of eIF4A for translation initiation was found to be directly proportional to the complexity of the mRNA 5′UTR region. Polysome profiling and ribosome foot-printing experiments confirmed that the eIF4A1 dependent translatome is enriched for mRNAs with a complex 5′UTR, those characterized by long length, GGC repeats, and the presence of G-quadruplexes [79]. Although eIF4A1 can resolve most 5′UTR regions with secondary RNA structures, it has also been reported that very long 5′UTRs with multiple stem-loops are resistant to eIF4A and that additional RNA helicases are needed to support cap-dependent translation [32]. PDCD4 is an intracellular translation inhibitor that suppresses eIF4A1 activity. PDCD4 competes with the binding site of eIF4A1 with eIF4G and eIF4B, thereby reducing overall translation [74]. Loss or reduced expression of PDCD4 has been implicated in the development and progression of a variety of aggressive human cancers, including lymphoma. Mechanistically, PDCD4 is regulated by mTOR-S6K1 signaling and the $SCF^{\beta\text{-}TRCP}$(Skp1–Cullin1–F-box protein-Beta-transducin repeats-containing proteins) ubiquitin ligase in response to the activation of the mTOR pathway by mitogens [80]. eIF4A3 is functionally distinct from eIF4A1 and eIF4A2, despite sharing 60% homology [81]. eIF4A3 is likely not involved in translation control, as it principally resides in the nucleus, where it forms a key component of the exon junction complex and plays a major role in the nonsense-mediated mRNA decay [82].

eIF4A-dependent translatome analyses have shown that eIF4A-dependent mRNAs include those that contribute to cell proliferation, cell cycle progression, cell survival, and angiogenesis [3]. eIF4A facilitates the translation of a wide spectrum of oncogenes such as MYC, NOTCH1, MYB, Cyclin-Dependent Kinase 6 (CDK6), mouse double minute 2 homolog (MDM2), Cyclin D3 (CCND3), ETS1, ADAM Metallopeptidase Domain 10 (ADAM10), Lymphoid Enhancer Binding Factor 1 (LEF1), B-cell lymphoma/leukemia 10 (BCL10), mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), ADP Ribosylation Factor 6 (ARF6), Cyclin D1 (CCND1), Rho Associated Coiled-Coil Containing Protein Kinase 1 (ROCK1), baculoviral IAP repeat-containing 5 (BIRC5) or survivin (survival), MCL1 and BCL2 (anti-apoptotic factors), Rho kinase1 (ROCK1, cell migration), SIN1 (part of mTORC2

complex, cell migration), Mucin1-C (MUC1-C), and Cyclin D3 (proliferation) among others for efficient ribosome scanning and their translation [83]. Given the functional scope of genes augmented with eIF4A expression, the protein is found to be increased in several human malignancies (Table 2). It is also important to note that the co-factors like eIF4B, eIF4H (see below), and PDCD4 which modulates eIF4A's enzymatic activity are also observed to be perturbed in numerous cancers.

Given its high importance in oncogenic regulation, several independent investigators identified natural products that inhibit eIF4A activity (Table 5). One of the best-studied is Hippuristanol, isolated from the coral *Isis hippuris*. It inhibits the activity of both eIF4A1 and eIF4A2 without affecting the activity of helicases outside of the eIF4A family.

Table 5. List of inhibitors against eIF4A.

Small molecules	Mechanism	*In vitro* IC_{50}	*In vivo* models	Clinical development	References
Elatol	Inhibit eIF4A1-mediated ATP hydrolysis *in vitro*	1–5 μM	DLBCL xenografts in SCID mice	Pre-Clinical	[195]
Elisabatin A	Inhibit eIF4A1-mediated ATP hydrolysis *in vitro*	55 μM	—	Pre-Clinical	[196]
Prostaglandin, 15d-PGJ2	Interferes with eIF4A: eIF4G interaction	100 μM	Mouse CT-26 s.c. tumor model, HL-60 leukemia xenograft model	Pre-Clinical	[197, 198]
6-aminocholestanol	Inhibits ATPase and helicase activity	>100 μM	—	Pre-Clinical	[199]
Sanguinarine	Inhibits ATPase and helicase activity	40 μM	LoVo cell xenografts in nude mice	Pre-Clinical	[200, 201]
Hippuristanol	Allosteric inhibitor of eIF4A	200–400 nM	BCBL-1 Cells xenograft in SCID mice	Pre-Clinical	[202, 203]

(*Continued*)

Table 5. (*Continued*)

Small molecules	Mechanism	*In vitro* IC_{50}	*In vivo* models	Clinical development	References
Silvestrol	Interferes in RNA binding domain	5 nM	Fibrotic pancreatic tumors graft s.c. in C57Bl/6J mice	Pre-Clinical	[204, 205]
Patamine	Inhibits ATPase and helicase activity	2 nM	Human cancer xenograft models in NCr mice	Pre-Clinical	[206, 207]
eFT226	Interferes with eIF4A association in eIF4F complex formation	1–20 nM	In development	Phase I	[208]
CR-1-31-B	Interferes in RNA binding domain	5 nM	Pancreatic cancer cells in athymic nude mice	Pre-Clinical	[90, 209]

This compound binds to the C-terminal domain of eIF4A, directly adjacent to the ATP-binding site and distant from the RNA-binding site, acting as an allosteric inhibitor of eIF4A. Studies have shown that it blocks eIF4A1 helicase activity by locking it into a closed conformation. Hippuristanol has been reported to suppress tumor growth in a mouse model of T-cell leukemia, as well as to chemosensitize Myc-driven tumors to DNA damaging agents in an Eμ-Myc mouse model [84]. Another well-studied molecule is Silvestrol which belongs to a class of compounds called rocaglamides, isolated from species of the Aglaia genus of plants [85]. Silvestrol induces binding of eIF4A to mRNA in a sequence-independent manner and has been shown to target the activity of eIF4A both *in vitro* and *in vivo* [86]. Rocaglamides also bind to prohibitin 1 and 2, which inhibit the CRaf/MEK/ERK/Mnk1 signaling pathway, making it difficult to relate the activity of these compounds as eIF4A specific inhibitors [87]. Silvestrol has been shown to significantly inhibit the synthesis of proteins that drive tumor growth such as CCND1, BCL2, MYC, and

NOTCH in tumor cell lines [86]. Rocaglamides and DDR (DNA Damage Response) decreased the levels of multiple signaling proteins important for tumor growth and survival, leading to G2-M cell-cycle arrest and activation of executioner caspases [88]. eIF4A inhibition shows selective repression of multiple critical oncoproteins in addition to MYC in B-cell lymphoma including NIMA Related Kinase 2 (NEK2), MCL1, Aurora Kinase A (AURKA), Polo Like Kinase 1 (PLK1), and several transcription factors that are generally considered undruggable [86].

Combining eIF4A inhibitors with BRAFi/MEKi can prevent resistance of $BRAF^{V600E}$ melanoma to targeted therapies [89]. The underlying mechanism of action shown by Chan *et al.*, revealed that G-quadruplexes in 5′-UTRs mediate selective suppression of translation downstream of eIF4A inhibition by the rocaglate Silvestrol. Alternatively, eIF4A clamping to polypurine sequences underlies the effects of the rocaglate Rocaglamide A. The functional gene subsets whose translation is strongly dependent on eIF4A in pancreatic ductal adenocarcinoma (PDA) include those involved in central carbon metabolism [90]. In a mouse model of melanoma, Signal Transducer And Activator Of Transcription 1 (STAT1)-mediated transcription of Programmed death-ligand 1 (PD-L1) was induced by interferon-gamma via upregulation of STAT1 translation. The translation of STAT1 was demonstrated to be inhibited by both Silvestrol and Pateamine, as well as siRNA targeting of eIF4A [91]. Consistent with its reported induction of innate immunity, silvestrol effectively treated melanoma in immune-competent mice, but not in immune-compromised mice such as nude mice and CD8+ T cell-depleted mice. This mechanism involves the suppression of cell-cycle feedback response and overcoming resistance to CDK4/6 inhibition in cancer [92]. Pateamine A is another small molecule of natural origin known to inhibit eIF4A1. It is isolated from the sea sponge *Mycale hentscheli* and was first characterized as a selective agent against tumor cells [93]. Pateamine A is characterized as an inhibitor of cap-dependent translation initiation that specifically targets eIF4A [94]. Pateamine A increases the affinity of eIF4A for ATP and RNA by inducing dimerization, which allows non-specific interaction of eIF4A–RNA. As a result, it sequesters eIF4A from the eIF4F complex [95]. The anti-tumor activity of Pateamine A and its simplified analog has been demonstrated both *in vitro* as well as *in vivo* models [83]. The

synthetic rocaglate, CR-1-31-B (CR-31), selectively reduced the viability of PDA organoids relative to their normal counterparts. CR-31 suppresses tumor growth *in vivo* and extends the survival of genetically engineered murine models of PDA [90]. Recently, a small molecule developed by Effector Therapeutics, eFT226, is a potent and selective eIF4A1 inhibitor that promotes eIF4A binding to specific mRNA sequences and interferes with the assembly of the eIF4F initiation complex. eFT226 selectively inhibits translation of mRNAs containing longer 5′-UTRs, an increased frequency of uORFs (upstream open reading frame), and polypurine and/or G-quadruplex recognition motifs. eFT226 is currently being evaluated in a Phase 1/2 clinical trial in patients with solid tumors[96].

5. eIF4B

The translation initiation factor eIF4B was identified and subsequently purified decades ago and shown to have translation-stimulating activity *in vitro*. Later, a detailed biochemical investigation established that it functionally interacts with the eIF4F complex and potentiates eIF4A RNA helicase activities [97]. The protein also interacts with PABP, and this interaction facilitates circularization of the mRNA. This is followed by the subsequent recruitment of the 43S preinitiation complex (PIC) composed of the small ribosomal subunit (the 40S), the ternary complex (eIF2/met-tRNA/GTP), and several initiation factors (eIF1, eIF1A, eIF3, and eIF5) [98]. Finally, this complex scans the untranslated region (UTR) of the mRNA until a start codon is recognized [99].

One of the most notable features of eIF4B is that the protein is the least evolutionary conserved translation initiation factor. Despite low sequence homology, the protein encompasses an N-terminal region, RNA recognition motif (RRM), middle DRYG/7-repeats region, and C-terminal Arginine Rich motif (ARM). eIF4B binds to the 40S subunit in a manner dependent on both the NTD and 7-repeats domains of the protein and independently of the RRM. NTD and the 7-repeats domain of eIF4B are required for robust mRNA recruitment to the PIC, suggesting these ribosome binding activities are critical for translation [100]. Interestingly, the RNA-Recognition Motif (RRM) was dispensable for stress responses. However, ribosomal binding by the N-terminal Domain (NTD) promoted

growth in response to various stressors, revealing that eIF4B regulates mRNA loading and scanning as a part of the PIC, rather than by activating mRNPs before ribosome binding [101]. Therefore, eIF4B acts through different mechanisms to stimulate translation initiation: 1) by enhancing the ATPase and helicase activities of eIF4A in linearizing the mRNA for scanning [102] and 2) by facilitating the recruitment of the 43S PIC [103]. Indeed, recent ribosome crystallization noted that protein was recruited near the P-site of the ribosome [99]. However, more detailed high-resolution images are needed to decipher the spatial positioning of eIF4B in an active ribosomal complex.

Although the precise mechanisms of action of eIF4B on the enhanced helicase activity of eIF4F are not fully understood, modulation of eIF4B expression in mammalian cells is positively correlated with the translation of mRNAs encompassing highly structured 5′UTRs [104, 105]. eIF4B can modulate the affinity for ATP and RNA by inducing conformational changes in eIF4A [106, 107], suggesting that the protein might act in the selection of substrate for eIF4A helicase activity. Additionally, eIF4B can enhance the efficiency of this process by coupling the ATP hydrolysis to duplex unwinding, thus avoiding redundant, energy-consuming events [102]. A direct interaction between eIF4A and eIF4B in the presence of RNA and an ATP analog has been established via the C terminal region of eIF4B [108, 109]. Importantly, mammalian eIF4B dimerizes and binds to eIF3a via its DRYG repeats, thus providing the main link between the eIF4F-loaded mRNA and the 43S PIC [103]. These data provide evidence that eIF4B participates in the recruitment and assembly of the PIC on mRNAs.

eIF4B is involved in the translation of numerous proliferative and anti-apoptotic mRNAs with highly structured 5′UTR impacting cell growth and survival [100, 110]. eIF4B RNA-IP analysis revealed eIF4B complexes mRNAs encoding fatty acid synthase (FASN), BCL2, XIAP, cMYC, BCL6, and poly [ADP-ribose] polymerase 1 (PARP-1), all of which are *bona fide* potent oncogenes. Interestingly, eIF4B protein interacts with its mRNA to enhance its protein level [100]. Moreover, the binding of 14-3-3 sigma tumor suppressor to eIF4B in late mitosis regulates translation, indicating direct involvement of eIF4B in the regulation of cell cycle [111]. Also, eIF4B is cleaved by a caspase-dependent mechanism

upon activation of the tumor necrosis factor pathway, suggesting a role in preventing apoptosis [112]. eIF4B expression and phosphorylation are de-regulated in many cancers (Table 2). Not surprisingly, abnormal protein levels are observed in multiple cancers, including Breast, DLBCL, ABL-positive CML, T-CLL, Kaposi Sarcoma, and others. A microarray study found eIF4B mRNA to be upregulated in a panel of 22 patients after they became resistant to combined cisplatin and fludarabine treatment in gastric cancer [113]. In a proteomic study, eIF4B was shown to be down-regulated following wild-type p53 knockdown in an NPC (nasopharyngeal carcinoma) cell line [114]. In T cell lymphoblastic leukemia/lymphoma, eIF4B mRNA was found to be up-regulated in a genome-wide study comparing the mouse model of thymic tumors to the untransformed thymus [115]. In non-small cell lung cancer (NSCLC), chromosomal aberrations in the 12q13 region encompassing the eIF4B gene are well described. Especially, eIF4B expression is significantly higher in NSCLC tumors showing this kind of alteration [116]. Although the mRNA level of eIF4B was not found to be enhanced in DLBCL and FL patients, the protein was noted to be upregulated in both cancer types [110].

Owing to its critical role in regulating the translational machinery, a number of labs have interrogated the post-translational regulation of this protein. eIF4B Ser^{422} phosphorylation is at the intersection of two major oncogenic pathways: MAP kinases and the AKT/mTOR pathway [117]. The protein was shown to be phosphorylated by multiple kinases, including p90RSK, p70S6K, PIM kinase, and Akt. These phosphorylation events promote augmented global translation, enhanced proliferation, and overexpression of specific oncoproteins such as MYC or BCL2 [117]. Activation of eIF4B and subsequent c-MYC induction potentiates arsenic-induced transformation [118]. Activation of Laminin γ2 by eIF4B is found in pre-malignant oral dysplasia, where eIF4B was found to be activated by the MEK/ERK kinase pathway [119]. In prostatic carcinoma, eIF4B phosphorylation by Pim2 leads to resistance to apoptosis [120]. eIF4B phosphorylation was also observed following activation of the mTOR pathway in lung cancer [121]. The synergistic cytotoxic effect of mTOR and MEK inhibitors in NSCLC cell lines is correlated with a significant decrease in eIF4B phosphorylation [122]. Apart from phosphorylation, the protein is also known to be regulated by ubiquitin-mediated signaling. The Gartenhaus lab recently identified eIF4B as a downstream mediator of

Table 6. Small molecule modulator of eIF4B activity.

Small molecules	**Mechanism**	***In vitro* IC_{50}**	***In vivo* model**	**Clinical development**	**References**
Mitoxantrone	USP11 inhibitor	3.15 μM	Nude mouse lung cancer xenograft model	Phase II	[110, 210]
AZD1208	Pan PIM Kinase	0.4 nM	MM and CLL xenograft	Phase I	[124]
PIM447 (LGH447)	Pan PIM Kinase	9 pM	DLBCL cell lines	Phase II	[123]

FASN-driven tumor growth. Depletion of FASN resulted in ubiquitin-mediated degradation of eIF4B. Mechanistically, it was found that FASN activates the mTOR/p70S6Kinase pathway, resulting in enhanced interaction between USP11 and its deubiquitinate substrate eIF4B. This augmented recruitment of eIF4B-USP11 on the TIC underlies, in part, the sustained oncogenic-translation seen in DLBCL [110]. Indeed, chemical treatment with Mitoxantrone, a USP11 non-specific inhibitor, significantly reduced eIF4B as well as eIF4B-dependent genes in DLBCL [110]. Apart from USP11, targeting PIM Kinase in multiple cancers has also been shown to decrease the eIF4B phosphorylation and thus its activity [123, 124] (Table 6).

Although the protein has been observed to be critical for several malignancies, the physiological role of eIF4B as well as its impact on tumor development and progression *in vivo* is still evolving. There is emerging evidence that eIF4B regulates both cap-dependent as well as IRES-dependent pathways, providing further impetus to fully define the specific genes regulated by eIF4B [110]. While the protein has been characterized for almost the past half-century, there is, unfortunately, no crystal structure yet to assist in drug design in order to consider it a druggable target.

6. eIF4H

Eukaryotic initiation factor 4H (eIF4H) plays an important role in cellular homeostasis [125], however, the precise functional landscape eIF4H is

still not as well characterized as other initiation factors. The gene encodes two splice variants leading to the expression of two protein isoforms (25.2 kDa and 27.3 kDa). The systematic role of eIF4H was described employing eIF4H null mice, which displayed both growth retardation and severe neurological deformities. The clinical relevance is further supported as the gene resides on chromosome 7q11, a region critical to Williams-Beuren Syndrome, a fatal congenital disorder associated with neurodevelopmental as well as cardiovascular abnormalities [126].

William Merrick and colleagues first coined the term eIF4H while studying globin synthesis under *in vitro* conditions and identified a protein with ~25 kDa molecular mass, which can stimulate the translational capacities of eIF4B and eIF4F [127]. The eIF4H protein shares high sequence homology with eIF4B. The protein encodes an RNA recognition motif (RRM), however, it lacks the regulatory C-terminal region phosphorylation site, suggesting that the protein might be constitutively active. eIF4H, as with eIF4B, is found to be expressed ubiquitously [128]. Both proteins form complexes with eIF4A1 at overlapping binding sites, rendering their binding to eIF4A1 mutually exclusive [109]. eIF4H enhances the ATP-dependent RNA unwinding activity of eIF4A, which unwinds secondary structures at the 5′-untranslated regions in translation initiation [129]. Both eIF4B and eIF4H stimulate ATP binding to eIF4A, RNA-dependent ATPase activity, ATP-dependent RNA binding, and the helicase activity of eIF4A [72]. eIF4B and eIF4H also stabilize partially unwound substrates and/or prevent mRNA reannealing, activities that further facilitate RNA restructuring during initiation [125]. eIF4AI/eIF4H complex exhibits an abundance of persistent, repetitive cycles of unwinding over extended periods [130]. Interestingly, using a cap-dependent cross-linking strategy, it was observed that eIF4H cross-links with mRNA up to 50 nucleotides away from the cap complex, suggesting that the protein might behave as an RNA chaperone in the absence of ATP hydrolysis [131].

Given the vital intracellular role of eIF4H in augmenting eIF4A's RNA helicase activity, there is growing interest in defining its potential oncogenic role (Table 2). Wu *et al*. reported that eIF4H isoform 1 (~27 kDa) is overexpressed in primary esophageal cancer compared to isoform 2 (~25 kDa). Further phenotypic evaluation using genetic modifications revealed that isoform 1, but not isoform 2, is critical for tumorigenesis as it regulates canonical eIF4A-dependent genes [132]. Interestingly,

Vaysse *et al*. noted that the protein level of both isoforms was enhanced in lung carcinomas. They noted that both the isoforms were critical for augmenting cell proliferative genes, including Cyclin D1, cMYC, B-cell lymphoma-extra large (BCL-XL), and Cellular Inhibitor of Apoptosis Protein 1 (CIAP-1) [125]. Varying results from these two studies may be due, in part to different cell types interrogated. The tumorigenic role of eIF4H was further supported by the observation that the protein is a *bona fide* transcriptional substrate of enhanced NFKB activity. eIF4H gene expression and protein content positively correlated with the level of p65 DNA binding activity in numerous cancer cell lines, including breast cancer (MDA-MB-231, MCF7), neuroblastoma (SH-SY5Y), glioblastoma (U251, D54), and B-cell lymphoma (MC3, DeFew). Correlation between p65/NF-kB activity and eIF4H expression levels in a panel of tumor cell lines suggests that the sustained NF-kB activity in these cells can promote cell growth by up-regulating the eIF4H-dependent translation initiation [129]. Apart from transcriptional regulation, eIF4H gene expression is also controlled by post-transcriptional modifications. Tumor suppressor miRNA-519d, which is depleted in lung adenocarcinoma, inhibits cell proliferation and invasion by regulating eIF4H expression levels [133]. Further, the expression level of eIF4H was also observed to be enhanced in chronic myeloid leukemia patient samples [133]. Additionally, the expression level of eIF4H was also noted to be upregulated in breast cancer [134]. Herpes simplex virus, a known etiologic agent in several oncogenic events leading to fatal diseases like Burkett's lymphoma, nasopharyngeal cancer, and others, utilizes eIF4H in conjunction with Vhs protein to amplify virion particles as well as to activate cellular proliferative genes [135]. In the aggregate, this protein appears to be critical for tumor cell survival and represents a promising therapeutic target. However, a lack of data regarding its direct RNA substrate(s) as well as the protein's post-translational regulation hinders progress towards that end.

7. Concluding remarks

The study of pro-tumorigenic consequences of dysregulated translation initiation has moved into the main arena of cancer research. With both technological and methodological advances, our grasp of the critical relationship between altered translational output and cellular transformation

has expanded enormously. Significantly, there is growing interest from researchers across the cancer research spectrum to interrogate and define the details of translational dysregulation driven by the core components of the translational apparatus described above. With our enhanced understanding, several new frontiers have opened to study the regulation of translation initiation. Apart from canonical cap-dependent translation, various modes of non-canonical translation such as leaky scanning, re-initiation, ribosome shunting, IRES (ITAFs)-driven translation, and m6A-mediated ribosome loading are emerging as alternative mechanisms associated with various stress-driven events [136]. Since cancer cells often proliferate under numerous stress-driven conditions, including hypoxia, nutrient availability, proteotoxic or genotoxic stress, understanding these non-canonical translation processes will be vital to developing novel therapeutic interventions. Significantly, there is a rapidly growing body of literature demonstrating their deregulation and contribution to oncogenesis and maintenance of the tumor phenotype [136].

Modifying the translational output in tumor cells appears to be an essential driver in cancer progression which is directly correlated with the hyperactivation of cellular signaling cascades. Indeed, the well-studied PI3K-mTOR pathway directly regulates the mRNA translational fate of the cancer cell. However, our understanding of the impact of other oncogenic signaling pathways, including Wnt, MAPK, and bioenergetic sensors like LKB1 (Liver Kinase B1) and AMPK's (AMP Kinase) on direct regulation of translational apparatus is still underexplored.

Finally, we must look beyond the eIF4F complex in translational initiation regulation to fully appreciate the complexity of translational regulation. Increasingly, independent investigators have reported that other translation initiation proteins like eIF3 complex, eIF1, eIF2, eIF5, and eIF6 are perturbed in cancer cells [137]. Defining the molecular and functional underpinning of these translational control circuits would provide clarity *vis a vis* how cancer cells proliferate and survive even under unfavorable conditions. Looking ahead, based on these recent observations, investigators can now delve deeply to delineate the dynamic positive convergence between oncogenic signaling and the regulatory components of the translational machinery. Ultimately, the oncogenic addiction of the

transformed cell to robust translation may well serve as the Achilles heel for novel targeted therapies.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Schwanhausser B, *et al.* (2011) Global quantification of mammalian gene expression control. *Nature*, **473**(7347), 337–342.
2. Truitt ML and Ruggero D. (2016) New frontiers in translational control of the cancer genome. *Nature Reviews Cancer*, **16**(5), 288–304.
3. Pelletier J, *et al.* (2015) Targeting the eIF4F translation initiation complex: A critical nexus for cancer development. *Cancer Research*, **75**(2), 250–263.
4. Browning KS, *et al.* (1987) Identification of a new protein synthesis initiation factor from wheat germ. *Journal of Biological Chemistry*, **262**(2), 538–541.
5. Browning KS, *et al.* (1989) Evidence that the 59-kDa protein synthesis initiation factor from wheat germ is functionally similar to the 80-kDa initiation factor 4B from mammalian cells. *Journal of Biological Chemistry*, **264**(15), 8491–8494.
6. Hentze MW. (1997) eIF4G: A multipurpose ribosome adapter? *Science*, **275**(5299), 500–501.
7. Lamphear BJ, *et al.* (1995) Mapping of functional domains in eukaryotic protein synthesis initiation factor 4G (eIF4G) with picornaviral proteases. Implications for cap-dependent and cap-independent translational initiation. *Journal of Biological Chemistry*, **270**(37), 21975–21983.
8. Prevot D, Darlix JL and Ohlmann T. (2003) Conducting the initiation of protein synthesis: The role of eIF4G. *Biology of the Cell*, **95**(3-4), 141–156.

9. Korneeva NL, *et al.* (2005) Interaction between the NH2-terminal domain of eIF4A and the central domain of eIF4G modulates RNA-stimulated ATPase activity. *Journal of Biological Chemistry*, **280**(3), 1872–1881.
10. Pyronnet S, *et al.* (1999) Human eukaryotic translation initiation factor 4G (eIF4G) recruits mnk1 to phosphorylate eIF4E. *The EMBO Journal*, **18**(1), 270–279.
11. Dobrikov M, *et al.* (2011) phosphorylation of eukaryotic translation initiation factor 4G1 (eIF4G1) by protein kinase C{alpha} regulates eIF4G1 binding to Mnk1. *Molecular and Cellular Biology*, **31**(14), 2947–2959.
12. Borman AM, *et al.* (1997) elF4G and its proteolytic cleavage products: Effect on initiation of protein synthesis from capped, uncapped, and IRES-containing mRNAs. *RNA*, **3**(2), 186–196.
13. Yanagiya A, *et al.* (2009) Requirement of RNA binding of mammalian eukaryotic translation initiation factor 4GI (eIF4GI) for efficient interaction of eIF4E with the mRNA cap. *Molecular and Cellular Biology*, **29**(6), 1661–1669.
14. Reinert LS, *et al.* (2006) MCT-1 protein interacts with the cap complex and modulates messenger RNA translational profiles. *Cancer Research*, **66**(18), 8994–9001.
15. Villa N, *et al.* (2013) Human eukaryotic initiation factor 4G (eIF4G) protein binds to eIF3c, -d, and -e to promote mRNA recruitment to the ribosome. *Journal of Biological Chemistry*, **288**(46), 32932–32940.
16. Raught B, *et al.* (2000) Serum-stimulated, rapamycin-sensitive phosphorylation sites in the eukaryotic translation initiation factor 4GI. *The EMBO Journal*, **19**(3), 434–444.
17. Muller D, *et al.* (2013) 4E-BP restrains eIF4E phosphorylation. *Translation (Austin)*, **1**(2), e25819.
18. Wang Q and Yang HS. (2018) The role of Pdcd4 in tumour suppression and protein translation. *Biology of the Cell*.
19. Comtesse N, *et al.* (2007) Frequent overexpression of the genes FXR1, CLAPM1 and EIF4G located on amplicon 3q26-27 in squamous cell carcinoma of the lung. *International Journal of Cancer*, **120**(12), 2538–2544.
20. Silvera D, *et al.* (2009) Essential role for eIF4GI overexpression in the pathogenesis of inflammatory breast cancer. *Nature Cell Biology*, **11**(7), 903–908.
21. Liang S, *et al.* (2014) Decreased expression of EIF4A1 after preoperative brachytherapy predicts better tumor-specific survival in cervical cancer. *International Journal of Gynecological Cancer*, **24**(5), 908–915.
22. Jaiswal PK, *et al.* (2019) Eukaryotic Translation Initiation Factor 4 Gamma 1 (EIF4G1): a target for cancer therapeutic intervention? *Cancer Cell International*, **19**, 224.
23. Tan FH, *et al.* (2019) mTOR signalling in head and neck cancer: Heads up. *Cells*, **8**(4).
24. Ramirez-Valle F, *et al.* (2008) eIF4GI links nutrient sensing by mTOR to cell proliferation and inhibition of autophagy. *Journal of Cell Biology*, **181**(2), 293–307.

25. Badura M, *et al.* (2012) DNA damage and eIF4G1 in breast cancer cells reprogram translation for survival and DNA repair mRNAs. *Proceedings of the National Academy of Sciences of the United States*, **109**(46), 18767–18772.
26. Mazan-Mamczarz K., *et al.* (2014) Down-regulation of eIF4GII by miR-520c-3p represses diffuse large B cell lymphoma development. *PLoS Genetics*, **10**(1), e1004105.
27. Imataka H, Olsen HS and Sonenberg N. (1997) A new translational regulator with homology to eukaryotic translation initiation factor 4G. *The EMBO Journal*, **16**(4), 817–825.
28. Yamanaka S, *et al.* (1997) A novel translational repressor mRNA is edited extensively in livers containing tumors caused by the transgene expression of the apoB mRNA-editing enzyme. *Genes & Development*, **11**(3), 321–333.
29. Henis-Korenblit S, *et al.* (2002) The caspase-cleaved DAP5 protein supports internal ribosome entry site-mediated translation of death proteins. *Proceedings of the National Academy of Sciences of the United States*, **99**(8), 5400–5405.
30. de la Parra C, *et al.* (2018) A widespread alternate form of cap-dependent mRNA translation initiation. *Nature Communications*, **9**(1), 3068.
31. Lee SH and McCormick F. (2006) p97/DAP5 is a ribosome-associated factor that facilitates protein synthesis and cell proliferation by modulating the synthesis of cell cycle proteins. *The EMBO Journal*, **25**(17), 4008–4019.
32. Marash L, *et al.* (2008) DAP5 promotes cap-independent translation of Bcl-2 and CDK1 to facilitate cell survival during mitosis. *Molecular Cell*, **30**(4), 447–459.
33. Moerke NJ, *et al.* (2007) Small-molecule inhibition of the interaction between the translation initiation factors eIF4E and eIF4G. *Cell*, **128**(2), 257–267.
34. Fan S, *et al.* (2010) The eIF4E/eIF4G interaction inhibitor 4EGI-1 augments TRAIL-mediated apoptosis through c-FLIP Down-regulation and DR5 induction independent of inhibition of cap-dependent protein translation. *Neoplasia*, **12**(4), 346–356.
35. Feng Y, *et al.* (2015) SBI-0640756 attenuates the growth of clinically unresponsive melanomas by disrupting the eIF4F translation initiation complex. *Cancer Research*, **75**(24), 5211–5218.
36. Herzog LO, *et al.* (2020) Targeting eIF4F translation initiation complex with SBI-756 sensitises B lymphoma cells to venetoclax. *British Journal of Cancer*.
37. Miyakawa S, *et al.* (2006) RNA aptamers to mammalian initiation factor 4G inhibit cap-dependent translation by blocking the formation of initiation factor complexes. *RNA*, **12**(10), 1825–1834.
38. Frydryskova K, *et al.* (2016) Distinct recruitment of human eIF4E isoforms to processing bodies and stress granules. *BMC Molecular Biology*, **17**(1), 21.
39. Landon AL, *et al.* (2014) MNKs act as a regulatory switch for eIF4E1 and eIF4E3 driven mRNA translation in DLBCL. *Nature Communications*, **5**, 5413.
40. Carroll M and Borden KL. (2013) The oncogene eIF4E: Using biochemical insights to target cancer. *Journal of Interferon & Cytokine Research*, **33**(5), 227–238.

41. Uttam S, *et al.* (2018) eIF4E-dependent translational control: A central mechanism for regulation of pain plasticity. *Frontiers in Genetics*, **9**, 470.
42. Bhat M, *et al.* (2015) Targeting the translation machinery in cancer. *Nature Reviews Drug Discovery*, **14**(4), 261–278.
43. Rousseau D, *et al.* (1996) Translation initiation of ornithine decarboxylase and nucleocytoplasmic transport of cyclin D1 mRNA are increased in cells overexpressing eukaryotic initiation factor 4E. *Proceedings of the National Academy of Sciences of the United States*, **93**(3), 1065–1070.
44. Thoreen CC, *et al.* (2012) A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature*, **485**(7396), 109–113.
45. Hsieh AC, *et al.* (2012) The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature*, **485**(7396), 55–61.
46. Iadevaia V, *et al.* (2008) All translation elongation factors and the e, f, and h subunits of translation initiation factor 3 are encoded by 5′-terminal oligopyrimidine (TOP) mRNAs. *RNA*, **14**(9), 1730–1736.
47. Fonseca BD, *et al.* (2018) LARP1 on TOP of ribosome production. *Wiley Interdisciplinary Reviews: RNA*, e1480.
48. Feoktistova K, *et al.* (2013) Human eIF4E promotes mRNA restructuring by stimulating eIF4A helicase activity. *Proceedings of the National Academy of Sciences of the United States*, **110**(33), 13339–13344.
49. Martin F, *et al.* (2011) Cap-assisted internal initiation of translation of histone H4. *Molecular Cell*, **41**(2), 197–209.
50. Cohen N, *et al.* (2001) PML RING suppresses oncogenic transformation by reducing the affinity of eIF4E for mRNA. *The EMBO Journal*, **20**(16), 4547–4559.
51. Topisirovic I, *et al.* (2009) Molecular dissection of the eukaryotic initiation factor 4E (eIF4E) export-competent RNP. *The EMBO Journal*, **28**(8), 1087–1098.
52. Liu W, *et al.* (2011) Structural basis for nematode eIF4E binding an m(2,2,7)G-Cap and its implications for translation initiation. *Nucleic Acids Research*, **39**(20), 8820–8832.
53. Sorrells DL, *et al.* (1998) Detection of eIF4E gene amplification in breast cancer by competitive PCR. *Annals of Surgical Oncology*, **5**(3), 232–237.
54. Sorrells DL, *et al.* (1999) Competitive PCR to detect eIF4E gene amplification in head and neck cancer. *Head & Neck*, **21**(1), 60–65.
55. Jones RM, *et al.* (1996) An essential E box in the promoter of the gene encoding the mRNA cap-binding protein (eukaryotic initiation factor 4E) is a target for activation by c-myc. *Molecular and Cellular Biology*, **16**(9), 4754–4764.
56. Lin CJ, *et al.* (2008) c-Myc and eIF4F are components of a feedforward loop that links transcription and translation. *Cancer Research*, **68**(13), 5326–5334.
57. Lynch M, *et al.* (2005) hnRNP K binds a core polypyrimidine element in the eukaryotic translation initiation factor 4E (eIF4E) promoter, and its regulation of eIF4E contributes to neoplastic transformation. *Molecular and Cellular Biology*, **25**(15), 6436–6453.

58. Khanna-Gupta A, *et al.* (2012) Up-regulation of translation eukaryotic initiation factor 4E in nucleophosmin 1 haploinsufficient cells results in changes in CCAAT enhancer-binding protein alpha activity: Implications in myelodysplastic syndrome and acute myeloid leukemia. *Journal of Biological Chemistry*, **287**(39), 32728–32737.
59. Topisirovic I, *et al.* (2003) Aberrant eukaryotic translation initiation factor 4E-dependent mRNA transport impedes hematopoietic differentiation and contributes to leukemogenesis. *Molecular and Cellular Biology*, **23**(24), 8992–9002.
60. Topisirovic I, *et al.* (2003) The proline-rich homeodomain protein, PRH, is a tissue-specific inhibitor of eIF4E-dependent cyclin D1 mRNA transport and growth. *The EMBO Journal*, **22**(3), 689–703.
61. Siddiqui N and Sonenberg N. (2015) Sonenberg, signalling to eIF4E in cancer. *Biochemical Society Transactions*, **43**(5), 763–772.
62. Joshi S and Platanias LC. (2014) Mnk kinase pathway: Cellular functions and biological outcomes. *World Journal of Biological Chemistry*, **5**(3), 321–333.
63. Lu C, *et al.* (2016) Targeting translation: eIF4E as an emerging anti-cancer drug target. *Expert Reviews in Molecular Medicine*, **18**, e2.
64. Wendel HG, *et al.* (2004) Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature*, **428**(6980), 332–337.
65. Topisirovic I, Ruiz-Gutierrez M and Borden KL. (2004) Phosphorylation of the eukaryotic translation initiation factor eIF4E contributes to its transformation and mRNA transport activities. *Cancer Research*, **64**(23), 8639–8642.
66. Wendel HG, *et al.* (2007) Dissecting eIF4E action in tumorigenesis. *Genes & Development*, **21**(24), 3232–3237.
67. Graff JR, *et al.* (2007) Therapeutic suppression of translation initiation factor eIF4E expression reduces tumor growth without toxicity. *Journal of Clinical Investigation*, **117**(9), 2638–2648.
68. Borden KL and Culjkovic-Kraljacic B. (2010) Ribavirin as an anti-cancer therapy: Acute myeloid leukemia and beyond? *Leukemia & Lymphoma*, **51**(10), 1805–1815.
69. Diab S, *et al.* (2014) Discovery of 5-(2-(phenylamino)pyrimidin-4-yl)thiazol-2(3H)-one derivatives as potent Mnk2 inhibitors: Synthesis, SAR analysis and biological evaluation. *ChemMedChem*, **9**(5), 962–972.
70. Grifo JA, *et al.* (1983) New initiation factor activity required for globin mRNA translation. *Journal of Biological Chemistry*, **258**(9), 5804–5810.
71. Ray BK, *et al.* (1983) Role of mRNA competition in regulating translation: Further characterization of mRNA discriminatory initiation factors. *Proceedings of the National Academy of Sciences of the United States*, **80**(3), 663–667.
72. Rogers GW Jr, Komar AA and Merrick WC. (2002) eIF4A: The godfather of the DEAD box helicases. *Progress in Nucleic Acid Research and Molecular Biology*, **72**, 307–331.

73. Lu WT, *et al.* (2014) The diverse roles of the eIF4A family: You are the company you keep. *Biochemical Society Transactions*, **42**(1), 166–172.
74. Andreou AZ and Klostermeier D. (2013) The DEAD-box helicase eIF4A: Paradigm or the odd one out? *RNA Biology*, **10**(1), 19–32.
75. Galicia-Vazquez G, *et al.* (2012) A cellular response linking eIF4AI activity to eIF4AII transcription. *RNA*, **18**(7), 1373–1384.
76. Meijer HA, *et al.* (2013) Translational repression and eIF4A2 activity are critical for microRNA-mediated gene regulation. *Science*, **340**(6128), 82–85.
77. Fabian MR, *et al.* (2011) miRNA-mediated deadenylation is orchestrated by GW182 through two conserved motifs that interact with CCR4-NOT. *Nature Structural & Molecular Biology*, **18**(11), 1211–1217.
78. Heerma van Voss MR, van Diest PJ and Raman V. (2017) Targeting RNA helicases in cancer: The translation trap. *Biochimica et Biophysica Acta — Reviews on Cancer*, **1868**(2), 510–520.
79. Jin HY and Xiao C. (2018) An integrated polysome profiling and ribosome profiling method to investigate in vivo translatome. *Methods in Molecular Biology*, **1712**, 1–18.
80. Chang JH, *et al.* (2009) Crystal structure of the eIF4A-PDCD4 complex. *Proceedings of the National Academy of Sciences of the United States*, **106**(9), 3148–3153.
81. Li Q, *et al.* (1999) Eukaryotic translation initiation factor 4AIII (eIF4AIII) is functionally distinct from eIF4AI and eIF4AII. *Molecular and Cellular Biology*, **19**(11), 7336–7346.
82. Chan CC, *et al.* (2004) eIF4A3 is a novel component of the exon junction complex. *RNA*, **10**(2), 200–209.
83. Raza F, Waldron JA and Quesne JL. (2015) Translational dysregulation in cancer: eIF4A isoforms and sequence determinants of eIF4A dependence. *Biochemical Society Transactions*, **43**(6), 1227–1233.
84. Cencic R and Pelletier J. (2016) Hippuristanol — A potent steroid inhibitor of eukaryotic initiation factor 4A. *Translation (Austin)*, **4**(1), e1137381.
85. Pan L, *et al.* (2014) Rocaglamide, silvestrol and structurally related bioactive compounds from Aglaia species. *Natural Product Reports*, **31**(7), 924–939.
86. Zhang X, *et al.* (2020) Targeting translation initiation by synthetic rocaglates for treating MYC-driven lymphomas. *Leukemia*, **34**(1), 138–150.
87. Polier G, *et al.* (2012) The natural anti-cancer compounds rocaglamides inhibit the Raf-MEK-ERK pathway by targeting prohibitin 1 and 2. *Chemistry & Biology*, **19**(9), 1093–1104.
88. Chang LS, *et al.* (2020) Targeting protein translation by rocaglamide and didesmethylrocaglamide to treat MPNST and other sarcomas. *Molecular Cancer Therapeutics*, **19**(3), 731–741.
89. Shen S, *et al.* (2019) An epitranscriptomic mechanism underlies selective mRNA translation remodelling in melanoma persister cells. *Nature Communications*, **10**(1), 5713.

90. Chan K, *et al.* (2019) eIF4A supports an oncogenic translation program in pancreatic ductal adenocarcinoma. *Nature Communications*, **10**(1), 5151.
91. Pal I, *et al.* (2019) Targeting translation of mRNA as a therapeutic strategy in cancer. *Current Hematologic Malignancy Reports*, **14**(4), 219–227.
92. Cerezo M, *et al.* (2018) Translational control of tumor immune escape via the eIF4F-STAT1-PD-L1 axis in melanoma. *Nature Medicine*, **24**(12), 1877–1886.
93. Northcote RJ. (1991) Metabolic parameters: How important are pharmacologically-induced changes? *Journal of Hypertension, Supplement*, **9**(7), S21–S25.
94. Low WK, *et al.* (2005) Inhibition of eukaryotic translation initiation by the marine natural product pateamine A. *Molecular Cell*, **20**(5), 709–722.
95. Bordeleau ME, *et al.* (2006) RNA-mediated sequestration of the RNA helicase eIF4A by Pateamine A inhibits translation initiation. *Chemistry & Biology*, **13**(12), 1287–1295.
96. Thompson PA, *et al.* (2017) Pre-clinical evaluation of eFT226, a novel, potent and selective eIF4A inhibitor with anti-tumor activity in B-cell malignancies. *Blood*, **130**(Supplement 1), 1530–1530.
97. Rozen F, *et al.* (1990) Bidirectional RNA helicase activity of eucaryotic translation initiation factors 4A and 4F. *Molecular and Cellular Biology*, **10**(3), 1134–1144.
98. Jackson RJ, Hellen CU, and Pestova TV. (2010) The mechanism of eukaryotic translation initiation and principles of its regulation. *Nature Reviews Molecular Cell Biology*, **11**(2), 113–127.
99. Kozak M. (2002) Pushing the limits of the scanning mechanism for initiation of translation. *Gene*, **299**(1-2), 1–34.
100. Shahbazian D, *et al.* (2010) eIF4B controls survival and proliferation and is regulated by proto-oncogenic signaling pathways. *Cell Cycle*, **9**(20), 4106–4109.
101. Liu X, Moshiri H and Walker S. (2019) Binding to the ribosome by eukaryotic initiation factor 4B drives yeast translational control in response to urea. *bioRxiv*, 819672.
102. Ozes AR, *et al.* (2011) Duplex unwinding and ATPase activities of the DEAD-box helicase eIF4A are coupled by eIF4G and eIF4B. *Journal of Molecular Biology*, **412**(4), 674–687.
103. Methot N, Song MS and Sonenberg N. (1996) A region rich in aspartic acid, arginine, tyrosine, and glycine (DRYG) mediates eukaryotic initiation factor 4B (eIF4B) self-association and interaction with eIF3. *Molecular and Cellular Biology*, **16**(10), 5328–5334.
104. Horvilleur E, *et al.* (2014) A role for eukaryotic initiation factor 4B overexpression in the pathogenesis of diffuse large B-cell lymphoma. *Leukemia*, **28**(5), 1092–1102.
105. Shahbazian D, *et al.* (2006) The mTOR/PI3K and MAPK pathways converge on eIF4B to control its phosphorylation and activity. *The EMBO Journal*, **25**(12), 2781–2791.
106. Bi X, Ren J and Goss DJ. (2000) Wheat germ translation initiation factor eIF4B affects eIF4A and eIFiso4F helicase activity by increasing the ATP binding affinity of eIF4A. *Biochemistry*, **39**(19), 5758–5765.

107. Marintchev A, *et al.* (2009) Topology and regulation of the human eIF4A/4G/4H helicase complex in translation initiation. *Cell*, **136**(3), 447–460.
108. Nielsen KH, *et al.* (2011) Synergistic activation of eIF4A by eIF4B and eIF4G. *Nucleic Acids Research*, **39**(7), 2678–2689.
109. Rozovsky N, Butterworth AC and Moore MJ. (2008) Interactions between eIF4AI and its accessory factors eIF4B and eIF4H. *RNA*, **14**(10), 2136–2148.
110. Kapadia B, *et al.* (2018) Fatty Acid Synthase induced S6Kinase facilitates USP11-eIF4B complex formation for sustained oncogenic translation in DLBCL. *Nature Communications*, **9**(1), 829.
111. Wilker EW, *et al.* (2007) 14-3-3sigma controls mitotic translation to facilitate cytokinesis. *Nature*, **446**(7133), 329–332.
112. Jeffrey IW, *et al.* (2002) Inhibition of protein synthesis in apoptosis: Differential requirements by the tumor necrosis factor alpha family and a DNA-damaging agent for caspases and the double-stranded RNA-dependent protein kinase. *Cancer Research*, **62**(8), 2272–2280.
113. Kim HK, *et al.* (2011) A gene expression signature of acquired chemoresistance to cisplatin and fluorouracil combination chemotherapy in gastric cancer patients. *PLoS One*, **6**(2), e16694.
114. Sun Y, *et al.* (2007) Identification of differential proteins in nasopharyngeal carcinoma cells with p53 silence by proteome analysis. *FEBS Letters*, **581**(1), 131–139.
115. Lin YW and Aplan PD. (2007) Gene expression profiling of precursor T-cell lymphoblastic leukemia/lymphoma identifies oncogenic pathways that are potential therapeutic targets. *Leukemia*, **21**(6), 1276–1284.
116. Liang Y, *et al.* (2013) Analysis of 20 genes at chromosome band 12q13: RACGAP1 and MCRS1 overexpression in nonsmall-cell lung cancer. *Genes, Chromosomes & Cancer*, **52**(3), 305–315.
117. Shahbazian D, *et al.* (2010) Control of cell survival and proliferation by mammalian eukaryotic initiation factor 4B. *Molecular and Cellular Biology*, **30**(6), 1478–1485.
118. Zhang Y, *et al.* (2011) Activation and up-regulation of translation initiation factor 4B contribute to arsenic-induced transformation. *Molecular Carcinogenesis*, **50**(7), 528–538.
119. Degen M, *et al.* (2012) MAPK/ERK-dependent translation factor hyperactivation and dysregulated laminin gamma2 expression in oral dysplasia and squamous cell carcinoma. *The American Journal of Pathology*, **180**(6), 2462–2478.
120. Ren K, *et al.* (2013) The over-expression of Pim-2 promote the tumorigenesis of prostatic carcinoma through phosphorylating eIF4B. *Prostate*, **73**(13), 1462–1469.
121. Gu X, *et al.* (2013) Integration of mTOR and estrogen-ERK2 signaling in lymphangioleiomyomatosis pathogenesis. *Proceedings of the National Academy of Sciences of the United States*, **110**(37), 14960–14965.
122. Zou ZQ, *et al.* (2012) The novel dual PI3K/mTOR inhibitor GDC-0941 synergizes with the MEK inhibitor U0126 in non-small cell lung cancer cells. *Molecular Medicine Reports*, **5**(2), 503–508.

123. Peters TL, *et al.* (2016) Control of translational activation by PIM kinase in activated B-cell diffuse large B-cell lymphoma confers sensitivity to inhibition by PIM447. *Oncotarget*, **7**(39), 63362–63373.
124. Cervantes-Gomez F, *et al.* (2019) PIM kinase inhibitor, AZD1208, inhibits protein translation and induces autophagy in primary chronic lymphocytic leukemia cells. *Oncotarget*, **10**(29), 2793–2809.
125. Vaysse C, *et al.* (2015) Key contribution of eIF4H-mediated translational control in tumor promotion. *Oncotarget*, **6**(37), 39924–39940.
126. Capossela S, *et al.* (2012) Growth defects and impaired cognitive-behavioral abilities in mice with knockout for Eif4h, a gene located in the mouse homolog of the Williams-Beuren syndrome critical region. *The American Journal of Pathology*, **180**(3), 1121–1135.
127. Richter-Cook NJ, *et al.* (1998) Purification and characterization of a new eukaryotic protein translation factor. Eukaryotic initiation factor 4H. *Journal of Biological Chemistry*, **273**(13), 7579–7587.
128. Kulak NA, *et al.* (2014) Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. *Nature Methods*, **11**(3), 319–324.
129. Fiume G, *et al.* (2013) Eukaryotic initiation factor 4H Is under transcriptional control of p65/NF-kappaB. *PLoS One*, **8**(6), e66087.
130. Sun Y, *et al.* (2012) The eukaryotic initiation factor eIF4H facilitates loop-binding, repetitive RNA unwinding by the eIF4A DEAD-box helicase. *Nucleic Acids Research*, **40**(13), 6199–6207.
131. Lindqvist L, Imataka H and Pelletier J. (2008) Cap-dependent eukaryotic initiation factor-mRNA interactions probed by cross-linking. *RNA*, **14**(5), 960–969.
132. Wu D, *et al.* (2011) An alternative splicing isoform of eukaryotic initiation factor 4H promotes tumorigenesis in vivo and is a potential therapeutic target for human cancer. *International Journal of Cancer*, **128**(5), 1018–1030.
133. Bai Y, *et al.* (2017) Overexpression of miR-519d in lung adenocarcinoma inhibits cell proliferation and invasion via the association of eIF4H. *Tumour Biology*, **39**(3), 1010428317694566.
134. Singh M, Trivedi R and Mishra DP. (2017) The eukaryotic translation initiation factor 4H regulates proliferation, migration, and invasion in cancer cells. *AACR*.
135. Doepker RC, *et al.* (2004) Herpes simplex virus virion host shutoff protein is stimulated by translation initiation factors eIF4B and eIF4H. *Journal of Virology*, **78**(9), 4684–4699.
136. Sriram A, Bohlen J and Teleman AA. (2018) Translation acrobatics: How cancer cells exploit alternate modes of translational initiation. *EMBO Reports*, **19**(10).
137. Chu J, *et al.* (2016) Translation initiation factors: Reprogramming protein synthesis in cancer. *Trends in Cell Biology*, **26**(12), 918–933.
138. Sokabe M, Fraser CS and Hershey JW. (2012) The human translation initiation multi-factor complex promotes methionyl-tRNAi binding to the 40S ribosomal subunit. *Nucleic Acids Research*, **40**(2), 905–913.

139. Nag N, *et al.* (2016) eIF1A/eIF5B interaction network and its functions in translation initiation complex assembly and remodeling. *Nucleic Acids Research*, **44**(15), 7441–7456.
140. Acker MG, *et al.* (2006) Interaction between eukaryotic initiation factors 1A and 5B is required for efficient ribosomal subunit joining. *Journal of Biological Chemistry*, **281**(13), 8469–8475.
141. Asano K, *et al.* (2000) A multifactor complex of eukaryotic initiation factors, eIF1, eIF2, eIF3, eIF5, and initiator tRNA(Met) is an important translation initiation intermediate in vivo. *Genes & Development*, **14**(19), 2534–2546.
142. Beilsten-Edmands V, *et al.* (2015) eIF2 interactions with initiator tRNA and eIF2B are regulated by post-translational modifications and conformational dynamics. *Cell Discovery*, **1**, 15020.
143. Kashiwagi K, *et al.* (2016) Crystal structure of eukaryotic translation initiation factor 2B. *Nature*, **531**(7592), 122–125.
144. LeFebvre AK, *et al.* (2006) Translation initiation factor eIF4G-1 binds to eIF3 through the eIF3e subunit. *Journal of Biological Chemistry*, **281**(32), 22917–22932.
145. Valasek L, *et al.* (2004) Interactions of eukaryotic translation initiation factor 3 (eIF3) subunit NIP1/c with eIF1 and eIF5 promote pre-initiation complex assembly and regulate start codon selection. *Molecular and Cellular Biology*, **24**(21), 9437–9455.
146. Zhao P, *et al.* (2017) Eukaryotic translation initiation factor 4G (eIF4G) coordinates interactions with eIF4A, eIF4B, and eIF4E in binding and translation of the barley yellow dwarf virus 3′ cap-independent translation element (BTE). *Journal of Biological Chemistry*, **292**(14), 5921–5931.
147. Korneeva NL, *et al.* (2001) Characterization of the two eIF4A-binding sites on human eIF4G-1. *Journal of Biological Chemistry*, **276**(4), 2872–2879.
148. Papadopoulos E, *et al.* (2014) Structure of the eukaryotic translation initiation factor eIF4E in complex with 4EGI-1 reveals an allosteric mechanism for dissociating eIF4G. *Proceedings of the National Academy of Sciences of the United States*, **111**(31), E3187–E3195.
149. Pestova TV, *et al.* (2001) Molecular mechanisms of translation initiation in eukaryotes. *Proceedings of the National Academy of Sciences of the United States*, **98**(13), 7029–7036.
150. Jennings MD, *et al.* (2013) eIF2B promotes eIF5 dissociation from eIF2*GDP to facilitate guanine nucleotide exchange for translation initiation. *Genes & Development*, **27**(24), 2696–2707.
151. Singh CR, *et al.* (2006) An eIF5/eIF2 complex antagonizes guanine nucleotide exchange by eIF2B during translation initiation. *The EMBO Journal*, **25**(19), 4537–4546.
152. Klinge S, *et al.* (2011) Crystal structure of the eukaryotic 60S ribosomal subunit in complex with initiation factor 6. *Science*, **334**(6058), 941–948.

153. Shuda M, *et al.* (2000) Enhanced expression of translation factor mRNAs in hepatocellular carcinoma. *Anticancer Research*, **20**(4), 2489–2494.
154. Chen Y, *et al.* (2010) Autoantibodies to tumor-associated antigens combined with abnormal alpha-fetoprotein enhance immunodiagnosis of hepatocellular carcinoma. *Cancer Letters*, **289**(1), 32–39.
155. Dai L, *et al.* (2017) Targeting EIF4F complex in non-small cell lung cancer cells. *Oncotarget*, **8**(33), 55731–55735.
156. Lyu S, *et al.* (2020) High expression of eIF4A2 is associated with a poor prognosis in esophageal squamous cell carcinoma. *Oncology Letters*, **20**(5), 177.
157. Modelska A, *et al.* (2015) The malignant phenotype in breast cancer is driven by eIF4A1-mediated changes in the translational landscape. *Cell Death & Disease*, **6**, e1603.
158. Eberle J, Krasagakis K and Orfanos CE. (1997) Translation initiation factor eIF-4A1 mRNA is consistently overexpressed in human melanoma cells in vitro. *International Journal of Cancer*, **71**(3), 396–401.
159. Rico C, *et al.* (2012) Pharmacological targeting of mammalian target of Rapamycin inhibits ovarian granulosa cell tumor growth. *Carcinogenesis*, **33**(11), 2283–2292.
160. Yang J, *et al.* (2013) eIF4B phosphorylation by pim kinases plays a critical role in cellular transformation by Abl oncogenes. *Cancer Research*, **73**(15), 4898–908.
161. Heikkinen T, *et al.* (2013) Eukaryotic translation initiation factor 4E (eIF4E) expression is associated with breast cancer tumor phenotype and predicts survival after anthracycline chemotherapy treatment. *Breast Cancer Research Treatment*, **141**(1), 79–88.
162. Holm N, *et al.* (2008) A prospective trial on initiation factor 4E (eIF4E) overexpression and cancer recurrence in node-negative breast cancer. *Annals of Surgical Oncology*, **15**(11), 3207–3215.
163. Byrnes K, *et al.* (2006) High eIF4E, VEGF, and microvessel density in stage I to III breast cancer. *Annals of Surgery*, **243**(5), 684–690; discussion 691–692.
164. Rosenwald IB, *et al.* (1993) Elevated levels of cyclin D1 protein in response to increased expression of eukaryotic initiation factor 4E. *Molecular and Cellular Biology*, **13**(12), 7358–7363.
165. Ruggero D, *et al.* (2004) The translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in lymphomagenesis. *Nature Medicine*, **10**(5), 484–486.
166. Crew JP, *et al.* (2000) Eukaryotic initiation factor-4E in superficial and muscle invasive bladder cancer and its correlation with vascular endothelial growth factor expression and tumour progression. *British Journal of Cancer*, **82**(1), 161–166.
167. Tejada S, *et al.* (2009) Eukaryotic initiation factors (eIF) 2alpha and 4E expression, localization, and phosphorylation in brain tumors. *Journal of Histochemistry & Cytochemistry*, **57**(5), 503–512.
168. Lee JW, *et al.* (2005) eIF-4E expression is associated with histopathologic grades in cervical neoplasia. *Human Pathology*, **36**(11), 1197–1203.

169. Yang SX, *et al.* (2007) Expression levels of eIF4E, VEGF, and cyclin D1, and correlation of eIF4E with VEGF and cyclin D1 in multi-tumor tissue microarray. *Oncology Reports*, **17**(2), 281–287.
170. Wang XL, *et al.* (2012) Detection of eukaryotic translation initiation factor 4E and its clinical significance in hepatocellular carcinoma. *World Journal of Gastroenterology*, **18**(20), 2540–2544.
171. Rosenwald IB, *et al.* (2003) Expression of translation initiation factor eIF-2alpha is increased in benign and malignant melanocytic and colonic epithelial neoplasms. *Cancer*, **98**(5), 1080–1088.
172. Franklin S, *et al.* (1999) Detection of the proto-oncogene eIF4E in larynx and hypopharynx cancers. *Archives of Otorhinolaryngology-Head & Neck Surgery*, **125**(2), 177–182.
173. Nathan CO, *et al.* (1997) Detection of the proto-oncogene eIF4E in surgical margins may predict recurrence in head and neck cancer. *Oncogene*, **15**(5), 579–584.
174. Hsu HS, *et al.* (2011) MDM2 is overexpressed and regulated by the eukaryotic translation initiation factor 4E (eIF4E) in human squamous cell carcinoma of esophagus. *Annals of Surgical Oncology*, **18**(5), 1469–1477.
175. Salehi Z and Mashayekhi F (2006) Expression of the eukaryotic translation initiation factor 4E (eIF4E) and 4E-BP1 in esophageal cancer. *Clinical Biochemistry*, **39**(4), 404–409.
176. Fan S, *et al.* (2009) Phosphorylated eukaryotic translation initiation factor 4 (eIF4E) is elevated in human cancer tissues. *Cancer Biology & Therapy*, **8**(15), 1463–1469.
177. Furic L, *et al.* (2010) eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. *Proceedings of the National Academy of Sciences of the United States*, **107**(32), 14134–14139.
178. Yoshizawa A, *et al.* (2010) Overexpression of phospho-eIF4E is associated with survival through AKT pathway in non-small cell lung cancer. *Clinical Cancer Research*, **16**(1), 240–248.
179. Noske A, *et al.* (2008) Activation of mTOR in a subgroup of ovarian carcinomas: Correlation with p-eIF-4E and prognosis. *Oncology Reports*, **20**(6), 1409–1417.
180. Liang S, *et al.* (2013) Upregulation of the eIF4E signaling pathway contributes to the progression of gastric cancer, and targeting eIF4E by perifosine inhibits cell growth. *Oncology Reports*, **29**(6), 2422–2430.
181. Bauer C, *et al.* (2002) Overexpression of the eukaryotic translation initiation factor 4G (eIF4G-1) in squamous cell lung carcinoma. *International Journal of Cancer*, **98**(2), 181–185.
182. Tu L, *et al.* (2010) Over-expression of eukaryotic translation initiation factor 4 gamma 1 correlates with tumor progression and poor prognosis in nasopharyngeal carcinoma. *Molecular Cancer*, **9**, 78.

183. Wu M, *et al.* (2016) Anti-cancer effect of cap-translation inhibitor 4EGI-1 in human glioma U87 cells: Involvement of mitochondrial dysfunction and ER stress. *Cellular Physiology and Biochemistry*, **40**(5), 1013–1028.
184. Cencic R, *et al.* (2011) Reversing chemoresistance by small molecule inhibition of the translation initiation complex eIF4F. *Proceedings of the National Academy of Sciences of the United States*, **108**(3), 1046–1051.
185. Urtishak KA, *et al.* (2019) Targeting EIF4E signaling with ribavirin in infant acute lymphoblastic leukemia. *Oncogene*, **38**(13), 2241–2262.
186. Jia Y, *et al.* (2010) Design, synthesis and evaluation of analogs of initiation factor 4E (eIF4E) cap-binding antagonist Bn7-GMP. *European Journal of Medicinal Chemistry*, **45**(4), 1304–13013.
187. Jacobson BA, *et al.* (2013) Targeting eukaryotic translation in mesothelioma cells with an eIF4E-specific antisense oligonucleotide. *PLoS One*, **8**(11), e81669.
188. Stelzer MK, *et al.* (2010) Rapamycin inhibits anal carcinogenesis in two pre-clinical animal models. *Cancer Prevention Research (Philadelphia, PA)*, **3**(12), 1542–1551.
189. Ito D, *et al.* (2006) In vivo anti-tumor effect of the mTOR inhibitor CCI-779 and gemcitabine in xenograft models of human pancreatic cancer. *International Journal of Cancer*, **118**(9), 2337–2343.
190. Mabuchi S, *et al.* (2007) RAD001 (Everolimus) delays tumor onset and progression in a transgenic mouse model of ovarian cancer. *Cancer Research*, **67**(6), 2408–2413.
191. Rivera VM, *et al.* (2011) Ridaforolimus (AP23573; MK-8669), a potent mTOR inhibitor, has broad anti-tumor activity and can be optimally administered using intermittent dosing regimens. *Molecular Cancer Therapeutics*, **10**(6), 1059–1071.
192. Francipane MG and Lagasse E. (2013) Selective targeting of human colon cancer stem-like cells by the mTOR inhibitor Torin-1. *Oncotarget*, **4**(11), 1948–1962.
193. Yin X, *et al.* (2019) TFE3 fusions escape from controlling of mTOR signaling pathway and accumulate in the nucleus promoting genes expression in Xp11.2 translocation renal cell carcinomas. *Journal of Experimental & Clinical Cancer Research*, **38**(1), 119.
194. Yu K, *et al.* (2010) Beyond rapalog therapy: Pre-clinical pharmacology and anti-tumor activity of WYE-125132, an ATP-competitive and specific inhibitor of mTORC1 and mTORC2. *Cancer Research*, **70**(2), 621–631.
195. Peters TL, *et al.* (2018) Target-Based Screening against eIF4A1 Reveals the Marine Natural Product Elatol as a Novel Inhibitor of Translation Initiation with In Vivo Antitumor Activity. *Clinical Cancer Research*, **24**(17), 4256–4270.
196. Tillotson J, *et al.* (2017) ATP-competitive, marine derived natural products that target the DEAD box helicase, eIF4A. *Bioorganic & Medicinal Chemistry Letters*, **27**(17), 4082–4085.

197. Yun SJ, *et al.* (2018) The mechanistic insight of a specific interaction between 15d-Prostaglandin-J2 and eIF4A suggests an evolutionary conserved role across species. *Biology Open*, **7**(11).
198. Shin SW, *et al.* (2009) 15d-PGJ2 induces apoptosis by reactive oxygen species-mediated inactivation of Akt in leukemia and colorectal cancer cells and shows in vivo anti-tumor activity. *Clinical Cancer Research*, **15**(17), 5414–5425.
199. Abdelkrim YZ, *et al.* (2018) The steroid derivative 6-aminocholestanol inhibits the DEAD-box helicase eIF4A (LieIF4A) from the Trypanosomatid parasite Leishmania by perturbing the RNA and ATP binding sites. *Molecular and Biochemical Parasitology*, **226**, 9–19.
200. Shen L and Pelletier J. (2020) Selective targeting of the DEAD-box RNA helicase eukaryotic initiation factor (eIF) 4A by natural products. *Natural Product Reports*, **37**(5), 609–616.
201. Zhu M, *et al.* (2020) Sanguinarine suppresses migration and metastasis in colorectal carcinoma associated with the inversion of EMT through the Wnt/beta-catenin signaling. *Clinical and Translational Medicine*, **10**(1), 1–12.
202. Waldron JA, Raza F. and Le Quesne J. (2018) eIF4A alleviates the translational repression mediated by classical secondary structures more than by G-quadruplexes. *Nucleic Acids Research*, **46**(6), 3075–3087.
203. Ishikawa C, *et al.* (2013) Hippuristanol reduces the viability of primary effusion lymphoma cells both in vitro and in vivo. *Marine Drugs*, **11**(9), 3410–3424.
204. Cunningham TA, Chapman E and Schatz JH. (2018) eIF4A inhibition: Ready for primetime? *Oncotarget*, **9**(85), 35515–35516.
205. Muller D, *et al.* (2019) eIF4A inhibition circumvents uncontrolled DNA replication mediated by 4E-BP1 loss in pancreatic cancer. *JCI Insight*, **4**(21).
206. Bordeleau ME, *et al.* (2006) Functional characterization of IRESes by an inhibitor of the RNA helicase eIF4A. *Nature Chemical Biology*, **2**(4), 213–220.
207. Kuznetsov G, *et al.* (2009) Potent in vitro and in vivo anti-cancer activities of des-methyl, des-amino pateamine A, a synthetic analogue of marine natural product pateamine A. *Molecular Cancer Therapeutics*, **8**(5), 1250–1260.
208. Thompson PA, *et al.* (2021) Targeting oncogene mRNA translation in B-cell malignancies with eFT226, a potent and selective inhibitor of eIF4A. *Molecular Cancer Therapeutics*, **20**(1), 26–36.
209. Naineni SK, *et al.* (2020) A comparative study of small molecules targeting eIF4A. *RNA*, **26**(5), 541–549.
210. Rentsch KM, *et al.* (1997) Comparative pharmacokinetic and cytotoxic analysis of three different formulations of mitoxantrone in mice. *British Journal of Cancer*, **75**(7), 986–992.

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Chapter 8

The mTOR Complexes: Coordinating Metabolism and Translation

Nikhil Patel and Estela Jacinto*

Department of Biochemistry and Molecular Biology,
Rutgers Univ.-Robert Wood Johnson Medical School,
683 Hoes Lane, Piscataway, NJ 08854.

Abstract

The mTOR signaling cascade controls cell growth and metabolism and its deregulation underlies a variety of metabolic disorders. The mTOR pathway is characterized by two unique mTOR complexes: mTORC1 and mTORC2. mTORC1 is activated in the presence of nutrients to promote cell growth and proliferation while mTORC2 responds to nutrient fluctuations in order to restore metabolic homeostasis and maintain cell survival. Due to the pivotal role of the mTORCs in cell growth and survival, mTOR signaling is often upregulated in many cancers. mTOR is involved in many aspects of anabolic metabolism but the function of mTORC1 in protein synthesis is perhaps the most well studied. In this review, we will discuss how both mTORC1 and mTORC2 control protein synthesis. We begin with an in-depth analysis of the two multiprotein complexes and the specific upstream and downstream proteins involved in their signaling. In particular, we elaborate on how mTORC1

*Corresponding author's email: jacintes@rwjms.rutgers.edu

regulates translation via its targets S6K1 and 4E-BP1. We then discuss how both mTORC1 and mTORC2 could be involved in the regulation of other translation regulators such as RNA binding proteins and RSK, and elaborate on the role of mTOR in ribosome biogenesis. Finally, we review how mTOR also controls the processes that provide building blocks for protein synthesis including amino acids, ribose, and nucleotides. We also consider the role of mTOR in controlling catabolic processes such as autophagy and the ubiquitin proteasome system which are critical for providing intracellular nutrient sources as well as maintaining cellular proteostasis. This review provides a thorough background on how mTOR can function as a molecular rheostat to control global protein translation in response to nutrient availability.

Key words: mTORC1, mTORC2, translation, protein synthesis, metabolism

1. Introduction

The story of TOR (target of rapamycin) began in 1964, when a team of scientists took an expedition to the Easter Island, also known as Rapa Nui. They collected soil samples which led to the isolation of a macrolide compound from *Streptomyces hygroscopicus* that exhibited remarkable antifungal properties, later named rapamycin (clinically referred to as sirolimus). Rapamycin was found to be a powerful immunosuppressant that also stopped cancer cell proliferation. Rapamycin's mechanism of action remained an enigma until 1991, when Michael Hall and colleagues used genetic screens in yeast to identify TOR1 and TOR2. Rapamycin complexes with prolyl isomerase FKBP12 to form a heterodimer that allosterically binds to the FKBP-rapamycin-binding (FRB) domain of TOR to exert an inhibitory effect [1]. Following Hall's discovery of the yeast TOR components, their mammalian counterparts were discovered and aptly named mammalian target of rapamycin, or mTOR [2–4]. mTOR is an atypical serine/threonine kinase in the PI3K-related kinase (PIKK) family of lipid kinases. It forms the catalytic subunit of two functionally and biochemically distinct multiprotein complexes named mTORC1 and mTORC2 (Fig. 1). Both mTOR complexes contain the core mTOR component, mLST8 (mammalian lethal with SEC13 protein 8) [5], and

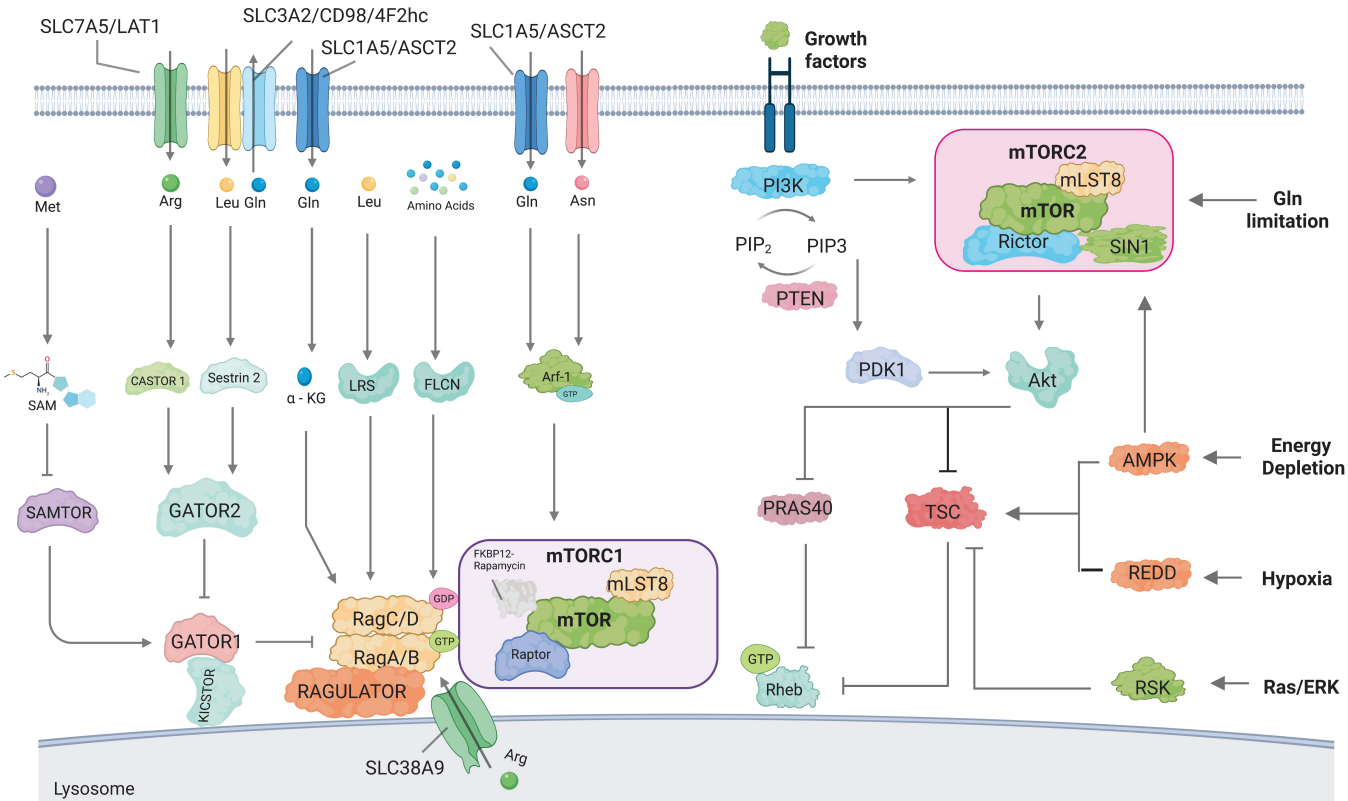

Figure 1. mTOR forms two distinct protein complexes, mTORC1 and mTORC2.

mTORC1 is activated by nutrients such as amino acids via Rag-dependent and -independent mechanisms to promote anabolic metabolism and cell growth. mTORC2 activation is enhanced by PI3K signaling and nutrient limitation to promote cell growth and/or survival.

DEPTOR (DEP domain-containing protein 6), a negative regulator of mTOR [6]. mTORC1 is distinguished from mTORC2 by the presence of Raptor (regulatory-associated protein of mTOR), a protein responsible for complex localization and substrate recruitment, [7, 8] and PRAS40 (proline-rich Akt substrate of 40 kDa), an insulin-responsive mTORC1 inhibitor [9, 10]. The mTORC2-specific components are Rictor (rapamycin insensitive companion of mTOR) [11], SIN1 (stress-activated protein kinase (SAPK)-interacting protein 1) [12], and Protor (protein observed with rictor) [13]. The mTOR signaling pathway regulates cellular metabolism and growth, and is implicated in various disorders like cancer and diabetes. Since its discovery, many studies have been aimed at elucidating how mTOR reprograms metabolism and nutrient uptake, which is critical for cell growth and survival [14, 15]. mTOR inhibitors are used to target metabolic reprogramming in cancer cells, preventing tumor growth [16].

2. mTORC1 regulation

mTORC1 is activated in the presence of nutrients to promote anabolism while suppressing autophagy [17]. The archetypal mechanism of mTORC1 activation is recruitment to the surface of the lysosome, a hub for degrading and recycling macromolecules and nutrients, where it is activated by the Ras-related GTP binding proteins (Rags). The Rag proteins, a family of four small GTPases that form obligate heterodimers between RagA or RagB with RagC or RagD, are tethered to the lysosomal surface via association with the pentameric Ragulator comprised of LAMTOR1/p18, LAMTOR2/p14, LAMTOR3/MP1, LAMTOR4/c7ORF59, and LAMTOR5/HBXIP. In physiological conditions, RagA/B is bound to GTP and RagC/D is bound to GDP to foster mTORC1 activity. The Ragulator serves as a scaffold, but also functions as a guanine nucleotide exchange factor (GEF) for RagA/B [18] by sensing intra-lysosomal amino acids through a mechanism dependent on lysosomal v-ATPase (vacuolar H^+-ATPase) complex and loading RagA/B with GTP [19]. Its reciprocal GTPase-activating protein (GAP) is the GATOR1 heterotrimeric complex (consisting of DEPDC5, Npr12, and Npr13) that inhibits mTORC1 [20]. The KICSTOR complex (comprised of Kaptin, ITFG2, c12orf66, and SZT2) is required for tethering GATOR1 to the lysosomal surface for

nutrient dependent control of mTORC1 [21]. GATOR2 by contrast is a pentameric complex (consisting of Mios, WDR24, WDR59, Seh1L, and Sec13) that functions as a positive regulator of mTORC1 activity by interacting with GATOR1 at the surface of the lysosome [20]. The activation of mTORC1 by Rags requires GTP-bound RagA/B and GDP-bound RagC/D. Leucyl-tRNA synthetase (LRS), the main sensor of intracellular leucine levels, acts as a GAP towards RagD in the presence of leucine to promote mTORC1 activity [22]. The folliculin (FLCN) tumor suppressor and its binding partners FNIP1/2 have GAP activity towards RagC/D to trigger mTORC1 activation [23]. Additionally, FLCN is implicated in lysosomal positioning. During growth-limiting conditions, FLCN promotes peri-nuclear clustering of lysosomes via Rab-34 to attenuate mTORC1 activity. Reciprocally, lysosomes disperse to the cell periphery during nutrient replete conditions to bolster mTORC1 activity [24]. Rag protein activity is determined by their guanine nucleotide state; in nutrient replete conditions, activated Rags harbor GTP-bound Rag A/B and GDP-bound Rag C/D. These nucleotide states allow Rags to interact with the Raptor scaffolding component of mTORC1, facilitating its translocation to the lysosomal surface for activation by Rheb (Ras homolog enriched in the brain) [25]. This prototypical mode of mTORC1 activation requires both growth factors and nutrients; growth factors culminate in the activation of small GTPase Rheb, while amino acids regulate the heterodimeric Rag GTPases, ensuring that cell growth is tightly linked to nutrient supply.

Specific amino acids activate mTORC1 via Rag-dependent and -independent mechanisms. Arginine and Leucine bind to CASTOR1 and Sestrin2, respectively, to promote mTORC1 activation [26]. Cellular arginine sensor for mTORC1 complex (CASTOR1) is a sensor of cytosolic arginine that binds and inhibits GATOR2 during arginine starvation. The presence of arginine causes the CASTOR1-GATOR2 heterodimer to dissociate, leading to GATOR2-mediated mTORC1 activation [27]. Additionally, SLC38A9 is an arginine gated amino acid transporter intimately connected with the Rag-Ragulator-v-ATPase complex that affects GTP loading of RagA/RagB, thus providing an input for inside-out lysosomal arginine sensing by mTORC1 [28]. Much like CASTOR1, monomeric Sestrin2 is a leucine sensor upstream of mTORC1 that binds and

inhibits GATOR2 during leucine starvation. Leucine addition causes dissociation of Sestrin2-GATOR2 heterodimers, potentiating mTORC1 activity [29]. Leucine also augments mTORC1 activity via its catabolite acetyl-coenzyme A independently of Sestrin2 [30]. Glutamine, an amino acid that is utilized as a nitrogen and energy source in highly proliferative cells, also influences mTORC1 signaling. Glutamine import by SLC1A5 has been proposed as the rate-limiting step for mTORC1 activation. The heterodimeric SLC7A5/SLC3A2 transporter leverages glutamine efflux to increase leucine uptake, which facilitates mTORC1 activation [31]. In addition, glutamine and asparagine signal to mTORC1 through Arf1 in the absence of the Rag GTPases [32]. Depletion of asparagine using asparaginase, which also has glutaminase activity, inhibits mTOR signaling in patients with acute myeloid leukemia (AML) [33]. Lastly, Methionine is sensed as S-adenosylmethionine (SAM), indicating the mTORC1 is not only sensitive to amino acids, but to their metabolic products as well; the binding of SAM to SAMTOR disrupts the SAMTOR-GATOR1 heterodimer, thus activating mTORC1 [34].

The activation of mTORC1 by intracellular amino acids is potentiated by growth factor signaling. Insulin and Insulin-like growth factor (IGF) bind to receptor tyrosine kinases (RTKs) that trigger Phosphoinositide 3-kinase (PI3K) activation. PI3K signals are connected to mTORC1 signaling through the heterotrimeric tumor suppressor tuberous sclerosis complex (composed of TSC1, TSC2, and TBC1D7) [35, 36]. *TSC1* and *TSC2* genes serve to make the proteins hamartin (TSC1) and tuberin (TSC2), respectively. TSC2 inhibits mTORC1 activity by functioning as a GTPase-activating protein (GAP) for the small GTPase Rheb [37], which allosterically binds to mTOR and causes realignment of several active site residues to enhance catalytic activity [38]. Mutations that render TSC1/2 nonfunctional are the molecular basis of tuberous sclerosis complex disorder, in which constitutive mTOR activation results in benign tumor-like lesions in the body. Multiple signaling pathways converge on TSC1/2 to modulate mTORC1 activity. Akt phosphorylates TSC2 at Ser939 and Thr1462 to inhibit TSC1/2, thus enhancing mTORC1 activation [39]. Ras is also wired to TSC1/2 signaling, via p90 ribosomal S6 kinase (RSK) that inhibits tuberin (TSC2) through phosphorylation at Ser1798, resulting in increased mTORC1 signaling to its effector S6K1 [40]. ERK1/2-mediated

phosphorylation of TSC2 at Ser530 and Ser664 leads to TSC1-TSC2 dissociation, markedly impairing its ability to inhibit mTORC1 signaling [41]. In the presence of limited nutrients and energy, TSC2 is phosphorylated to inhibit mTORC1 activity. During energy depletion, the AMP-activated protein kinase (AMPK) phosphorylates TSC2 at Thr1271 and Ser1387 to enhance its activity, coupling mTOR activity to cellular energy status [42]. In addition, AMPK directly phosphorylates Raptor at Ser722 and Ser792, which promotes its association with inhibitory 14-3-3 proteins and subsequent mTORC1 inactivation [43]. GSK3β, an effector of the Wnt pathway, modulates mTORC1 activation by phosphorylating TSC2 at Ser1375, Ser1379, and Ser1383 in a manner that requires AMPK-priming phosphorylation [44]. During hypoxia, the hypoxia-inducible transcription factor (HIF) promotes expression of several hypoxia-response genes, including regulated in development and DNA damage response 1 (REDD1), which suppresses mTORC1 by releasing TSC2 from its association with inhibitory 14-3-3 proteins [45]. Finally, the DNA damage-response pathway inhibits mTORC1 through transcription of p53 target genes such as AMPKβ, PTEN, and TSC2, all of which increase TSC1/2 activity [46]. Interestingly, TSC2 phosphorylation does not affect its GAP activity towards small GTPase Rheb, but rather its spatial localization [47]. During starvation, TSC2 anchors to the lysosome in close proximity to mTORC1, while growth factor and nutrient replenishment promotes TSC2 dissociation from the lysosomal membrane to allow accumulation of GTP-bound Rheb and subsequent mTORC1 activation [48, 49].

3. mTORC2 regulation

While mTORC1 promotes cell growth and metabolism, mTORC2 is indispensable for regulating cell survival in response to growth factors and nutrient fluctuations by phosphorylating members of the AGC (Protein Kinase A, PKG, PKC) family of protein kinases. Elucidating the function of mTORC2 has been challenging due to the lack of specific mTORC2 inhibitors and its complex regulation of cellular functions. mTORC2 is characterized by its insensitivity to acute rapamycin treatment. The insensitivity of mTORC2 is due to Rictor occluding the FRB domain, where the

FKBP12-rapamycin heterodimer normally binds [50]. The prolonged presence of rapamycin will eventually inhibit mTORC2 activity, presumably by binding to the nascent and free mTOR pool, preventing mTORC2 assembly [51].

mTORC2 activity is enhanced by the presence of growth factors like insulin, which activates the PI3K pathway [52]. Insulin and Insulin-like growth factor (IGF) bind to receptor tyrosine kinases that activate PI3K via the insulin receptor substrate-1/2 (IRS-1/2). PI3K catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3) [53]. PTEN is a tumor suppressor protein that hydrolyzes PIP3 into PIP2, acting antagonistically to PI3K [54]. PIP3 functions to tether signaling molecules to the membrane compartment. It recruits 3-phosphoinositide-dependent kinase 1 (PDK1) to the plasma membrane via its Pleckstrin Homology (PH) domain, where it can colocalize with Akt and phosphorylate its activation loop (Thr308) upon PI3K activation [55]. In addition, PI3K-generated PIP3 binds SIN1 via its PH domain, relieving SIN1-PH autoinhibition on the mTOR kinase domain, triggering mTORC2 activation at the plasma membrane [52, 56]. A hallmark of mTORC2 activation is phosphorylation of Akt at the allosteric site, Ser473, which is nestled in the hydrophobic motif (HM) [57]. Ser473 phosphorylation enhances Akt maximal activity against certain substrates like FoxO1 and GSK3β. In $SIN1^{-/-}$ cells, Akt can still phosphorylate many of its substrates, including TSC2, indicating that mTORC2-dependent phosphorylation at Ser473 is dispensable for phosphorylation against certain substrates [12]. In addition, insulin/PI3K signaling promotes association of mTORC2 with ribosomes [58, 59]. This colocalization of mTORC2 and actively translating ribosomes increases Akt phosphorylation at Ser473 and also promotes cotranslational phosphorylation of Akt at the turn motif, Thr450, to increase stabilization of the nascent Akt polypeptide [58, 59]. Active Akt mediates phosphorylation of tuberous sclerosis complex 2 (TSC2) to diminish its GAP activity towards Rheb, allowing accumulation of Rheb-GTP, potentiating mTORC1 activation [60]. Increased mTORC1 signaling, such as during inactivation of TSC, feeds back to insulin/PI3K signaling to reduce mTORC2/Akt signaling. Downregulation of mTORC1 signaling such as by rapamycin treatment can upregulate PI3K/mTORC2 signals [61]. S6K can also

phosphorylate rictor at Thr1135 to modulate mTORC2 signaling [62, 63]. Thus, there is crosstalk between mTORC1 and mTORC2 signaling. While mTORC2/Akt may potentiate mTORC1 signals via TSC, mTORC1 signals attenuate mTORC2 signals at the level of RTK/PI3K signaling.

In addition to growth factor signaling through the insulin/PI3K axis, mTORC2 responds to nutrient and metabolite fluctuations. Phosphorylation of the mTORC2 substrate Akt is enhanced during withdrawal of glutamine, suggesting that mTORC2 responds to glutamine limitation [64]. In addition, mTORC2 activity is triggered during glucose deprivation via AMPK-mediated phosphorylation of mTOR and possibly rictor [64, 65]. The activation of mTORC2 during glutamine and glucose depletion is essential for mTORC2 to maintain flux through the hexosamine biosynthetic pathway (HBP). The HBP consumes glutamine and glucose to generate uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), which is utilized for the glycosylation of proteins and lipids [66]. Additionally, Sestrin2 has been found to activate mTORC2 but suppress mTORC1, aligning with other studies that indicate tuberous sclerosis complex and AMPK activate mTORC2 but inhibit mTORC1 [65, 67]. Although Sestrin2 is an established AMPK activator, the Sestrin2-induced mTORC2 activation could occur independently of AMPK. The molecular basis of this mechanism requires GATOR2, which acts as a bridge between Sestrin2 and rictor, promoting mTORC2 activation [68]. In addition, Sestrin3 interacts with and activates mTORC2, triggering Akt phosphorylation at Ser473 [69]. Interestingly, amino acid addition also bolsters mTORC2 activity, as evidenced by rapid phosphorylation of Akt Thr308 and Ser473 [70]. A recent study found that both mTORC1 and mTORC2 are activated in response to exogenous fatty acids via the *de novo* synthesis of phosphatidic acid (PA). PA interacts with the FRB domain of the mTOR protein, stabilizing both multiprotein complexes [71]. Another study found that the inhibitor of nuclear factor κ-B kinase (IKK) interacts with rictor to promote mTORC2 activity [72]. Additionally, crosstalk between the MAPK and mTOR pathway has been established by superoxide anion stimulus leading to enhanced Ras-SIN1 interaction, which correlates with increased mTORC2 signaling [73]. Finally, mTORC2 activity is enhanced by calcium release from the endoplasmic reticulum and ammonium from glutaminolysis [74]. As evidenced by this diverse group

of regulators, mTORC2 integrates signals from nutrients, metabolites, stress, and growth signals to promote cell survival. mTORC2 signaling is also regulated by mTORC1 through two negative feedback loops involving insulin/PI3K signaling. S6K1 suppresses mTORC2 activation through phosphorylation dependent degradation of insulin receptor substrate I (IRS1) [75]. In addition, mTORC1-mediated phosphorylation stabilizes Grb10, leading to feedback inhibition of the PI3K and ERK/MAPK pathways [76]. Together, these findings indicate that mTORC2 responds to nutrient fluctuations and stress conditions and could function to restore metabolic homeostasis, mTORC1 activation, and promote cell survival during unfavorable growth conditions.

A conserved function of mTORC2 among mammals is the phosphorylation of AGC kinases, like PKCα, SGK1, and Akt [77]. The most well characterized substrate of mTORC2, however, is Akt. During growth factor/insulin stimulation, Akt promotes cell survival through the phosphorylation and inhibition of FoxO1/3a, GSK3β, and TSC2 [12]. mTORC2 also phosphorylates PKC at a TOR interaction motif (TIM) and thus promotes PDK1-mediated phosphorylation at the activation loop and autophosphorylation at the hydrophobic motif site [78]. Lastly, mTORC2 phosphorylates SGK1 (serum- and glucocorticoid-induced protein kinase 1) at Ser422 to control ion transport and growth [79].

4. Therapeutic strategies targeting mTOR

The first generation of mTOR inhibitors are a group of rapamycin derivatives called rapalogs. These allosteric mTOR inhibitors have been approved by the FDA for the treatment of certain cancers [80]. Temsirolimus was approved for treating advanced renal cell carcinoma in 2007, followed by everolimus in 2009 [81]. These rapalogs had less success than anticipated in clinical trials. This subpar performance is attributed to their allosteric binding, which blocks the phosphorylation of some but not all targets of mTOR. For example, S6K1 activity is completely abolished during acute rapamycin treatment, while 4E-BP1 phosphorylation is relatively unscathed [82]. This weakened inhibition of 4E-BP1 activity may contribute to the poor antiproliferative effect of rapalogs. In addition, mTORC1-specific inhibition by the rapalogs relieves the negative

feedback on PI3K/Akt signaling, paradoxically increasing cell survival. Hence, enhanced Akt signaling observed in cancer patients treated with everolimus could explain the rapalogs' cytostatic effect [83]. mTORC1 inhibition also serves to promote autophagy, which may be beneficial for nutrient-starved tumors in poorly vascularized locations. Due to the rapalogs' limited clinical success, second generation ATP-competitive catalytic mTOR inhibitors were soon developed. Unlike rapamycin, these inhibitors directly compete with ATP in the catalytic site of mTOR, providing broader and sustained inhibition of both mTORC1 and mTORC2. Despite their success in halting cell growth and proliferation during preclinical trials, their increased toxicity during clinical trials raised concerns [84]. Additionally, mutations in the mTOR kinase domain conferred resistance to mTOR kinase inhibitors in clinical practice. Dual PI3K/mTOR inhibitors have also been engineered in order to have broader inhibition of PI3K and mTOR signaling. While the pre-clinical studies were promising, clinical trials have led to modest results and toxicity [80]. The dual PI3K/mTOR inhibitors can concomitantly activate distinct signaling cascades such as the JAK2/STAT5 pathway, which mediates resistance [85]. In 2016, a third generation mTOR inhibitor, named Rapalink-1, was developed. Rapalink-1 is a bivalent molecule linking a rapamycin-FRB binding element to a second generation TOR Kinase inhibitor (TORKi) using a polyethylene glycol cross-linker of optimal length. This third-generation inhibitor overcomes mutations that diminish FRB binding or hyperactivate the kinase domain [86]. Rapalink-1 has been shown to significantly delay growth of PDX models of bone-metastatic prostate cancer and alter tumor heterogeneity [87].

In addition to treating cancer, mTOR inhibitors have shown promising effects against neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD) [88]. Hyperactivated mTORC1 signaling has been linked to cognitive severity in AD patients [89]. The dual PI3K/mTOR inhibitor NVP-BEZ235 was found to preserve memory and reduce microglial activation in a transgenic mouse model of AD [90]. The mTOR pathway can also contribute to the pathogenesis of PD, which results in accumulation of α-synuclein-containing inclusions called Lewy bodies, as well as mitochondrial dysfunction. Suppression of mTOR/p70S6K signaling enhances clearance of A53T α-synuclein via autophagy

induction [91]. Additionally, rapamycin treatment restores mitophagy in PD models with a PARKIN mutation by promoting PGC1α-TFEB signaling [92]. Understanding how the mTOR pathway contributes to pathogenesis of neurodegenerative disorders may lead to novel therapeutic strategies.

The mTOR pathway has also been recognized as a key mediator of aging — a process characterized by successive metabolic alterations that increases vulnerability to disease and death. Inhibition of mTORC1 extends lifespan in organisms ranging from yeast [93] to mammals [94]. Reduced mTORC1 signaling can arise from rapamycin, caloric restriction, or genetic mutations. Interestingly, rapamycin treatment has been found to increase not just lifespan, but also healthspan — the length of time an organism is free from disease [95]. This indicates that mTOR suppression may reverse aging hallmarks, such as deregulated nutrient sensing, cellular senescence, loss of proteostasis, and mitochondrial dysfunction [96]. Consistent with this, loss of S6K1 extends lifespan in mammals, suggesting that restriction of protein synthesis can slow aging. 4E-BP also prolongs lifespan upon dietary restriction in Drosophila by translational regulation of nuclear-encoded mitochondrial genes [97]. mTORC1 inhibition also induces autophagy, which is the indiscriminate bulk slaughtering of large proteins and organelles at autolysosomes, allowing aged cells to regenerate molecular precursors and clear waste [98]. The mTORC2/Akt signaling axis is also implicated in aging. One study found that haploinsufficiency of Akt1 extends lifespan in mice [99]. Additionally, depletion of Rictor significantly decreases male, but not female lifespan; this may explain why mTOR inhibition has greater efficacy in extending lifespan in females [100]. Although mTOR inhibitors have shown promising geroprotective effects, chronic dosing can result in glucose intolerance and immunosuppression. Recent reports have found that anti-aging benefits can be achieved with lower and intermittent dosing, which can extend lifespan without causing insulin resistance and immunosuppression [101].

5. S6K1: coordinating global translation

The 70-kDa ribosomal protein S6 kinase (p70S6K) is a serine/threonine kinase that belongs to the AGC (Protein Kinase A, PKG, PKC) family of

protein kinases. The S6K family consists of two genes – *S6K1* and *S6K2*, which share over 70% homology [102]. The S6Ks have five domains: N-terminal regulatory domain, catalytic domain, linker domain, autoinhibitory domain, and the C-terminal domain. Phosphorylation of four serine/threonine-proline residues (Ser411, Ser418, Ser421, and Ser424) in the autoinhibitory domain relieves autoinhibition of the kinase domain [103], allowing subsequent phosphorylation by mTORC1 at Thr389 in the HM site via the TOR signaling (TOS) motif in the N-terminal regulatory domain [104] (Fig. 2). This primes S6K1 for subsequent PDK1-mediated

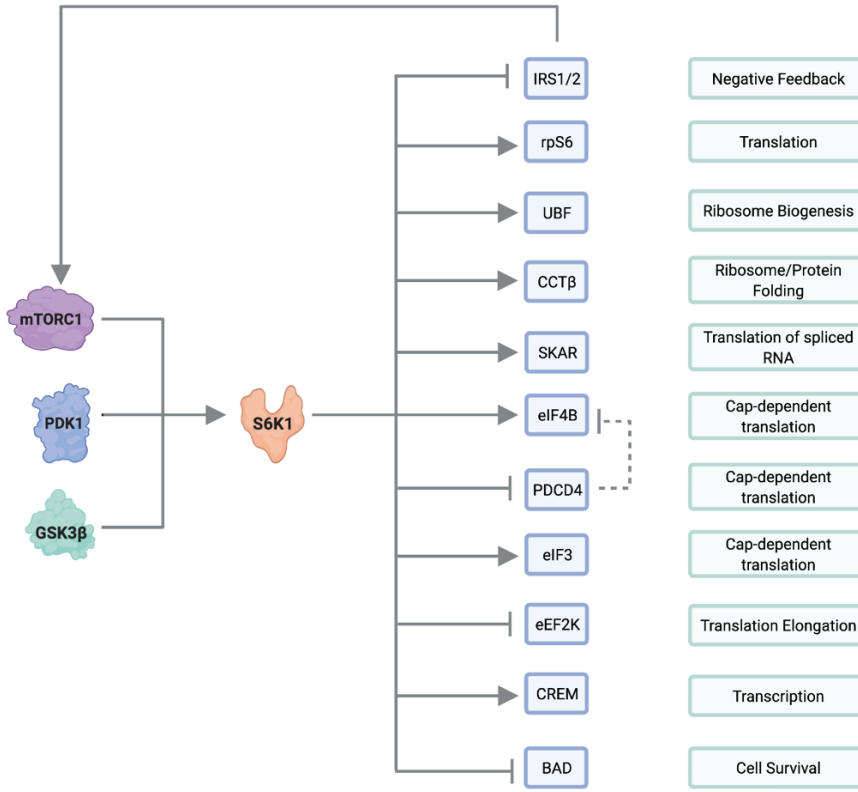

Figure 2. S6K1 is phosphorylated at the activation loop by PDK1 and at allosteric sites by mTORC1 and GSK3b.

S6K1 has numerous targets involved in translation.

phosphorylation at the activation loop (Thr229) [105]. In addition, GSK3β promotes S6K phosphorylation at the turn motif (Ser371) in response to growth factor signaling, which also contributes to its full activation [106]. mTORC1-dependent phosphorylation of S6K1 at Th389 promotes its nuclear localization during mid-G1 phase of the cell cycle [107], where it can interact with the transcription factor TBR2 [108]. SIRT1 and SIRT2 inhibits C-terminal acetylation of S6K1, thus promoting Thr389 phosphorylation *in vitro*, indicating that crosstalk between mTOR and sirtuin pathways regulates S6K1 activity [109].

S6K1 phosphorylates the ribosomal protein rpS6, a component of the 40S subunit, at Ser240/Ser244, which is involved in regulation of cell growth and ribosome biogenesis [110, 111]. Phosphorylation of rpS6 is critical for cell size and growth as evidenced by the defective growth in $rpS6^{-/-}$ cells [112]. S6K1 targets a number of substrates that regulate protein synthesis. S6K1 regulates ribosome biogenesis by phosphorylating the transcription factor UBF, which promotes RNA Polymerase I-mediated transcription of genes that produce 47S rRNA [113]. Additionally, S6K1 converges on the chaperonin complex subunit CCTβ phosphorylation at Ser260 to aid protein folding of ribosomes and polypeptides [114]. S6K1 enhances nuclear export and translation efficiency of spliced mRNAs by phosphorylating SKAR at Ser383 and Ser385, and cap-dependent splicing through CBP80 phosphorylation [115–117]. S6K1 also exerts control of cap-dependent translation through several mechanisms. It phosphorylates eIF4B at Ser422, which is an auxiliary factor that stimulates eIF4A's helicase activity of unwinding and resolving inhibitory secondary structures in the 5′ UTR of mRNA [118]. S6K1 also phosphorylates the tumor suppressor programmed cell death protein 4 (PDCD4), which normally inhibits eIF4B activity, however, S6K1-mediated phosphorylation of PDCD4 at Ser67 results in its rapid degradation via ubiquitin ligase βTRCP, which augments cap-dependent translation [119]. Lastly, S6K1/2 phosphorylates eIF3 during nutrient replete conditions to stimulate Paip1 (PABP-interacting protein)-eIF3 association, which promotes cap-dependent translation initiation [120]. S6K1 mediates translational elongation by inactivating the eukaryotic elongation factor 2 (eEF2) kinase (eEF2K) via phosphorylation at Ser366 [121]. eEF2K is a negative regulator of protein synthesis that phosphorylates and inhibits eEF2, a GTPase that shifts the growing peptidyl from A site to P site in the ribosome.

Increased S6K1 activity or expression is often found in cancer due to its various roles in promoting expression of genes as well as phosphorylation of proteins that are involved in growth, proliferation, or survival. S6K1 facilitates gene transcription via phosphorylation of transcriptional regulators. Upon mitogen stimulation, S6K1 phosphorylates CREM (cAMP responsive element modulator) at Ser117 and ERα (estrogen receptor α) at Ser167 [122, 123] to promote transcription of specific genes. S6K1 also promotes the translation of mRNA that become upregulated in cancer as well as normal highly proliferating cells. Due to the increased need of these cells for nutrients, they enhance expression of key enzymes involved in metabolism. For example, TCA cycle intermediates are replenished through glutamine-dependent anaplerosis in order to satisfy energy requirements during glucose limitation. This process requires Glutaminase (GLS) to deaminate glutamine into glutamate and Glutamate Dehydrogenase (GDH) to deaminate glutamate into α-ketoglutarate. S6K1 enhances MYC-dependent translation of the GLS mRNA through eIF4B phosphorylation at Ser422, which is critical for unwinding inhibitory secondary structures in the 5′-UTR. Therefore, S6K1, via modulating translation of GLS, rewires glutamine metabolism to support cell growth and proliferation [124]. S6K1 is also implicated in cancer by phosphorylating and inhibiting the pro-apoptotic protein BAD (Bcl-2-associated death promoter) [125]. Hence, S6K1 play multiple roles in protein synthesis and regulation to promote growth, proliferation, and cell survial.

6. 4E-BP1 inhibits eIF4F complex and cap-dependent translation

Eukaryotic nuclear-encoded mRNAs contain a cap made of modified guanosine (7-methyl guanosine) at their 5′ends. Cap-dependent translation requires two steps: attachment of the 43S preinitiation complex to the mRNA and cap-mediated recruitment of the eIF4F complex. Assembly of the 43S pre-initiation complex (PIC) involves association of the 40S ribosomal subunit with eIF1, eIF1a, and eIF3, followed by binding of the ternary eIF2-GTP-tRNAiMet complex to the ribosomal P site, and addition of eIF5 factors. This newly formed 43S PIC is capable of recognizing the AUG start codon in an open reading frame (ORF) once it is attached

to the mRNA [126]. At the mRNA, interaction of the eIF4F complex with the 5′ 7-methylguanosine 5′-triphosphate (m^7GTP) mRNA cap is necessary for recruitment of the 43S PIC. The heterotrimeric eIF4F complex is composed of the DEAD (Asp-Glu-Ala-Asp)-box helicase eIF4A, cap binding subunit eIF4E, and large scaffolding protein eIF4G. eIF4G mediates eIF4E-eIF4A interaction and also associates with poly(A)-binding protein (PABP). The PABP binds to poly(A) tails, bringing the 5′ and 3′ termini in close proximity to form a loop [127]. The eIF4F ternary complex facilitates the recruitment of the 43S PIC to mRNA by binding to the 5′-m^7GTP cap via eIF4E, unwinding inhibitory secondary structures with eIF4A helicase activity, and recruiting/stabilizing the 43S PIC through eIF4G-eIF3 interaction [128]. Upon binding to the mRNA, the PIC begins scanning through the 5′-untranslated region (UTR) in search of the start codon. Once found, the initiation factors dissociate and the large 60S subunit is recruited to form the 80S ribosome and translation ensues.

Reprogramming mRNA translation during stress and starvation occurs via 4E-BP1/S6K1 signaling and phosphorylation of eIF2α. Due to the presence of a common binding motif, eIF4E binding proteins (4E-BPs) interfere with eIF4F assembly by competing with eIF4G for binding to the conserved hydrophobic patch on eIF4E, thus blocking the eIF4E-eIF4G interaction [129]. 4E-BP1 is regulated post-translationally via phosphorylation by mTORC1, which facilitates its dissociation from eIF4E, allowing cap-dependent translation to proceed. 4E-BPs contain a C-terminus extreme TOS motif that promotes its interaction with mTORC1 [130] for hierarchical phosphorylation at Thr37 and Thr46, which are priming sites for subsequent phosphorylation at Thr70 and Ser65. Phosphorylation of 4E-BPs during physiological conditions restores eIF4E-eIF4G interaction and eIF4F complex assembly [131]. In quiescent cells, 4E-BPs are kept in a hypo-phosphorylated state to prevent eIF4F assembly. The aberrant phosphorylation of 4E-BP1 controverts the apparently linear architecture of mTORC1 signaling, indicating that cross talks with additional kinases are involved in 4E-BP phosphorylation, including Pim-2 (Ser65), GSK3β (Thr36/46), Casein kinase 1ε (Thr41/50), and CDK1 (Thr70 and Ser83) [132–135]. Additionally, eIF4F function is also promoted by S6K1-dependent phosphorylation of eIF4B and PDCD4, which triggers eIF4A helicase activity [118, 119].

Furthermore, S6K1-mediated phosphorylation of MAD1 at Ser145 promotes MYC-mediated transcription. The MYC transcription factor, a frequently overexpressed oncogene in cancers, promotes transcription of all three components of the eIF4F complex, suggesting another axis of mTOR-dependent control of mRNA translation [136].

The integrated stress response propagates stress signals that impinge on eIF2α to regulate cap-dependent translation. The availability of the eIF2-GTP-tRNAiMet complex necessary for 43S PIC formation is influenced by environmental stress. During physiological conditions, the GTP-charged ternary complex is ubiquitous to promote translation. Under stress, the regulatory α subunit of heterotrimeric eIF2 is phosphorylated by HR1 (heme-regulated eIF2 α kinase), PKR (double-stranded RNA-dependent protein kinase), GCN2 (general control non-depressible-2), and PERK (PKR-like ER Kinase) at Ser51 to inhibit GTP exchange, thus depleting eIF2α-GTP-tRNAiMet availability and repressing translation [137].

Not all mRNAs are similarly selected by the eIF4F complex. Studies have shown that eIF4F is involved in the modulation of specific mRNAs, called "eIF4E-sensitive" mRNAs [138]. These mRNAs are characterized by a long and highly structured 5′ UTR, which makes them more dependent on the helicase activity of eIF4A for efficient translation, and thus highly sensitive to eIF4E level fluctuation in the cell [139]. This includes mRNAs that encode for proteins with a central role in cell survival and proliferation, such as cyclins [140], ornithine decarboxylase [141], VEGF [142], and MYC [143]. In addition, another group of mTOR-regulated mRNAs controlled through eIF4F contain 5′ terminal oligopyrimidine (TOP) motifs. This cis-regulatory motif comprises a C or U residue, immediately after the 5′-m^7GTP cap, followed by an uninterrupted tract of 4–14 pyrimidines (12 on average) [144]. The highly conserved 5′-TOP motif is found on mRNAs that encode for components of the translation apparatus, such as initiation factors, all 79 ribosomal proteins, and PABPs that control global protein synthesis and ribosome biogenesis [145]. The translation of 5′ TOP mRNAs is very sensitive to stress; hypoxia, amino acid deprivation, and cell cycle arrest all rapidly repress 5′ TOP mRNA translation, so resources to support protein synthesis are redirected to resolve stress-induced damage [144]. Replenishment of growth factors

and nutrients stimulates translation of TOP mRNAs through mTOR signaling. Although the exact mechanisms are unclear, there are two lines of evidence that indicate mTORC1 is the major regulator of 5′ TOP mRNA translation: 1) second generation ATP-competitive mTOR kinase inhibitors specifically repress TOP mRNA translation [146] and 2) constitutive activation of mTORC1 by either knocking out TSC1/2 or overexpressing Rheb activates translation of TOP mRNAs [147].

7. LARP1 and other RNA-binding proteins

The translation of mRNA is controlled by RNA binding proteins (RBP) that either enhance or inhibit the utilization of mRNA depending on environmental conditions. The RBPs function in several aspects of mRNA regulation including RNA processing and modification, localization, and translation. They primarily bind to sequence or structural motifs in RNA via RNA-binding domains to form ribonucleoprotein (RNP) complexes. Here, we will give examples of RBPs that have been linked to mTOR signaling.

The La related protein 1 (LARP1) is a *bona fide* regulator of 5′-TOP mRNA translation, although incongruous studies as to whether LARP1 is a TOP activator or repressor have yet to be reconciled (Fig. 3). LARP1 is a functionally nebulous RNA-binding protein containing a PABP-interacting domain, DM15 region, and La-Module comprised of an La Motif (LaM) and RNA Recognition Motif (RRM) [148]. Recent studies indicate that only the highly conserved C-terminal DM15 region is required for LARP1 to recognize the 5′-TOP motif [149]. The positively charged DM15 region consists of three tandem HEAT-like helix-turn-helix regions that facilitates interaction with the negatively-charged phosphate backbone of the mRNA. Lys-915 stabilizes the γ-phosphate of the 5′-m^7GTP cap, and Arg-879 and Arg-847 recognize the invariant cytidine in the first position of 5′-TOP mRNAs [150]. LARP1 represses cap-dependent translation of 5′-TOP mRNAs downstream of mTORC1. The recognition of the 5′-TOP motif via LARP1's DM15 region impedes eIF4E's access to the cap, thereby preventing eIF4F complex assembly and halting global translation [151]. Several other groups have since

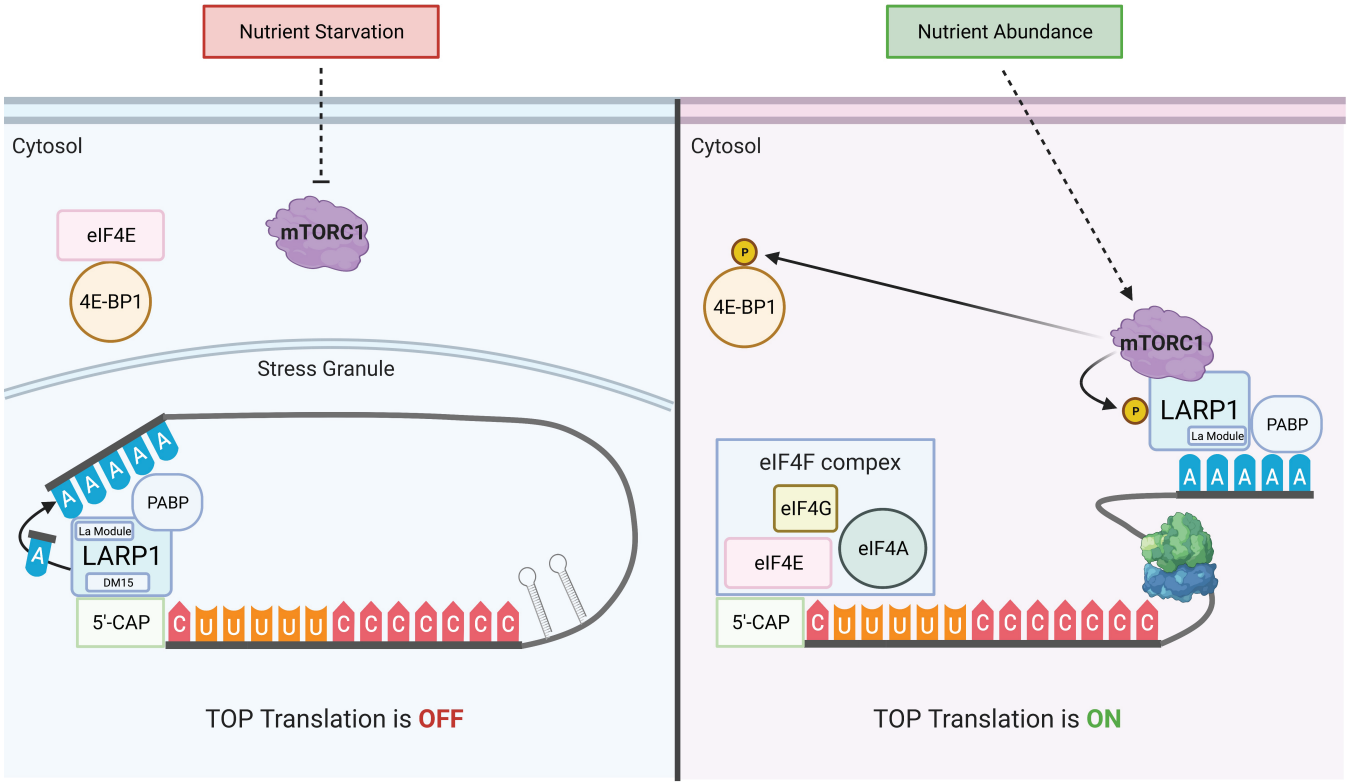

Figure 3. mTORC1 modulates the RNA binding protein, LARP1. LARP1 is a regulator of 5′TOP mRNA translation.

corroborated LARP1's role as a repressor of 5′-TOP translation [150, 152]. Other studies contradict the repressive role of LARP1 — Aoki *et al*. determined that LARP1 binds to the poly(A) tail of mRNA transcripts to increase steady state levels of mature 5′-TOP transcripts [153]. For example, while LARP1 destabilizes the BIK mRNA, it interacts with the 3′ UTR to stabilize the BCL2 mRNA to drive cell survival and ovarian cancer progression [154]. The mechanism of how LARP1 stabilizes some mRNAs and represses others still remains elusive. Fonseca *et al.* also discovered that LARP1 physically associates with mTORC1, and that this interaction is mediated by Raptor [151]. LARP1 contains over 26 rapamycin-sensitive phospho-sites [155], but only two residues, Ser774 and Ser766, were phosphorylated *in vitro* by mTORC1 [156]. Interestingly, both of these residues were located near the DM15 region required for recognition of 5′-TOP motif [149].

An attractive model of LARP1-dependent control of 5′-TOP mRNAs posits that LARP1 assumes different conformations in response to nutrient abundant and starved conditions. During nutrient starvation and concurrent mTORC1 inhibition, active 4E-BPs destabilize eIF4F formation so that hypophosphorylated LARP1 can bind to the 5′-TOP mRNA cap via its DM15 domain, repressing translation initiation by blocking the binding of eIF4E. Interestingly, hypophosphorylated LARP1 also promotes TOP transcript stability by maintaining the length of their poly(A) tails [157]. Additionally, LARP1's capacity to simultaneously bind PABP and the TOP motif *in vitro* via its La-Module [158] suggests that LARP1 may be involved in the circularization of repressed transcripts [159], a conformation that occurs in stress granules [160]. This coincides with a recent finding that LARP1 sequesters 5′-TOP transcripts from decapping and decay factors, and anchors them within stress granules and P-bodies to prevent degradation [161]. Thus, LARP1 functions to repress 5′-TOP translation and promote transcript stabilization during nutrient deplete conditions. In nutrient abundant conditions, mTORC1 phosphorylates LARP1, causing loss of repressive DM15 binding to the 5′-m^7GTP cap but maintaining La-Module association with the 3′ UTR to serve as a scaffold for mTORC1-dependent induction of translation initiation [155]. This model suggests that LARP1 switches between two phosphorylation states to increase or decrease stability of 5′-TOP mRNAs under mTORC1's

command. A recent study using Torin1 to inhibit both mTOR complexes also identified LARP1 to have increased binding to TOP mRNAs along with other mRNAs in a RNA protein interaction network study [162]. Findings in this study confirmed a role for LARP1 in translational repression and that it interacts directly with PABPC1. Another RNA binding protein, TRIM25, an E3 ubiquitin ligase, also had increased RNA binding upon mTOR inhibition. Other proteins that have reduced interaction with mRNA following Torin1 treatment included ribosomal proteins and associated factors such as Serpiine mRNA binding protein 1 (SERBP1). Since Torin1 inhibits both mTORC1 and mTORC2, future studies should address whether each of the mTORCs could have distinct effects on RNA binding.

mTOR also regulates another LARP family member, LARP6. LARP6 binds to collagen mRNA and is involved in modulating collagen mRNA translation [163]. The mTORC2 substrate, Akt, phosphorylates LARP6 at Ser451, a priming phosphorylation. Akt inhibition or the expression of phosphodeficient mutant LARP6-S451A, reduces collagen production [164]. mTORC1 also phosphorylates LARP6 on Ser348 and Ser409 to enhance LARP6 interaction with the accessory protein STRAP (serine-threonine kinase receptor-associated protein) and thus coordinate the translation of collagen mRNAs. The phosphorylation also mitigates LARP6 accumulation at the ER membrane and thus facilitates its nuclear redistribution to allow further collagen mRNA regulation.

Another RBP regulated by mTORC2 is IMP1 (IGF2 mRNA-binding protein 1/*MYC* coding region determinant-binding protein, CRD-BP). mTORC2 cotranslationally phosphorylates IMP1, which is involved in IGF2 splicing and translation [165]. MP1 phosphorylation enhances IMP1 binding to the IGF-2 leader 3 5′untranslated region, which is necessary for IGF2-leader 3 mRNA translational initiation by internal ribosomal entry.

The RBPs also modulate mTOR signaling reciprocally. PDCD4, which binds with eIF4A and inhibits its helicase activity [166, 167], modulates mTORC2. Loss of PDCD4, a tumor invasion suppressor, increases mTORC2 activity via promoting SIN1 translation [168]. The PDCD4-mediated regulation of SIN1 occurs via eIF4A. Increased SIN1 translation in the absence of PDCD4 promotes tumor invasion. PDCD4 also associates with rictor and negatively regulates Akt phosphorylation [169]. In renal

cancer cells, this complex formation is reduced due to increased miR-21 expression, which downregulates PDCD4, leading to increased Akt phosphorylation.

The RNA-binding protein CLUH (clustered mitochondria homolog) serves to coordinate translation of mitochondrial proteins with mTOR signaling and mitophagy in response to nutrient availability [170]. Under unfavorable conditions, CLUH inhibits mTORC1 signaling and translation of mRNAs involved in mitochondrial protein synthesis while promoting translation of mRNAs involved in mitochondrial catabolic and ketogenic pathways in hepatocytes. The RBP GRSF1 (G-rich RNA sequence-binding factor 1), which is also involved in maintaining mitochondrial homeostasis, also influence mTOR signaling. Loss of GRSF impairs the function of respiratory complexes I and IV, leading to increased oxidative stress and mTOR activation. The increased mTORC1 activation is likely involved in inducing gene transcription of *IL-6*, which is a pro-inflammatory factor. However, it is not clear how mTORC1 is activated under growth suppressive conditions. Whether mTORC2 could be involved during this response needs to be examined.

Together, these findings unravel how both mTORC1 and mTORC2 could control translation via modulation of RBPs. They also indicate how RBPs could in turn impact mTOR signaling via direct regulation of translation of mTORC components and/or indirectly via control of expression of key proteins that affect mTOR signaling.

8. RSK: Integrating mTOR and MAPK signals

The 90 kDa ribosomal S6 kinase (RSK) family is a group of serine/threonine kinases that consist of four isoforms: RSK1, RSK2, RSK3, and RSK4 [171]. RSK isoforms are normally present in the cytosol, but upon growth factor stimulation, they translocate to the nucleus [172]. Interestingly, RSK4, which is functionally distinct from the other RSK kinases, does not significantly accumulate in the nucleus following Ras/MAPK stimulation [173]. RSK contains two structurally and functionally distinct kinase domains. The D-type docking motif at the C-terminal kinase domain (CTKD) receives an activating signal from ERK1/2, which is transmitted to the N-terminal kinase domain (NTKD)

that is responsible for phosphorylating substrates. The CTKD belongs to the CAMK (Ca^{2+}/calmodulin-dependent protein kinase) family and the NTKD belongs to the AGC family of protein kinases [174]. Due to its NTKD domain, there is significant overlap between RSK and other AGC protein kinase targets. RSK phosphorylates rpS6, suggesting its putative role in translational regulation [175]. Despite S6K1 and S6K2 being the predominant rpS6 kinases, RSK phosphorylates rpS6 at Ser235/236 to promote the binding of rpS6 to the 5′-m^7GTP cap, suggesting that the Ras/ERK signaling cascade is implicated in translation preinitiation complex assembly [176]. Additionally, RSK phosphorylates eIF4B at Ser422 to promote its interaction with eIF3, which is essential for recruiting the 43S PIC to the mRNA strand [177]. RSK also stimulates eIF4A helicase activity, which is essential for translation of mRNAs with highly structured 5′-UTRs, by phosphorylating the tumor suppressor PDCD4 at Ser76 and Ser457 to promote its degradation [178]. Lastly, RSK is implicated in the translation elongation phase by phosphorylating elongation factor 2 kinase (eEF2K) at Ser366, which subsequently phosphorylates and inactivates eEF2 [121]. Although RSK and S6Ks share considerable target overlap, they regulate substrates with different kinetics. The precise conditions as to how these substrates are similarly or distinctly regulated by RSK and S6K remain to be elucidated. RSK also phosphorylates CCTβ, a subunit of the chaperonin containing TCP-1 (CCT) complex [114], suggesting that in addition to the direct control of translation regulators, it is involved in modulating protein folding. The RSK-targeted site in CCTβ has defective phosphorylation in mTORC2-disrupted cells [179]. Indeed, mTORC2 is required for optimal phosphorylation of RSK at the HM site during serum stimulation or nutrient withdrawal, although the kinase activity of mTOR does not seem to be required for this phosphorylation [179]. Instead, mTORC2 could provide a scaffold to enhance RSK HM phosphorylation presumably at the membrane. Since the RSK NTKD is conserved among AGC kinases, how mTORC1 or mTORC2 could modulate RSK deserves further investigation. In turn, RSK positively regulates mTORC1 signaling in the presence of growth signals via phosphorylation and repression of TSC [40]. Altogether, these studies reveal how RSK could integrate signals from mTOR and MAPK to control translation in response to environmental stimuli.

9. Control of ribosome biogenesis

Ribosome biogenesis is an energy-demanding and nutrient-dependent process that facilitates the augmented translation accompanying cell growth, survival, and proliferation. This process begins in the nucleolus, which contains several hundred copies of ribosomal DNA (rDNA) genes that encode for the 47S pre-rRNA transcript. The 47S pre-rRNA transcript is subsequently cleaved to generate the mature 18S, 28S, and 5.8S rRNAs. Additionally, a portion of chromosome 1 in the nucleoplasm contains several hundred copies of the 5S rDNA gene [180]. All three RNA Polymerases are required to transcribe the 47S rRNA, 5S rRNAs, and necessary ribosomal proteins (RPs) to form the small 40S and large 60S subunits. The 40S subunit consists of the 18S rRNA and 33 RPs, while the 60S subunit is composed of the 28S, 5.8S, and 5S rRNA and 43 RPs [181]. In the cytoplasm, these 40S and 60S subunits coalesce in the presence of mRNA and initiator tRNA to form the mature 80S eukaryotic ribosome [180]. It is well established that cancer cells display increased rates of ribosome biogenesis and that oncogenic pathways, like the mTOR signaling cascade, positively regulate several steps of ribosome production (Fig. 4).

The first stage and rate-limiting step of rRNA synthesis is RNA polymerase 1-mediated transcription of 47S rDNA [182]. Upstream Binding Factor (UBF) first binds to the core promoter element of the 47S rDNA, circularizing the promoter into a nucleosome-like structure. Selectivity factor (SL)-1 then recognizes and binds the promoter [183]. Finally, the UBF-SL1 association recruits TIF-IA and RNA Polymerase I to the promoter to form the pre-initiation complex. The formation of this complex is assisted by the binding of the MYC:MAX heterodimer to the upstream E-box elements and recruitment of the histone acetyltransferase (HAT) complex. Conversely, this complex can be inhibited by p53 or pRB/p130 [184, 185]. Depending on nutrient status, TOR1 in yeast shuttles dynamically between the nucleus and cytoplasm to control RNA Polymerase I activity [186]. mTORC1 stimulates transcription of the 47S rDNA by phosphorylating the UBF carboxyl-terminal activation domain, thus promoting association of UBF and SL-1 at the promoter [113]. Additionally, mTORC1 phosphorylates TIF-IA at Ser44/Ser199, which is essential for

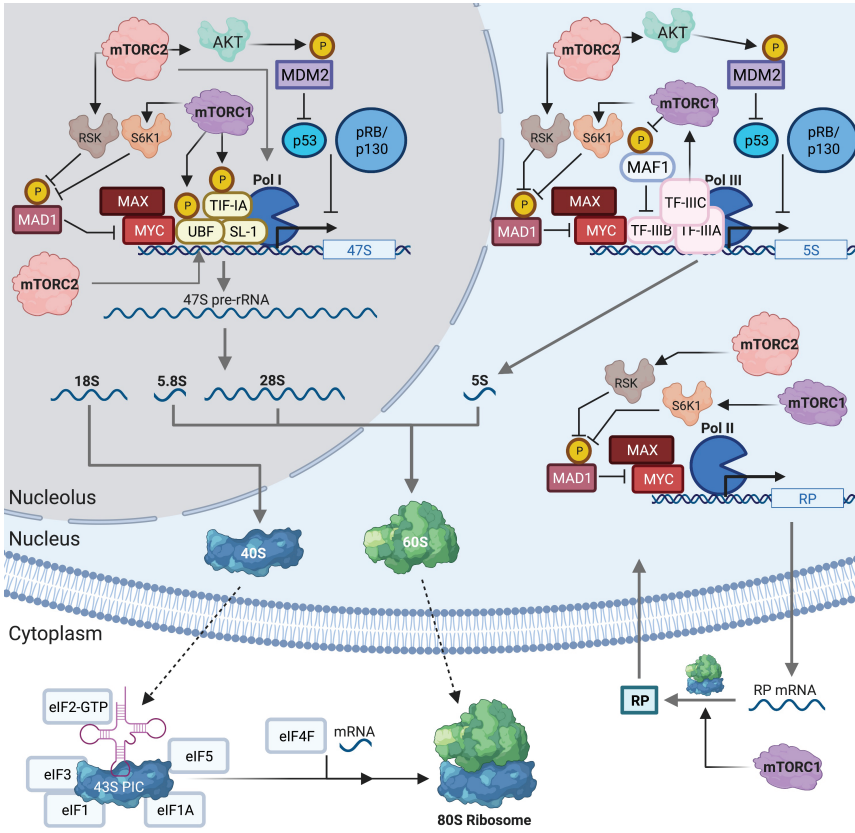

Figure 4. mTORC1 and mTORC2 are involved in various aspects of ribosome biogenesis.

recruiting RNA Polymerase I to the rDNA promoter. Phosphorylation at Ser44 is positively regulated by mTORC1 while Ser199 undergoes dephosphorylation upon serum stimulation, indicating that both kinases and phosphatases downstream of mTORC1 regulate TIF-IA. During mTORC1 inhibition, RNA Polymerase I is no longer recruited to the promoter and a significant amount of TIF-IA translocates to the cytoplasm [187]. mTORC2 has also been linked to the induction of ribosome biogenesis. During epithelial- mesenchymal transition (EMT), ribosome biogenesis is activated along with the dissociation of nuclear chromatin

remodeling complex (NoRC) from rDNA, increased expression and association of PolI, UBF, and the EMT-promoting transcription factor Snail1, with rDNA and enhanced nucleolar recruitment of Rictor [188]. These findings are consistent with the role of mTORC2 in EMT and its association with ribosomes [58, 59, 189].

In the next stage of ribosome biogenesis, RNA Polymerase III synthesizes the 5S rRNA and tRNAs. TF-IIIA, TF-IIIB, TF-IIIC, and RNA Polymerase III bind to the 5S rDNA promoter to form the preinitiation complex [190]. This complex is assisted by the association of TF-IIIB with MYC and recruitment of the HAT complex [191]. Conversely, the preinitiation complex can be inhibited by p53 or the pRB/p130 complex [192]. The ancillary TF-IIIC appears to be involved in recruiting mTORC1 to the promoter via its TOR-signaling (TOS) motif [193]. It is posited that TF-IIIC recruits mTORC1 to phosphorylate MAF1, a key repressor of RNA Polymerase III activity. Unphosphorylated MAF1 has increased nuclear retention, where it serves to inhibit TF-IIIB complex formation. mTORC1-mediated MAF1 phosphorylation at Ser75, Ser60, Thr64, and Ser68 during advantageous growth conditions facilitates its nuclear export, thereby promoting RNA Polymerase III-dependent transcription of 5S rDNA [194].

Following transcription of the 47S and 5S transcripts, RNA Polymerase II begins transcribing RPs with assistance from MYC:MAX heterodimer and HAT complex [180, 184]. After processing, the transcripts are exported to the cytoplasm for translation and subsequently imported to the nucleus for rRNA maturation and assembly [195]. The synthesis of RPs is tightly regulated by nutrient availability and mitogen or growth factor stimulation. It is believed that this delicate translational control hinges on the mTOR signaling axis [196]. All RP mRNAs contain a 5′-TOP motif, allowing cells to swiftly coordinate the production of proteins involved in the translation apparatus. Akt phosphorylates LARP6 and mTORC1 phosphorylates LARP1 to favor the translation of 5′-TOP mRNAs that are essential for ribosome biogenesis [164, 197]. Additionally, mTORC1 is implicated in the translation of 5′-TOP mRNAs by relieving 4E-BP1-mediated inhibition of eIF4F [147]. The nascent RPs are imported to the nucleus where chaperone proteins facilitate their proper incorporation into the growing ribosome subunits to stabilize rRNA folding [198].

mTORC1 also exerts indiscriminate control over general rDNA transcription via two distinct mechanisms. In one mechanism, S6K1 and RSK have been shown to phosphorylate MAD1 on Ser145 upon growth factor stimulation to promote ribosome biogenesis. MAD1 is a member of the MYC/MAX/MAD family that inhibits MYC-mediated transcription of rDNA by competing with MYC for heterodimerization with MAX. Phosphorylation at Ser145 accelerates MAD1 ubiquitination and degradation, thus promoting MYC-mediated transcription [136]. In a separate mechanism, Akt phosphorylates and stabilizes MDM2 at Ser166 and Ser186. This enhances MDM2s nuclear localization where it interacts with p300 and inhibits p19ARF, resulting in the degradation of p53 (a critical repressor of ribosome biogenesis) [199].

mTOR also controls rRNA processing, ribosome assembly, and maturation. In line with these functions, mTORC1 components are also found in the nucleolus. Inhibition of mTORC1 by rapamycin prevents the processing of pre-rRNA [200]. The assembly of eukaryotic ribosomes involves more than 200 assembly factors that includes several RNA-binding proteins, nucleases, RNA helicases, GTPases, and ATPases. The assembly and/or quantity of pre-ribosomes are sensitive to mTOR inhibition or amino acid levels [201]. The expression of the ribosome assembly factor URB1 correlates with increased raptor expression in colorectal cancer cells [202]. The ribosome biogenesis regulatory 1 homolog (RRS1) protein, which interacts with RPF2 to regulate the maturation of the 60S ribosomal subunit, enhances mTOR/Akt signaling [203]. Inhibiting Akt diminishes the proliferation and invasion of RRS1-overexpressing retinoblastoma (RB) cells. It is not clear, however, how RRS1 modulates mTOR/Akt signaling.

10. Coordinating protein synthesis with nutrient uptake and metabolism

Highly proliferating cells undergo metabolic reprogramming in order to enhance nutrient uptake, generate energy, and synthesize macromolecules necessary for growth and proliferation. The synthesis of proteins, including ribosome biogenesis and translation, is an energy-demanding process in the cell and thus requires abundant supply of nutrients including amino

acids, glucose (to generate energy and ribose), and nucleotides (to synthesize RNA) (Fig. 5). The transport of essential and non-essential amino acids is modulated by mTORC1 by mechanisms involving control of transcription and/or trafficking of amino acid transporters [204–206]. mTORC2 is directly involved in amino acid transport by phosphorylation of the cystine-glutamate antiporter xCT, which coordinates glutamate efflux with cystine influx [207]. While amino acids, particularly essential ones, are acquired from the diet, non-essential amino acids can be synthesized intracellularly. mTORC1 regulates the expression of amino acid synthetase such as the asparagine synthetase (ASNS) and arginosuccinate synthetase-1 (ASS1) [204, 208]. mTORC1, via the transcription factor ATF4, also controls *de novo* serine-glycine biosynthesis [209]. mTOR also indirectly controls amino acid biosynthesis via its role in modulating

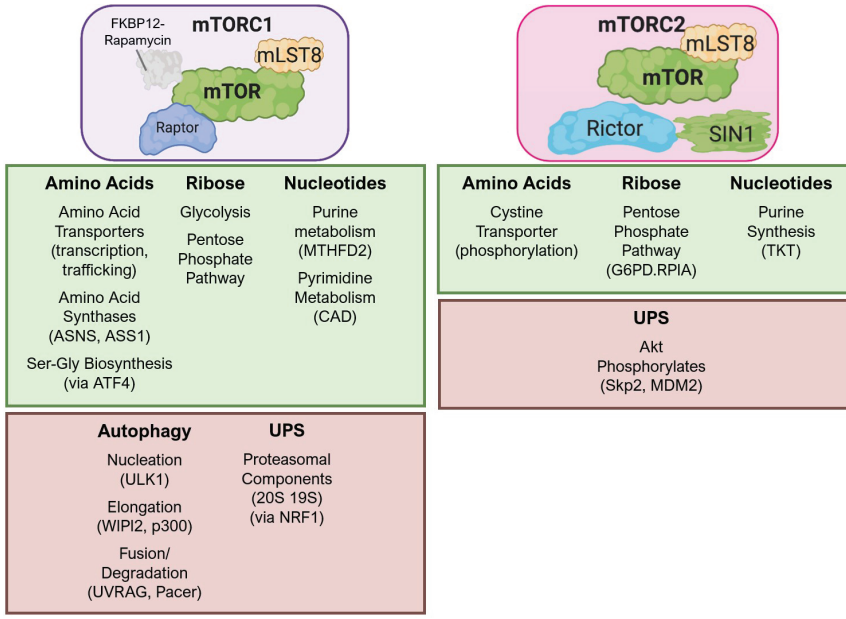

Figure 5. The mTOR complexes modulate metabolic processes to provide intermediates/metabolites required for protein synthesis.

They enhance the availability of key nutrients by controlling amino acid, ribose, and nucleotide metabolism (green box) and also control intracellular levels to maintain proteostasis by modulating autophagy and the ubiquitin proteasome system (UPS).

other metabolic pathways, such as glycolysis, the TCA cycle, and pentose phosphate pathway, that provide intermediates necessary for generating specific amino acids.

Ribosome biogenesis is a rapacious consumer of nucleotides and ribose. rRNA makes up almost 90% of the total RNA in a cell. In addition to controlling the synthesis of rRNA and RPs, mTORC1 is responsible for supplying precursors to support ongoing rates of ribosome biogenesis. mTORC1 modulates the expression of genes involved in the pentose phosphate pathway (PPP), which produces the backbone ribose-5-phosphate for synthesis of nucleic acids [210, 211]. mTORC1-dependent activation of SREBP increases flux through the oxidative branch of the pentose phosphate pathway (PPP), which generates NADPH and intermediary metabolites for nucleotide biosynthesis [210, 212]. mTORC1 is responsive to the depletion of purine nucleotides in a manner analogous to its sensing of amino acids [213]. mTORC1 increases ATF4-dependent expression of MTHFD2 (methylenetetrahydrofolate dehydrogenase 2), which stimulates the mitochondrial tetrahydrofolate (mTHF) cycle that provides one carbon units for purine synthesis [214]. Purine nucleotides, such as ATP and GTP, are used as sources of energy for protein synthesis. In particular, GTP is preferentially incorporated into rRNA and tRNA [215]. In addition, S6K1 phosphorylates CAD (carbamoyl-phosphate synthetase, aspartate transcarbamoylase, and dihydroorotase) at Ser1859, which catalyzes the first three steps of *de novo* pyrimidine biosynthesis [216]. mTORC2, via PI3K/Akt signaling, also modulates the PPP and nucleotide synthesis. Akt modulates the rate-limiting enzyme of the PPP, G6PD, and the ribose-5-phosphate isomerase A (RPIA) [217, 218]. It also enhances purine synthesis via phosphorylation of transketolase (TKT), a key enzyme of the non-oxidative branch of the PPP [219, 220]. In yeast, PPP enzymes have been shown to physically associate with TORC2, highlighting a role for TORC2 in this metabolic pathway.

In addition to promoting *de novo* synthesis of nutrients that are required for protein synthesis, mTOR also exerts control over intracellular nutrient sources such as from autophagy and the ubiquitin-proteasome system (UPS), processes that are crucial to maintain cellular proteostasis. While autophagy is the indiscriminate bulk slaughtering of large proteins and organelles at the autolysosomes, the UPS is a selective system in

which specific proteins are recognized and tagged for proteasomal degradation. Central to both processes is ubiquitination, the conjugation of the 76-amino acid 8.5 kDa ubiquitin protein to lysine residues [221]. The term autophagy encompasses macroautophagy, microautophagy, and chaperone-mediated autophagy [222]. Macroautophagy (hereafter autophagy) is the self-degradation process that is critical for cells to respond to nutrient stress and growth factor depletion. Autophagy is divided into five stages: induction, nucleation, elongation, lysosomal fusion, and degradation. Induction of autophagosome formation is governed by the ULK1-ATG13-FIP200 kinase complex, which is under tight control by mTORC1 and AMPK in response to fluctuations in energy and nutrient status [223]. Scarcity of amino acids inactivates mTORC1 and relieves inhibition of the ULK1 complex, which then translocates to ER subdomains. During the nucleation stage, the activated ULK1 complex prompts the PIK3C3 lipid kinase to synthesize phosphatidylinositol 3-phosphate (PI3P) and form the omegasome, which are ER compartments where the remaining core machinery are recruited. This includes the transmembrane protein ATG9 [224] and the two ubiquitin-like (Ubl) conjugation systems (ATG12-ATG5-ATG16L and LC3-PE) [225] that foster the covalent attachment of small ubiquitin-like protein LC3 to phosphatidylethanolamine to drive the expansion of the autophagosome membrane. mTORC1 is implicated in this elongation step by targeting WIPI2 [226] and the acetyltransferase p300 [227]. Once elongation is complete, the autophagosomes move along microtubules in a dynein-dependent manner and cluster near the microtubule-organizing center by the nucleus, where they fuse with lysosomes [228] with assistance from SNARE proteins [229]. This fusion and subsequent degradation stage is regulated by mTORC1-dependent control of UVRAG [230] and Pacer [231]. From these findings, it appears that mTORC1 directly targets components of the autophagy machinery to negatively regulate autophagic flux.

As a central coordinator of protein generation, it is fitting that the mTOR pathway also regulates protein demise through the UPS. Since mTOR is anabolic and the 26S proteasome is catabolic, it is tempting to assume they function in an opposing manner, however, the actual relationship is more intricate. There is accumulating evidence that proteasomal activity corresponds to mTORC1 activity in order to maintain quality

control during low fidelity global translation. mTORC1 activation predisposes the cell to ER stress, which triggers the UPR. This stress may arise from the increased synthesis of certain proteins not being accompanied by a corresponding increase in binding partners or chaperone proteins [232]. As the UPS is a cornerstone of the endoplasmic reticulum-associated protein degradation (ERAD) pathway, increased proteasomal activity may restore homeostasis in response to constitutive mTORC1 activation [233]. Zhang *et al*. found that mTORC1 activates NRF1 to promote the expression of mRNAs encoding subunits of the 20S and 19S proteasomal components. Consensus sterol regulatory elements (SREs) are conserved in the promoter of NRF1 genes, suggesting that SREBP1 is downstream of mTORC1 in the induction of NRF1 activity. Therefore, mTORC1 promotes the translation of proteasomal components to ensure quality control and supply substrates for sustained protein synthesis [234]. In line with these findings, amino acid starvation (associated with mTORC1 inhibition) enhances polyubiquitination of the proteasome, resulting in stress-induced autophagy of the proteolytic machinery via p62 [235]. The correlation of proteophagy with mTORC1 repression indicates that these two pathways are intimately linked to maintain adequate flux of proteins in the cell. Consistent with this, an early study found that rapamycin treatment of lymphocytes suppressed the expression of proteasome activator PA28 [236].

In addition to controlling prevalence of proteasome subunits, mTOR signaling also targets proteins for degradation by the UPS. Akt phosphorylates the substrate-binding unit of the SCF^{Skp2} ligase, Skp2, at Ser72 to promote its translocation to the cytosol [237], where it targets the cell cycle regulators p21 [238] and p27 [239] for proteasomal destruction. Akt also phosphorylates and stabilizes MDM2 at Ser166 and Ser186, enhancing its E3 ubiquitin ligase activity towards p53 [199]. Hence, the pro-proliferative effects of Akt are mediated in part by E3 ubiquitin ligases. Finally, many substrates of the cullin-RING E3 ubiquitin ligase require phosphorylation before they are ubiquitinated. mTORC1 phosphorylates DEPTOR for degradation, which concomitantly augments mTOR signaling in a positive feedback loop [240]. During prolonged IGF stimulation, mTORC1 phosphorylates IRS1 at Ser422 to prime it for $SCF^{\beta TrCP}$-mediated ubiquitination, constituting a potent negative feedback loop in

mTOR signaling [241]. mTORC1 also phosphorylates WIPI2 at Ser395 to facilitate its ubiquitination by E3 ubiquitin ligase HUWE1, resulting in diminished autophagic flux, which may indirectly serve to increase proteasomal activity [226]. In summary, mTOR signaling has opposing effects on proteasomal abundance depending on cellular circumstances.

The mTOR pathway also governs protein quality control via the Unfolded Protein Response (UPR). Proper folding requires chaperones and foldases, while maturation involves signal sequence cleavage, N-linked glycosylation, isomerization, and reduction of disulfide bridges. Under certain circumstances, misfolded and unfolded proteins accumulate in the ER lumen. If they cannot be fixed by ER chaperones, they are sent to the ERAD pathway for proteolysis [242]. The Unfolded Protein Response (UPR) is a cytoprotective response to prolonged ER stress conditions via transcriptional induction of molecular chaperones, however, the UPR pathway may lead to apoptosis during extensive and severe ER stress [243]. The UPR is a trinity of signaling cascades that originates in the ER. The growing presence of misfolded proteins triggers the UPR via the dissociation of the molecular chaperone BiP from the ER membrane-embedded receptors PERK, IRE1α, and ATF6α, each of which impart distinct but overlapping cell fate signals. The UPR relieves ER Stress by halting global translation, synthesizing ER chaperone proteins to increase the protein-folding capacity of the ER, and upregulating the ERAD pathway to clear terminally misfolded proteins from the ER [244]. Similar to the mTOR pathway, the UPR is sensitive to oxygen and nutrient level fluctuations. Glucose starvation is a popular method of eliciting the UPR because it precipitously decreases ATP levels, causing failure of ER Ca^{2+} pumps [245]. This starvation condition activates mTORC2 and promotes flux through the hexosamine biosynthesis pathway (HBP) via modulation of the rate-limiting enzyme of this pathway, GFAT1 [64, 246]. The HBP generates the metabolite, UDP-GlcNAc, which is required for proper protein folding via *N*-glycosylation of secretory and membrane proteins. Additionally, hypoxia is another trigger of UPR signaling by inhibition of disulfide bond formation or upregulation of GSK3β [247]. The interplay between mTOR signaling and the UPR is complex with multiple layers of regulation. ATF6α promotes cell survival by upregulating Rheb in an Akt independent fashion, which activates mTORC1 [248]. Similarly, PERK

increases Akt activity via phosphorylation of its substrate diacylglycerol (DAG) [249]. The increased mTORC1 activity may contribute to eventual Akt inhibition via suppressive phosphorylation of IRS1 and mTORC2 by S6K1. PERK-CHOP mediated transcription of TRB3 also inhibits Akt during prolonged ER Stress [250]. Lastly, ER stress leads to GSK3β-mediated phosphorylation of Rictor at Ser1235, which inhibits Akt activity and contributes to the UPR apoptotic signaling [251]. From these studies, it appears that ER stress causes early activation of mTORC1 and mTORC2 activity and eventual inhibition, which elicits apoptotic UPR signaling.

In addition to controlling the UPR, the mTOR pathway exerts control over the Integrated Stress Response (ISR), which is an evolutionarily conserved signaling network that helps the cell adapt to different forms of stress. The ISR restores homeostasis by reprogramming gene expression via several downstream transcription factors. Four specialized kinases (PERK, GCN2, PKR, and HRI) sense these various stresses and converge by phosphorylating the α subunit of eIF2 at Ser51 [137]. eIF2 is a heterotrimeric translation initiation factor that forms the ternary eIF2-GTP-tRNAiMet complex. eIF2 phosphorylation blocks eIF2B GEF activity, resulting in global inhibition of protein synthesis. Simultaneously, phosphorylation of eIF2 also stimulates expression of certain mRNAs with short inhibitory upstream open reading frames (uORFs) in their 5′ UTRs to restore homeostasis. If the stress is not eliminated, the ISR regresses into an apoptotic signaling cascade [252]. The ISR has multiple layers of crosstalk with both mTOR complexes. Its regulation of mTORC2 is highly dependent on the temporal dimension of stress. In response to ER stress, the PERK branch upregulates PI3K activity, and, by extension, mTORC2 activity in both a p-eIF2α dependent [253] and independent manner [249]. While p-eIF2α induced mTORC2 activity promotes pro-survival signaling to combat stress, it concomitantly promotes tumor survival during oxidative stress [254]. Prolonged p-eIF2α signaling can attenuate Akt activity, leading to death of human leukemia cells [255]. Sustained p-eIF2α signaling also activates GSK3β, which further inhibits mTORC2 activity [256]. From these studies, it appears that the ISR induces early activation of the mTORC2/Akt signaling axis to combat acute stress, but eventually suppresses it in response to prolonged stress.

The ISR and mTORC1 also have several layers of cross regulation. In PTEN-deficient cells, hyperactivated mTORC1 phosphorylates eIF2β at Ser2 and Ser67 to promote protein phosphatase 1 (PP1) mediated dephosphorylation of eIF2α [257]. In TSC-deficient cells, hyperactive mTORC1 activity exceeds the protein folding capacity of the cell and triggers ER stress. mTORC1-driven ER stress elicits a unique UPR, where the PERK-eIF2α branch is selectively upregulated to promote cell survival [258]. This pro-survival response during ER stress is antithetical to PERK-eIF2α inhibition in TSC-deficient cells during oxidative stress, which promotes cell death [259]. During amino acid starvation, mTORC1 inhibition recruits protein phosphatase 6 (PP6) to dephosphorylate GCN2, promoting its activation [260]. In *Saccharomyces cerevisiae*, TAP42 is involved in rapamycin-induced GCN2 Ser577 dephosphorylation to promote GCN2 activation and subsequent phosphorylation of eIF2α at Ser51 [261]. In fission yeast grown in the presence of nitrogen and amino acids, TOR2 as part of TORC1 is responsible for preventing eIF2α phosphorylation by suppressing GCN2 activity [262]. While mTORC1 can suppress GCN2, GCN2 also exerts an inhibitory effect on mTORC1. Upon leucine or arginine deprivation, GCN2 can inhibit mTORC1 activity in a manner that requires eIF2α phosphorylation but is independent of ATF4 [263]. Upon amino acid starvation, GCN2 upregulates the stress-responsive transcription factor ATF4 to induce expression of Sestrin2, which sustains repression of mTORC1 by blocking its lysosomal localization [264]. Moreover, GCN2-eIF2α inhibition increases mTORC1 activity in cells and precludes the Amino Acid Deprivation Response (AADR) in response to Asparaginase treatment [265]. It appears that communication between the mTOR pathway and the ISR is bidirectional and is highly dependent on the type of stress used to elicit a response.

11. Conclusions and future directions

mTOR has multi-faceted roles in translation. It modulates the activity, expression and function of proteins involved in translation initiation, RNA stability and processing, and ribosome biogenesis in response to nutrient availability and stress conditions. Related to its function in translation, it also regulates the availability of macromolecules, such as amino acids and

nucleotides, that are utilized for protein synthesis. In turn, mTOR responds to the intracellular levels of nutrients. While mTORC1 activity is enhanced by abundance of specific amino acids (e.g., leucine and glutamine), mTORC2 activity is augmented during limitation of amino acids such as glutamine [64]. How mTORC1 responds to amino acid levels has been the subject of many investigations and reveal the involvement of different amino acid sensors that modulate mTORC1 activation in Rag-dependent or independent manner [26]. How mTORC2 is modulated by amino acid starvation remains to be elucidated. Since translation is dependent not only on the availability of amino acid but also on glucose and nucleotides, which are utilized for RNA synthesis, mTOR signaling is also influenced by these nutrients. Glucose starvation downregulates mTORC1 via AMPK-mediated mechanisms that modulate raptor and TSC2 phosphorylation [42, 43]. In contrast, glucose limitation upregulates mTORC2 via AMPK phosphorylation of mTOR [64, 65]. Purine nucleotide levels, specifically adenylate, are sensed by mTORC1 via TSC and Rheb, independently of AMPK [213]. Future studies should elucidate the specific mechanisms involved in mTORC1 and mTORC2 regulation by these different nutrients leading to control of translation. How availability of nutrients critical for translation coordinate the different processes that control translation and how they influence translation of specific mRNAs would also need further investigation.

Increased activation of mTOR signaling, leading to deregulated translation, underlies a variety of pathological conditions including cancer. Rapamycin and its analogues (rapalogs), which are allosteric inhibitors, are used as immunosuppressants and are also approved for the treatment of specific types of cancer [80]. Rapalogs and mTOR ATP-competitive inhibitors (TORKis), which target the mTOR active site, are undergoing clinical trials to treat a variety of malignancies. It is noteworthy that rapamycin only inhibits some functions of mTORC1, such as phosphorylation of S6K1, but does not effectively block phosphorylation of 4E-BP1 [82, 266]. mTOR inhibitors, including dual PI3K/mTOR inhibitors, while more potent in suppressing both mTORC1 and mTORC2 in pre-clinical studies, have undesirable toxic side effects in phase I clinical studies for several types of cancer [80]. Studies are ongoing to improve their pharmacokinetic properties and to devise better strategies to combine mTOR

inhibition with other targeted therapies, chemotherapeutic agents, and immunotherapy. Diseases linked to translation defects such as ribosomopathies, which are characterized by defective ribosome biogenesis, could also benefit from restoring optimal mTOR signaling. Activating mTOR in ribosomopathies via treatment with L-leucine in Diamond-Blackfan anemia syndrome (DBA) and 5q-syndrome, promoted mRNA translation and alleviated anemia in animal models and patients [267, 268]. These findings highlight how the metabolic functions of mTOR are intertwined with its control of protein synthesis. A better understanding of how mTORC1 and mTORC2 signaling orchestrates translation in different tissues in response to nutrient requirements and availability along with distinct gene expression is needed to improve therapeutic strategies for cancer and a variety of diseases linked to translation deregulation.

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Abbreviations

αKG	α-ketoglutarate
4EBP1	4E-binding protein 1
Ac-CoA	acetyl-coenzyme A
ACL	ATP citrate lyase
AGC kinases	Protein kinase A (PKA), PKG, PKC
AMPK	AMP-activated protein kinase
Arf1	ADP-ribosylation factor 1
ASNS	asparagine synthetase
ASS1	argininosuccinate synthetase-1
ATF4	Activating Transcription Factor 4
ATG13/ATG14	Autophagy related
BAD	Bcl-2-associated death promoter
BCAA	branched-chain amino acids (isoleucine, leucine and valine)

CAD	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydro-orotase
CCT	chaperonin containing tailless complex polypeptide
CLUH	clustered mitochondria homolog
CPT1	carnitine palmitoyltransferase I
CREM	cyclic AMP responsive element modulator
DAP1	Death associated protein 1
DEPDC5	DEP domain containing 5
DEPTOR	DEP domain-containing mTOR-interacting protein
eIF4B	eukaryotic translation initiation factor 4B
EMT	epithelial-to-mesenchymal transition
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
FA	fatty acids
FAO	fatty acid oxidation
FASN	Fatty acid synthase
FKBP12	12-kDa FK506-binding protein
FLCN	folliculin
FNIP1 and FNIP2	folliculin interacting protein 1 and 2
FOXK1	Forkhead box K1
FOXO	forkhead box O
FRB domain	FKBP12/rapamycin binding domain
G6PD	glucose-6-phosphate dehydrogenase
GAP	GTPase-activating protein
GEF	guanine nucleotide exchange factor
GlcNAc	*N*-Acetylglucosamine
GLUT1	glucose transporter 1
GSK3β	glycogen synthase kinase 3 beta
GTP	Guanosine-5′-triphosphate
HAT	histone acetyltransferase
HBP	hexosamine biosynthesis pathway
HIF	hypoxia-inducible factor
HM	hydrophobic motif
IGF1R	insulin-like growth factor 1 receptor
IKK	inhibitor of nuclear factor k-B kinase
IMP1	IGF2 mRNA binding protein 1
IMPDH2	Inosine-5′-monophosphate dehydrogenase 2

IRS-1	insulin receptor substrate-1
LAMTOR2	Late Endosomal/Lysosomal Adaptor, MAPK and mTOR Activator 2
LARP1/6	La related protein 1/6
LATS1/LATS2	LArge Tumor Suppressor 1.2
MAM	mitochondria-associated ER membranes
MAPK	mitogen-activated protein kinase
MEFs	Murine Embryonic Fibroblasts
MTHFD2	methylenetetrahydrofolate dehydrogenase 2
mTOR	mechanistic target of rapamycin
mTORC1	mTOR complex 1
mTORC2	mTOR complex 2
NAD	Nicotinamide adenine dinucleotide
NADK	nicotinamide adenine dinucleotide kinase
NADPH	nicotinamide adenine dinucleotide phosphate
NPRL2	nitrogen permease regulator 2-like protein
OGT	O-GlcNAc transferase
OXPHOS	oxidative phosphorylation
PABP	poly(A)-binding protein
PDCD4	programmed cell death 4 protein
PDK	pyruvate dehydrogenase kinase
PDK1	phosphoinositide-dependent kinase 1
PH	pleckstrin homology domain
PI3K	phosphatidylinositol-3-kinase
PIP3	phosphatidylinositol 3,4,5-trisphosphate
PKC	protein kinase C
PLA2	phospholipase A2
Poly-Q	polyglutamine
PPP	pentose phosphate pathway
PRAS40	proline-rich Akt substrate of 40 kDa
Protor	Protein observed with rictor
PRPP	phosphoribosyl pyrophosphate
PRPS2	phosphoribosyl pyrophosphate synthetase 2
PTEN	Phosphatase and tensin homolog
Rag	Ras-related GTP binding protein
RAPTOR	regulatory-associated protein of mTOR

REDD1	regulated in development and DNA damage response 1
Rheb	Ras homolog enriched in brain
ROS	reactive oxygen species
RBP	RNA binding protein
RNP	ribonucleoprotein complexes
RPIA	ribose-5-phosphate isomerase A
RSK	p90 ribosomal S6 kinase
RTK	receptor tyrosine kinase
S6K	ribosomal protein S6 kinase
SAM	S-adenosylmethionine
SAMTOR	SAM sensor upstream of mTORC1
SIN1	stress-activated protein kinase (SAPK) interacting protein 1
SIRT1	Sirtuin 1
SKAR	S6 kinase 1 Aly/REF-like target
SLC	Solute carrier
SREBP	sterol regulatory element binding protein
TCA	tricarboxylic acid cycle
THF	tetrahydrofolate
TKT	transketolase
TM	turn motif
TNBC	triple negative breast cancer
TOP	5′terminal oligopyrimidine
TOS	TOR signaling motif
TSC	tuberous sclerosis complex
UBF	Upstream Binding Factor
UDP-GlcNAc	uridine-5-diphosphate-N-acetylglucosamine
ULK1	unc-51 Like Autophagy Activating Kinase 1
UMP	uridine monophosphate
UPR	unfolded protein response
UPS	ubiquitin proteasome system
URB1	URB1 Ribosome Biogenesis Homolog
UTR	untranslated region
WD40	Trp Asp repeats
WDR24	WD repeat-containing protein 24

References

1. Heitman J, Movva NR, and Hall MN. (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*, **253**(5022), 905–909.
2. Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, *et al.* (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature*, **369**(6483), 756–758.
3. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P and Snyder SH. (1994) RAFT1: A mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell*, **78**(1), 35–43.
4. Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, Wiederrecht G, *et al.* (1995) Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. *Journal of Biological Chemistry*, **270**(2), 815–822.
5. Kim DH, Sarbassov DD, Ali SM, Latek RR, Guntur KV, Erdjument-Bromage H, *et al.* (2003) GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Molicular Cell*, **11**(4), 895–904.
6. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, *et al.* (2009) DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell*, **137**(5), 873–886.
7. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, *et al.* (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell*, **110**(2), 177–189.
8. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, *et al.* (2002) mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*, **110**(2), 163–175.
9. Wang L, Harris TE, Roth RA and Lawrence JC Jr. (2007) PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding. *Journal of Biological Chemistry*, **282**(27), 20036–20044.
10. Oshiro N, Takahashi R, Yoshino K, Tanimura K, Nakashima A, Eguchi S, *et al.* (2007) The proline-rich Akt substrate of 40 kDa (PRAS40) is a physiological substrate of mammalian target of rapamycin complex 1. *Journal of Biological Chemistry*, **282**(28), 20329–20339.
11. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, *et al.* (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Current Biology*, **14**(14), 1296–1302.
12. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, *et al.* (2006) SIN1/MIP1 Maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell*, **127**(1), 125–137.
13. Pearce LR, Huang X, Boudeau J, Pawlowski R, Wullschleger S, Deak M, *et al.* (2007) Identification of protor as a novel rictor-binding component of mTOR complex-2. *Biochemical Journal*, **405**(3), 513–522.

14. Shimobayashi M and Hall MN. (2014) Making new contacts: The mTOR network in metabolism and signalling crosstalk. *Nature Reviews Molecular Cell Biology*, **15**(3), 155–162.
15. Saxton RA and Sabatini DM. (2017) mTOR signaling in growth, metabolism, and disease. *Cell*, **168**(6), 960–976.
16. Mossmann D, Park S and Hall MN. (2018) mTOR signalling and cellular metabolism are mutual determinants in cancer. *Nature Reviews Cancer*, **18**(12), 744–757.
17. Kim J and Guan KL. (2019) mTOR as a central hub of nutrient signalling and cell growth. *Nature Cell Biology*, **21**(1), 63–71.
18. Bar-Peled L, Schweitzer LD, Zoncu R and Sabatini DM. (2012) Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. *Cell*, **150**(6), 1196–1208.
19. Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y and Sabatini DM. (2011) mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. *Science*, **334**(6056), 6786–83.
20. Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, *et al.* (2013) A Tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science*, **340**(6136), 1100–1106.
21. Wolfson RL, Chantranupong L, Wyant GA, Gu X, Orozco JM, Shen K, *et al.* (2017) KICSTOR recruits GATOR1 to the lysosome and is necessary for nutrients to regulate mTORC1. *Nature*, **543**(7645), 438–442.
22. Kim JH, Lee C, Lee M, Wang H, Kim K, Park SJ, *et al.* (2017) Control of leucine-dependent mTORC1 pathway through chemical intervention of leucyl-tRNA synthetase and RagD interaction. *Nature Communications*, **8**(1), 732.
23. Tsun ZY, Bar-Peled L, Chantranupong L, Zoncu R, Wang T, Kim C, *et al.* (2013) The folliculin tumor suppressor is a GAP for the RagC/D GTPases that signal amino acid levels to mTORC1. *Molecular Cell*, **52**(4), 495–505.
24. Starling GP, Yip YY, Sanger A, Morton PE, Eden ER and Dodding MP. (2016) Folliculin directs the formation of a Rab34-RILP complex to control the nutrient-dependent dynamic distribution of lysosomes. *EMBO Reports*, **17**(6), 823–841.
25. Kim E, Goraksha-Hicks P, Li L, Neufeld TP and Guan KL. (2008) Regulation of TORC1 by Rag GTPases in nutrient response. *Nature Cell Biology*, **10**(8), 935–945.
26. Wolfson RL and Sabatini DM. (2017) The dawn of the age of amino acid sensors for the mTORC1 pathway. *Cell Metabolism*, **26**(2), 301–309.
27. Saxton RA, Chantranupong L, Knockenhauer KE, Schwartz TU and Sabatini DM. (2016) Mechanism of arginine sensing by CASTOR1 upstream of mTORC1. *Nature*, **536**(7615), 229–233.
28. Rebsamen M, Pochini L, Stasyk T, de Araujo ME, Galluccio M, Kandasamy RK, *et al.* (2015) SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1. *Nature*, **519**(7544), 477–481.
29. Saxton RA, Knockenhauer KE, Wolfson RL, Chantranupong L, Pacold ME, Wang T, *et al.* (2016) Structural basis for leucine sensing by the Sestrin2-mTORC1 pathway. *Science*, **351**(6268), 53–58.

30. Son SM, Park SJ, Lee H, Siddiqi F, Lee JE, Menzies FM, *et al.* (2019) Leucine Signals to mTORC1 via Its Metabolite Acetyl-Coenzyme A. *Cell Metabolism*, **29**(1), 192–201 e7.
31. Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, Nyfeler B, *et al.* (2009) Bidirectional transport of amino acids regulates mTOR and autophagy. Cell, **136**(3), 521–534.
32. Meng D, Yang Q, Wang H, Melick CH, Navlani R, Frank AR, *et al.* (2020) Glutamine and asparagine activate mTORC1 independently of Rag GTPases. *Journal of Biological Chemistry*, **295**(10), 2890–2899.
33. Willems L, Jacque N, Jacquel A, Neveux N, Maciel TT, Lambert M, *et al.* (2013) Inhibiting glutamine uptake represents an attractive new strategy for treating acute myeloid leukemia. *Blood*, **122**(20), 3521–3532.
34. Gu X, Orozco JM, Saxton RA, Condon KJ, Liu GY, Krawczyk PA, *et al.* (2017) SAMTOR is an S-adenosylmethionine sensor for the mTORC1 pathway. *Science*, **358**(6364), 813–818.
35. Dibble CC, Elis W, Menon S, Qin W, Klekota J, Asara JM, *et al.* (2012) TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. *Molecular Cell*, **47**(4), 535–546.
36. Manning BD, Tee AR, Logsdon MN, Blenis J and Cantley LC. (2002) Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Molecular Cell*, **10**(1), 151–162.
37. Tee AR, Manning BD, Roux PP, Cantley LC and Blenis J. (2003) Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Current Biology*, **13**(15), 1259–1268.
38. Yang H, Jiang X, Li B, Yang HJ, Miller M, Yang A, *et al.* (2017) Mechanisms of mTORC1 activation by RHEB and inhibition by PRAS40. *Nature*, **552**(7685), 368–373.
39. Inoki K, Li Y, Zhu T, Wu J and Guan KL. (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nature Cell Biology*, **4**(9), 648–657.
40. Roux PP, Ballif BA, Anjum R, Gygi SP and Blenis J. (2004) Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. *Proceedings of the National Academy of Sciences of the United States*, **101**(37), 13489–13494.
41. Ma L, Chen Z, Erdjument-Bromage H, Tempst P and Pandolfi PP. (2005) Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell*, **121**(2), 179–193.
42. Inoki K, Zhu T and Guan KL. (2003) TSC2 mediates cellular energy response to control cell growth and survival. *Cell*, **115**(5), 577–590.
43. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, *et al.* (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Molecular Cell*, **30**(2), 214–226.

44. Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, *et al.* (2006) TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell*, **126**(5), 955–968.
45. DeYoung MP, Horak P, Sofer A, Sgroi D and Ellisen LW. (2008) Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. *Genes & Development*, **22**(2), 239–251.
46. Feng Z, Hu W, de Stanchina E, Teresky AK, Jin S, Lowe S, *et al.* (2007) The regulation of AMPK beta1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-AKT-mTOR pathways. *Cancer Research*, **67**(7), 3043–3053.
47. York B, Lou D and Noonan DJ. (2006) Tuberin nuclear localization can be regulated by phosphorylation of its carboxyl terminus. Mol *Cancer Research*, **4**(11), 885–897.
48. Cai SL, Tee AR, Short JD, Bergeron JM, Kim J, Shen J, *et al.* (2006) Activity of TSC2 is inhibited by AKT-mediated phosphorylation and membrane partitioning. *Journal of Cell Biology*, **173**(2), 279–289.
49. Menon S, Dibble CC, Talbott G, Hoxhaj G, Valvezan AJ, Takahashi H, *et al.* (2014) Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. *Cell*, **156**(4), 771–785.
50. Gaubitz C, Oliveira TM, Prouteau M, Leitner A, Karuppasamy M, Konstantinidou G, *et al.* (2015) Molecular basis of the rapamycin insensitivity of target of rapamycin complex 2. *Molecular Cell*, **58**(6), 977–988.
51. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, *et al.* (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Molecular Cell*, **22**(2), 159–168.
52. Liu P, Gan W, Chin YR, Ogura K, Guo J, Zhang J, *et al.* (2015) PtdIns(3,4,5) P3-dependent activation of the mTORC2 kinase complex. *Cancer Discovery*, **5**(11), 1194–1209.
53. Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC and Abraham RT. (2017) The PI3K pathway in human disease. *Cell*, **170**(4), 605–635.
54. Maehama T and Dixon JE. (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *Journal of Biological Chemistry*, **273**(22), 13375–13378.
55. Currie RA, Walker KS, Gray A, Deak M, Casamayor A, Downes CP, *et al.* (1999) Role of phosphatidylinositol 3,4,5-trisphosphate in regulating the activity and localization of 3-phosphoinositide-dependent protein kinase-1. *Biochemical Journal*, 337 (Pt 3)(Pt 3), 575–583.
56. Gan X, Wang J, Su B and Wu D. (2011) Evidence for Direct Activation of mTORC2 Kinase Activity by Phosphatidylinositol 3,4,5-Trisphosphate. *Journal of Biological Chemistry*, **286**(13), 10998–1002.
57. Sarbassov DD, Guertin DA, Ali SM and Sabatini DM. (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science, **307**(5712), 1098–1101.

58. Oh WJ, Wu CC, Kim SJ, Facchinetti V, Julien LA, Finlan M, *et al.* (2010) mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent Akt polypeptide. *EMBO Journal*, **29**(23), 3939–3951.
59. Zinzalla V, Stracka D, Oppliger W and Hall MN. (2011) Activation of mTORC2 by association with the ribosome. *Cell*, **144**(5), 7577–68.
60. Inoki K, Li Y, Xu T and Guan KL. (2003) Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes & Development*, **17**(15), 1829–1834.
61. Harrington LS, Findlay GM and Lamb RF. (2005) Restraining PI3K: mTOR signalling goes back to the membrane. *Trends in Biochemical Sciences*, **30**(1), 35–42.
62. Dibble CC, Asara JM and Manning BD. (2009) Characterization of Rictor phosphorylation sites reveals direct regulation of mTOR complex 2 by S6K1. *Molecular Cell Biology*, **29**(21), 5657–5670.
63. Julien LA, Carriere A, Moreau J and Roux PP. (2010) mTORC1-activated S6K1 phosphorylates Rictor on threonine 1135 and regulates mTORC2 signaling. *Molecular Cell Biology*, **30**(4), 908–921.
64. Moloughney JG, Kim PK, Vega-Cotto NM, Wu CC, Zhang S, Adlam M, *et al.* (2016) mTORC2 Responds to Glutamine Catabolite Levels to Modulate the Hexosamine Biosynthesis Enzyme GFAT1. *Molecular Cell*, **63**(5), 811–826.
65. Kazyken D, Magnuson B, Bodur C, Acosta-Jaquez HA, Zhang D, Tong X, *et al.* (2019) AMPK directly activates mTORC2 to promote cell survival during acute energetic stress. *Science Signaling*, **12**(585).
66. Hanover JA, Chen W and Bond MR. (2018) O-GlcNAc in cancer: An Oncometabolism-fueled vicious cycle. *Journal of Bioenergetics and Biomembranes*, **50**(3), 155–173.
67. Huang J and Manning BD. (2009) A complex interplay between Akt, TSC2 and the two mTOR complexes. *Biochemical Society Transactions*, **37**(Pt 1), 217–222.
68. Kowalsky AH, Namkoong S, Mettetal E, Park HW, Kazyken D, Fingar DC, *et al.* (2020) The GATOR2-mTORC2 axis mediates Sestrin2-induced AKT Ser/Thr kinase activation. *Journal of Biological Chemistry*, **295**(7), 1769–1780.
69. Tao R, Xiong X, Liangpunsakul S and Dong XC. (2015) Sestrin 3 protein enhances hepatic insulin sensitivity by direct activation of the mTORC2-Akt signaling. *Diabetes*, **64**(4), 1211–1223.
70. Tato I, Bartrons R, Ventura F and Rosa JL. (2011) Amino acids activate mammalian target of rapamycin complex 2 (mTORC2) via PI3K/Akt signaling. *Journal of Biological Chemistry*, **286**(8), 6128–6142.
71. Menon D, Salloum D, Bernfeld E, Gorodetsky E, Akselrod A, Frias MA, *et al.* (2017) Lipid sensing by mTOR complexes via de novo synthesis of phosphatidic acid. *Journal of Biological Chemistry*, **292**(15), 6303–6311.
72. Xu Y, Lai E, Liu J, Lin J, Yang C, Jia C, *et al.* (2013) IKK interacts with rictor and regulates mTORC2. *Cell Signaling*, **25**(11), 2239–2245.

73. Lone MU, Miyan J, Asif M, Malik SA, Dubey P, Singh V, *et al.* (2019) Direct physical interaction of active Ras with mSIN1 regulates mTORC2 signaling. *BMC Cancer*, **19**(1), 1236.
74. Merhi A, Delree P and Marini AM. (2017) The metabolic waste ammonium regulates mTORC2 and mTORC1 signaling. *Scientific Rreports*, **7**, 44602.
75. Shah OJ, Wang Z and Hunter T. (2004) Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. *Current Biology*, **14**(18), 1650–1656.
76. Yu Y, Yoon SO, Poulogiannis G, Yang Q, Ma XM, Villen J, *et al.* (2011) Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. *Science*, **332**(6035), 1322–1326.
77. Su B and Jacinto E. (2011) Mammalian TOR signaling to the AGC kinases. *Critical Reviews in Biochemistry and Molecular Biology*, **46**(6), 527–547.
78. Baffi TR, Lorden G, Wozniak JM, Feichtner A, Yeung W, Kornev AP, *et al.* (2021) mTORC2 controls the activity of PKC and Akt by phosphorylating a conserved TOR interaction motif. *Science Signaling*, **14**(678).
79. Garcia-Martinez JM and Alessi DR. (2008) mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochemical Journal*, **416**(3), 375–385.
80. Magaway C, Kim E and Jacinto E. Targeting mTOR and metabolism in cancer: Lessons and innovations. *Cells*, **8**(12), 1–51.
81. Easton JB and Houghton PJ. (2006) mTOR and cancer therapy. *Oncogene*, **25**(48), 6436–6446.
82. Choo AY, Yoon SO, Kim SG, Roux PP and Blenis J. (2008) Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. *Proceedings of the National Academy of Sciences of the United States*, **105**(45), 17414–1749.
83. Tabernero J, Rojo F, Calvo E, Burris H, Judson I, Hazell K, *et al.* (2008) Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: A phase I tumor pharmacodynamic study in patients with advanced solid tumors. *Journal of Clinical Oncology*, **26**(10), 1603–1610.
84. Benjamin D, Colombi M, Moroni C and Hall MN. (2011) Rapamycin passes the torch: A new generation of mTOR inhibitors. *Nature Reviews Drug Discovery*, **10**(11), 868–880.
85. Britschgi A, Andraos R, Brinkhaus H, Klebba I, Romanet V, Müller U, *et al.* (2012) JAK2/STAT5 inhibition circumvents resistance to PI3K/mTOR blockade: A rationale for cotargeting these pathways in metastatic breast cancer. *Cancer Cell*, **22**(6), 796–811.
86. Rodrik-Outmezguine VS, Okaniwa M, Yao Z, Novotny CJ, McWhirter C, Banaji A, *et al.* (2016) Overcoming mTOR resistance mutations with a new-generation mTOR inhibitor. *Nature*, **534**(7606), 272–276.

87. La Manna F, De Menna M, Patel N, Karkampouna S, De Filippo MR, Klima I, *et al.* (2020) Dual-mTOR inhibitor rapalink-1 reduces prostate cancer patient-derived xenograft growth and alters tumor heterogeneity. *Frontiers in Oncology*, **10**, 1012.
88. de Mello NP, Orellana AM, Mazucanti CH, de Morais Lima G, Scavone C and Kawamoto EM. (2019) Insulin and autophagy in neurodegeneration. *Frontiers in Neuroscience*, **13**, 491.
89. Sun YX, Ji X, Mao X, Xie L, Jia J, Galvan V, *et al.* (2014) Differential activation of mTOR complex 1 signaling in human brain with mild to severe Alzheimer's disease. *Journal of Alzheimer's Disease*, **38**(2), 437–444.
90. Bellozi PMQ, Gomes GF, de Oliveira LR, Olmo IG, Vieira É LM, Ribeiro FM, *et al.* (2019) NVP-BEZ235 (Dactolisib) has protective effects in a transgenic mouse model of Alzheimer's disease. *Frontiers in Pharmacology*, **10**, 1345.
91. Jiang TF, Zhang YJ, Zhou HY, Wang HM, Tian LP, Liu J, *et al.* (2013) Curcumin ameliorates the neurodegenerative pathology in A53T α-synuclein cell model of Parkinson's disease through the downregulation of mTOR/p70S6K signaling and the recovery of macroautophagy. *Journal of Neuroimmune Pharmacology*, **8**(1), 356–369.
92. Siddiqui A, Bhaumik D, Chinta SJ, Rane A, Rajagopalan S, Lieu CA, *et al.* (2015) Mitochondrial quality control via the PGC1α-TFEB signaling pathway is compromised by Parkin Q311X mutation but independently restored by Rapamycin. *Journal of Neuroscience*, **35**(37), 12833–12844.
93. Kaeberlein M, Powers RW, 3rd, Steffen KK, Westman EA, Hu D, Dang N, *et al.* (2005) Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science*, **310**(5751), 1193–1196.
94. Wu JJ, Liu J, Chen EB, Wang JJ, Cao L, Narayan N, *et al.* (2013) Increased mammalian lifespan and a segmental and tissue-specific slowing of aging after genetic reduction of mTOR expression. *Cell Reports*, **4**(5), 913–920.
95. Bitto A, Ito TK, Pineda VV, LeTexier NJ, Huang HZ, Sutlief E, *et al.* (2016) Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice. *Elife*, **5**.
96. López-Otín C, Blasco MA, Partridge L, Serrano M and Kroemer G. (2013) The hallmarks of aging. *Cell*, **153**(6), 1194–1217.
97. Zid BM, Rogers AN, Katewa SD, Vargas MA, Kolipinski MC, Lu TA, *et al.* (2009) 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in Drosophila. *Cell*, **139**(1), 149–160.
98. Tóth ML, Sigmond T, Borsos E, Barna J, Erdélyi P, Takács-Vellai K, *et al.* (2008) Longevity pathways converge on autophagy genes to regulate life span in Caenorhabditis elegans. *Autophagy*, **4**(3), 330–338.
99. Nojima A, Yamashita M, Yoshida Y, Shimizu I, Ichimiya H, Kamimura N, *et al.* (2013) Haploinsufficiency of akt1 prolongs the lifespan of mice. *PLoS One*, **8**(7), e69178.

100. Lamming DW, Mihaylova MM, Katajisto P, Baar EL, Yilmaz OH, Hutchins A, *et al.* (2014) Depletion of Rictor, an essential protein component of mTORC2, decreases male lifespan. *Aging Cell*, **13**(5), 911–917.
101. Arriola Apelo SI, Neuman JC, Baar EL, Syed FA, Cummings NE, Brar HK, *et al.* (2016) Alternative rapamycin treatment regimens mitigate the impact of rapamycin on glucose homeostasis and the immune system. *Aging Cell*, **15**(1), 28–38.
102. Fenton TR and Gout IT. (2011) Functions and regulation of the 70kDa ribosomal S6 kinases. *International Journal of Biochemistry & Cell Biology*, **43**(1), 47–59.
103. Dufner A and Thomas G. (1999) Ribosomal S6 kinase signaling and the control of translation. *Experimental Cell Research*, **253**(1), 100–109.
104. Schalm SS and Blenis J. (2002) Identification of a Conserved Motif Required for mTOR Signaling. *Current Biology*, **12**(8), 632–639.
105. Tavares MR, Pavan IC, Amaral CL, Meneguello L, Luchessi AD and Simabuco FM. (2015) The S6K protein family in health and disease. *Life Science*, **131**, 1–10.
106. Shin S, Wolgamott L, Yu Y, Blenis J and Yoon SO. (2011) Glycogen synthase kinase (GSK)-3 promotes p70 ribosomal protein S6 kinase (p70S6K) activity and cell proliferation. *Proceedings of the National Academy of Sciences of the United States*, **108**(47), E1204–E1213.
107. Rosner M and Hengstschläger M. (2011) Nucleocytoplasmic localization of p70 S6K1, but not of its isoforms p85 and p31, is regulated by TSC2/mTOR. *Oncogene*, **30**(44), 4509–4522.
108. Kosach V, Shkarina K, Kravchenko A, Tereshchenko Y, Kovalchuk E, Skoroda L, *et al.* (2018) Nucleocytoplasmic distribution of S6K1 depends on the density and motility of MCF-7 cells in vitro. *F1000Research*, **7**, 1332.
109. Hong S, Zhao B, Lombard DB, Fingar DC and Inoki K. (2014) Cross-talk between sirtuin and mammalian target of rapamycin complex 1 (mTORC1) signaling in the regulation of S6 kinase 1 (S6K1) phosphorylation. *Journal of Biological Chemistry*, **289**(19), 13132–13141.
110. Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, Nir T, *et al.* (2005) Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes & Development*, **19**(18), 2199–21211.
111. Chauvin C, Koka V, Nouschi A, Mieulet V, Hoareau-Aveilla C, Dreazen A, *et al.* (2014) Ribosomal protein S6 kinase activity controls the ribosome biogenesis transcriptional program. *Oncogene*, **33**(4), 474–483.
112. Shima H, Pende M, Chen Y, Fumagalli S, Thomas G and Kozma SC. (1998) Disruption of the p70(s6k)/p85(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase. *EMBO Journal*, **17**(22), 6649–6659.
113. Hannan KM, Brandenburger Y, Jenkins A, Sharkey K, Cavanaugh A, Rothblum L, *et al.* (2003) mTOR-dependent regulation of ribosomal gene transcription requires S6K1 and is mediated by phosphorylation of the carboxy-terminal activation domain of the nucleolar transcription factor UBF. *Molecular Cell Biology*, **23**(23), 8862–8877.

114. Abe Y, Yoon SO, Kubota K, Mendoza MC, Gygi SP and Blenis J. (2009) p90 ribosomal S6 kinase and p70 ribosomal S6 kinase link phosphorylation of the eukaryotic chaperonin containing TCP-1 to growth factor, insulin, and nutrient signaling. *Journal of Biological Chemistry*, **284**(22), 14939–14948.
115. Ma XM, Yoon SO, Richardson CJ, Julich K and Blenis J. (2008) SKAR links pre-mRNA splicing to mTOR/S6K1-mediated enhanced translation efficiency of spliced mRNAs. *Cell*, **133**(2), 303–313.
116. Richardson CJ, Bröenstrup M, Fingar DC, Jülich K, Ballif BA, Gygi S, *et al.* (2004) SKAR is a specific target of S6 kinase 1 in cell growth control. *Current Biology*, **14**(17), 1540–1549.
117. Wilson KF, Wu WJ and Cerione RA. (2000) Cdc42 stimulates RNA splicing via the S6 kinase and a novel S6 kinase target, the nuclear cap-binding complex. *Journal of Biological Chemistry*, **275**(48), 37307–37310.
118. Raught B, Peiretti F, Gingras AC, Livingstone M, Shahbazian D, Mayeur GL, *et al.* (2004) Phosphorylation of eucaryotic translation initiation factor 4B Ser422 is modulated by S6 kinases. *EMBO Journal*, **23**(8), 1761–1769.
119. Dorrello NV, Peschiaroli A, Guardavaccaro D, Colburn NH, Sherman NE and Pagano M. (2006) S6K1- and betaTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth. *Science*, **314**(5798), 467–471.
120. Martineau Y, Wang X, Alain T, Petroulakis E, Shahbazian D, Fabre B, *et al.* (2014) Control of Paip1-eukayrotic translation initiation factor 3 interaction by amino acids through S6 kinase. *Molecular Cell Biology*, **34**(6), 1046–1053.
121. Wang X, Li W, Williams M, Terada N, Alessi DR and Proud CG. (2001) Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase. Embo J, **20**(16), 4370–4379.
122. de Groot RP, Ballou LM and Sassone-Corsi P. (1994) Positive regulation of the cAMP-responsive activator CREM by the p70 S6 kinase: An alternative route to mitogen-induced gene expression. *Cell*, **79**(1), 81–91.
123. Yamnik RL, Digilova A, Davis DC, Brodt ZN, Murphy CJ and Holz MK. (2009) S6 kinase 1 regulates estrogen receptor alpha in control of breast cancer cell proliferation. *Journal of Biological Chemistry*, **284**(10), 6361–6369.
124. Csibi A, Lee G, Yoon SO, Tong H, Ilter D, Elia I, *et al.* (2014) The mTORC1/S6K1 pathway regulates glutamine metabolism through the eIF4B-dependent control of c-Myc translation. *Current Biology*, **24**(19), 2274–2280.
125. Harada H, Andersen JS, Mann M, Terada N and Korsmeyer SJ. (2001) p70S6 kinase signals cell survival as well as growth, inactivating the pro-apoptotic molecule BAD. *Proceedings of the National Academy of Sciences of the United States*, **98**(17), 9666–9670.
126. Jackson RJ, Hellen CU and Pestova TV. (2010) The mechanism of eukaryotic translation initiation and principles of its regulation. *Nature Reviews Molecular Cell Biology*, **11**(2), 113–127.

127. Parsyan A, Svitkin Y, Shahbazian D, Gkogkas C, Lasko P, Merrick WC, *et al.* (2011) mRNA helicases: The tacticians of translational control. *Nature Reviews Molecular Cell Biology*, **12**(4), 235–245.
128. Sonenberg N and Hinnebusch AG. (2009) Regulation of translation initiation in eukaryotes: Mechanisms and biological targets. *Cell*, **136**(4), 731–745.
129. Pause A, Belsham GJ, Gingras AC, Donzé O, Lin TA, Lawrence JC Jr., *et al.* (1994) Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. *Nature*, **371**(6500), 762–767.
130. Schalm SS, Fingar DC, Sabatini DM and Blenis J. (2003) TOS motif-mediated raptor binding regulates 4E-BP1 multisite phosphorylation and function. *Current Biology*, **13**(10), 797–806.
131. Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, *et al.* (1999) Regulation of 4E-BP1 phosphorylation: A novel two-step mechanism. *Genes & Development*, **13**(11), 1422–1437.
132. Fox CJ, Hammerman PS, Cinalli RM, Master SR, Chodosh LA and Thompson CB. (2003) The serine/threonine kinase Pim-2 is a transcriptionally regulated apoptotic inhibitor. *Genes & Development*, **17**(15), 1841–1854.
133. Shin S, Wolgamott L, Tcherkezian J, Vallabhapurapu S, Yu Y, Roux PP, *et al.* (2014) Glycogen synthase kinase-3β positively regulates protein synthesis and cell proliferation through the regulation of translation initiation factor 4E-binding protein 1. *Oncogene*, **33**(13), 1690–1699.
134. Shin S, Wolgamott L, Roux PP and Yoon SO. (2014) Casein kinase 1ε promotes cell proliferation by regulating mRNA translation. *Cancer Research*, **74**(1), 201–211.
135. Shuda M, Velásquez C, Cheng E, Cordek DG, Kwun HJ, Chang Y, *et al.* (2015) CDK1 substitutes for mTOR kinase to activate mitotic cap-dependent protein translation. *Proceedings of the National Academy of Sciences of the United States*, **112**(19), 5875–5882.
136. Zhu J, Blenis J and Yuan J. (2008) Activation of PI3K/Akt and MAPK pathways regulates Myc-mediated transcription by phosphorylating and promoting the degradation of Mad1. *Proceedings of the National Academy of Sciences of the United States*, **105**(18), 6584–6589.
137. Wek RC. (2018) Role of eIF2α kinases in translational control and adaptation to cellular stress. *Cold Spring Harbor Perspectives in Biology*, **10**(7).
138. Koromilas AE, Lazaris-Karatzas A and Sonenberg N. (1992) mRNAs containing extensive secondary structure in their 5′ non-coding region translate efficiently in cells overexpressing initiation factor eIF-4E. *EMBO Journal*, **11**(11), 4153–4158.
139. Rubio CA, Weisburd B, Holderfield M, Arias C, Fang E, DeRisi JL, *et al.* (2014) Transcriptome-wide characterization of the eIF4A signature highlights plasticity in translation regulation. *Genome Biology*, **15**(10), 476.
140. Rosenwald IB, Kaspar R, Rousseau D, Gehrke L, Leboulch P, Chen JJ, *et al.* (1995) Eukaryotic translation initiation factor 4E regulates expression of cyclin D1 at

transcriptional and post-transcriptional levels. *Journal of Biological Chemistry*, **270**(36), 21176–21180.

141. Fagan RJ, Lazaris-Karatzas A, Sonenberg N and Rozen R. (1991) Translational control of ornithine aminotransferase. Modulation by initiation factor eIF-4E. *Journal of Biological Chemistry*, **266**(25), 16518–1623.
142. Kevil CG, De Benedetti A, Payne DK, Coe LL, Laroux FS and Alexander JS. (1996) Translational regulation of vascular permeability factor by eukaryotic initiation factor 4E: Implications for tumor angiogenesis. *International Journal of Cancer*, **65**(6), 785–790.
143. Zimmer SG, DeBenedetti A and Graff JR. (2000) Translational control of malignancy: The mRNA cap-binding protein, eIF-4E, as a central regulator of tumor formation, growth, invasion and metastasis. *AntiCancer Research*, **20**(3a), 1343–13451.
144. Meyuhas O. (2000) Synthesis of the translational apparatus is regulated at the translational level. *European Journal of Biochemistry*, **267**(21), 6321–6330.
145. Yamashita R, Suzuki Y, Takeuchi N, Wakaguri H, Ueda T, Sugano S, *et al.* (2008) Comprehensive detection of human terminal oligo-pyrimidine (TOP) genes and analysis of their characteristics. *Nucleic Acids Research*, **36**(11), 3707–3715.
146. Huo Y, Iadevaia V, Yao Z, Kelly I, Cosulich S, Guichard S, *et al.* (2012) Stable isotope-labelling analysis of the impact of inhibition of the mammalian target of rapamycin on protein synthesis. *Biochemical Journal*, **444**(1), 141–151.
147. Patursky-Polischuk I, Stolovich-Rain M, Hausner-Hanochi M, Kasir J, Cybulski N, Avruch J, *et al.* (2009) The TSC-mTOR pathway mediates translational activation of TOP mRNAs by insulin largely in a raptor- or rictor-independent manner. *Molecular Cell Biology*, **29**(3), 640–649.
148. Tcherkezian J, Cargnello M, Romeo Y, Huttlin EL, Lavoie G, Gygi SP, *et al.* (2014) Proteomic analysis of cap-dependent translation identifies LARP1 as a key regulator of 5′TOP mRNA translation. *Genes & Development*, **28**(4), 357–371.
149. Lahr RM, Mack SM, Héroux A, Blagden SP, Bousquet-Antonelli C, Deragon JM, *et al.* (2015) The La-related protein 1-specific domain repurposes HEAT-like repeats to directly bind a 5′TOP sequence. *Nucleic Acids Research*, **43**(16), 8077–8088.
150. Lahr RM, Fonseca BD, Ciotti GE, Al-Ashtal HA, Jia JJ, Niklaus MR, *et al.* (2017) La-related protein 1 (LARP1) binds the mRNA cap, blocking eIF4F assembly on TOP mRNAs. *Elife*, **6**.
151. Fonseca BD, Zakaria C, Jia JJ, Graber TE, Svitkin Y, Tahmasebi S, *et al.* (2015) La-related protein 1 (LARP1) represses terminal oligopyrimidine (TOP) mRNA translation downstream of mTOR complex 1 (mTORC1). *Journal of Biological Chemistry*, **290**(26), 15996–6020.
152. Philippe L, Vasseur JJ, Debart F and Thoreen CC. (2018) La-related protein 1 (LARP1) repression of TOP mRNA translation is mediated through its cap-binding domain and controlled by an adjacent regulatory region. *Nucleic Acids Research*, **46**(3), 1457–1469.

153. Aoki K, Adachi S, Homoto M, Kusano H, Koike K and Natsume T. (2013) LARP1 specifically recognizes the 3′ terminus of poly(A) mRNA. *FEBS Letters*, **587**(14), 2173–2178.
154. Hopkins TG, Mura M, Al-Ashtal HA, Lahr RM, Abd-Latip N, Sweeney K, *et al.* (2016) The RNA-binding protein LARP1 is a post-transcriptional regulator of survival and tumorigenesis in ovarian cancer. *Nucleic Acids Research*, **44**(3), 1227–1246.
155. Hong S, Freeberg MA, Han T, Kamath A, Yao Y, Fukuda T, *et al.* (2017) LARP1 functions as a molecular switch for mTORC1-mediated translation of an essential class of mRNAs. *Elife*, **6**.
156. Kang SA, Pacold ME, Cervantes CL, Lim D, Lou HJ, Ottina K, *et al.* (2013) mTORC1 phosphorylation sites encode their sensitivity to starvation and rapamycin. *Science*, **341**(6144), 1236566.
157. Ogami K, Oishi Y, Nogimori T, Sakamoto K and Hoshino S-I. (2019) LARP1 facilitates translational recovery after amino acid refeeding by preserving long poly(A)-tailed TOP mRNAs. *bioRxiv*, 716217.
158. Al-Ashtal HA, Rubottom CM, Leeper TC and Berman AJ. (2019) The LARP1 La-Module recognizes both ends of TOP mRNAs. *RNA Biology*, 1–11.
159. Afonina ZA, Myasnikov AG, Shirokov VA, Klaholz BP and Spirin AS. (2014) Formation of circular polyribosomes on eukaryotic mRNA without cap-structure and poly(A)-tail: A cryo electron tomography study. *Nucleic Acids Research*, **42**(14), 9461–9469.
160. Adivarahan S, Livingston N, Nicholson B, Rahman S, Wu B, Rissland OS, *et al.* (2018) Spatial organization of single mRNPs at different stages of the gene expression pathway. *Molecular Cell*, **72**(4), 727–738.e5.
161. Wilbertz JH, Voigt F, Horvathova I, Roth G, Zhan Y and Chao JA. (2019) Single-molecule imaging of mRNA localization and regulation during the integrated stress response. *Molecular Cell*, **73**(5), 946–958.e7.
162. Smith EM, Benbahouche NEH, Morris K, Wilczynska A, Gillen S, Schmidt T, *et al.* (2021) The mTOR regulated RNA-binding protein LARP1 requires PABPC1 for guided mRNA interaction. *Nucleic Acids Research*, **49**(1), 458–478.
163. Zhang Y and Stefanovic B. (2017) mTORC1 phosphorylates LARP6 to stimulate type I collagen expression. *Scientific Reports*, **7**, 41173.
164. Zhang Y and Stefanovic B. (2016) Akt mediated phosphorylation of LARP6; critical step in biosynthesis of type I collagen. *Scientific Reports*, **6**, 22597.
165. Dai N, Christiansen J, Nielsen FC and Avruch J. (2013) mTOR complex 2 phosphorylates IMP1 cotranslationally to promote IGF2 production and the proliferation of mouse embryonic fibroblasts. *Genes & Development*, **27**(3), 301–312.
166. Loh PG, Yang HS, Walsh MA, Wang Q, Wang X, Cheng Z, *et al.* (2009) Structural basis for translational inhibition by the tumour suppressor Pdcd4. *EMBO Journal*, **28**(3), 274–285.

167. Yang HS, Jansen AP, Komar AA, Zheng X, Merrick WC, Costes S, *et al.* (2003) The transformation suppressor Pdcd4 is a novel eukaryotic translation initiation factor 4A binding protein that inhibits translation. *Molecular Cell Biology*, **23**(1), 26–37.
168. Wang Q, Zhu J, Wang YW, Dai Y, Wang YL, Wang C, *et al.* (2017) Tumor suppressor Pdcd4 attenuates Sin1 translation to inhibit invasion in colon carcinoma. *Oncogene*, **36**(45), 6225–6234.
169. Bera A, Das F, Ghosh-Choudhury N, Kasinath BS, Abboud HE and Choudhury GG. (2014) microRNA-21-induced dissociation of PDCD4 from rictor contributes to Akt-IKKbeta-mTORC1 axis to regulate renal cancer cell invasion. *Experimental Cell Research*, **328**(1), 99–117.
170. Pla-Martin D, Schatton D, Wiederstein JL, Marx MC, Khiati S, Kruger M, *et al.* (2020) CLUH granules coordinate translation of mitochondrial proteins with mTORC1 signaling and mitophagy. *EMBO Journal*, **39**(9), e102731.
171. Romeo Y, Zhang X and Roux PP. (2012) Regulation and function of the RSK family of protein kinases. *Biochemical Journal*, **441**(2), 553–569.
172. Chen RH, Sarnecki C and Blenis J. (1992) Nuclear localization and regulation of erk- and rsk-encoded protein kinases. *Molecular Cell Biology*, **12**(3), 915–927.
173. Dümmler BA, Hauge C, Silber J, Yntema HG, Kruse LS, Kofoed B, *et al.* (2005) Functional characterization of human RSK4, a new 90-kDa ribosomal S6 kinase, reveals constitutive activation in most cell types. *Journal of Biological Chemistry*, **280**(14), 13304–13314.
174. Fisher TL and Blenis J. (1996) Evidence for two catalytically active kinase domains in pp90rsk. *Molecular Cell Biology*, **16**(3), 1212–1219.
175. Erikson E and Maller JL. (1985) A protein kinase from Xenopus eggs specific for ribosomal protein S6. *Proceedings of the National Academy of Sciences of the United States*, **82**(3), 742–746.
176. Roux PP, Shahbazian D, Vu H, Holz MK, Cohen MS, Taunton J, *et al.* (2007) RAS/ERK signaling promotes site-specific ribosomal protein S6 phosphorylation via RSK and stimulates cap-dependent translation. *Journal of Biological Chemistry*, **282**(19), 14056–14064.
177. Shahbazian D, Roux PP, Mieulet V, Cohen MS, Raught B, Taunton J, *et al.* (2006) The mTOR/PI3K and MAPK pathways converge on eIF4B to control its phosphorylation and activity. *EMBO Journal*, **25**(12), 2781–2791.
178. Galan JA, Geraghty KM, Lavoie G, Kanshin E, Tcherkezian J, Calabrese V, *et al.* (2014) Phosphoproteomic analysis identifies the tumor suppressor PDCD4 as a RSK substrate negatively regulated by 14-3-3. *Proceedings of the National Academy of Sciences of the United States*, **111**(29), E2918–E2927.
179. Chou PC, Rajput S, Zhao X, Patel C, Albaciete D, Oh WJ, *et al.* (2020) mTORC2 Is involved in the induction of RSK phosphorylation by serum or nutrient starvation. *Cells*, **9**(7).
180. Pelletier J, Thomas G and Volarević S. (2018) Ribosome biogenesis in cancer: New players and therapeutic avenues. *Nature Reviews Cancer*, **18**(1), 51–63.

181. Kressler D, Hurt E and Bassler J. (2010) Driving ribosome assembly. *Biochimica et Biophysica Acta*, **1803**(6), 673–683.
182. Mayer C and Grummt I. (2006) Ribosome biogenesis and cell growth: mTOR coordinates transcription by all three classes of nuclear RNA polymerases. *Oncogene*, **25**(48), 6384–6391.
183. Hempel WM, Cavanaugh AH, Hannan RD, Taylor L and Rothblum LI. (1996) The species-specific RNA polymerase I transcription factor SL-1 binds to upstream binding factor. *Molecular Cell Biology*, **16**(2), 557–563.
184. van Riggelen J, Yetil A and Felsher DW. (2010) MYC as a regulator of ribosome biogenesis and protein synthesis. *Nature Reviews Cancer*, **10**(4), 301–309.
185. Cavanaugh AH, Hempel WM, Taylor LJ, Rogalsky V, Todorov G and Rothblum LI. (1995) Activity of RNA polymerase I transcription factor UBF blocked by Rb gene product. *Nature*, **374**(6518), 177–180.
186. Li H, Tsang CK, Watkins M, Bertram PG and Zheng XF. (2006) Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. *Nature*, **442**(7106), 1058–1061.
187. Mayer C, Zhao J, Yuan X and Grummt I. (2004) mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. *Genes & Development*, **18**(4), 423–434.
188. Prakash V, Carson BB, Feenstra JM, Dass RA, Sekyrova P, Hoshino A, *et al.* (2019) Ribosome biogenesis during cell cycle arrest fuels EMT in development and disease. *Nature Communications*, **10**(1), 2110.
189. Lamouille S, Connolly E, Smyth JW, Akhurst RJ and Derynck R. (2012) TGF-beta-induced activation of mTOR complex 2 drives epithelial-mesenchymal transition and cell invasion. *Journal of Cell Science*, **125**(Pt 5), 1259–1273.
190. Braun BR, Kassavetis GA and Geiduschek EP. (1992) Bending of the Saccharomyces cerevisiae 5S rRNA gene in transcription factor complexes. *Journal of Biological Chemistry*, **267**(31), 22562–22569.
191. Gomez-Roman N, Grandori C, Eisenman RN and White RJ. (2003) Direct activation of RNA polymerase III transcription by c-Myc. *Nature*, **421**(6920), 290–294.
192. White RJ, Trouche D, Martin K, Jackson SP and Kouzarides T. (1996) Repression of RNA polymerase III transcription by the retinoblastoma protein. *Nature*, **382**(6586), 88–90.
193. Kantidakis T, Ramsbottom BA, Birch JL, Dowding SN and White RJ. (2010) mTOR associates with TFIIIC, is found at tRNA and 5S rRNA genes, and targets their repressor Maf1. *Proceedings of the National Academy of Sciences of the United States*, **107**(26), 11823–11828.
194. Shor B, Wu J, Shakey Q, Toral-Barza L, Shi C, Follettie M, *et al.* (2010) Requirement of the mTOR kinase for the regulation of Maf1 phosphorylation and control of RNA polymerase III-dependent transcription in cancer cells. *Journal of Biological Chemistry*, **285**(20), 15380–15392.

195. Zemp I and Kutay U. (2007) Nuclear export and cytoplasmic maturation of ribosomal subunits. *FEBS Letters*, **581**(15), 2783–2793.
196. Caldarola S, Amaldi F, Proud CG and Loreni F. (2004) Translational regulation of terminal oligopyrimidine mRNAs induced by serum and amino acids involves distinct signaling events. *Journal of Biological Chemistry*, **279**(14), 13522–13531.
197. Fonseca BD, Lahr RM, Damgaard CK, Alain T and Berman AJ. (2018) LARP1 on TOP of ribosome production. *Wiley Interdisciplinary Reviews: RNA*, e1480.
198. Pillet B, Mitterer V, Kressler D and Pertschy B. (2017) Hold on to your friends: Dedicated chaperones of ribosomal proteins: Dedicated chaperones mediate the safe transfer of ribosomal proteins to their site of pre-ribosome incorporation. *Bioessays*, **39**(1), 1–12.
199. Zhou BP, Liao Y, Xia W, Zou Y, Spohn B and Hung MC. (2001) HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. *Nature Cell Biology*, **3**(11), 973–82.
200. Iadevaia V, Zhang Z, Jan E and Proud CG. (2012) mTOR signaling regulates the processing of pre-rRNA in human cells. *Nucleic Acids Research*, **40**(6), 2527–2539.
201. Abetov DA, Kiyan VS, Zhylkibayev AA, Sarbassova DA, Alybayev SD, Spooner E, *et al.* (2019) Formation of mammalian pre-ribosomes proceeds from intermediate to composed state during ribosome maturation. *Journal of Biological Chemistry*.
202. Wang T, Zhang WS, Wang ZX, Wu ZW, Du BB, Li LY, *et al.* (2020) RAPTOR promotes colorectal cancer proliferation by inducing mTORC1 and upregulating ribosome assembly factor URB1. *Cancer Medicine*, **9**(4), 1529–1543.
203. Yan X, Wu S, Liu Q and Zhang J. (2020) RRS1 promotes retinoblastoma cell proliferation and invasion via activating the AKT/mTOR signaling pathway. *BioMed Research International*, **2020**, 2420437.
204. Peng T, Golub TR and Sabatini DM. (2002) The immunosuppressant rapamycin mimics a starvation-like signal distinct from amino acid and glucose deprivation. *Molecular and Cellular Biology*, **22**(15), 5575–5584.
205. Rosario FJ, Powell TL and Jansson T. (2016) Mechanistic target of rapamycin (mTOR) regulates trophoblast folate uptake by modulating the cell surface expression of FR-α and the RFC. *Science Reports*, **6**, 31705.
206. Park Y, Reyna-Neyra A, Philippe L and Thoreen CC. (2017) mTORC1 balances cellular amino acid supply with demand for protein synthesis through post-transcriptional control of ATF4. *Cell Reports*, **19**(6), 1083–1090.
207. Gu Y, Albuquerque CP, Braas D, Zhang W, Villa GR, Bi J, *et al.* (2017) mTORC2 regulates amino acid metabolism in cancer by phosphorylation of the cystine-glutamate Antiporter xCT. *Molecular Cell*, **67**(1), 128–138.e7.
208. Toda K, Kawada K, Iwamoto M, Inamoto S, Sasazuki T, Shirasawa S, *et al.* (2016) Metabolic alterations caused by KRAS mutations in colorectal cancer contribute to cell adaptation to glutamine depletion by upregulation of asparagine synthetase. *Neoplasia*, **18**(11), 654–665.

209. Selvarajah B, Azuelos I, Platé M, Guillotin D, Forty EJ, Contento G, *et al.* (2019) mTORC1 amplifies the ATF4-dependent de novo serine-glycine pathway to supply glycine during TGF-β(1)-induced collagen biosynthesis. *Science Signaling*, **12**(582).
210. Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, *et al.* (2010) Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Molecular Cell*, **39**(2), 171–183.
211. Yecies JL, Zhang HH, Menon S, Liu S, Yecies D, Lipovsky AI, *et al.* (2011) Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. *Cell Metabolism*, **14**(1), 21–32.
212. Laplante M and Sabatini DM. (2009) An emerging role of mTOR in lipid biosynthesis. *Current Biology*, **19**(22), R1046–R1052.
213. Hoxhaj G, Hughes-Hallett J, Timson RC, Ilagan E, Yuan M, Asara JM, *et al.* (2017) The mTORC1 signaling network senses changes in cellular purine nucleotide levels. *Cell Reports*, **21**(5), 1331–1346.
214. Ben-Sahra I, Hoxhaj G, Ricoult SJH, Asara JM and Manning BD. (2016) mTORC1 induces purine synthesis through control of the mitochondrial tetrahydrofolate cycle. *Science*, **351**(6274), 728–733.
215. Kofuji S, Hirayama A, Eberhardt AO, Kawaguchi R, Sugiura Y, Sampetrean O, *et al.* (2019) IMP dehydrogenase-2 drives aberrant nucleolar activity and promotes tumorigenesis in glioblastoma. *Nature Cell Biology*, **21**(8), 1003–1014.
216. Ben-Sahra I, Howell JJ, Asara JM and Manning BD. (2013) Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. *Science*, **339**(6125), 1323–1328.
217. Cheng J, Huang Y, Zhang X, Yu Y, Wu S, Jiao J, *et al.* (2020) TRIM21 and PHLDA3 negatively regulate the crosstalk between the PI3K/AKT pathway and PPP metabolism. *Nature Communications*, **11**(1), 1880.
218. Evert M, Calvisi DF, Evert K, De Murtas V, Gasparetti G, Mattu S, *et al.* (2012) V-AKT murine thymoma viral oncogene homolog/mammalian target of rapamycin activation induces a module of metabolic changes contributing to growth in insulin-induced hepatocarcinogenesis. *Hepatology*, **55**(5), 1473–1484.
219. Saha A, Connelly S, Jiang J, Zhuang S, Amador DT, Phan T, *et al.* (2014) Akt phosphorylation and regulation of transketolase is a nodal point for amino acid control of purine synthesis. *Molecular Cell*, **55**(2), 264–276.
220. Kliegman JI, Fiedler D, Ryan CJ, Xu YF, Su XY, Thomas D, *et al.* (2013) Chemical genetics of rapamycin-insensitive TORC2 in S. cerevisiae. *Cell Reports*, **5**(6), 1725–1736.
221. Ciechanover A and Kwon YT. (2017) Protein quality control by molecular chaperones in neurodegeneration. *Frontiers in Neuroscience*, **11**, 185.
222. Codogno P and Meijer AJ. (2005) Autophagy and signaling: Their role in cell survival and cell death. *Cell Death & Differentiation*, **12**(Suppl 2), 1509–1518.
223. Ganley IG, Lam du H, Wang J, Ding X, Chen S and Jiang X. (2009) ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. *Journal of Biological Chemistry*, **284**(18), 12297–12305.

224. Young AR, Chan EY, Hu XW, Köchl R, Crawshaw SG, High S, *et al.* (2006) Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *Journal of Cell Science*, **119**(Pt 18), 3888–3900.
225. Ohsumi Y. (2001) Molecular dissection of autophagy: Two ubiquitin-like systems. *Nature Reviews Molecular Cell Biology*, **2**(3), 211–216.
226. Wan W, You Z, Zhou L, Xu Y, Peng C, Zhou T, *et al.* (2018) mTORC1-regulated and HUWE1-mediated WIPI2 degradation controls autophagy flux. *Molecular Cell*, **72**(2), 303–315.e6.
227. Wan W, You Z, Xu Y, Zhou L, Guan Z, Peng C, *et al.* (2017) mTORC1 phosphorylates acetyltransferase p300 to regulate autophagy and lipogenesis. *Molecular Cell*, **68**(2), 323–335.e6.
228. Monastyrska I, Rieter E, Klionsky DJ and Reggiori F. (2009) Multiple roles of the cytoskeleton in autophagy. *Biological Reviews of the Cambridge Philosophical Society*, **84**(3), 431–448.
229. Nair U, Jotwani A, Geng J, Gammoh N, Richerson D, Yen WL, *et al.* (2011) SNARE proteins are required for macroautophagy. *Cell*, **146**(2), 290–302.
230. Munson MJ, Allen GF, Toth R, Campbell DG, Lucocq JM and Ganley IG. (2015) mTOR activates the VPS34-UVRAG complex to regulate autolysosomal tubulation and cell survival. *EMBO Journal*, **34**(17), 2272–2290.
231. Cheng X, Ma X, Zhu Q, Song D, Ding X, Li L, *et al.* (2019) Pacer is a mediator of mTORC1 and GSK3-TIP60 signaling in regulation of autophagosome maturation and lipid metabolism. *Molecular Cell*, **73**(4), 788–802.e7.
232. Ozcan U, Ozcan L, Yilmaz E, Duvel K, Sahin M, Manning BD, *et al.* (2008) Loss of the tuberous sclerosis complex tumor suppressors triggers the unfolded protein response to regulate insulin signaling and apoptosis. *Molecular Cell*, **29**(5), 541–551.
233. Olzmann JA, Kopito RR and Christianson JC. (2013) The mammalian endoplasmic reticulum-associated degradation system. *Cold Spring Harbor Perspectives in Biology*, **5**(9).
234. Zhang Y, Nicholatos J, Dreier JR, Ricoult SJ, Widenmaier SB, Hotamisligil GS, *et al.* (2014) Coordinated regulation of protein synthesis and degradation by mTORC1. *Nature*, **513**(7518), 440–443.
235. Cohen-Kaplan V, Livneh I, Avni N, Fabre B, Ziv T, Kwon YT, *et al.* (2016) p62- and ubiquitin-dependent stress-induced autophagy of the mammalian 26S proteasome. *Proceedings of the National Academy of Sciences of the United States*, **113**(47), E7490-e9.
236. Wang X, Omura S, Szweda LI, Yang Y, Bérard J, Seminaro J, *et al.* (1997) Rapamycin inhibits proteasome activator expression and proteasome activity. *European Journal of Immunology*, **27**(11), 2781–2786.
237. Gao D, Inuzuka H, Tseng A, Wei W. (2009) Akt finds its new path to regulate cell cycle through modulating Skp2 activity and its destruction by APC/Cdh1. *Cell Division*, **4**, 11.

238. Bornstein G, Bloom J, Sitry-Shevah D, Nakayama K, Pagano M and Hershko A. (2003) Role of the SCFSkp2 ubiquitin ligase in the degradation of p21Cip1 in S phase. *Journal of Biological Chemistry*, **278**(28), 25752–25757.
239. Carrano AC, Eytan E, Hershko A and Pagano M. (1999) SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nature Cell Biology*, **1**(4), 193–199.
240. Gao D, Inuzuka H, Tan MK, Fukushima H, Locasale JW, Liu P, *et al.* (2011) mTOR drives its own activation via SCF(βTrCP)-dependent degradation of the mTOR inhibitor DEPTOR. *Molecular Cell*, **44**(2), 290–303.
241. Yoneyama Y, Inamitsu T, Chida K, Iemura SI, Natsume T, Maeda T, *et al.* (2018) Serine phosphorylation by mTORC1 promotes IRS-1 degradation through SCFβ-TRCP E3 ubiquitin ligase. *iScience*, **5**, 1–18.
242. Wang WA, Groenendyk J and Michalak M. (2014) Endoplasmic reticulum stress associated responses in cancer. *Biochimica et Biophysica Acta*, **1843**(10), 2143–2149.
243. Hotamisligil GS. (2010) Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell*, **140**(6), 900–917.
244. Hetz C, Chevet E and Oakes SA. (2015) Proteostasis control by the unfolded protein response. *Nature Cell Biology*, **17**(7), 829–838.
245. Moore CE, Omikorede O, Gomez E, Willars GB and Herbert TP. (2011) PERK activation at low glucose concentration is mediated by SERCA pump inhibition and confers preemptive cytoprotection to pancreatic β-cells. *Molecular Endocrinology*, **25**(2), 315–326.
246. Moloughney JG, Vega-Cotto NM, Liu S, Patel C, Kim PK, Wu CC, *et al.* (2018) mTORC2 modulates the amplitude and duration of GFAT1 Ser-243 phosphorylation to maintain flux through the hexosamine pathway during starvation. *Journal of Biological Chemistry*, **293**(42), 16464–16478.
247. Wouters BG, Koritzinsky M. (2008) Hypoxia signalling through mTOR and the unfolded protein response in cancer. *Nature Reviews Cancer*, **8**(11), 851–864.
248. Schewe DM and Aguirre-Ghiso JA. (2008) ATF6alpha-Rheb-mTOR signaling promotes survival of dormant tumor cells in vivo. *Proceedings of the National Academy of Sciences of the United States*, **105**(30), 10519–10524.
249. Bobrovnikova-Marjon E, Pytel D, Riese MJ, Vaites LP, Singh N, Koretzky GA, *et al.* (2012) PERK utilizes intrinsic lipid kinase activity to generate phosphatidic acid, mediate Akt activation, and promote adipocyte differentiation. *Molecular Cell Biology*, **32**(12), 2268–2278.
250. Ohoka N, Yoshii S, Hattori T, Onozaki K and Hayashi H. (2005) TRB3, a novel ER stress-inducible gene, is induced via ATF4-CHOP pathway and is involved in cell death. *EMBO Journal*, **24**(6), 1243–1255.
251. Chen CH, Shaikenov T, Peterson TR, Aimbetov R, Bissenbaev AK, Lee SW, *et al.* (2011) ER stress inhibits mTORC2 and Akt signaling through GSK-3β-mediated phosphorylation of rictor. *Science Signaling*, **4**(161), ra10.

252. Costa-Mattioli M and Walter P. (2020) The integrated stress response: From mechanism to disease. *Science*, **368**(6489).
253. Kazemi S, Mounir Z, Baltzis D, Raven JF, Wang S, Krishnamoorthy JL, *et al.* (2007) A novel function of eIF2alpha kinases as inducers of the phosphoinositide-3 kinase signaling pathway. *Molecular Biology of the Cell*, **18**(9), 3635–3644.
254. Rajesh K, Krishnamoorthy J, Kazimierczak U, Tenkerian C, Papadakis AI, Wang S, *et al.* (2015) Phosphorylation of the translation initiation factor eIF2α at serine 51 determines the cell fate decisions of Akt in response to oxidative stress. *Cell Death & Disease*, **6**(1), e1591.
255. Li LJ, Chai Y, Guo XJ, Chu SL and Zhang LS. (2018) Effects of endoplasmic reticulum stress on autophagy and apoptosis of human leukemia cells via inhibition of the PI3K/AKT/mTOR signaling pathway. *Molecular Medicine Report*, **17**(6), 7886–7892.
256. Baltzis D, Pluquet O, Papadakis AI, Kazemi S, Qu LK and Koromilas AE. (2007) The eIF2alpha kinases PERK and PKR activate glycogen synthase kinase 3 to promote the proteasomal degradation of p53. *Journal of Biological Chemistry*, **282**(43), 31675–31687.
257. Gandin V, Masvidal L, Cargnello M, Gyenis L, McLaughlan S, Cai Y, *et al.* (2016) mTORC1 and CK2 coordinate ternary and eIF4F complex assembly. *Nature Communications*, **7**, 11127.
258. Kang YJ, Lu MK and Guan KL. (2011) The TSC1 and TSC2 tumor suppressors are required for proper ER stress response and protect cells from ER stress-induced apoptosis. *Cell Death & Differentiation*, **18**(1), 133–144.
259. Krishnamoorthy J, Tenkerian C, Gupta J, Ghaddar N, Wang S, Darini C, *et al.* (2018) Downregulation of PERK activity and eIF2α serine 51 phosphorylation by mTOR complex 1 elicits pro-oxidant and pro-death effects in tuberous sclerosis-deficient cells. *Cell Death & Disease*, **9**(3), 254.
260. Wengrod J, Wang D, Weiss S, Zhong H, Osman I, Gardner LB. (2015) Phosphorylation of eIF2α triggered by mTORC1 inhibition and PP6C activation is required for autophagy and is aberrant in PP6C-mutated melanoma. *Science Signaling*, **8**(367), ra27.
261. Cherkasova VA and Hinnebusch AG. (2003) Translational control by TOR and TAP42 through dephosphorylation of eIF2alpha kinase GCN2. *Genes & Development*, **17**(7), 859–872.
262. Valbuena N, Rozalén AE and Moreno S. (2012) Fission yeast TORC1 prevents eIF2α phosphorylation in response to nitrogen and amino acids via Gcn2 kinase. *Journal of the Cell Science*, **125**(Pt 24), 5955–5959.
263. Averous J, Lambert-Langlais S, Mesclon F, Carraro V, Parry L, Jousse C, *et al.* (2016) GCN2 contributes to mTORC1 inhibition by leucine deprivation through an ATF4 independent mechanism. *Scientific Reports*, **6**, 27698.

264. Ye J, Palm W, Peng M, King B, Lindsten T, Li MO, *et al.* (2015) GCN2 sustains mTORC1 suppression upon amino acid deprivation by inducing Sestrin2. *Genes & Development*, **29**(22), 2331–2336.
265. Bunpo P, Dudley A, Cundiff JK, Cavener DR, Wek RC and Anthony TG. (2009) GCN2 protein kinase is required to activate amino acid deprivation responses in mice treated with the anti-cancer agent L-asparaginase. *Journal of Biological Chemistry*, **284**(47), 32742–32749.
266. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, *et al.* (2009) An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *Journal of Biological Chemistry*, **284**(12), 8023–8032.
267. Boultwood J, Yip BH, Vuppusetty C, Pellagatti A and Wainscoat JS. (2013) Activation of the mTOR pathway by the amino acid (L)-leucine in the 5q- syndrome and other ribosomopathies. *Advances in Biological Regulation*, **53**(1), 8–17.
268. Pospisilova D, Cmejlova J, Hak J, Adam T and Cmejla R. (2007) Successful treatment of a Diamond-Blackfan anemia patient with amino acid leucine. *Haematologica*, **92**(5), e66–e67.

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Chapter 9

Therapeutic Potential of Inhibiting mTOR Signaling in Neoplasia

Predrag Jovanovic[1,2,*] and Ivan Topisirovic[1,2,3,4,**]

[1]*Lady Davis Institute, Sir Mortimer B. Davis Jewish General Hospital, Montreal, Canada*
[2]*McGill University, Dept. of Medicine, Division of Experimental Medicine, Montreal, Canada*
[3]*McGill University, Dept. Biochemistry, Montreal, Canada*
[4]*McGill University, Gerald Bronfman Dept. of Oncology, Montreal, Canada*

Abstract

Mechanistic target of rapamycin (mTOR) kinase is a key coordinator of anabolic cellular processes and a subunit of two multimeric complexes — mTORC1 and mTORC2. Its activity is crucial for normal physiology but is also frequently upregulated in cancer. This rendered mTOR an appealing target for anti-cancer drugs. In this chapter, we discuss several generations of mTOR inhibitors (mTORi) and their therapeutic potential. The first class of mTORi are allosteric inhibitors which include rapamycin and its analogs. Although they showed promising preclinical results, rapamycin and rapalogs were only modestly successful when used in oncological indications. Subsequent development

* Corresponding author's email: predrag.jovanovic@mail.mcgill.ca
** Corresponding author's email: ivan.topisirovic@mcgill.ca

of active-site mTORi marked the second generation of these drugs. In recent years, a third generation of mTORi was developed by combining features of the first two classes. These compounds are more potent and specific in their effects than the first two generations of mTORi. Still in preclinical studies, this last class of mTORi inherits the hopes and expectations, while simultaneously addressing the pitfalls and shortcomings of its predecessors.

1. Introduction

Rapamycin is a naturally occurring antibiotic discovered in a soil sample from Easter Island [1]. It was determined that rapamycin binds a 289kDa protein, later identified as an evolutionarily conserved serine/threonine kinase [2–4]. Now known as the mechanistic target of rapamycin (mTOR), this kinase has a critical role in regulating key cellular processes such as growth, proliferation, protein synthesis, cytoskeletal organization, and ribosomal biogenesis. In response to environmental cues, such as nutrient availability, growth factors, and oxygen levels, mTOR coordinates major anabolic pathways, including protein synthesis as well as lipid and nucleotide synthesis. This evolutionarily conserved kinase is part of two distinct complexes — mTOR complex 1 (mTORC1) and 2 (mTORC2). The two complexes share certain subunits (mTOR, MTOR Associated Protein LST8 Homolog — mLST8, DEP Domain Containing MTOR Interacting Protein — Deptor), while others are specific to mTORC1 (regulatory-associated protein of mTOR — Raptor, 40kDa proline-rich AKT substrate — PRAS40) or mTORC2 (rapamycin insensitive companion of TOR — Rictor, mammalian stress-activated protein kinase-interacting protein — mSIN1, proline-rich protein 5 — Protor) [5–11].

Hormones and growth factors, such as insulin or insulin-like growth factor-1 (IGF-1) initiate the activation of phosphatidylinositol 3-kinase (PI3K) (Fig. 1). At the cell membrane, activated PI3K mediates the conversion of phosphatidylinositol 4,5 bisphosphate (PIP2) to phosphatidylinositol 3,4,5 triphosphate (PIP3), leading to recruitment of phosphoinositide-dependent kinase 1 (PDK1), which activates protein kinase B (PKB) also known as AKT [12]. The reverse conversion of PIP3 to PIP2 is orchestrated by the phosphatase and tensin homolog (PTEN),

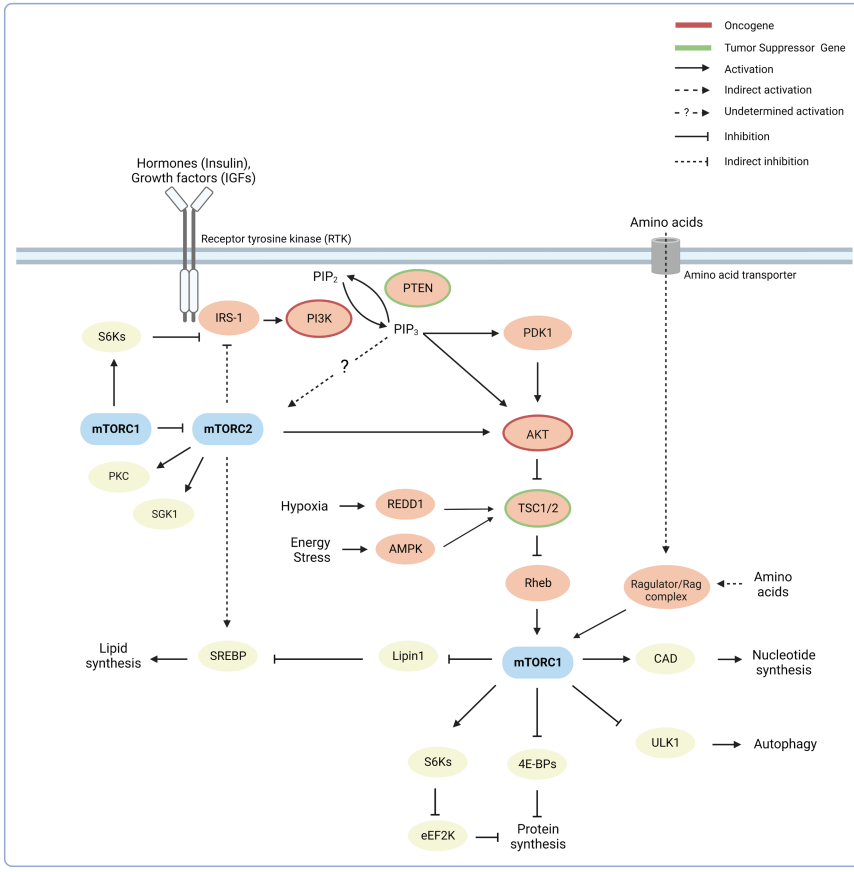

Figure 1. Schematic representation of mTOR signaling.

mTOR kinase is part of two distinct complexes (shown in blue). Its activity is modulated by a variety of intracellular and extracellular cues which are sensed by regulators upstream of mTOR (shown in orange). RTK activation leads to recruitment of the IRS-1 scaffold protein which binds PI3K. Subsequent conversion of PIP2 to PIP3 in the membrane by PI3K allows for recruitment of PDK1. PTEN catalyzes the reverse reaction. PDK1 activates AKT which alleviates TSC1/2 mediated inhibition of Rheb-mTORC1. Downstream, mTORC1 orchestrates multiple anabolic processes through serine/threonine phosphorylation of a plethora of substrates (shown in yellow). mTORC2 activation is less characterized but also dependent on upstream PI3K signaling. Abbreviations: mTOR: mechanistic target of rapamycin; RTK: receptor tyrosine kinase; IRS-1: insulin receptor substrate 1; PI3K: phosphatidylinositol 3-kinase; PIP2: phosphatidylinositol 4,5 bisphosphate; PIP3: phosphatidylinositol 3,4,5 triphosphate; PTEN: phosphatase and tensin homolog;

Figure 1. (*Continued*) PDK1: phosphoinositide-dependent kinase 1; TSC: tuberous sclerosis complex; Rheb: Ras homolog enriched in brain; REDD1: protein regulated in development and DNA damage response 1; AMPK: AMP-activated protein kinase; 4E-BP: 4E-binding protein; S6K: S6 kinase; eEF2K: eEF2 kinase; ULK1: autophagy promoting factors unc-51-like kinase 1; CAD: carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase; SREBP: sterole regulatory element binding protein; PKC: protein kinase C; SGK1: serum- and glucocorticoid-induced kinase 1. Created with BioRender.com.

a tumor suppressor that antagonizes PI3K [13]. AKT phosphorylates tuberous sclerosis complex 2 (TSC2), a GTPase activating protein (GAP) which inhibits mTORC1 by inactivating its direct upstream regulator, the Ras homolog enriched in brain (Rheb) GTP-binding protein [14]. Phosphorylation of TSC2 by AKT leads to its inhibition, and subsequent relief of inhibition on mTORC1, thus increasing mTORC1 signaling [14]. Once activated, mTORC1 orchestrates several anabolic processes. Of these, protein synthesis is coordinated to a large extent by p70 ribosomal protein S6 kinases (S6Ks; S6K1 and 2) and eukaryotic translation initiation factor 4E binding proteins (4E-BPs; 4E-BP1 to 3) [15]. mTORC1 activity is commonly assessed through the phosphorylation levels of these two substrates, at sites Thr 37/46, Ser 65/70 on p-4E-BPs and Thr 389 and Thr 388 on S6K1 and S6K2, respectively, in humans (Fig. 1). In addition to growth factors which is the most relevant to cancer, mTORC1 is modulated in response to a plethora of other stimuli including amino acid availability, oxygen levels, and energy status. Mechanisms of mTORC1 activation in response to these stimuli have been covered in a number of excellent reviews [16–20].

Regulation of mTORC2 is less understood than that of mTORC1. However, it is appreciated that upstream PI3K signaling may mediate its activation in response to growth factors [21]. Phosphorylation of Akt on Ser 473 is most notably used as a readout of mTORC2 activity. Other mTORC2 substrates include protein kinase C (PKC) family members, as well as serum- and glucocorticoid-induced kinase 1 (SGK1) [22].

Aberrant activation of mTORC1 signaling in cancer is commonly caused by mutations in upstream regulators, many of which are oncogenes (e.g., PIK3CA, AKT) or tumor suppressors (e.g., PTEN, TSC2) (Fig. 1) [23]. Hence, it is not surprising that mTOR signaling is so often

deregulated in cancer [24]. Considering this, targeting mTOR signaling has generated immense interest. Here, we will review the several classes of mTOR inhibitors, including their discovery, mechanism of action, and use as anti-cancer therapies.

2. First generation of mTOR inhibitors

The first generation of mTOR inhibitors includes rapamycin and its analogs, that are commonly referred to as rapalogs. Rapamycin binds to its intracellular receptor protein, the immunophilin FK 506-binding protein of 12kDa (FKBP12), creating a complex that targets the FKBP12-rapamycin binding (FRB) domain of mTOR [25, 26]. This impedes the interaction of mTOR with Raptor, leading to specific allosteric inhibition of mTORC1 [27], but not mTORC2 where Rictor blocks access to the FRB domain [28]. As such, mTORC2 is not susceptible to acute rapamycin inhibition, but can be negatively affected under chronic treatment due to inhibition of *de novo* mTORC2 complex assembly, in a cell type-specific manner [29]. More recently, it became apparent that rapamycin does not inhibit all mTORC1 outputs equally. Namely, the smaller 4E-binding proteins (4E-BPs) preferentially escape inhibition as opposed to the larger mTORC1 substrates such as ribosomal protein S6 kinases (S6Ks) [30, 31].

Initial characterization of rapamycin revealed that it exhibits anti-fungal [1, 32], immunosuppressive [33], and anti-tumor properties [34]. Suppressive effects of rapamycin on mTORC1 signaling, cellular growth, and proliferation set high hopes for its use in cancer therapy [35]. The U.S. Food and Drug Administration (FDA) had initially approved the use of rapamycin as an immunosuppressant, which was then clinically employed to prevent organ rejection in patients undergoing transplants [36, 37]. However, in its original form, rapamycin showed poor water solubility [38]. To repurpose rapamycin as an anticancer drug, its pharmacological properties had to be improved. This inspired the development of its analogs (referred to as “rapalogs”) (ABT-578/zotarolimus; AP23573/ridofarolimus; RAD001/everolimus, CCI-779/temsirolimus), which show improved stability and water-solubility while maintaining the core mechanism of action.

The first rapalog to be clinically evaluated was temsirolimus. When given to patients with refractory renal cell carcinoma (RCC) in a phase 2

trial, it showed promising results as a monotherapy [39]. Subsequently, in a phase 3 trial of 626 patients with untreated RCC, temsirolimus was shown be more effective than the standard of care, interferon α. It prolonged the overall survival (OS) (temsirolimus monotherapy median OS time 10.9 months, 8.4 months in combination therapy, 7.3 months in interferon α monotherapy), as well as progression-free survival (PFS) (temsirolimus monotherapy median PFS time 3.8 months, 3.7 months in combination treated, and 1.9 months in interferon α treated group, as determined by the site investigators' assessment, and 5.5, 4.7, and 3.1 months, respectively, as determined by independent radiologists) [40]. Temsirolimus was also generally well tolerated. Likewise, in a phase 3 study including 162 patients with relapsed/refractory mantle cell lymphoma (MCL), temsirolimus significantly improved PFS compared with investigator's choice therapy [41].

Everolimus was assessed as an anti-cancer agent in several clinical trials. The RECORD (Renal cell Cancer treatment with Oral RAD-001) trials investigated the effects of this rapalog in patients with advanced RCC. RECORD-1 study showed that although everolimus monotherapy was associated with prolonged PFS compared to placebo, this effect was not mirrored in the OS [42]. Subsequently, the phase 2 RECORD-2 trial demonstrated that the efficacy and safety of everolimus was similar to that of interferon α-2a, when they are combined with the humanized anti-VEGF monoclonal antibody becavizumab as a first-line therapy [43]. Finally, the RECORD-3 phase 2 trial noted that everolimus should not be given before the RTK inhibitor sunitinib, but rather after, again leaving the standard of care unchanged [44].

The BOLERO (Breast cancer trials of oral everolimus) studies examined the effects of everolimus in patients with advanced breast cancer. When combined with an aromatase inhibitor, everolimus improved PFS but not OS of patients with HER2-negative hormone-receptor positive patients. Similarly, if given to patients with refractory HER2-positive advanced breast cancers, everolimus prolonged PFS, albeit from 5.78–7.0 months. An improvement of overall survival was not observed [45].

The effects of everolimus on prolonging PFS were also notable in patients with gastropancreatic neuroendocrine tumors (NETs), as demonstrated by the RADIANT (RAD-001 in advanced neuroendocrine tumor) trials. Its anti-tumor properties were consistent in both phase 2 and 3

trials, increasing objective response rate and PFS of patients which showed no response to chemotherapy [46, 47].

Patients suffering from tuberous sclerosis (TSC) develop benign tumors, referred to as hamartomas [48]. These tissue malformations are usually harmless but have the potential to develop into significant morbidities [48]. The underlying phenotype is driven by aberrant mTORC1 signaling and dysregulated cell growth caused by the mutation of tuberous sclerosis complex 1 and 2 (TSC1 or TSC2, also known as hamartin and tuberin, respectively) (Fig. 1) [49]. Several trials were thus carried out to examine the effects of everolimus in TSC patients with pediatric or adult subependymal giant cell astrocytoma (SEGA). From the initial small sample study, everolimus was a successful antitumor agent, causing a significant reduction of SEGA in a large percentage of the patient population, with mainly mild to moderate side-effects [50]. The benefits were maintained with prolonged treatment, with no additional side-effects. A follow-up phase 3 trial, EXIST-1 (Examining everolimus in a study of tuberous sclerosis complex-1), of 117 patients confirmed these results. Subsequently, the EXIST-2 trial also showed that everolimus is successful it treating renal angiomyolipomas, reducing the lesion volume with no major toxicity [51]. The success of these trials are reflected in the approval of everolimus for treatment of both SEGA and angiomyolipoma in TSC patients [48]. Overall, the first generation of mTORi has shown success in only a limited number of cancers, even when the drugs were used in combination therapies. The benefits were found to be mostly modest and even the sporadic successes were generally reflected in the PFS and not OS. Decades later from the initial high hopes set for rapamycin and its analogs, the track record is mostly filled with unmet expectations while success stories appear to be the exceptions.

Several recent studies highlighted molecular mechanisms that potentially explain less than expected clinical efficacy of rapalogs in clinical applications. For instance, rapamycin induces compensatory signaling by interfering with several negative feedback loops which act to prevent pathway overactivation. By inhibiting the phosphorylation of p70S6K1, downstream of mTORC1, a negative feedback loop to insulin receptor substrate 1 (IRS1) which prevents PI3K/Akt overactivation is lost (Fig. 1) [52–54]. Another negative feedback loop that is disrupted by rapamycin

treatment is the p70S6K1/IRS/Ras/Raf axis, which results in activation of MEK/ERK signaling [55]. Furthermore, rapamycin can also induce an elevation in the levels of several receptor tyrosine kinases (RTKs), including IGF-1 receptor (IGF-1R), human EGF receptor 2 (HER2), and insulin receptor [56]. Considering that mTORC1 is a suppressor of autophagy, rapalogs can also cause inadvertent activation of autophagy, which may have protective effects for cancer cells [57–61]. Importantly, rapamycin is unable to significantly suppress phosphorylation of 4E-BPs (Fig. 2), which are major mediators of mTORC1 on proliferation in mammalian cells [62].

The limited results in the clinical setting, combined with the unwanted mechanisms of resistance, led to the eventual development of the second generation of mTOR inhibitors.

3. Second generation of mTOR inhibitors

The second generation of mTOR inhibitors is comprised of compounds that directly target the active-site of the kinase and thus inhibit both mTORC1 and mTORC2. They are also referred to as TOR kinase inhibitors (TORKi). The initial representative of this class of drugs was PP242, which successfully blocked phosphorylation of mTORC2 specific substrates (Ser 473 on p-Akt) as well as rapamycin resistant mTORC1 targets Thr 37/46 on p-4E-BPs (Fig. 2). The latter is likely responsible for the superior efficacy of TORKi as compared to allosteric mTORi, at least in preclinical studies [63, 64]. The effects were noted both *in vitro* and *in vivo* [64]. A number of subsequent TORKi that were developed include Torin1, Torin2, INK128/MLN0128 (now referred to as TAK-228), Ku-0063794, AZD2014, AZD8055, Palomid 529, GDC-0349, ATG-008 (CC-223), OSI-027, WYE354, WYE312, WYE687, WAY600, XL388. Several of these have entered phase 1 and 2 clinical trials.

Currently, active trials are ongoing only for AZD2014 (total of 12), TAK-228 (total of 20), and ATG-008 (total of 4) (clinicaltrials.gov) (Table 1). Unfortunately, a phase 2 trial in patients with VEGF-refractory metastatic RCC AZD2014 showed worse results in PFS and OS than everolimus, with no significant difference in adverse effects [65].

Similarly, TAK-228 (sapanisertib) had inferior outcome in PFS and OS than everolimus in patients with clear-cell renal cell carcinoma, even

Table 1. Ongoing clinical trials of second generation mTOR inhibitors.

Drug ID	Clinical trial ID	Disease	Clinical trial phase	Outcomes
AZD2014	NCT02599714	Advanced and metastatic Breast cancer	I	N/A
	NCT03071874	Meningioma	II	N/A
	NCT02583542	Breast cancer, Lung cancer	I, II	N/A
	NCT01597388	Advanced and metastatic Breast cancer	I	Dosing regiments were tolerable. Clinical benefit was observed in nearly half of patients.
	NCT02398747	Advanced Solid Malignancies	I	N/A
	NCT02216786	Breast cancer	II	N/A
	NCT02208375	Endometrial, Breast, Ovarian, primary Peritoneal, Fallopian tube cancers	I, II	N/A
	NCT02730923	Endometrial cancer	I, II	N/A
	NCT02546661	Invasive Bladder cancer	I	N/A
	NCT03366103	Lung cancer and relapsed solid tumors	I, II	N/A
	NCT02664935	Lung cancer	II	Recruiting
	NCT03334617	Lung cancer	II	Recruiting
	NCT02987959	Soft tissue sarcoma	II	Two out of six enrolled patients displayed serious adverse effects. No results reflecting clinical benefit are posted thus far.
	NCT03193853	Breast cancer	II	N/A

(*Continued*)

Table 1. (*Continued*)

Drug ID	Clinical trial ID	Disease	Clinical trial phase	Outcomes
TAK-228	NCT03154294	Advanced Solid tumors	I	N/A
	NCT02244463	Thyroid cancer	II	N/A
	NCT02575339	Liver cancer	I, II	N/A
	NCT02142803	Brain, Endometrial, Ovarian, Fallopian tube, primary Peritoneal, Uterine cancers	I	N/A
	NCT02503722	Lung cancer	I	N/A
	NCT02133183	Brain cancer	I	N/A
	NCT02893930	Pancreatic Neuroendocrine cancer	II	N/A
	NCT02484430	Leukemia	II	N/A
	NCT03047213	Bladder cancer	II	N/A
	NCT02601209	Sarcoma	I, II	No clinical benefit was demonstrated compared to standard treatment at interim analysis.
	NCT03097328	Renal cell carcinoma	II	Recruiting
	NCT03648489	Ovarian, Fallopian tube, primary Peritoneal cancers	II	Recruiting
	NCT04250545	Brain cancer, Lung cancer	I	Recruiting
	NCT03017833	Advanced and metastatic Solid tumors	I	Recruiting
	NCT02159989	Liver, Ovarian, Pancreatic Neuroendocrine cancers	I	Recruiting

Table 1. (*Continued*)

Drug ID	Clinical trial ID	Disease	Clinical trial phase	Outcomes
	NCT03430882	Advanced and Metastatic Solid tumors	I	Recruiting
	NCT04479306	Lung cancer	I	Recruiting
	NCT02465060	Various (total of 49) refractory cancers	II	Recruiting
ATG-008	NCT02031419	Lymphoma	I	N/A
	NCT04518137	Advanced Solid tumors	II	Recruiting
	NCT03591965	Hepatocellular carcinoma	II	Recruiting
	NCT04337463	Advanced Solid tumors	II	Recruiting

when combined with the PI3K inhibitor MLN1117 [66]. In a small cohort of 33 patients with refractory/relapsed hematological malignancies which include multiple myeloma, non-Hodgkin lymphoma, and Waldenström's macroglobulinemia, TAK-228 induced stable disease in 18 patients. One patient exhibited a minor response (defined by 25–50% reduction of serum monoclonal IgM with no new symptoms or signs of active disease), while another had a partial response (defined by ≥50% reduction of serum monoclonal IgM, ≥50% decrease in adenopathy/organomegaly, and no new symptoms or signs of active disease) [67]. Future studies involving TAK-228 were then envisioned as potential combination therapies. The second phase 1 trial of TAK-228 assessed its effects as a combination therapy with paclitaxel, with/without trastuzumab, in 67 patients suffering from advanced solid and HER2+ neoplasms [68]. These included breast, lung, endometrial, esophageal, and ovarian carcinomas where the paclitaxel/TAK-228 combination was given to all participants, while trastuzumab was added only for HER2+ patients. Out of 54 patients in whom the response could be measured, eight achieved a partial response, while six had stable disease [68]. In a phase 2 trial of patients with advanced or

metastatic breast cancer treated with aromatase inhibitors, TAK-228 showed improved PFS in combination with estrogen receptor inhibitor fulvestrant, compared to fulvestrant monotherapy. The results for OS showed a similar trend but were not conclusive due to a smaller number of participants. Finally, in a phase 2 study in patients with endometrial cancer, TAK-228 showed improved PFS in combination with paclitaxel compared to the monotherapies. Regrettably, these results were not reflected in the OS. To date, none of the second generation mTORi are FDA approved.

Cell models showed the effectiveness of TORKi in restricting growth, even in cases of intrinsic and acquired resistance to allosteric mTOR inhibitors [69, 70]. However, resistance mechanisms to TORKi have been reported as well. Similar to resistance mechanisms to rapalogs, these include RTK elevation [71-74] and MEK/ERK signaling upregulation. Additionally, alterations in the eIF4E/4E-BP1 ratio [75-78] and compensatory increases in glutamine metabolism [79, 80] represent additional mechanisms that may attenuate the anti-neoplastic efficacy of TORKi. Finally, it has been shown that TORKi may induce metabolic dormancy [81, 82]. These findings underline the need for further studies to provide a molecular basis for more efficient mTOR-targeted therapies and rational combination of mTOR inhibitors with other therapeutic modalities.

4. Third generation of mTOR inhibitors

A new class of mTOR inhibitors is represented by RapaLink-1. This third generation of mTORi was developed with the aim of overcoming resistance mechanisms that emerge with the use of first- and second-generation drugs. Treating MCF-7 breast cancer cells with either first generation mTORi, rapamycin, or second generation TORKi, AZD8055, for three months allowed for mapping of the mutations that convey resistance to these drugs [83]. The TORKi resistant (TKi-R) cells developed point mutations in the catalytic site (M2327I), while two rapamycin resistant (RR) clones carried point mutations in the FRB domain (A2034V and F2108L) [83]. Of note, although the RR clones showed impaired ability to bind rapamycin, the TKi-R mutation did not affect drug binding but

caused an unusual upregulation in intrinsic kinase activity [83]. Notably, these mutations were found to be clinically relevant as the F2108L mTOR mutation was reported in a patient who relapsed after everolimus treatment [84]. Similarly, the M2327I mutation was previously found in five patients with different types of solid tumors [85]. Interestingly, a molecular model of mTOR in complex with rapamycin-FKBP12 revealed the juxtaposition between the rapamycin and TORKi binding sites [83]. This was leveraged during the synthesis of the RapaLink-1, a novel bivalent mTOR inhibitor, which consists of both a rapamycin-FRB binding element and a TORKi element, tethered together via a linker region [83].

RapaLink-1 is more potent than both first and second generation mTORi in reducing phosphorylation of downstream mTORC1 (Thr 37/46 p-4E-BPs) and mTORC2 (Ser 473 p-Akt) substrates (Fig. 2) [86]. It also attenuates the resistance of breast cancer cells to mTORi *in vitro* and *in vivo*, regardless of whether it arose from FRB or kinase domain mutations [83]. Owing to its association with FKBP12, RapaLink-1 showed better accumulation within cells, compared to TAK-228, and was able to cross the blood-brain barrier in a glioblastoma model [86]. Here, the compound showed greater reduction in xenograft tumor growth compared to rapamycin and TAK-228, although with a lower inhibition of mTORC2 specific p-Akt Ser 473 and eventual tumor regrowth [86].

More recently, in a patient-derived xenograft model of prostate cancer, RapaLink-1 was shown to effectively block mTORC1/2 signaling and delay tumor growth [87]. Furthermore, RapaLink-1 also caused a reduction in stem cell markers and outgrowth of bone metastatic organoids [87]. Likewise, reduced stemness was reported in breast cancer cells treated with RapaLink-1, with effects superior to those of MLN0128 treated cells [88]. This was accompanied by decreased anchorage-independent growth, reduced viability, and diminished TGFβ-induced resistance to chemotherapies [88].

To further the effort of achieving fine-tuned specificity of mTOR inhibitors, another group of bivalent mTORi which target mTORC1 but not mTORC2 was developed [89]. These compounds were generated with consideration that mTORC2 inhibition is responsible for certain mechanisms of resistance, such as RTK induction, and adverse effects,

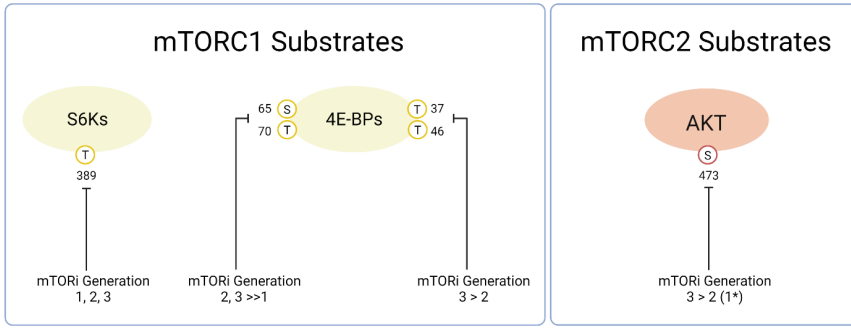

Figure 2. Diagram demonstrating sensitivity of mTORC1 and mTORC2 outputs to the three generations of mTOR inhibitors (mTORi).

The mTORC1 specific threonine (T) 37/46 phosphorylation of 4E-BP is insensitive to first generation of mTORi but suppressed by second generation mTORi, and more potently by third generation mTORi. Serine (S) 65 and T70 4E-BP phosphorylation is inhibited by second and third generation mTORi to a much greater extent than the first generation mTORi. S6K phosphorylation on T389 is similarly sensitive to all generations of mTORi. Phosphorylation of S473 on AKT, downstream of mTORC2, is highly susceptible to inhibition by second and third generation mTORi, although the latter is more potent. It is responsive to first generation mTORi only under prolonged treatment (*). Created with BioRender.com.

such as hyperglycemia [56]. In addition to maintaining mTORC1 specificity, they are successful in inhibiting its rapamycin-resistant outputs as well (Fig. 2). Representatives of this class of mTORC1 specific mTORi, e.g., RMC-4627 or BiS-13x, induced tumor regression comparable to pan-mTORi both *in vitro* and *in vivo*, without unwanted effects of RTK induction and hyperglycemia [89]. Moreover, their effects on the translatome (i.e., collection of all actively translated mRNAs in the cells) was comparable to that observed with active-site mTOR inhibitors [89].

5. Summary

Although mTOR inhibitors have been in development for decades, only the first generation is approved for clinical use. The FDA and European Medicines Agency (EMA) have approved everolimus and temsirolimus for the treatment of certain types of cancers, which include advanced RCC

[42], pediatric or adult SEGA with TSC [50], as well as selected NETs [90] and hormone receptor-positive, HER2-negative breast cancer in post-menopausal women [45, 91, 92].

Persistently improving design, based on an expanding understanding of mTOR biology, has inspired development of the second and third class of mTORi. The generation of bivalent mTORi has epitomized the effort of achieving a specific and sustained inhibition of this kinase complex. Following the recurring pattern of drug development, these mTORi also address the adaptive resistance issues of its predecessor drugs.

However, the use of RapaLink-1 as a monotherapy in pre-clinical models demonstrates tumor regrowth even upon satisfactory mTOR inhibition [86]. In this light, recent work has shown that chronic mTORi treatment may not only be insufficient, but even detrimental, as it promotes the plasticity of breast cancer cells [93]. Accordingly, metabolic plasticity of cancer cells has been shown to attenuate the effects of mTOR-targeted therapies [94]. This re-emphasizes the need to improve understanding of the relative contributions of a plethora of mTOR outputs that may contribute to tumor recurrence and resistance in a context-dependent manner. It also suggests that further research is warranted to provide molecular basis for rational use of mTORi as powerful tools in combination with other anti-oncogenic drugs. Such therapies may help turn a cytostatic response to a cytotoxic one.

Lastly, it should be noted that one of the major difficulties in mTOR targeted cancer therapies is assessing the efficacy of signaling inhibition within the tumor [95]. Therefore, defining reliable biomarkers that attest to the levels of mTOR inhibition is of great importance and interest.

6. Limitations

It must be mentioned that the sections above focus only on the potential of mTOR inhibition in cancer therapies. Aside from cancer, mTOR inhibitors are successfully used in drug-eluting stents in order to treat coronary artery disease [96, 97], as well as to prevent rejection of transplanted solid organs [36]. Their use is also being considered as a prevention of graft-versus-host disease in hematopoietic stem cell transplantation [98]. There

is also a noteworthy interest in using mTOR inhibitors to treat the detrimental effects of aging [99, 100]. Finally, modulators of mTOR activity are also explored for a wide range of neurological disorders including epilepsy, autism, stroke, and neurodegenerative disorders [101].

References

1. Vézina C and Kudelski A. (1975) Rapamycin (AY-22,989), a new antifungal antibiotic. I. taxonomy of the producing streptomycete and isolation of the active principle. *The Journal of Antibiotics*, **28**(10), 721–726.
2. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P and Snyder SH. (1994) RAFT1: A mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell*, **78**(1), 35–43.
3. Brown EJ, Albers MW, Bum Shin T, Ichikawa K, Keith CT, Lane WS, *et al.* (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature*, **369**(6483), 756–758.
4. Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, Wiederrecht G, *et al.* (1995) Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. *Journal of Biological Chemistry*, **270**(2), 815–822.
5. Dowling RJ, Topisirovic I, Fonseca BD and Sonenberg N. (2010) Dissecting the role of mTOR: Lessons from mTOR inhibitors. *Biochimica et Biophysica Acta*, **1804**(3), 433–439.
6. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, *et al.* (2009) DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell*, **137**(5), 873–886.
7. Frias MA, Thoreen CC, Jaffe JD, Schroder W, Sculley T, Carr SA, *et al.* (2006) mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. *Current Biology*, **16**(18), 1865–1870.
8. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, *et al.* (2006) SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell*, **127**(1), 125–137.
9. Pearce LR, Huang X, Boudeau J, Pawlowski R, Wullschleger S, Deak M, *et al.* (2007) Identification of protor as a novel rictor-binding component of mTOR complex-2. *Biochemical Journal*, **405**(3), 513–522.
10. Thedieck K, Polak P, Kim ML, Molle KD, Cohen A, Jeno P, *et al.* (2007) PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. *PLoS One*, **2**(11), e1217.
11. Woo SY, Kim DH, Jun CB, Kim YM, Haar EV, Lee SI, *et al.* (2007) PRR5, a novel component of mTOR complex 2, regulates platelet-derived growth factor receptor

beta expression and signaling. *Journal of Biological Chemistry*, **282**(35), 25604–25612.
12. Dibble CC and Cantley LC. (2015) Regulation of mTORC1 by PI3K signaling. *Trends in Cell Biology*, **25**(9), 545–555.
13. Maehama T and Dixon JE. (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *Journal of Biological Chemistry*, **273**(22), 13375–13378.
14. Inoki K, Li Y, Zhu T, Wu J and Guan KL. (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nature Cell Biology*, **4**(9), 648–657.
15. Hay N and Sonenberg N. (2004) Upstream and downstream of mTOR. *Genes & Development*, **18**(16), 1926–1945.
16. Saxton RA and Sabatini DM. (2017) mTOR signaling in growth, metabolism, and disease. *Cell*, **168**(6), 960–976.
17. Kim J and Guan KL. (2019) mTOR as a central hub of nutrient signalling and cell growth. *Nature Cell Biology*, **21**(1), 63–71.
18. Laplante M and Sabatini DM. (2009) mTOR signaling at a glance. *Journal of Cell Science*, **122**(Pt 20), 3589–3594.
19. Fernandes SA and Demetriades C. (2021) The multifaceted role of nutrient sensing and mTORC1 signaling in physiology and aging. *Frontiers in Aging*, **2**.
20. Sengupta S, Peterson TR and Sabatini DM. (2010) Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Molecular Cell*, **40**(2), 310–322.
21. Fu W and Hall MN. (2020) Regulation of mTORC2 Signaling. *Genes (Basel)*, **11**(9).
22. Oh WJ and Jacinto E. (2011) mTOR complex 2 signaling and functions. *Cell Cycle*, **10**(14), 2305–2316.
23. Yuan TL and Cantley LC. (2008) PI3K pathway alterations in cancer: Variations on a theme. *Oncogene*, **27**(41), 5497–5510.
24. Liu GY and Sabatini DM. (2020) mTOR at the nexus of nutrition, growth, ageing and disease. *Nature Reviews Molecular Cell Biology*, **21**(4), 183–203.
25. Chen J, Zheng XF, Brown EJ and Schreiber SL. (1995) Identification of an 11-Kda Fkbp12-rapamycin-binding domain within the 289-Kda Fkbp12-rapamycin-associated protein and characterization of a critical serine residue. *Proceedings of the National Academy of Sciences of the United States*, **92**(11), 4947–4951.
26. Choi JW, Chen J, Schreiber SL and Clardy J. (1996) Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. *Science*, **273**(5272), 239–242.
27. Oshiro N, Yoshino K, Hidayat S, Tokunaga C, Hara K, Eguchi S, *et al.* (2004) Dissociation of raptor from mTOR is a mechanism of rapamycin-induced inhibition of mTOR function. *Genes Cells*, **9**(5), 497.

28. Gaubitz C, Oliveira TM, Prouteau M, Leitner A, Karuppasamy M, Konstantinidou G, *et al.* (2015) Molecular basis of the rapamycin insensitivity of target of rapamycin complex 2. *Molecular Cell*, **58**(6), 977–988.
29. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, *et al.* (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Molecular Cell*, **22**(2), 159–168.
30. Choo AY, Yoon SO, Kim SG, Roux PP and Blenis J. (2008) Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. *Proceedings of the National Academy of Sciences of the United States*, **105**(45), 17414–17419.
31. Aylett CH, Sauer E, Imseng S, Boehringer D, Hall MN, Ban N, *et al.* (2016) Architecture of human mTOR complex 1. *Science*, **351**(6268), 48–52.
32. Heitman J, Movva NR and Hall MN. (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*, **253**(5022), 905–909.
33. Martel RR, Klicius J and Galet S. (1977) Inhibition of immune-response by rapamycin, a new antifungal antibiotic. *Canadian Journal of Physiology and Pharmacology*, **55**(1), 48–51.
34. Eng CP, Sehgal SN and Vezina C. (1984) Activity of rapamycin (Ay-22,989) against transplanted tumors. *The Journal of Antibiotics*, **37**(10), 1231–1237.
35. Petroulakis E, Mamane Y, Le Bacquer O, Shahbazian D and Sonenberg N. (2006) mTOR signaling: Implications for cancer and anticancer therapy. *British Journal of Cancer*, **94**(2), 195–199.
36. Waldner M, Fantus D, Solari M and Thomson AW. (2016) New perspectives on mTOR inhibitors (rapamycin, rapalogs and TORKinibs) in transplantation. *British Journal of Clinical Pharmacology*, 1158–1170.
37. van Gelder T, Fischer L, Shihab F and Shipkova M. (2017) Optimizing everolimus exposure when combined with calcineurin inhibitors in solid organ transplantation. *Transplantation Reviews*, **31**(3), 151–157.
38. Simamora P, Alvarez JM and Yalkowsky SH. (2001) Solubilization of rapamycin. *International Journal of Pharmaceutics*, **213**(1–2), 25–29.
39. Atkins MB, Hidalgo M, Stadler WM, Logan TF, Dutcher JP, Hudes GR, *et al.* (2004) Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *Journal of Clinical Oncology*, **22**(5), 909–918.
40. Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, *et al.* (2007) Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *New England Journal of Medicine*, **356**(22), 2271–2281.
41. Hess G, Smith SM, Berkenblit A and Coiffier B. (2009) Temsirolimus in mantle cell lymphoma and other non-Hodgkin lymphoma subtypes. *Seminars in Oncology*, **36**(Suppl 3), S37–S45.
42. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, *et al.* (2008) Efficacy of everolimus in advanced renal cell carcinoma: A double-blind, randomised, placebo-controlled phase III trial. *The Lancet*, **372**(9637), 449–456.

43. Ravaud A, Barrios CH, Alekseev B, Tay MH, Agarwala SS, Yalcin S, *et al.* (2015) RECORD-2: Phase II randomized study of everolimus and bevacizumab versus interferon α-2a and bevacizumab as first-line therapy in patients with metastatic renal cell carcinoma. *Annals of Oncology*, **26**(7), 1378–1384.
44. Knox JJ, Barrios CH, Kim TM, Cosgriff T, Srimuninnimit V, Pittman K, *et al.* (2017) Final overall survival analysis for the phase II RECORD-3 study of first-line everolimus followed by sunitinib versus first-line sunitinib followed by everolimus in metastatic RCC. *Annals of Oncology,* **28**(6), 1339–1345.
45. André F, O'Regan R, Ozguroglu M, Toi M, Xu B, Jerusalem G, *et al.* (2014) Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3), A randomised, double-blind, placebo-controlled phase 3 trial. *The Lancet Oncology*, **15**(6), 580–591.
46. Yao YC, Lombard-Bohas C, Baudin E, Kvols LK, Rougier P, Ruszniewski P, *et al.* (2010) Daily oral everolimus activity in patients with metastatic pancreatic neuroendocrine tumors after failure of cytotoxic chemotherapy: A phase II trial. *Journal of Clinical Oncology*, **28**(1), 69–76.
47. Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, *et al.* (2011) Everolimus for advanced pancreatic neuroendocrine tumors. *New England Journal of Medicine*, **364**(6), 514 –523.
48. Wataya-Kaneda M, Uemura M, Fujita K, Hirata H, Osuga K, Kagitani-Shimono K, *et al.* (2017) Tuberous sclerosis complex: Recent advances in manifestations and therapy. *International Journal of Urology*, **24**(9), 681–691.
49. Sampson JR. (2009) Therapeutic targeting of mTOR in tuberous sclerosis. *Biochemical Society Transactions*, **37**(1), 259–264.
50. Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, *et al.* (2010) Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *New England Journal of Medicine*, **363**(19), 1801–1811.
51. Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E, *et al.* (2013) Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): A multicentre, randomised, double-blind, placebo-controlled trial. *The Lancet*, **381**(9869), 817–824.
52. Shi Y, Yan H, Frost P, Gera J and Lichtenstein A. (2005) Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade. *Molecular Cancer Therapeutics*, **4**(10), 1533–1540.
53. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, *et al.* (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Research*, **66**(3), 1500–1508.
54. Tamburini J, Chapuis N, Bardet V, Park S, Sujobert P, Willems L, *et al.* (2008) Mammalian target of rapamycin (mTOR) inhibition activates phosphatidylinositol 3-kinase/Akt by up-regulating insulin-like growth factor-1 receptor signaling in

acute myeloid leukemia: Rationale for therapeutic inhibition of both pathways. *Blood*, **111**(1), 379–382.

55. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, *et al.* (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *Journal of Clinical Investigation*, **118**(9), 3065–3074.
56. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, *et al.* (2011) AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. *Cancer Cell*, **19**(1), 58–71.
57. Bray K, Mathew R, Lau A, Kamphorst JJ, Fan J, Chen J, *et al.* (2012) Autophagy suppresses RIP kinase-dependent necrosis enabling survival to mTOR inhibition. *PLoS ONE*, **7**(7).
58. Xie X, White EP and Mehnert JM. (2013) Coordinate Autophagy and mTOR Pathway Inhibition Enhances Cell Death in Melanoma. *PLoS One*, **8**(1).
59. Lee HO, Mustafa A, Hudes GR and Kruger WD. (2015) Hydroxychloroquine destabilizes phospho-S6 in human renal carcinoma cells. *PLoS One*, **10**(7).
60. Simioni C, Cani A, Martelli AM, Zauli G, Alameen AM, Ultimo S, *et al.* (2015) The novel dual PI3K/mTOR inhibitor NVP-BGT226 displays cytotoxic activity in both normoxic and hypoxic hepatocarcinoma cells. *Oncotarget*, **6**(19), 17147–17160.
61. Yang X, Niu B, Wang L, Chen M, Kang X, Wang L, *et al.* (2016) Autophagy inhibition enhances colorectal cancer apoptosis induced by dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235. *Oncology Letters*, **12**(1), 102–106.
62. Dowling RJ, Topisirovic I, Alain T, Bidinosti M, Fonseca BD, Petroulakis E, *et al.* (2010) mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. *Science*, **328**(5982), 1172–1176.
63. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, *et al.* (2009) An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *Journal of Biological Chemistry*, **284**(12), 8023–8032.
64. Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, *et al.* (2009) Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. *PLoS Biology*, **7**(2), e38.
65. Powles T, Wheater M, Din O, Geldart T, Boleti E, Stockdale A, *et al.* (2016) A randomised phase 2 study of AZD2014 versus everolimus in patients with VEGF-refractory metastatic clear cell renal cancer. *European Urology*, **69**(3), 450–456.
66. MLN0128 and MLN0128 + MLN1117 compared with everolimus in the treatment of adults with advanced or metastatic clear-cell renal cell carcinoma. https://ClinicalTrials.gov/show/NCT02724020.
67. Ghobrial IM, Siegel DS, Vij R, Berdeja JG, Richardson PG, Neuwirth R, *et al.* (2016) TAK-228 (formerly MLN0128), an investigational oral dual TORC1/2 inhibitor: A phase I dose escalation study in patients with relapsed or refractory multiple myeloma, non-Hodgkin lymphoma, or Waldenström's macroglobulinemia. *American Journal of Hematology*, **91**(4), 400 –5.

68. Burris HA, Kurkjian CD, Hart L, Pant S, Murphy PB, Jones SF, *et al.* (2017) TAK-228 (formerly MLN0128), an investigational dual TORC1/2 inhibitor plus paclitaxel, with/without trastuzumab, in patients with advanced solid malignancies. *Cancer Chemotherapy and Pharmacology*, **80**(2), 261–273.
69. Vandamme T, Beyens M, De Beeck KO, Dogan F, Van Koetsveld PM, Pauwels P, *et al.* (2016) Long-term acquired everolimus resistance in pancreatic neuroendocrine tumours can be overcome with novel PI3K-AKT-mTOR inhibitors. *British Journal of Cancer*, **114**(6), 650–658.
70. Hassan B, Akcakanat A, Sangai T, Evans KW, Adkins F, Eterovic AK, *et al.* (2014) Catalytic mTOR inhibitors can overcome intrinsic and acquired resistance to allosteric mTOR inhibitors. *Oncotarget*, **5**(18), 8544–8557.
71. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, Poulikakos PI, Scaltriti M, Moskatel E, *et al.* (2011) mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. *Cancer Discovery*, **1**(3), 248–259.
72. Cirstea D, Santo L, Hideshima T, Eda H, Mishima Y, Nemani N, *et al.* (2014) Delineating the mTOR kinase pathway using a dual TORC1/2 inhibitor, AZD8055, in multiple myeloma. *Molecular Cancer Therapeutics*, **13**(11), 2489–2500.
73. Wei F, Zhang Y, Geng L, Zhang P, Wang G and Liu Y. (2015) mTOR inhibition induces EGFR feedback activation in association with its resistance to human pancreatic cancer. *International Journal of Molecular Sciences*, **16**(2), 3267–3282.
74. Fujishita T, Kojima Y, Kajino-Sakamoto R, Taketo MM and Aoki M. (2017) Tumor microenvironment confers mTOR inhibitor resistance in invasive intestinal adenocarcinoma. *Oncogene*, **36**(46), 6480–6489.
75. Alain T, Morita M, Fonseca BD, Yanagiya A, Siddiqui N, Bhat M, *et al.* (2012) eIF4E/4E-BP ratio predicts the efficacy of mTOR targeted therapies. *Cancer Research*, **72**(24), 6468–6476.
76. Martineau Y, Azar R, Müller D, Lasfargues C, El Khawand S, Anesia R, *et al.* (2014) Pancreatic tumours escape from translational control through 4E-BP1 loss. *Oncogene*, **33**(11), 1367–1374.
77. Mallya S, Fitch BA, Lee JS, So L, Janes MR and Fruman DA. (2014) Resistance to mTOR kinase inhibitors in lymphoma cells lacking 4EBP1. *PLoS ONE*, **9**(2).
78. Cope CL, Gilley R, Balmanno K, Sale MJ, Howarth KD, Hampson M, *et al.* (2014) Adaptation to mTOR kinase inhibitors by amplification of eIF4E to maintain cap-dependent translation. *Journal of Cell Science*, **127**(4), 788–800.
79. Tanaka K, Sasayama T, Irino Y, Takata K, Nagashima H, Satoh N, *et al.* (2015) Compensatory glutamine metabolism promotes glioblastoma resistance to mTOR inhibitor treatment. *Journal of Clinical Investigation*, **125**(4), 1591–1602.
80. Guo L, Zhou B, Liu Z, Xu Y, Lu H, Xia M, *et al.* (2016) Blockage of glutaminolysis enhances the sensitivity of ovarian cancer cells to PI3K/mTOR inhibition involvement of STAT3 signaling. *Tumor Biology*, **37**(8), 11007–1115.
81. Morita M, Gravel SP, Chenard V, Sikstrom K, Zheng L, Alain T, *et al.* (2013) mTORC1 controls mitochondrial activity and biogenesis through 4E-BP-dependent translational regulation. *Cell Metabolism*, **18**(5), 698–711.

82. Gandin V, Masvidal L, Hulea L, Gravel SP, Cargnello M, McLaughlan S, *et al.* (2016) nanoCAGE reveals 5' UTR features that define specific modes of translation of functionally related MTOR-sensitive mRNAs. *Genome Research*, **26**(5), 636–648.
83. Rodrik-Outmezguine VS, Okaniwa M, Yao Z, Novotny CJ, McWhirter C, Banaji A, *et al.* (2016) Overcoming mTOR resistance mutations with a new-generation mTOR inhibitor. *Nature*, **534**(7606), 272–276.
84. Wagle N, Grabiner BC, Van Allen EM, Amin-Mansour A, Taylor-Weiner A, Rosenberg M, *et al.* (2014) Response and acquired resistance to everolimus in anaplastic thyroid cancer. *New England Journal of Medicine*, **371**(15), 1426–1433.
85. Grabiner BC, Nardi V, Birsoy K, Possemato R, Shen K, Sinha S, *et al.* (2014) A diverse array of cancer-associated MTOR mutations are hyperactivating and can predict rapamycin sensitivity. *Cancer Discovery*, **4**(5), 554–563.
86. Fan Q, Aksoy O, Wong RA, Ilkhanizadeh S, Novotny CJ, Gustafson WC, *et al.* (2017) A kinase inhibitor targeted to mTORC1 drives regression in glioblastoma. *Cancer Cell*, **31**(3), 424–435.
87. La Manna F, De Menna M, Patel N, Karkampouna S, De Filippo MR, Klima I, *et al.* (2020) Dual-mTOR inhibitor rapalink-1 reduces prostate cancer patient-derived xenograft growth and alters tumor heterogeneity. *Frontiers in Oncology*, **10**, 1012.
88. Katsuno Y, Meyer DS, Zhang Z, Shokat KM, Akhurst RJ, Miyazono K, *et al.* (2019) Chronic TGF-beta exposure drives stabilized EMT, tumor stemness, and cancer drug resistance with vulnerability to bitopic mTOR inhibition. *Science Signaling*, **12**(570).
89. Lee BJ, Boyer JA, Burnett GL, Thottumkara AP, Tibrewal N, Wilson SL, *et al.* (2021) Selective inhibitors of mTORC1 activate 4EBP1 and suppress tumor growth. *Nature Chemical Biology*.
90. Pavel M, Unger N, Borbath I, Ricci S, Hwang TL, Brechenmacher T, *et al.* (2016) Safety and QOL in patients with advanced NET in a phase 3b expanded access study of everolimus. *Targeted Oncology*, **11**(5), 667–675.
91. Baselga J, Campone M, Piccart M, Burris Iii HA, Rugo HS, Sahmoud T, *et al.* (2012) Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *New England Journal of Medicine*, **366**(6), 520–529.
92. Piccart M, Hortobagyi GN, Campone M, Pritchard KI, Lebrun F, Ito Y, *et al.* (2014) Everolimus plus exemestane for hormonereceptor- positive, human epidermal growth factor receptor-2-negative advanced breast cancer: Overall survival results from BOLERO-2. *Annals of Oncology*, **25**(12), 2357–2362.
93. Jewer M, Lee L, Leibovitch M, Zhang G, Liu J, Findlay SD, *et al.* (2020) Translational control of breast cancer plasticity. *Nature Communications*, **11**(1), 2498.
94. Hulea L, Gravel SP, Morita M, Cargnello M, Uchenunu O, Im YK, *et al.* (2018) Translational and HIF-1alpha-dependent metabolic reprogramming underpin

metabolic plasticity and responses to kinase inhibitors and biguanides. *Cell Metabolism*, **28**(6), 817–832 e8.

95. O'Reilly T and McSheehy PM. (2010) Biomarker development for the clinical activity of the mTOR inhibitor everolimus (RAD001): Processes, limitations, and further proposals. *Translational Oncology*, **3**(2), 65–79.
96. Doggrell SA. (2004) Sirolimus- or paclitaxel-eluting stents to prevent coronary artery restenosis. *Expert Opinion on Pharmacotherapy*, **5**(11), 2209–2220.
97. Kalra A, Rehman H, Khera S, Thyagarajan B, Bhatt DL, Kleiman NS, *et al.* (2017) New-generation coronary stents: Current data and future directions. *Current Atherosclerosis Reports*, **19**(3).
98. Feng Y, Chen X, Cassady K, Zou Z, Yang S, Wang Z, *et al.* (2020) The role of mTOR inhibitors in hematologic disease: From bench to bedside. *Frontiers in Oncology*, 10, 611690.
99. Evangelisti C, Cenni V and Lattanzi G. (2016) Potential therapeutic effects of the MTOR inhibitors for preventing ageing and progeria-related disorders. *British Journal of Clinical Pharmacology*, 1229–1244.
100. Papadopoli D, Boulay K, Kazak L, Pollak M, Mallette F, Topisirovic I, *et al.* (2019) mTOR as a central regulator of lifespan and aging. *F1000Research*, **8**.
101. Crino PB. (2016) The mTOR signalling cascade: Paving new roads to cure neurological disease. *Nature Reviews Neurology*, **12**(7), 379–392.

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Chapter 10

Roles of MicroRNAs in Hematologic Malignancies

MinJung Kim[1], Christian L. Eberly[1], Curt I. Civin[1,2]*

[1]*Center for Stem Cell Biology & Regenerative Medicine, Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201*
[2]*Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD 21201*

Abstract

MicroRNAs are small non-coding RNAs that modulate many cellular processes including hematopoiesis, a highly regulated developmental process responsible for the generation of all mature blood cells from hematopoietic stem cells. Dysregulated microRNA expression is involved mechanistically in hematologic malignancies, generally by inhibiting differentiation, enhancing proliferation, or increasing survival of the malignant cells. Although most mature microRNAs are generated by the canonical pathway using the RNA-induced silencing complex (RISC), isomiRs, microRNA isoforms with small variations in length and sequence of mature microRNAs, are generated by alternate DROSHA/DICER cleavages or non-template base additions. In addition, adenosine deaminase acting on RNA (ADAR)-mediated RNA

*Corresponding author's email: ccivin@som.umaryland.edu

editing in primary microRNA transcripts, precursor microRNAs, or target mRNAs, could affect the biogenesis and target mRNA recognition of microRNAs. In this review, we highlight how these non-canonical mechanisms expand the diversity and potential targets of microRNAs to provide more intricate control of gene expression in normal and malignant cells. We also discuss exosomal microRNAs (exomiRs), their impact on hematologic malignancies, and their role as potential biomarkers. Finally, we briefly summarize microRNA-related clinical research studies in hematologic malignancies to highlight the potential therapeutic applications in cancer treatment.

1. Hematologic malignancies

Hematopoiesis is the process of generating all the mature functional cells of the blood and immune system (Fig. 1). Since hematopoietic cells are relatively short-lived, proper regulation of hematopoietic stem-progenitor cell (HSPC) quiescence, self-renewal, proliferation, and differentiation is essential to maintain homeostasis of the blood and immune systems throughout life as well as to respond to acute and chronic systemic challenges such as hypoxia, bleeding, and infection [1–4].

Hematologic malignancies, also referred to as blood cancers, are classified into three broad categories on the basis of clinical manifestations: leukemias, lymphomas, and myelomas. All three types of hematologic malignancies are composed of growth-dysregulated blood-forming cells with increased proliferation, enhanced survival, and/or blocks in differentiation, as well as other abnormal hallmarks of cancer [5–7]. In leukemias, morphologically immature leukemic blast cells outgrow normal HSPCs to predominate in the bone marrow, the home of normal hematopoiesis, and circulate throughout the body in the blood. Based historically on the rate of disease progression and more recently on molecular aberrations, leukemias are categorized as either acute or chronic. Based on the expression of morphologic features, genomic alterations and expression of proteins and mRNAs that serve as lineage markers, leukemias are further categorized as either myeloid or lymphoid [8–12]. In lymphomas, expanded

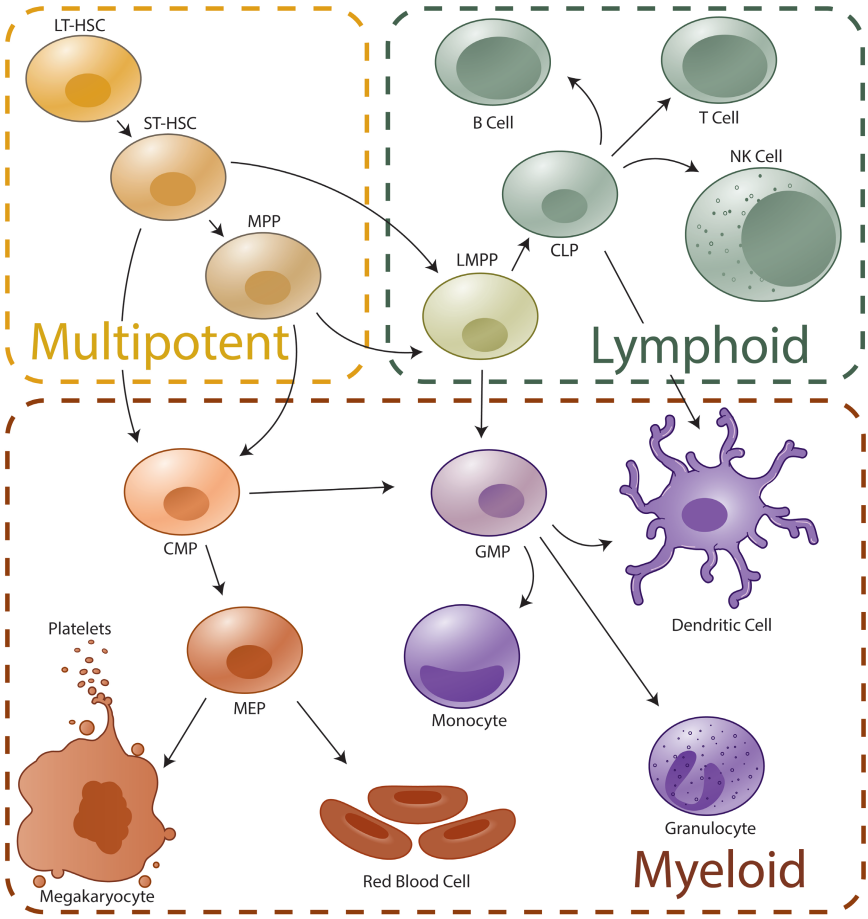

Figure 1. Schematic representation of normal hematopoiesis.

Hematopoiesis is a highly regulated developmental process from which rare hematopoietic stem cells (HSCs) give rise to increasingly more lineage-restricted progenitor cells that enable the vast expansion of cell numbers while generating the repertoire of short-lived mature blood cells constituting the blood and immune system. Rare long-term (LT) HSCs generate short-term (ST) HSCs with limited self-renewal capacity that generate multipotent progenitors (MPPs) and more lineage-restricted progenitors. Common myeloid progenitors (CMPs) generate megakaryocyte-erythrocyte progenitors (MEPs) which ultimately lead to the generation of red blood cells (RBCs) and platelets. Granulocyte-monocyte progenitors (GMPs) yield granulocytes (eosinophils, neutrophils, basophils) and monocytes. Lymphoid-primed multipotent progenitors (LMPPs) generate common lymphoid progenitors (CLPs), which in turn generate lymphocytes (B cells and T cells), natural killer cells (NK), and dendritic cells.

populations of cells derived from lymphoid progenitor/precursors, typically beyond the stage of antigen receptor rearrangement, accumulate in lymph nodes and other lymphoid tissues. Lymphomas are classified as Hodgkin lymphoma (HL) or Non-Hodgkin lymphoma (NHL), with NHLs subclassified as Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), lymphoblastic lymphoma, and anaplastic large cell lymphoma (ALCL) [13–15]. In multiple myeloma (MM), a clone of abnormal plasma cells secreting an idiotypic antibody (sometimes in quantities that cause clinically consequential sludging of blood in small blood vessels) accumulate in the bone marrow and frequently form tumors in bone [16–18].

2. MicroRNAs

Discovered in *C. elegans* in the early 1990s, microRNAs are short single-stranded RNA molecules that have emerged as important regulators of gene expression across diverse species. MicroRNAs predominantly function post-transcriptionally to reduce gene expression by hybridizing to a cognate mRNA in its 3′ untranslated region (UTR) to inhibit translation or trigger mRNA degradation. The human genome contains over 1,900 annotated hairpin precursors and 2,654 mature microRNA sequences [19]. MicroRNAs were first implicated in human disease nearly two decades ago, when it was found that the chromosomal band 13q14.3 deletion observed in more than 50% of chronic lymphocytic leukemia (CLL) patients deleted the genomic DNA encoding the microRNAs, miR-15 and miR-16. MiR-15/miR-16 deletion occurs in the smallest chromosomal deletions observed in CLL patients indicating that loss of these tumor suppressor microRNAs (TS-miRs) is important in the genesis of this molecular subtype of CLL. Conversely, the sufficiency of a single oncogenic microRNA (oncomiR) to cause cancer was demonstrated when transgenic mice overexpressing miR-155 in lymphoid cells developed lymphoid leukemia-lymphomas [20] without additional genetic alterations.

MicroRNAs are instrumental in regulating nearly all phases of hematopoiesis. Notably, microRNA-mediated modulation of key hematopoietic transcription factors shapes lineage commitment throughout myeloid and lymphoid development, controls developmental timing of HSPC proliferation versus differentiation, and reinforces cell fate commitment by

enhancing or repressing alternative gene transcription programs [21]. Thus, it is not surprising that dysregulated microRNA expression can lead to hematologic malignancies by blocking cell differentiation trajectory/capacity, enhancing cell proliferation, or increasing cell survival. Numerous studies have implicated connections between common genomic abnormalities in hematologic malignancies and altered microRNA expression [22, 23]. Here, in order to stimulate further interrogation of the complex regulatory roles of microRNAs in hematologic malignancies, we seek to draw attention to several pertinent but less appreciated roles for microRNAs and microRNA biology and suggest investigation of their potential contributions to development, progression, and therapeutic resistance of hematologic malignancies. We also highlight the expanding role of exosomal microRNAs (exomiRs) and ongoing efforts to utilize microRNAs as biomarkers and therapeutic targets.

Most mature microRNAs are generated via the canonical pathway initiated by polymerase II-mediated transcription of miRNA primary transcripts (pri-miRs) (Fig. 2). In the nucleus, pri-miRs are processed by the type III RNA nuclease Drosha complexed with DGCR8 and cleaved to generate an ~70 base "precursor miRNAs (pre-miRs)." After export to the cytoplasm by Ran-GTP-dependent exportin-5, pre-miRs are further processed by the type III RNAase Dicer to generate a mature microRNA of ~22 nucleotides. Mature microRNAs are loaded onto the Argonaute (AGO) complex to form the RNA-induced Silencing Complex (RISC). The microRNA guide strand is retained by AGO and directs RISC to target mRNAs, primarily via a "seed sequence" comprised nucleotides 2–8 of the mature microRNA. Non-canonical targeting mechanisms also exist [24, 25]. The exact length of mature microRNAs varies depending on Dicer cut site selection, which is determined by the relative cut site efficiencies and positioning of Dicer active site by accessory proteins such as PACT and TRBP which influence pre-miR dsRNA structure [26]. The generation of multiple species of mature microRNAs from a single pre-miR arm results in microRNA variants referred to as "isomiRs" [27] (Fig. 3). IsomiRs can also be generated by post-processing non-templated base addition [28] and their generation adds another level of complexity to microRNA biogenesis and functional interrogation. Alterations at the 5′ end of mature microRNAs generate isomiRs with distinct seed sequences

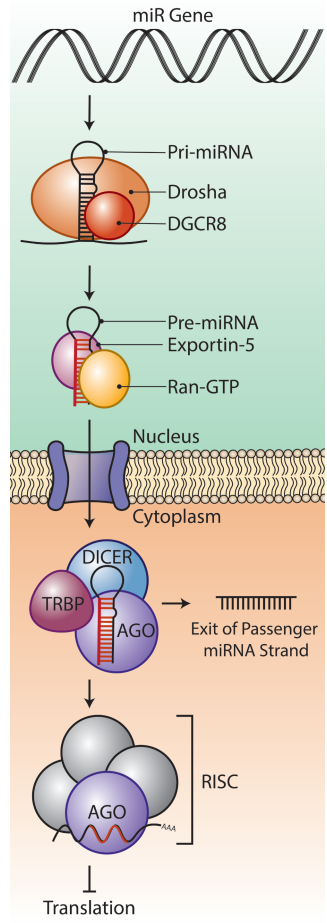

Figure 2. Schematic representation of the conventional microRNA biogenesis.

Pri-miRNAs are transcribed from miRNA genes by RNA polymerase II and processed by the Drosha-DGCR8 complex to form pre-miRNAs, which are exported into the cytoplasm by Ran-GTP-dependent exportin-5. Pre-miRNAs undergo cleavage by the DICER complex to produce mature microRNAs that are loaded into AGO and guide the RISC complex to the cognate mRNA targets to inhibit translation or trigger mRNA degradation. Abbreviations: Pri-miRNA: primary microRNA; DGCR8: microprocessor complex subunit DiGeorge syndrome critical region gene 8; Pre-miRNA: precursor microRNA; Ran-GTP: Ras-related nuclear protein GTPase; TRBP: transactivation response element RNA-binding protein; AGO: Argonaute; miRNA: mature microRNA; RISC: RNA-induced silencing complex.

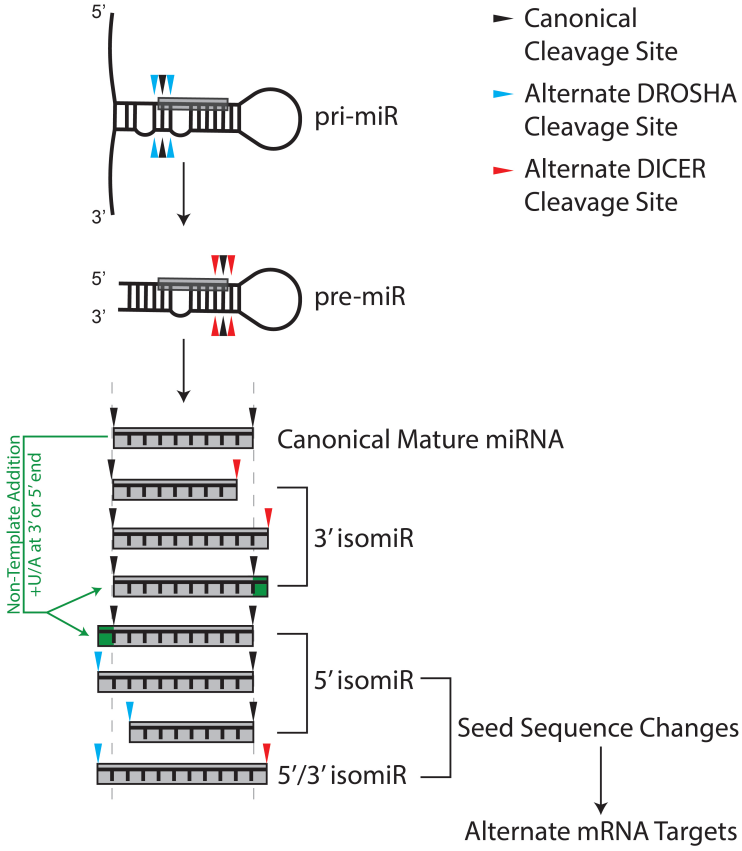

Figure 3. Schematic representation of the isomiR biogenesis.

IsomiRs are mature microRNA variants that can be generated from a single pri-miR and/or pre-miR arm. For example, alternate DROSHA cleavages (blue arrows), alternate DICER cleavages (red arrows), or non-templated base additions (green). The seed sequences of the canonical mature microRNA and 3′ isomiRs are the same. In contrast, 5′ isomiRs will generally have altered seed sequences and distinct target mRNAs.

and mRNA target specificities. Reference microRNAs annotated in miRBase database often differ from the isomiRs detected as abundant in lymphoblastoid cell lines and patient samples. Analysis of patient-derived lymphoblastoid cell lines revealed that for almost half of pre-miR arms analyzed, the most prevalent isomiR did not match the reference sequence

in miRbase [29]. Even more striking, over 10% of the isomiRs had altered 5′ ends. The analysis further revealed gender-dependent differences in isomiR expression profiles [29], raising the possibility that isomiR expression profiles may contribute to sex differences in CLL progression and treatment response [30].

IsomiR generation is a regulated process in cells. For example, stimulation of K562 myeloid leukemia cells with PMA to trigger cell differentiation selectively altered isomiR production for a subset of microRNAs, including miR-22, modulating miR-22 target inhibition efficiency [31]. MiR-22 functions as a tumor suppressor in acute myeloid leukemia (AML) by targeting multiple oncogenes, including FLT3, MYCBP, and CRTC to impair CREB and MYC pathways [32], and MM by targeting LIG3 [33], but the regulation and functional role of miR-22 isomiRs has not been interrogated. IsomiR expression patterns can distinguish AML from normal tissue and 31 other cancers in The Cancer Genome Atlas (TCGA) [34]. Combined with the potential impact of isomiRs on microRNA function, these observations indicate that isomiR contribution to hematologic malignancies warrants further investigation.

Although the major mechanism of microRNA function is post-transcriptional inhibition of gene expression by binding to mRNAs in the 3′ UTR to block translation or trigger mRNA degradation, alternative functions and mechanisms of action have been reported. Multiple studies have shown that microRNA binding to either the 5′ UTR or open reading frame (ORF) can also inhibit mRNA translation, greatly expanding potential mRNA targets of microRNAs [35–38]. In addition, some microRNAs can stimulate, rather than inhibit, the translation of specific mRNA targets. The impact of a given microRNA depends on multiple factors, including cell type, cell state, mRNA structure, miRNP complex components, and microRNA subcellular localization. The same microRNA may repress or activate distinct mRNAs within the same cell. In addition, the consequence of a microRNA can change depending on cellular conditions. This has been shown for miR-369 regulation of TNFalpha mRNA, which switches from repression to activation upon serum starvation [39–41]. Both microRNA-mediated repression and stimulation are dependent upon cis- and trans-acting mRNA-regulatory factors. MicroRNA-mediated inhibition of translation requires the P-body subunit

GW182 and canonical translation factors such as PABP and a poly(A) tail [42–45]. In contrast, microRNA-mediated activation requires FXR1, non-canonical translation factors PARN and P97, and shortened poly(A) tails [46, 47]. MicroRNA-mediated activation also requires recruitment to the nucleus, adding new significance to the contentious topic of nuclear translation recently revived following compelling new data by several groups [41, 48, 49]. MicroRNAs can also oscillate between translational repression and stimulation of specific mRNA targets, depending on cell type, cell state, and status of trans-acting factors. Canonical cap-dependent translation initiation is inhibited by many stresses, such as hypoxia, nutrient limitation, proteotoxic stress, or genotoxic stress. Since cancer cells are often exposed to these stresses, they rely on alternative modes of translation initiation for protein synthesis and cell growth [50]. MicroRNA-mediated inhibition of mTOR in the THP1 acute monocytic leukemia cell line activates translation of a subset of mRNAs [47], including activation of CD207 mRNA translation by miR-16 and TNFalpha by miR-369. Given that certain antineoplastic drugs kill cancer cells by downregulating canonical translation, it will be important to understand the contribution of microRNA-mediated translation activation in the development of chemoresistance [51].

MiR-10a is highly overexpressed in AML patients harboring NPM1 mutations and can inhibit the expression of key tumor suppressors such as the p53 regulator MDM4, Rb regulator RB1CC1, p21 regulator TFAP2C and p53, thereby impairing the p53/Rb network opposing cancer growth [52, 53]. In chronic myeloid leukemias (CMLs), miR-10a inhibits USF2 expression to promote cell proliferation [54]. In contrast, miR-10a enhances translation 5′-terminal oligopyrimidine (TOP)-containing mRNAs [55] which primarily encode ribosomal proteins. Manipulation of miR-10a levels in mouse embryonic stem cells (ES cells) indicated that endogenous miR-10 is rate-limiting for global protein synthesis [55]. Overexpression of miR-10a also bypassed mTOR- and PI3K-mediated repression of TOP mRNAs in response to cellular stress due to amino acid starvation [56, 57], implicating miR-10a as a regulator of cellular stress responses [55]. Stimulation of translation by miR-10a requires its binding to a cis-regulatory element downstream of the TOP motif and cooperation with distinct RNA binding proteins (RNPs) [58–60].

In CML blast cells, miR-328 indirectly stimulates CEBPA translation by acting as a sponge for hnRNP E2 RNA binding protein and thereby preventing its binding to CEBPA mRNA [61]. When miR-328 levels drop during blast crisis phase, hnRNP E2 binds to the 5′UTR of CEBPA mRNA, repressing translation and thereby blocking blast cell differentiation. Restoration of miR-328 expression not only rescued blast cell differentiation by binding to hnRNP E2, but also impaired blast cell survival via traditional microRNA-mediated repression of PIM1 mRNA translation, leading to loss of the pro-survival kinase Pim1 [61]. MiR-328 function highlights the ability of microRNAs to simultaneously activate and repress translation of distinct mRNAs within the same cell of a hematologic malignancy.

MicroRNAs can also enhance translation from internal ribosome entry site (IRES) elements, as shown for miR-122 and Hepatitis C virus IRES [36]. Multiple IRES-containing genes such as *c-MYC* and *BCL2* play key roles in hematologic malignancies [51]. IRES-mediated translation of c-MYC is required for BCR-ABL to trigger progression of CML to blast crisis [62], but whether microRNAs play a role in stimulating MYC translation in blood cancers is unknown. MicroRNAs localized in mitochondria, referred to as "mito-miRs," stimulate the translation of mitochondrial RNAs. MiR-1, which promotes AML cell proliferation downstream of EVI1 activation [63], enhances mitochondrial RNA translation to promote cell proliferation during myogenesis [64]. Mito-miRs have the capacity to regulate energy production, ion homeostasis, lipid metabolism, and oxidative stress, suggesting they could play important roles in development, progression, and therapeutic responses of hematologic malignancies [65, 66].

MicroRNAs can also bind to DNA and regulate gene transcription. MicroRNAs can stimulate gene expression by a process referred to as RNA-based gene activation [67, 68]. MiR-205-5p stimulates the expression of *IL-24* and *IL-32* through direct sequence-specific binding to DNA sequences in their promoters leading to enhanced transcription due to increased RNA polymerase II association and accumulation of activating histone marks [69]. Overexpression of IL-24 [70] or IL-32 [71] is cytotoxic for CML cells, suggesting miR-205 may enhance therapeutic responses in CML, consistent with the observation that long non-coding

RNA (lncRNA) SNHG5 promotes imatinib resistance by acting as a sponge for endogenous miR-205-5p [72]. Alternatively, microRNAs can inhibit transcription. MiR-10a inhibits *HOXD4* transcription in breast cancer cells via induction of *de novo* H3K27me3 DNA methylation [73]. MiR-10a might function similarly in hematologic malignancies. In addition, some microRNAs contain transcription factor-binding sequences and therefore indirectly regulate gene transcription by functioning as decoys. Such a mechanism has been demonstrated for endogenous miR-939-mediated inhibition of NF-kB. Both mature and pre-miR-939 can bind to NF-kB, preventing the activation of downstream gene targets [74]. MiR-939 overexpression blocked NF-kB translocation to the nucleus and stimulation of pro-inflammatory cytokines in THP1 monocytic leukemia cells following lipopolysaccharide (LPS) treatment.

3. Regulation of microRNA expression and function

Hematologic malignancies are associated with altered microRNA expression patterns [75, 76]. Common chromosomal deletions lead to the loss of TS-miRs, including the miR-15/miR-16 cluster in chr13q deletion in B-cell CLL [77, 78] and miR-146 in chromosome 5q deletion in AML [79, 80]. The chr14q32 deletion in T cell malignancies deletes a region encompassing 52 microRNAs, including miR-203, which targets ABL and BCR-ABL fusion protein to inhibit tumor cell proliferation [81]. Expression of TS-miRs is also inhibited by additional mechanisms in hematologic malignancies. For example, in the absence of chr14q32 deletion, the expression of miR-15/miR-16, which target BCL2 to inhibit cell survival [82], are frequently reduced by defects in Drosha [83]. Additionally, a pri-miR-16-1 mutation that impairs Drosha processing has been observed in CLL patients and linked to B cell expansion in a mouse model of CLL [84, 85]. Aberrant microRNA expression in CLL is also caused by extensive promoter DNA hyper-methylation and hypo-methylation [86]. Hyper-methylation of the miR-124-1 promoter region is also observed in other leukemias and lymphomas, and epigenetic repression of the miR-124-1 locus leads to activation of the miR-124-1 target, CDK6

[87, 88]. Downregulating of miR-22 in AML is caused by TET1/GFI1/EZH2/SIN3A-mediated epigenetic repression and/or DNA copy-number loss. MYC inhibits TS-miRs, such as miR-26, miR-29, miR-30, and let-7 family members in lymphomas [80, 89–91].

In contrast, oncomiRs are frequently upregulated in hematologic malignancies. Amplification of chromosomal band 13q31-q32 in DLBCL leads to overexpression of the miR-17-92 cluster comprised of miR-17, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a [92]. Enforced expression of the miR-17-92 cluster and c-Myc cooperated to accelerate tumor development in a mouse B-cell lymphoma model [93]. The miR-17-92 cluster is also amplified at the genomic level in mixed lineage leukemia (MLL)-rearranged acute leukemias leading to overexpression of the encoded microRNAs [22, 94–98]. In CML, BCR-ABL and MYC stimulate miR-17-92 cluster expression [97]. MiR-21 is overexpressed in CLL [99], DLBCL [100], HL [101], and AML [102]. The ability of miR-21 to function as an oncomiR was confirmed by the observation that enforced expression of miR-21 in mice drove development of a pre-B cell lymphomas [103]. MYB overexpression in B-cell chronic lymphocytic leukemia (B-CLL) leads to activation of miR-155 expression with accumulation of activating histone marks at the miR-155 host gene (*MIR155HG)* promoter [104]. MiR-155 has emerged as one of the most significantly upregulated microRNAs in AMLs harboring the FLT3-ITD mutation, and plays a pivotal role in uncontrolled blast hyper-proliferation and survival. Upregulation of miR-155 in FLT3-ITD^{+} AML blasts is mediated by p-AKT inhibition of lncRNA splicing factor DDX3X. This results in increased unspliced *MIR155HG* mRNA being exported from the nucleus via NXF1, which allows subsequent miR-155 processing by the cytoplasmic DROSHA/DGCR8 complex [105].

RNA editing by adenosine deaminases acting on RNA (ADARs) has revealed previously unappreciated complexity in microRNA processing and function. ADARs convert adenosines (A) to inosines (I) (ultimately read as guanosines) in both coding and noncoding RNAs, and aberrant ADAR activity has been linked to hematologic malignancies [106]. Editing of double-stranded microRNA precursors can alter pri-miR and/or pre-miR processing and stability, RISC strand selection or mature microRNA targeting [107] (Fig. 4a). Conversely, ADAR-mediated editing

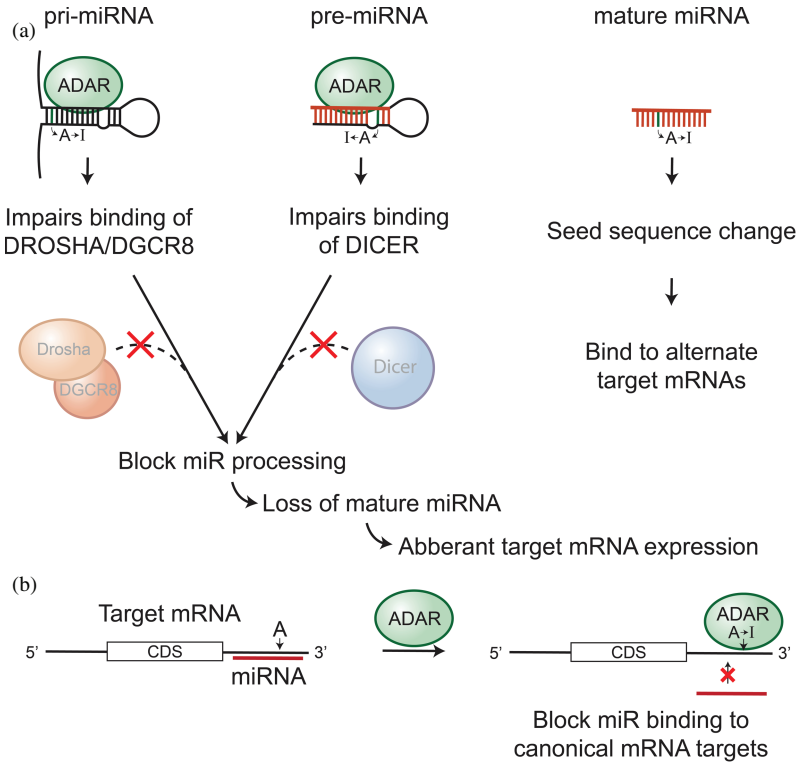

Figure 4. Schematic representation of the ADAR-mediated editing.

ADARs can convert adenosine to inosine in pri-miRNAs, pre-miRNAs, mature microRNAs, or target mRNAs. (a) ADAR-mediated editing during the microRNA biogenesis. ADAR-mediated editing of pri-miRNA or pre-miRNA could interfere with DROSHA/DGCR8 or DICER binding, respectively, inhibiting microRNA processing and reducing the levels of mature microRNA in cells. Reduced microRNA-mediated inhibition would facilitate protein synthesis of microRNA target genes. ADAR editing within mature microRNA seed sequences may alter which mRNAs are targeted. (b) ADAR-mediated editing of target mRNAs. ADAR-mediated editing of the microRNA recognition sites within mRNAs can block microRNA binding to its canonical mRNA targets leading to increased expression. (c) Human miR-155 function is regulated by ADAR-mediated editing by multiple mechanisms. ADAR-mediated editing of pri-miR-155 decreases mature miR-155 levels by blocking miR-155 processing. In addition, ADAR-mediated editing of the 3′UTR of MDM2 mRNA (at the genomic loci 68843722) disrupts the miR-155 recognition sites. In CML LSCs, ADAR activity leads to increased MDM2 mRNA levels and protein. MDM2 inhibits the expression of the tumor-suppressor protein p53 (TP53), causing CML progression.

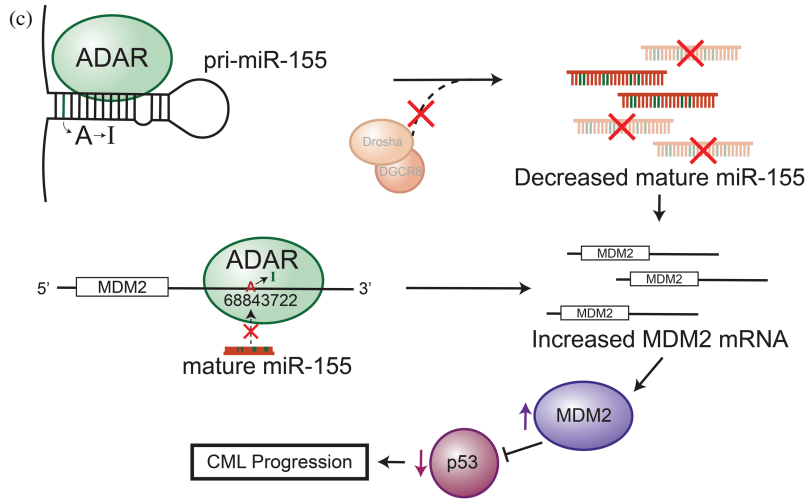

Figure 4. (*Continued*)

of a microRNA recognition sites in a target mRNA can render mRNAs insensitive to microRNA regulation [108] (Fig. 4b). The recognition of pri-miR hairpin structures by Drosha/DGCR8 can be affected by A-to-I editing, as was first demonstrated for pri-miR-142. Editing of pri-miR-142 at the +4 and +5 positions relative to the Drosha cut site at the 5′ end of the mature microRNA by ADAR1 and ADAR2 inhibits its cleavage by Drosha/DGCR8 [109]. Loss-of-function point mutations in miR-142 are observed in AML and previously reported to contribute to leukemic transformation through inappropriate HOXA9/10 expression in myeloid progenitors due to the loss of miR-142-mediated repression of ASH1 [110]. ADAR2-mediated editing of pri-miR-142 in AML significantly inhibits miR-142 expression and provides a novel diagnostic marker for AML [111]. Expression of ADAR1 is activated by inflammatory signaling in multiple cancers, including CML [112]. ADAR1 editing is stimulated by JAK2 [113], and ADAR1 activation by BCR-ABL and JAK2 drives the transformation of CML from its less aggressive chronic phase to the more highly life-threatening and therapy-resistant blast crisis phase [113]. ADAR1 editing of pri-let-7 blocks Drosha/DGCR8 processing leading to

reduced levels of miR-let-7, which in turn inhibits LIN28B expression. Increased levels of LIN28 enhances leukemic stem cell (LSC) self-renewal and transition of CML from chronic phase to blast crisis phase [113]. It is unknown whether pri-let-7 RNA editing occurs in MM or AML, where the LIN28/let-7 axis is critical for MYC-mediated cell proliferation [114, 115]. Similarly, editing of pri-miR-26a inhibits mature miR-26a production causing increased EZH2 expression which indirectly represses CDKN1A to regulate cell cycle transit and self-renewal [116]. ADARB1 RNA editing of pri-miR-15/pri-miR-16 is another mechanism by which miR-15/miR-16 expression is inhibited in CLL patients [83].

In addition to directly regulating microRNA levels or sequence, RNA editing by ADARs can also disrupt the function of microRNAs by editing their corresponding binding sites in cognate mRNA targets. In CML LSCs, the level of the antiapoptotic factor MDM2 is increased via two complementary RNA editing mechanisms [116] (Fig. 4c). First, ADAR1 editing of pri-miR-155 reduces mature miR-155 levels causing elevated levels of MDM2. Second, ADAR1 edits the 3′UTR of MDM2, disrupting the miR-155 recognition sites, further stabilizing MDM2 transcripts, which increases MDM2 protein levels. MDM2 inhibits p53 transcriptional activation contributing to enhanced CML propagation associated with disease progression to blast crisis phase [117].

The activity of microRNAs can also be modulated by repertoire of lncRNAs and circular RNAs (circRNAs) within a cell [118–120]. The interplay between non-coding RNAs can have important consequences in context of hematologic malignancies [121]. Cytoplasmic circular RNA *circ-APC* enhances expression of its host gene *APC* by acting as a sponge for miR-888. Downregulation of circ-APC in DLBCL cells leads to decreased APC expression and enhanced cell proliferation [122]. CircRNA-DLEU2, which is upregulated in AML, acts as a sponge for miR-496 leading to increased AML cell proliferation and survival [123]. LncRNA CRNDE facilitates APL development by functioning as a sponge for miR-181 family members thereby inhibiting cell differentiation and enhancing proliferation [124]. Designing circRNAs or lncRNAs targeting oncomiRs may provide an avenue for future therapeutic development.

4. Exosomal microRNA functions in hematologic malignancies

The bone marrow niche plays a critical role in the maintenance and function of HSPC via cell-cell contacts and secreted factors, including exosomes [125–129]. Hematologic malignancies remodel the niche to support cancer progression and perturbations in the niche can be sufficient to induce cancer development [130–133]. Exosomes are membrane-bound vesicles released by most cell types that play important roles in intercellular communication by transferring their cargo, which includes proteins, lipids, mRNAs, tRNAs, and microRNAs from one cell to another [134–136]. Numerous studies have reported altered exosome content in leukemias, lymphomas, and MMs compared to normal tissue [137–139]. Here we highlight exomiRs and their ability to impact diverse cell types in hematologic malignancies as examples of this rapidly expanding area of investigation.

Two major sources of exosomes affect blood cancer biology: cancer cell-derived exosomes and stromal cell-derived exosomes [140–144]. After their release, exosomes can be taken up by cancer cells, normal HSPCs, or bone marrow stromal cells (BMSCs), including bone marrow mesenchymal stem cells (BM-MSCs), bone marrow endothelial cells (BMECs), and BM-MSC descendent osteolineage cells (OLCs) to regulate cancer development, progression, and response to therapy. ExomiRs can be classified into five broad categories (Fig. 5): 1) oncomiRs released from and taken up by cancer cells to enhance cell growth and survival, 2) oncomiRs released from cancer cells and transferred to BMSCs to co-opt the BM microenvironment for the support of cancer cells, 3) oncomiRs transferred from BMSCs to blood cancer cells to induce cancer cell proliferation and promote drug resistance, 4) TS-miRs exported from cancer cells to allow them to escape microRNA-mediated growth inhibition, and 5) TS-miRs transferred from BM-MSCs to blood cancer cells to oppose cancer cell growth.

Cancer cell-derived exomiR-181a levels are increased in exosomes derived from pediatric acute lymphoblastic leukemia (ALL) patients and ALL cell lines compared to that of healthy individuals and correlated with ALL disease stages [145]. ALL-derived exosomes increase the expression

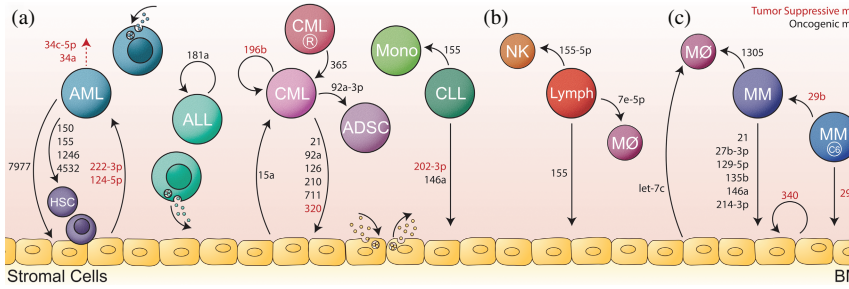

Figure 5. Schematic representation of the intercellular exosomal microRNA transfer in hematologic malignancies.

ExomiRs released from cancer cells or bone marrow stromal cells play a role in hematologic malignancies by modulating recipient target cell gene expression and phenotypes. (a) exomiRs in leukemias, (b) exomiRs in lymphomas, (c) exomiRs in multiple myelomas. Numbers denote the identity of exosomal microRNA. Tumor suppressive exomiRs are in red and oncogenic exomiRs are in black. Black arrows indicate the direction of exomiR movement between cells. The red dotted arrow indicates exomiR export as a mechanism to reduce intracellular levels. Abbreviations: HSC: hematopoietic stem cells; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; CML: chronic myeloid leukemia; CML®: drug-resistant chronic myeloid leukemia; ADSC: adipose-derived mesenchymal stem cells; CLL: chronic lymphoblastic leukemia; Mono: monocytes; Lymph: lymphoma; NK: natural killer cells; MØ: macrophages; MM: multiple myeloma; MMⓒ6: C6-ceramide-treated multiple myeloma; BM: bone marrow.

of proliferative and pro-survival genes while inhibiting the expression of pro-apoptotic genes in ALL cell lines [145], creating a positive regulatory loop to promote ALL growth. Treatment with Vincristine or Prednisone, two ALL standard chemotherapeutic agents, reduced miR-181a, leading to increased cell apoptosis in first time diagnosed leukemia cell lines, but not in relapsed leukemia cell lines with higher miR-181a levels [146], implicating miR-181a in modulating ALL therapeutic responses.

Remodeling of the BM niche to promote malignant development and progression is a common feature of blood cancers and exomiRs have been implicated in the process [138, 139]. Disruption of normal hematopoiesis due to niche alterations contributes to patient morbidity and mortality [6, 147, 148]. Elevated release of multiple oncogenic exomiRs from blood cancer cells can impact BM-MSCs, key components of the niche. AML-derived exomiR-7977 can downregulate STK4 which may further disrupt

the hematopoietic stem cell (HSC) supportive microenvironment by inactivating the Hippo-YAP signaling pathway, triggering increased BM-MSC proliferation due to the loss of contact inhibition [149]. MiR-7977 also reduces BM-MSC expression of multiple ligands important for normal HSPCs including JAG1, ANGPT1, SCF, and PCBP1 (a direct miR-7977 target) [150]. ExomiR-711 derived from CML cells directly downregulates expression of the adhesion molecule CD44 [151]. CLL-derived exosomes have elevated levels of exomiR-146a that downregulates USP16 to stimulate the transition of BM-MSCs into cancer-associated fibroblasts (CAFs), which promote tumor progression in CLL patients [152]. MM-derived exomiR-146a downregulates the Notch signaling inhibitor DAPT to increase the secretion of cytokines and chemokines (e.g., CXCL1, IL-6, IL-8, IP-10, MCP-1, and CCL5) that promote MM cell proliferation and migration [153], generating a positive feedback loop between cancer cells and niche MSCs to promote MM disease progression. MM-derived exosomes carrying miR-21 and miR-146a increased proliferation and CAF transformation of MSCs due to increased level of miR-21 as well as increased the secretion of IL-6 from MSCs due to increased level of miR-146a [154]. MM-derived exomiR-27b-3p and miR-214-3p promoted BM-MSC proliferation and inhibited apoptosis by regulating the FBXW7/MCL1/NOTCH1 and PTEN/AKT/GSK pathways, respectively, to enhance disease progression from monoclonal gammopathy of unknown significance (MGUS) to MM [155].

Blood cancer cell-derived oncogenic exomiRs can also regulate endothelial cell functions. CML-derived exomiR-92a promotes migration and angiogenic properties of human umbilical vein endothelial cells (HUVECs) by downregulating integrin-α5 expression [156]. ExomiR-126 decreases CXCL12 and VCAM1 expression, the direct targets of miR-126, thereby impairing CML cell migration towards and adhesion to HUVECs [157]. *In vivo*, reduced homing and migration to the bone marrow support increases leukemic blast mobilization into the bloodstream [158]. ExomiR-155 derived from Epstein Bar virus positive Burkitt's lymphoma cells enhanced pro-angiogenic capacity of endothelial cells by increasing VEGF-A expression [159]. Exposure of HUVECs to exosomes carrying high level of miR-210 derived from hypoxic CML cells increases angiogenic properties by downregulating the expression of

the anti-angiogenic factor EFNA3 [160], a previously validated miR-210 target [161]. Hypoxia in MM cells is known to contribute to MM progression by promoting stem cell-like properties and increasing drug resistance, as well as metastasis by activating the epithelial-mesenchymal transition (EMT) process [162, 163]. ExomiR-135b present in exosomes derived from hypoxia-resistant MM-cells stimulate HUVEC angiogenic properties *in vitro* by directly downregulating FIH-1 and increased the density of $CD31^+$ neo-vessels *in vivo*, implicating exomiR-135b in MM-associated angiogenesis [164].

Exosome-mediated communication between cancer cells and the niche is bidirectional. BM-MSCs can release exosomes with high levels of exomiR-15a that enhance CML cell growth by increasing BCL2 and inhibiting apoptosis as well as induces imatinib resistance in mice [141]. In addition to regulating cancer cells directly, exomiRs release form BM-MSCs can indirectly promote blood cancer by modulating the activity of other cell types important in disease progression. BM-MSC-derived exosomes carrying high level of let-7c can polarize macrophages toward the anti-inflammatory M2 state [165]. ExomiR-let-7c also increased proliferation, migration, and tube formation of myeloma vascular endothelial cells (MVECs) [165]. These observations suggest that in MM, BM-MSCs can be co-opted to release exosomes that promote disease progression via shifting macrophages toward a pro-oncogenic role and enhancing angiogenesis.

In addition to remodeling the hematopoietic niche, oncogenic exomiRs derived from cancer cells can directly modulate HSPC function. AML-derived exomiR-150 and exomiR-155 inhibit normal hematopoiesis by downregulating the critical hematopoietic transcription factor c-MYB levels in HSPCs [136]. AML-derived exomiR-4532 inhibits hematopoiesis via downregulating LDOC1, increasing JAK2 and STAT3 signaling in HSPCs [136,140], whereas exomiR-1246 derived from AML targets the mTOR subunit Raptor, inhibiting HSC protein synthesis and triggering quiescence [166].

Cancer cell-derived exomiRs can also impact additional blood cell types, such as monocytes, NK cells, or macrophages, to contribute to disease progression. CLL-derived exosomes carrying high level of miR-155 stimulate monocyte myeloid-derived suppressor cell (MDSC) induction

probably by downregulating SOCS1 expression [167]. MiR-155 levels in CLL-derived exosomes was decreased by vitamin D treatment, but whether reduced miR-155 levels contribute to reduced frequency of MDSC induction observed in CLL patients with higher vitamin D levels remains to be determined [167]. Exposure of NK cells obtained from healthy donors to exosomes derived from refractory and relapsed DLBCL patients increased the miR-155-5p [168]. High miR-155-5p levels are inversely related to mRNA levels of its known target SOCS-1 in NK cells of DLBCL patients. DLBCL-derived exomiR-155-5p decreased proliferation and cytotoxicity of NK cells of responsive DLBCL patients, leading to increase the recurrence risk of DLBCL [168]. ExomiR-7e-5p released from low-grade follicular lymphoma (FL) cells is taken up by macrophages, leading to inhibition of apoptosis by downregulating FasL expression and caspase signaling. During the transition to high-grade FL, c-MYC represses the expression of miR-7e-5p in lymphoma cells, leading to reduced levels in exosomes. FL-derived exosomes with low levels of miR-7e-5p induce macrophage apoptosis [169], which correlates with the transformation of FL to aggressive DLBCL and poor clinical outcomes. Exposure of a macrophage cell line to exomiR-1305 from hypoxia-resistant MM cells stimulates macrophage polarization, which is a pro-oncogenic M2-macrophage phenotype [170]. High levels of exomiR-1305 correlates with poor overall survival rates in MM patients [170]. Consistent with increased export of miR-1305, hypoxic MM cells have increased the levels of miR-1305 targets FGF2, IGF1, and MDM2, which further function to promote the MM oncogenic activities [170].

In addition to releasing oncogenic exomiRs, blood cancer cells can utilize exosomes as a mechanism to discard TS-miRs and bypass growth inhibition. The cellular levels of tumor suppressive miR-34c-5p and miR-34a are significantly decreased in AML LSCs compared to normal HSCs and low levels are associated with poor clinical outcomes and poor therapeutic efficacy in AML patients [171, 172]. In contrast, the levels of miR-34c-5p and miR-34a were increased in exosomes released from AML cells or AML patients [171, 172]. Enforced expression of miR-34c-5p in AML cells induced senescence *in vitro*, inhibited AML development *in vivo*, and promoted AML leukemia stem cell eradication in mice [171]. Overexpression of miR-34a in AML cells induced apoptosis by enhancing

the JAK1/STAT2/p53 signaling pathway and/or suppressing the expression of HDAC2, a direct target of miR-34a. Administration of miR-34a mimic extended mouse survival in xenograft mice [172]. Therefore, maintaining low levels of miR-34c-5p and miR-34a in AML LSCs through their removal by exosomes enhances leukemia growth [171, 172]. Tumor suppressive exomiR-202-3p released from CLL can be transferred into BM-MSCs [173]. High level of miR-202-3p expression in CLL-exosome-treated MSC cell line was inversely correlated with the expression of Sufu, a Hedgehog signaling inhibitor, and previously validated target of miR-202-3p [174], which is associated with poor prognosis in CLL patients [174].

Tumor suppressive exomiRs can also be released from the niche to impede malignant development and progression. In AML, the level of miR-222-3p and miR-124-5p were higher in exosomes released from AML BM-MSCs, as compared to normal BM-MSCs [175, 176]. BM-MSC-derived exomiR-222-3p and exomiR-124-5p were taken up by AML cells, where they inhibited cell proliferation, induced apoptosis, and induced cell cycle arrest at the G0/G1 phase by negatively regulating the IRF2/INPP4B signaling pathway or directly targeting SMC4 in AML cells, respectively [175, 176]. The level of miR-340 was decreased in BM-MSCs from older individuals, as compared to that in younger individuals [177]. Exosomes carrying high level of miR-340 isolated from BM-MSCs of young individuals inhibited angiogenesis of hypoxia-resistant MM cells *in vivo*, reduced the senescence of BMSCs, and decreased tube formation of HUVECs by downregulating the expression of c-MET and HGF, a cMET/HGF signaling pathway [177]. It suggests that exomiR-340 derived from young BMSCs functions as a tumor suppressive exomiR to inhibit the angiogenic effect in MM patients.

Certain exomiRs, including miR-92a-3p, miR-320, and miR-129-5p, also impact more distant cell types. CML-derived exosomes carrying high level of oncogenic miR-92a-3p were transferred into adipose-derived mesenchymal stem cells (ADSCs), and then inhibited adipogenic differentiation of ADSCs by downregulating the expression of C/EBPα, which in turn induced significant loss of body weight and body fat in mice [178]. It suggests that exomiR-92a-3p released from CML is partially responsible for cancer cachexia in CML patients. Interestingly, exomiR-320 has

dual functions as a TS-miR in donor CML cells and an oncomiR in recipient BM-MSCs. The miR-320 was sorted into exosomes in CML cells by RNA binding protein HNRNPA1 [179]. Then, exosomes carrying miR-320 was released from CML cells to transfer into BM-MSCs. This resulted in high miR-320 level in CML-exosomes, low cellular miR-320 level in donor CML cells, and high miR-320 level in recipient BM-MSCs. Exosomes isolated from CML xenograft mice or CML cells decreased the colony-forming capacity of CFU-F and osteogenic differentiation of BM-MSCs due to high level of miR-320. In addition, cellular miR-320 inhibited proliferation of CML cells and induced cell cycle arrest at the G1 phase by directly targeting BCR-ABL, whereas CML-derived exomiR-320 suppressed osteogenesis of recipient BM-MSCs by directly targeting β-catenin [179]. This study demonstrates that exosomes released from CML cells could be transferred into BM-MSCs to reconstruct BM niche to promote CML progression. Similarly, high level of MM-derived oncogenic exomiR-129-5p in MSCs inhibited osteogenic differentiation by downregulating SP1 and ALPL, which in turn promoted the disease progression in MM patients, especially from smoldering multiple myeloma (SMM) to MM [180].

Not unexpectedly, exomiRs can also modulate therapeutic responses of hematologic malignancies. Targeted inhibition of BCR-ABL kinase by imatinib revolutionized CML patient treatment, but development of drug resistance leads to disease recurrence [181, 182]. Imatinib-resistant CML cell-derived exosomes contain elevated levels of miR-365, which when taken up by imatinib-sensitive CML cells, downregulates the expression of BAX and inhibits apoptosis. Thus, imatinib-resistant CML cells could confer drug resistant to other leukemic cells via exomiR-365 [183].

The anti-cancer drug curcumin inhibits CML growth *in vitro* and *in vivo* concomitant with increased packaging of miR-21 into exosomes. The resulting reduction in cellular miR-21 levels resulted in increased expression of the tumor suppressor PTEN, a known miR-21 target, leading to reduced AKT phosphorylation and VEGF expression, decreased leukemia colony forming capacity, and impaired cell migration [184]. Exposure of HUVECs to curcumin-treated CML-derived exosomes carrying high level of miR-21 reduced the angiogenic capacity of HUVECs by directly targeting RhoB, which is involved in the regulation of cell growth [185]. Exosomes derived from curcumin-treated CML also contain high

level of anti-angiogenic proteins and low level of pro-angiogenic proteins, including MARCKS (a direct target of miR-21), that further modulate the endothelial cell function [185].

In CML patients treated with curcumin, the expression of TS-miR-196b was decreased in exosomes and increased in CML cells. The mRNA and protein expression of BCR-ABL, a hallmark of CML, was decreased in CML cells in conjunction with increased expression of miR-196b [184]. In response to C6-ceramide, a broad anticancer agent upon conversion to pro-apoptotic sphingolipid metabolites, the level of tumor suppressive miR-29b, was increased in exosomes released from MM cells as compare to that in control cells [186, 187]. Exosomes released from MM cells treated with C6-ceramide decreased cell proliferation and increased apoptosis of MM cells by upregulating cleaved-caspase3/caspase9/PARP [186]. C6-ceramide treated MM cell-derived exosomes carrying high level of miR-29b reduced cell proliferation, and angiogenic properties of HUVECs by downregulating the mRNA and protein expressions of AKT3, PI3K, and VEGFA [187]. These studies suggest that exomiR-29b released from MM cells after C6-ceramide treatment can suppress angiogenesis of BMECs via regulating the AKT signaling pathway to prevent the disease progression in MM patients.

Exosomes have been reported to be promising therapeutic targets in cancers as efficient and effective vehicle system to deliver small molecules including microRNAs to target cells [188, 189]. Understanding how oncogenic or tumor suppressive exomiRs modulate the gene expression and behavior of target cells will provide new insight into develop the therapeutic strategies to treat hematopoietic malignancies. ExomiR profiling analyses are revealing the utility of altered exomiR expression patterns in stratifying hematologic malignances disease and predicting therapeutic responses. Table 1 summarizes exomiRs with potential to be served as biomarkers to inform treatment.

5. Clinical translation of microRNAs in hematologic malignancies

MicroRNAs have been investigated for potential anti-cancer therapeutic approaches in various types of cancers [206]. As of now, there are 1,226

Table 1. Exosomal microRNA biomarkers.

Diseases	miRs	Expression	Ref
AML	miR-26a-5p	up in AML patients	190
	miR-101-3p	up in AML patients	
	miR-23-5p	down in AML patients	
	miR-339-3p	down in AML patients	
	miR-425-5p	down in AML patients	
	miR-10b	up in AML patients with poor prognosis	191
	miR-125b	up in AML patients with poor prognosis	192
	miR-532	up in AML patients with favorable prognosis	193
CML	miR-140-3p	up in CML patients with musculoskeletal pain	194
	miR-215	down in CML patients after IM treatment discontinuation	195
Lymphoma	miR-20a	up in HIV-1-infected cHL patients	196
	miR-21	up in HIV-1-infected cHL patients	
	miR-16	down in HIV-1-infected cHL patients	
	miR-99a-5p	up in chemoresistant DLBCL patients	197
	miR-125b-5p	up in chemoresistant DLBCL patients	
	miR-155	up in refractory/relapsed DLBCL patients	198
	miR-21-5p	up in drug-resistant NKTL patients	199
	miR-320e	up in drug-resistant NKTL patients	
	miR-4451	up in drug-resistant NKTL patients	
	miR-25-3p	up in NKTL patients	
	miR-222-3p	up in NKTL patients	
	miR-451a	down in DLBCL patients	200
Multiple myeloma	let-7b	down in MM patients	201
	miR-18a	down in MM patients	
	let-7c-5p	down in MM patients	202
	let-7d-5p	down in MM patients	
	miR-20a-5p	down in MM patients	
	miR-103-3p	down in MM patients	
	miR-140-3p	down in MM patients	
	miR-185-5p	down in MM patients	
	miR-425-5p	down in MM patients	
	miR-4505	up in MM patients	
	miR-4741	up in MM patients	
	let-7c-5p	down in MM patients	203
	let-7d-5p	down in MM patients	
	miR-140-3p	down in MM patients	

Table 1. (*Continued*)

Diseases	miRs	Expression	Ref
	miR-185-5p	down in MM patients	
	miR-425-5p	down in MM patients	
	miR-10a	up in MM patients	204
	miR-16	down in MM patients	
	miR-15a-5p	down in Bz-resistant MM patients	205
	miR-16-5p	down in Bz-resistant MM patients	
	miR-17-5p	down in Bz-resistant MM patients	
	miR-20a-5p	down in Bz-resistant MM patients	

Table 2. MicroRNA drugs in clinical trials.

MiR drugs	Functions	Diseases	Clinical Trial Number	Phase Status
MRX34	miR-34a mimic	Lymphoma	NCT01829971	Phase 1 (Terminated)
Cobomarsen (MRG-106)	miR-155 inhibitor	Lymphoma (MF-CTCL)	NCT03837457	Phase 2 (Terminated)
			NCT03713320	Phase 2 (Terminated)
			NCT02580552	Phase 1 (Completed)
LNA-i-miR-221	miR-221 inhibitor	Multiple Myeloma (Refractory)	NCT04811898	Phase 1 (Recruiting)

funded microRNA-related clinical research studies (including currently active and already completed/terminated studies) that are registered in the ClinicalTrials.gov database and conducted around the world. Of these studies, 34 microRNA-related clinical studies are involved in hematologic malignancies including 19 studies in leukemias, 10 studies in lymphomas, and five studies in MMs. Among 34 studies, five studies have conducted to treat patients with microRNA-based therapeutic drugs including MRX34 (miR-34a mimic), Cobomarsen (MRG-106; miR-155 inhibitor), and LNA-i-miR-221 (miR-221 inhibitor) (Table 2), whereas, in the rest of studies, drug-treated or non-treated patient samples were collected to identify new microRNA biomarkers or confirm the expression changes of previously reported microRNA biomarkers in response to drug treatments.

MiR-34a is a well-known TS-miR and its expression is decreased in many types of cancers including leukemia and lymphomas [206, 207]. MRX34 is a liposomal miR-34a mimic and was used for the first-in-class phase I clinical trials in patients with lymphoma, MM, primary liver cancer, small-cell carcinoma, melanoma, renal cell carcinoma, and non-small cell lung cancer [206, 208–210]. However, this study was terminated early due to unexpected severe immune-mediated toxicities in patients [206, 207, 211]. Cobomarsen and LNA-i-miR-221 are locked nucleic acid-modified oligonucleotide inhibitors of miR-155 and miR-221, respectively, which are well-studied oncomiRs [212–217]. Cobomarsen was used for three clinical trial studies (including one completed Phase 1 study and two terminated Phase 2 studies) in patients with lymphomas (MF-CTCL) [212, 213]. These two Phase 2 studies were terminated early due to diseases progression of patients according to the clinical trial criteria [213]. LNA-i-miR-221 will be used for the Phase 1 clinical trial study, which is currently recruiting participants, to treat patients with refractory MM.

MicroRNA-based therapeutics is novel promising strategies to treat cancer patients by administrating TS-miRs and/or targeting oncomiRs directly to cancer patients. However, there are concerns to using microRNAs as therapeutic drugs. First concern is their target specificity due to multiple targets of individual microRNAs [218]. Another concern is the lack of efficient microRNA delivery system, which is critical to deliver therapeutic microRNAs into target cells or tissues, due to their instability to RNases [219, 220]. The major therapeutic microRNA delivery systems that are currently developing include virus-mediated delivery (e.g., lentivirus, retrovirus, and adenovirus), non-viral delivery (e.g., liposomes and polymeric vector), and exosome/extracellular vesicle-mediated delivery systems [219, 220]. Therefore, further studies will be necessary to overcome these issues to use microRNA-based therapeutic drugs safely and effectively to treat cancer patients.

6. Conclusions

MicroRNAs are an evolutionarily conserved mechanism, regulating cell transcriptomes and proteomes. MicroRNAs play key roles in the

development, progression, and therapeutic responsiveness of blood cancers by regulating fundamental aspects of cell biology, including cell proliferation, survival, signaling, stress response, and metabolism. The presence of isomiRs and RNA editing greatly expand the diversity and potential targets of microRNAs, facilitating more nuanced control of gene expression. Understanding microRNA function therefore requires knowledge of isomiR expression patterns and levels, microRNA interactions with the cellular repertoire of RNA binding proteins and non-coding RNAs, as well as microRNA localization within a cell and packaging into exosomes. MicroRNAs can also influence phenoytpes and function of multiple cell types to shape blood cancer development, progression, and therapeutic responses. Combined with the recognition that microRNAs can regulate gene expression via interacting with DNA, RNA or protein elucidating the mechanisms by which microRNAs regulate blood cancers will be a key challenge. The application of CRISPR technologies to introduce cell-specific mutations into microRNAs or predicted microRNA target sites will provide powerful tools to dissect functional roles of microRNAs and identify relevant targets in context of physiologic and pathophysiologic expression levels.

List of abbreviations

Abbreviation	Definition
ABL	Abelson proto-oncogene
ADAR	Adenosine deaminases acting on RNA
ADARB1	Adenosine deaminase RNA-specific B1
ADSC	Adipose-derived mesenchymal stem cell
AGO	Argonaute
AKT (p-AKT)	Phospho-ATK serine/threonine kinase 3
AKT3	ATK Serine/Threonine kinase 3
ALCL	Anaplastic large cell lymphoma
ALL	Acute lymphoblastic leukemia
ALPL	Alkaline phosphatase, Liver
AML	Acute myeloid leukemia
ANGPT1	Angiopoietin 1
APL	Acute promyelocytic leukemia

ASH1 (ASH1L)	ASH1-like histone lysine methyltransferase
BAX	BCL2-associated X protein
BCL2	B-cell lymphoma 2
B-CLL	B-lymphocytic leukemia
BCR-ABL	Breakpoint cluster region gene-Abelson proto-oncogene
BL	Burkitt lymphoma
BM	Bone marrow
BMEC	Bone marrow endothelial cell
BM-MSC	Bone marrow mesenchymal stem cell
BMSC	Bone marrow stromal cell
Bz	Bortezomib
C. elegans	Caenorhabditis elegans
CAF	Cancer-associated fibroblast
CCL5	CC motif chemokine ligand 5
CDK6	Cyclin dependent kinase 6
CDKN1A	Cyclin-dependent kinase inhibitor 1A
CEBPA (C/EBPa)	CCAAT/enhancer-binding protein alpha
CFU-F	Colony-forming unit-Fibroblast
circRNA	Circular RNA
CLL	Chronic lymphocytic leukemia
CLP	Common lymphoid progenitor
CML	Chronic myeloid leukemia
CMP	Common myeloid progenitor
CREB	cAMP response element-binding protein
CRISPR	Clustered regularly interspaced short palindromic repeats
CRTC	CREB-regulated transcription coactivator
CXCL1	CXC motif chemokine ligand 1
CXCL12	C-X-C motif chemokine ligand 12
DAPT	Inhibitor of the Notch pathway
DDX3X	DEAD-box helicase 3, X-linked
DGCR8	Microprocessor complex subunit DiGeorge syndrome critical region gene 8
DLBCL	Diffuse large B-cell lymphoma
EFNA3	Ephrin A3

EMT	Epithelial-mesenchymal transition
ES cell	Embryonic stem cell
EVI1	Ecotropic viral integration site 1
ExomiR	Exosomal microRNA
EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit
FasL	Fas ligand
FBXW7	F-box and WD40 domain protein 7
FGF2	Fibroblast growth factor 2
FIH-1	Factor-inhibiting HIF-1
FL	Follicular lymphoma
FLT3	FMS-like tyrosine kinase 3
FLT3-ITD	FLT3 internal tandem duplications
FXR1	FMR1 autosomal homolog 1
GFI1	Growth factor-independent 1
GMP	Granulocyte-monocyte progenitor
GSK	Glycogen synthase kinase
GW182	Glycine-tryptophan protein of 182 kDa
H3K27me3	Trimethylation of lysine 27 of histone H3
HDAC2	Histone deacetylase 2
HGF	Hepatocyte growth factor
HL	Hodgkin lymphoma
hnRNP E2	Heterogeneous nuclear ribonucleoprotein E2
HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1
HOXA9/10	Homeobox A9/A10
HOXD4	Homeobox D4
HSC	Hematopoietic stem cell
HSPC	Hematopoietic stem-progenitor cell
HUVEC	Human umbilical vein endothelial cell
IGF1	Insulin like growth factor 1
IL-24	Interleukin 24
IL-32	Interleukin 32
IL-6	Interleukin 6
IL-8	Interleukin 8
INPP4B	Inositol polyphosphate-4-phosphatase, Type II, 105-kDa

IP-10	Interferon gamma-induced protein 10
IRES	Internal ribosome entry site
IRF2	Interferon regulatory factor 2
JAG1	Jagged 1
JAK1	Janus kinase 1
JAK2	Janus kinase 2
LDOC1	Leucine zipper protein downregulated in cancer 1
LIG3	DNA ligase 3
LIN28B	Lin28 homolog B
LMPP	Lymphoid-primed multipotent progenitor
lncRNA	Long non-coding RNA
LncRNA CRNDE	Colorectal neoplasia differentially expressed
LPS	Lipopolysaccharide
LSC	Leukemic stem cell
LT-HSC	Long-term hematopoietic stem cell
MARCKS	Myristoylated alanine-rich protein kinase C Substrate
MCL1	Myeloid cell leukemia 1
MCP-1	Monocyte chemoattractant protein-1
MDM2	MDM2 protooncogene
MDM4	MDM4 regulator of p53
MDSC	Monocyte myeloid-derived suppressor cell
MEP	Megakaryocyte-erythrocyte progenitor
MET (c-MET)	MET protooncogene
MF-CTCL	Mycosis fungoides-type cutaneous T-cell lymphoma
MGUS	Monoclonal gammopathy of unknown significance
miR	MicroRNA
miRNP	Ribonucleoproteins containing numerous microRNAs
mito-miR	Mitochondrial miRNA
MLL	Mixed lineage leukemia
MM	Multiple myeloma
MPP	Multipotent progenitor
MSC	Mesenchymal stem cell
mTOR	Mechanistic/mammalian target of rapamycin
MVEC	Myeloma vascular endothelial cell

MYB (c-MYB)	MYB protooncogene
MYC (c-MYC)	MYC protooncogene
MYCBP	MYC binding protein
NF-kB	Nuclear factor kappa-B
NHL	Non-Hodgkin lymphoma
NK cell	Natural killer cell
NKTL	Natural killer T cell lymphoma
NOTCH1	NOTCH receptor 1
NPM1	Nucleophosmin 1
NXF1	Nuclear RNA export factor 1
OLC	Osteolineage cell
OncomiR	Oncogenic microRNA
ORF	Open reading frame
PABP	Poly(A)-binding protein
PACT	PKR activating protein
PARN	Poly(A)-specific ribonuclease
PARP	Poly(ADP-ribose) polymerases
PCBP1	Poly(rC)-binding protein 1
PI3K	Phosphoinositide 3-kinase
PIM1	Pim1 oncogene
PMA	Phorbol 12-myristate 13-acetate
Pre-miR	Precursor miRNA transcript
Pri-miR	Primary miRNA transcript
PTEN	Phosphatase and tensin homolog
Ran-GTP	Ras-related nuclear protein GTPase
Rb	Retinoblastoma protein
RB1CC1	RB1-inducible coiled-coil 1
RBC	Red blood cell
RhoB	RAS homolog gene family, member B
RISC	RNA-induced silencing complex
RNP	RNA binding protein
SCF	Stem cell factor
SIN3A	SIN3 transcription regulator family member A
SMC4	Structural maintenance of chromosomes 4
SMM	smoldering multiple myeloma
SNHG5	Small nucleolar RNA host gene 5

SOCS1	Suppressor of cytokine signaling 1
SP1	Transcription factor SP1
STAT2	Signal transducer and activator of transcription 2
STAT3	Signal transducer and activator of transcription 3
ST-HSC	Short-term hematopoietic stem cell
STK4	Serine/Threonine protein kinase 4
Sufu	Sufu negative regulator of Hedgehog signaling
TCGA	The cancer genome atlas
TET1	TET methylcytosine dioxygenase 1
TFAP2C	Transcription factor AP2 gamma
TNFalpha	Tumor necrosis factor alpha
TOP	5′-terminal oligopyrimidine
TRBP	TAR RNA-binding protein
TS-miR	Tumor suppressor miR
USF2	Upstream transcription factor 2
USP16	Ubiquitin-specific protease 16
UTR	Untranslated region
VCAM1	Vascular cell adhesion molecule 1
VEGF-A (VEGFA)	Vascular endothelial growth factor A
YAP	Yes1-associated protein

References

1. Liggett LA and Sankaran VG. (2020) Unraveling hematopoiesis through the lens of genomics. *Cell,* **182**, 1384–1400.
2. Jaiswal S. (2020) Clonal hematopoiesis and nonhematologic disorders. *Blood,* **136**, 1606–1614.
3. Jaiswal S and Ebert BL. (2019) Clonal hematopoiesis in human aging and disease. *Science,* **366**.
4. Bao EL, Cheng AN and Sankaran VG. (2019) The genetics of human hematopoiesis and its disruption in disease. *EMBO Molecular Medicine,* **11**, e10316.
5. Taylor J, Xiao W and Abdel-Wahab O. (2017) Diagnosis and classification of hematologic malignancies on the basis of genetics. *Blood,* **130**, 410–423.
6. Mendez-Ferrer S, *et al.* (2020) Bone marrow niches in haematological malignancies. *Nature Reviews Cancer,* **20**, 285–298.
7. Han Z, Rosen ST and Querfeld C. (2020) Targeting microRNA in hematologic malignancies. *Current Opinion in Oncology,* **32**, 535–544.

8. Davis AS, Viera AJ and Mead MD. (2014) Leukemia: An overview for primary care. *American Family Physician,* **89**, 731–738.
9. Kantarjian H, *et al.* (2021) Acute myeloid leukemia: Current progress and future directions. *Blood Cancer Journal* **11**, 41.
10. Inaba H, Greaves M and Mullighan CG. (2013) Acute lymphoblastic leukaemia. *Lancet,* **381**, 1943–1955.
11. Jabbour E and Kantarjian H. (2018) Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. *American Journal of Hematology,* **93**, 442–459.
12. Hallek M, Shanafelt TD and Eichhorst B. (2018) Chronic lymphocytic leukaemia. *Lancet,* **391**, 1524–1537.
13. Ansell SM and Lin Y. (2020) Immunotherapy of lymphomas. *Journal of Clinical Investigation,* **130**, 1576–1585.
14. Mugnaini EN and Ghosh N. (2016) Lymphoma. *Primary Care,* **43**, 661–675.
15. Armitage JO, Gascoyne RD, Lunning MA and Cavalli F. (2017) Non-Hodgkin lymphoma. *Lancet,* **390**, 298–310.
16. Bazarbachi AH, Al Hamed R, Malard F, Harousseau JL and Mohty M. (2019) Relapsed refractory multiple myeloma: A comprehensive overview. *Leukemia,* **33**, 2343–2357.
17. Castaneda O and Baz R. (2019) Multiple myeloma genomics — A concise review. *Acta Medica Academica,* **48**, 57–67.
18. Palumbo A and Anderson K. (2011) Multiple myeloma. *New England Journal of Medicine,* **364**, 1046–1060.
19. Kozomara A, Birgaoanu M and Griffiths-Jones S. (2019) miRBase: From microRNA sequences to function. *Nucleic Acids Research,* **47**, D155–D162.
20. Costinean S, *et al.* (2006) Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proceedings of the National Academy of Sciences of the United States,* **103**, 7024–7029.
21. Kim M, Civin CI and Kingsbury TJ. (2019) MicroRNAs as regulators and effectors of hematopoietic transcription factors. *Wiley Interdisciplinary Reviews: RNA* **10**, e1537.
22. Calin GA, *et al.* (2004) MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. *Proceedings of the National Academy of Sciences of the United States,* **101**, 11755–11760.
23. Seckinger A, *et al.* (2015) miRNAs in multiple myeloma — A survival relevant complex regulator of gene expression. *Oncotarget,* **6**, 39165–39183.
24. Moore MJ, *et al.* (2015) miRNA-target chimeras reveal miRNA 3′-end pairing as a major determinant of Argonaute target specificity. *Nature Communications,* **6**, 8864.
25. Chi SW, Hannon GJ and Darnell RB. (2012) An alternative mode of microRNA target recognition. *Nature Structural & Molecular Biology,* **19**, 321–327.
26. Ha M and Kim VN. (2014) Regulation of microRNA biogenesis. *Nature Reviews Molecular Cell Biology,* **15**, 509–524.

27. Guo L and Chen F. (2014) A challenge for miRNA: Multiple isomiRs in miRNAomics. *Gene* **544**, 1–7.
28. Gebert LFR and MacRae IJ. (2019) Regulation of microRNA function in animals. *Nature Reviews Molecular Cell Biology,* **20**, 21–37.
29. Loher P, Londin ER and Rigoutsos I. (2014) IsomiR expression profiles in human lymphoblastoid cell lines exhibit population and gender dependencies. *Oncotarget* **5**, 8790–8802.
30. Molica S. (2006) Sex differences in incidence and outcome of chronic lymphocytic leukemia patients. *Leukemia & Lymphoma,* **47**, 1477–1480.
31. Muiwo P, Pandey P, Ahmad HM, Ramachandran SS and Bhattacharya A. (2018) IsomiR processing during differentiation of myelogenous leukemic cell line K562 by phorbol ester PMA. *Gene* **641**, 172–179.
32. Jiang X, *et al.* (2016) miR-22 has a potent anti-tumour role with therapeutic potential in acute myeloid leukaemia. *Nature Communications,* **7**, 11452.
33. Caracciolo D, *et al.* (2019) miR-22 suppresses DNA ligase III addiction in multiple myeloma. *Leukemia* **33**, 487–498.
34. Telonis AG, *et al.* (2017) Knowledge about the presence or absence of miRNA isoforms (isomiRs) can successfully discriminate amongst 32 TCGA cancer types. *Nucleic Acids Research,* **45**, 2973–2985.
35. Moretti F, Thermann R and Hentze MW. (2010) Mechanism of translational regulation by miR-2 from sites in the 5′ untranslated region or the open reading frame. *RNA* **16**, 2493–2502.
36. Roberts AP, Lewis AP and Jopling CL. (2011) miR-122 activates hepatitis C virus translation by a specialized mechanism requiring particular RNA components. *Nucleic Acids Research,* **39**, 7716–7729.
37. Zhou H and Rigoutsos I. (2014) MiR-103a-3p targets the 5′ UTR of GPRC5A in pancreatic cells. *RNA,* **20**, 1431–1439.
38. Tay Y, Zhang J, Thomson AM, Lim B and Rigoutsos I. (2008) MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature,* **455**, 1124–1128.
39. Buchan JR and Parker R. (2007) Molecular biology. The two faces of miRNA. *Science,* **318**, 1877–1878.
40. Vasudevan S, Tong Y and Steitz JA. (2007) Switching from repression to activation: MicroRNAs can up-regulate translation. *Science,* **318**, 1931–1934.
41. Truesdell SS, *et al.* (2012) MicroRNA-mediated mRNA translation activation in quiescent cells and oocytes involves recruitment of a nuclear microRNP. *Scientific Reports* **2**, 842.
42. Chekulaeva M and Filipowicz W. (2009) Mechanisms of miRNA-mediated post-transcriptional regulation in animal cells. *Current Opinion in Cell Biology,* **21**, 452–460.

43. Fabian MR, Sundermeier TR and Sonenberg N. (2010) Understanding how miRNAs post-transcriptionally regulate gene expression. *Progress in Molecular and Subcellular Biology* **50**, 1–20.
44. Jonas S and Izaurralde E. (2015) Towards a molecular understanding of microRNA-mediated gene silencing. *Nature Reviews Genetics* **16**, 421–433.
45. Rana TM. (2007) Illuminating the silence: Understanding the structure and function of small RNAs. *Nature Reviews Molecular Cell Biology,* **8**, 23–36.
46. Bukhari SI and Vasudevan S. (2017) FXR1a-associated microRNP: A driver of specialized non-canonical translation in quiescent conditions. *RNA Biology,* **14**, 137–145.
47. Bukhari SIA, *et al.* (2016) A specialized mechanism of translation mediated by FXR1a-associated microRNP in cellular quiescence. *Molecular Cell,* **61**, 760–773.
48. Yewdell JW and David A. (2013) Nuclear translation for immunosurveillance. *Proceedings of the National Academy of Sciences of the United States,* **110**, 17612–17613.
49. David A, *et al.* (2012) Nuclear translation visualized by ribosome-bound nascent chain puromycylation. *Journal of Cell Biology,* **197**, 45–57.
50. Sriram A, Bohlen J and Teleman AA. (2018) Translation acrobatics: How cancer cells exploit alternate modes of translational initiation. *EMBO Reports,* **19**.
51. Horvilleur E, *et al.* (2015) Cap-independent translation in hematological malignancies. *Frontiers in Oncology,* **5**, 293.
52. Vu TT, *et al.* (2020) miR-10a as a therapeutic target and predictive biomarker for MDM2 inhibition in acute myeloid leukemia. *Leukemia*.
53. Ovcharenko D, *et al.* (2011) miR-10a overexpression is associated with NPM1 mutations and MDM4 downregulation in intermediate-risk acute myeloid leukemia. *Experimental Hematology,* **39**, 1030–1042 e1037.
54. Agirre X, *et al.* (2008) Down-regulation of hsa-miR-10a in chronic myeloid leukemia CD34+ cells increases USF2-mediated cell growth. *Molecular Cancer Research,* **6**, 1830–1840.
55. Orom UA, Nielsen FC and Lund AH. (2008) MicroRNA-10a binds the 5′UTR of ribosomal protein mRNAs and enhances their translation. *Molecular Cell,* **30**, 460–471.
56. Meyuhas O. (2000) Synthesis of the translational apparatus is regulated at the translational level. *European Journal of Biochemistry,* **267**, 6321–6330.
57. Hamilton TL, Stoneley M, Spriggs KA and Bushell M. (2006) TOPs and their regulation. *Biochemical Society Transactions,* **34**, 12–16.
58. Zhu J, Hayakawa A, Kakegawa T and Kaspar RL. (2001) Binding of the La autoantigen to the 5′ untranslated region of a chimeric human translation elongation factor 1A reporter mRNA inhibits translation in vitro. *Biochimica et Biophysica Acta,* **1521**, 19–29.

59. Pellizzoni L, Lotti F, Rutjes SA and Pierandrei-Amaldi P. (1998) Involvement of the Xenopus laevis Ro60 autoantigen in the alternative interaction of La and CNBP proteins with the 5′UTR of L4 ribosomal protein mRNA. *Journal of Molecular Biology,* **281**, 593–608.
60. Crosio C, Boyl PP, Loreni F, Pierandrei-Amaldi P and Amaldi F. (2000) La protein has a positive effect on the translation of TOP mRNAs in vivo. *Nucleic Acids Research,* **28**, 2927–2934.
61. Eiring AM, *et al.* (2010) miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. *Cell,* **140**, 652–665.
62. Notari M *et al.* (2006) A MAPK/HNRPK pathway controls BCR/ABL oncogenic potential by regulating MYC mRNA translation. *Blood,* **107**, 2507–2516.
63. Gomez-Benito M, *et al.* (2010) EVI1 controls proliferation in acute myeloid leukaemia through modulation of miR-1-2. *British Journal of Cancer,* **103**, 1292–1296.
64. Zhang X *et al.* (2014) MicroRNA directly enhances mitochondrial translation during muscle differentiation. *Cell,* **158**, 607–619.
65. Macgregor-Das AM and Das S. (2018) A microRNA's journey to the center of the mitochondria. *American Journal of Physiology-Heart and Circulatory Physiology,* **315**, H206–H215.
66. Das S, Vasanthi HR and Parjapath R. (2017) MitomiRs keep the heart beating. *Advances in Experimental Medicine and Biology,* **982**, 431–450.
67. Vaschetto LM. (2018) miRNA activation is an endogenous gene expression pathway. *RNA Biology,* **15**, 826–828.
68. Liu H, *et al.* (2018) Nuclear functions of mammalian MicroRNAs in gene regulation, immunity and cancer. *Molecular Cancer,* **17**, 64.
69. Majid S, *et al.* (2010) MicroRNA-205-directed transcriptional activation of tumor suppressor genes in prostate cancer. *Cancer,* **116**, 5637–5649.
70. Wei X, *et al.* (2015) Targeting eradication of chronic myeloid leukemia using chimeric oncolytic adenovirus to drive IL-24 expression. *International Journal of Clinical and Experimental Pathology,* **8**, 3775–3784.
71. Cheon S, *et al.* (2011) Overexpression of IL-32alpha increases natural killer cell-mediated killing through up-regulation of Fas and UL16-binding protein 2 (ULBP2) expression in human chronic myeloid leukemia cells. *Journal of Biological Chemistry,* **286**, 12049–12055.
72. He B, Bai Y, Kang W, Zhang X and Jiang X. (2017) LncRNA SNHG5 regulates imatinib resistance in chronic myeloid leukemia via acting as a CeRNA against MiR-205-5p. *American Journal of Cancer Research,* **7**, 1704–1713.
73. Tan Y, *et al.* (2009) Transcriptional inhibiton of Hoxd4 expression by miRNA-10a in human breast cancer cells. *BMC Molecular Biology,* **10**, 12.
74. Cui C, Yu J, Huang S, Zhu H and Huang Z. (2014) Transcriptional regulation of gene expression by microRNAs as endogenous decoys of transcription factors. *Cellular Physiology and Biochemistry,* **33**, 1698–1714.

75. Ciccone, M and Calin, GA. (2015) MicroRNAs in myeloid hematological malignancies. *Current Genomics,* **16**, 336–348.
76. Starczynowski DT, *et al.* (2011) Genome-wide identification of human microRNAs located in leukemia-associated genomic alterations. *Blood,* **117**, 595–607.
77. Calin GA, *et al.* (2002) Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences of the United States,* **99**, 15524–15529.
78. Pekarsky, Y and Croce, CM. (2015) Role of miR-15/16 in CLL. *Cell Death & Differentiation,* **22**, 6–11.
79. Zhao JL and Starczynowski DT. (2014) Role of microRNA-146a in normal and malignant hematopoietic stem cell function. *Frontiers in Genetics,* **5**, 219.
80. Ali Syeda Z, Langden SSS, Munkhzul C, Lee M and Song SJ. (2020) Regulatory mechanism of microRNA expression in cancer. *International Journal of Molecular Sciences,* **21**.
81. Bueno MJ, *et al.* (2008) Genetic and epigenetic silencing of microRNA-203 enhances ABL1 and BCR-ABL1 oncogene expression. *Cancer Cell,* **13**, 496–506.
82. Cimmino A, *et al.* (2005) miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proceedings of the National Academy of Sciences of the United States,* **102**, 13944–13949.
83. Allegra D, *et al.* (2014) Defective DROSHA processing contributes to downregulation of MiR-15/-16 in chronic lymphocytic leukemia. *Leukemia,* **28**, 98–107.
84. Raveche ES, *et al.* (2007) Abnormal microRNA-16 locus with synteny to human 13q14 linked to CLL in NZB mice. *Blood,* **109**, 5079–5086.
85. Kasar S, *et al.* (2016) Alterations in the mir-15a/16-1 loci impairs its processing and augments B-1 expansion in de novo mouse model of chronic lymphocytic leukemia (CLL). *PLoS One,* **11**, e0149331.
86. Baer C, *et al.* (2012) Extensive promoter DNA hypermethylation and hypomethylation is associated with aberrant microRNA expression in chronic lymphocytic leukemia. *Cancer Research,* **72**, 3775–3785.
87. Liang YJ, *et al.* (2013) MiR-124 targets Slug to regulate epithelial-mesenchymal transition and metastasis of breast cancer. *Carcinogenesis,* **34**, 713–722.
88. Lujambio A, *et al.* (2007) Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. *Cancer Research,* **67**, 1424–1429.
89. Chang TC, *et al.* (2008) Widespread microRNA repression by Myc contributes to tumorigenesis. *Nature Genetics,* **40**, 43–50.
90. Molenaar JJ, *et al.* (2012) LIN28B induces neuroblastoma and enhances MYCN levels via let-7 suppression. *Nature Genetics,* **44**, 1199–1206.
91. Zhang X, *et al.* (2012) Coordinated silencing of MYC-mediated miR-29 by HDAC3 and EZH2 as a therapeutic target of histone modification in aggressive B-Cell lymphomas. *Cancer Cell,* **22**, 506–523.

92. Ota A, *et al.* (2004) Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. *Cancer Research,* **64**, 3087–3095.
93. He L, *et al.* (2005) A microRNA polycistron as a potential human oncogene. *Nature,* **435**, 828–833.
94. Mi S, *et al.* (2010) Aberrant overexpression and function of the miR-17-92 cluster in MLL-rearranged acute leukemia. *Proceedings of the National Academy of Sciences of the United States,* **107**, 3710–3715.
95. Hayashita Y, *et al.* (2005) A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Research,* **65**, 9628–9632.
96. Lu Y, Thomson JM, Wong HY, Hammond SM and Hogan BL. (2007) Transgenic over-expression of the microRNA miR-17-92 cluster promotes proliferation and inhibits differentiation of lung epithelial progenitor cells. *Developmental Biology,* **310**, 442–453.
97. Venturini L, *et al.* (2007) Expression of the miR-17-92 polycistron in chronic myeloid leukemia (CML) CD34+ cells. *Blood* **109**, 4399–4405.
98. Volinia S, *et al.* (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. *Proceedings of the National Academy of Sciences of the United States,* **103**, 2257–2261.
99. Fulci V, *et al.* (2007) Quantitative technologies establish a novel microRNA profile of chronic lymphocytic leukemia. *Blood,* **109**, 4944–4951.
100. Lawrie CH, *et al.* (2007) MicroRNA expression distinguishes between germinal center B cell-like and activated B cell-like subtypes of diffuse large B cell lymphoma. *International Journal of Cancer,* **121**, 1156–1161.
101. Navarro A, *et al.* (2008) MicroRNA expression profiling in classic Hodgkin lymphoma. *Blood,* **111**, 2825–2832.
102. Jongen-Lavrencic M, Sun SM, Dijkstra MK, Valk PJ and Lowenberg B. (2008) MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. *Blood,* **111**, 5078–5085.
103. Medina PP, Nolde M and Slack FJ. (2010) OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. *Nature,* **467**, 86–90.
104. Vargova K, *et al.* (2011) MYB transcriptionally regulates the miR-155 host gene in chronic lymphocytic leukemia. *Blood,* **117**, 3816–3825.
105. Nguyen LXT, *et al.* (2021) Cytoplasmic DROSHA and non-canonical mechanisms of MiR-155 biogenesis in FLT3-ITD acute myeloid leukemia. *Leukemia*.
106. Teoh PJ, Koh MY and Chng WJ. (2021) ADARs, RNA editing and more in hematological malignancies. *Leukemia,* **35**, 346–359.
107. Kawahara Y, *et al.* (2007) Redirection of silencing targets by adenosine-to-inosine editing of miRNAs. *Science,* **315**, 1137–1140.
108. Kume H, Hino K, Galipon J and Ui-Tei K. (2014) A-to-I editing in the miRNA seed region regulates target mRNA selection and silencing efficiency. *Nucleic Acids Research,* **42**, 10050–10060.

109. Yang W, *et al.* (2006) Modulation of microRNA processing and expression through RNA editing by ADAR deaminases. *Nature Structural & Molecular Biology,* **13**, 13–21.
110. Trissal, M. C. *et al.* (2018) MIR142 loss-of-function mutations derepress ASH1L to increase HOXA gene expression and promote leukemogenesis. *Cancer Research,* **78**, 3510–3521.
111. Zhang Y, Qian H, Xu J and Gao W. (2019) ADAR, the carcinogenesis mechanisms of ADAR and related clinical applications. *Annals of Translational Medicine,* **7**, 686.
112. Jiang Q, *et al.* (2013) ADAR1 promotes malignant progenitor reprogramming in chronic myeloid leukemia. *Proceedings of the National Academy of Sciences of the United States,* **110**, 1041–1046.
113. Zipeto MA, *et al.* (2016) ADAR1 activation drives leukemia stem cell self-renewal by impairing let-7 biogenesis. *Cell Stem Cell,* **19**, 177–191.
114. Manier S, *et al.* (2017) The LIN28B/let-7 axis is a novel therapeutic pathway in multiple myeloma. *Leukemia,* **31**, 853–860.
115. Roos, MM, Li M, Amara P and Chute JP. (2018) Pharmacologic targeting of LIN28/Let-7 in acute myeloid leukemia. *Blood,* **132**, 4072.
116. Jiang Q, *et al.* (2019) Hyper-editing of cell-cycle regulatory and tumor suppressor RNA promotes malignant progenitor propagation. *Cancer Cell,* **35**, 81–94 e87.
117. Trotta R, *et al.* (2003) BCR/ABL activates mdm2 mRNA translation via the La antigen. *Cancer Cell,* **3**, 145–160.
118. Rajappa A, Banerjee S, Sharma V and Khandelia P. (2020) Circular RNAs: Emerging role in cancer diagnostics and therapeutics. *Frontiers in Molecular Biosciences,* **7**, 577938.
119. Paralkar VR and Weiss MJ. (2013) Long noncoding RNAs in biology and hematopoiesis. *Blood,* **121**, 4842–4846.
120. Dahariya S, *et al.* (2019) Long non-coding RNA: Classification, biogenesis and functions in blood cells. *Molecular Immunology,* **112**, 82–92.
121. Dostalova MM, Krejcik Z, Szikszai K and Kundrat D. (2020) Circular RNAs in hematopoiesis with a focus on acute myeloid leukemia and myelodysplastic syndrome. *International Journal of Molecular Sciences,* **21**.
122. Hu Y, *et al.* (2019) A circular RNA from APC inhibits the proliferation of diffuse large B-cell lymphoma by inactivating Wnt/beta-catenin signaling via interacting with TET1 and miR-888. *Aging (Albany NY),* **11**, 8068–8084.
123. Wu DM, *et al.* (2018) Role of circular RNA DLEU2 in human acute myeloid leukemia. *Molecular Cell Biology,* **38**.
124. Ma X, *et al.* (2020) Oncogenic role of lncRNA CRNDE in acute promyelocytic leukemia and NPM1-mutant acute myeloid leukemia. *Cell Death Discovery,* **6**, 121.
125. Scadden DT. (2014) Nice neighborhood: Emerging concepts of the stem cell niche. *Cell,* **157**, 41–50.
126. Morrison SJ and Scadden DT. (2014) The bone marrow niche for haematopoietic stem cells. *Nature,* **505**, 327–334.

127. Baryawno N, *et al.* (2019) A cellular taxonomy of the bone marrow stroma in homeostasis and leukemia. *Cell,* **177**, 1915–1932 e1916.
128. Niazi V, *et al.* (2020) Communication between stromal and hematopoietic stem cell by exosomes in normal and malignant bone marrow niche. *Biomedicine & Pharmacotherapy,* **132**, 110854.
129. Butler JT, Abdelhamed S and Kurre P. (2018) Extracellular vesicles in the hematopoietic microenvironment. *Haematologica,* **103**, 382–394.
130. Arranz L, *et al.* (2014) Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms. *Nature,* **512**, 78–81.
131. Dong L, *et al.* (2016) Leukaemogenic effects of Ptpn11 activating mutations in the stem cell microenvironment. *Nature,* **539**, 304–308.
132. Kode A, *et al.* (2014) Leukaemogenesis induced by an activating beta-catenin mutation in osteoblasts. *Nature,* **506**, 240–244.
133. Raaijmakers MH, *et al.* (2010) Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature,* **464**, 852–857.
134. El-Saghir J, Nassar F, Tawil N and El-Sabban, M. (2016) ATL-derived exosomes modulate mesenchymal stem cells: Potential role in leukemia progression. *Retrovirology,* **13**, 73.
135. Moloudizargari M, Hekmatirad S, Mofarahe ZS and Asghari MH. (2021) Exosomal microRNA panels as biomarkers for hematological malignancies. *Current Problems in Cancer*, 100726.
136. Hornick NI, *et al.* (2016) AML suppresses hematopoiesis by releasing exosomes that contain microRNAs targeting c-MYB. *Science Signaling* **9**, ra88.
137. Boyiadzis M and Whiteside TL. (2015) Information transfer by exosomes: A new frontier in hematologic malignancies. *Blood Reviews,* **29**, 281–290.
138. Litwinska Z, Luczkowska K and Machalinski B. (2019) Extracellular vesicles in hematological malignancies. *Leukemia & Lymphoma,* **60**, 29–36.
139. Boyiadzis M and Whiteside TL. (2017) The emerging roles of tumor-derived exosomes in hematological malignancies. *Leukemia,* **31**, 1259–1268.
140. Zhao C, Du F, Zhao Y, Wang S and Qi L. (2019) Acute myeloid leukemia cells secrete microRNA-4532-containing exosomes to mediate normal hematopoiesis in hematopoietic stem cells by activating the LDOC1-dependent STAT3 signaling pathway. *Stem Cell Research & Therapy,* **10**, 384.
141. Zhang X, *et al.* (2020) Exosomes from bone marrow microenvironment-derived mesenchymal stem cells affect CML cells growth and promote drug resistance to tyrosine kinase inhibitors. *Stem Cells International,* **2020**, 8890201.
142. Merino-Gonzalez C, *et al.* (2016) Mesenchymal stem cell-derived extracellular vesicles promote angiogenesis: Potencial clinical application. *Frontiers in Physiology,* **7**, 24.
143. Bruno S, *et al.* (2013) Microvesicles derived from human bone marrow mesenchymal stem cells inhibit tumor growth. *Stem Cells and Development,* **22**, 758–771.

144. Ge X, *et al.* (2020) Exosomal transfer of LCP1 promotes osteosarcoma cell tumorigenesis and metastasis by activating the JAK2/STAT3 signaling pathway. *Molecular Therapy — Nucleic Acids,* **21**, 900–915.
145. Haque S and Vaiselbuh SR. (2020) Silencing of exosomal miR-181a reverses pPediatric acute lymphocytic leukemia cell proliferation. *Pharmaceuticals (Basel),* **13**.
146. Haque S and Vaiselbuh SR. (2020) Vincristine and prednisone regulates cellular and exosomal miR-181a expression differently within the first time diagnosed and the relapsed leukemia B cells. *Leukemia Research Reports* **14**, 100221.
147. Yu VW and Scadden DT. (2016) Hematopoietic stem cell and its bone marrow niche. *Current Topics in Developmental Biology,* **118**, 21–44.
148. Ghobrial IM, Detappe A, Anderson KC and Steensma DP. (2018) The bone-marrow niche in MDS and MGUS: Implications for AML and MM. *Nature Reviews Clinical Oncology,* **15**, 219–233.
149. Yoshida M, *et al.* (2019) miR-7977 inhibits the Hippo-YAP signaling pathway in bone marrow mesenchymal stromal cells. *PLoS One,* **14**, e0213220.
150. Horiguchi H, *et al.* (2016) Extracellular vesicle miR-7977 is involved in hematopoietic dysfunction of mesenchymal stromal cells via poly(rC) binding protein 1 reduction in myeloid neoplasms. *Haematologica,* **101**, 437–447.
151. Jiang, YH, *et al.* (2020) K562 cell-derived exosomes suppress the adhesive function of bone marrow mesenchymal stem cells via delivery of miR-711. *Biochemical and Biophysical Research Communications,* **521**, 584–589.
152. Yang Y, Li J and Geng Y. (2020) Exosomes derived from chronic lymphocytic leukaemia cells transfer miR-146a to induce the transition of mesenchymal stromal cells into cancer-associated fibroblasts. *Journal of Biochemistry,* **168**, 491–498.
153. De Veirman K, *et al.* (2016) Induction of miR-146a by multiple myeloma cells in mesenchymal stromal cells stimulates their pro-tumoral activity. *Cancer Letters,* **377**, 17–24.
154. Cheng Q, *et al.* (2017) Multiple myeloma-derived exosomes regulate the functions of mesenchymal stem cells partially via modulating miR-21 and miR-146a. *Stem Cells International,* **2017**, 9012152.
155. Frassanito MA, *et al.* (2019) Bone marrow fibroblasts overexpress miR-27b and miR-214 in step with multiple myeloma progression, dependent on tumour cell-derived exosomes. *Journal of Pathology,* **247**, 241–253.
156. Umezu T, Ohyashiki K, Kuroda M and Ohyashiki JH. (2013) Leukemia cell to endothelial cell communication via exosomal miRNAs. *Oncogene,* **32**, 2747–2755.
157. Taverna S, *et al.* (2014) Exosomal shuttling of miR-126 in endothelial cells modulates adhesive and migratory abilities of chronic myelogenous leukemia cells. *Molecular Cancer,* **13**, 169.
158. Gruszka AM, Valli D, Restelli C and Alcalay M. (2019) Adhesion deregulation in acute myeloid leukaemia. *Cells,* **8**.

159. Yoon C, *et al.* (2016) Delivery of miR-155 to retinal pigment epithelial cells mediated by Burkitt's lymphoma exosomes. *Tumour Biology,* **37**, 313–321.
160. Tadokoro H, Umezu T, Ohyashiki K, Hirano T and Ohyashiki JH. (2013) Exosomes derived from hypoxic leukemia cells enhance tube formation in endothelial cells. *Journal of Biological Chemistry,* **288**, 34343–34351.
161. Fasanaro, P. *et al.* (2008) MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. *Journal of Biological Chemistry,* **283**, 15878–15883.
162. Muz B, de la Puente P, Azab F, Luderer M and Azab AK. (2014) Hypoxia promotes stem cell-like phenotype in multiple myeloma cells. *Blood Cancer Journal,* **4**, e262.
163. Azab AK, *et al.* (2012) Hypoxia promotes dissemination of multiple myeloma through acquisition of epithelial to mesenchymal transition-like features. *Blood,* **119**, 5782–5794.
164. Umezu T, *et al.* (2014) Exosomal miR-135b shed from hypoxic multiple myeloma cells enhances angiogenesis by targeting factor-inhibiting HIF-1. *Blood,* **124**, 3748–3757.
165. Tian X, *et al.* (2021) Exosome-derived miR-let-7c promotes angiogenesis in multiple myeloma by polarizing M2 macrophages in the bone marrow microenvironment. *Leukemia Research,* **105**, 106566.
166. Abdelhamed S, *et al.* (2019) Extracellular vesicles impose quiescence on residual hematopoietic stem cells in the leukemic niche. *EMBO Reports,* **20**, e47546.
167. Bruns H, *et al.* (2017) CLL-cell-mediated MDSC induction by exosomal miR-155 transfer is disrupted by vitamin D. *Leukemia,* **31**, 985–988.
168. Zare N, Haghjooy Javanmard SH, Mehrzad V, Eskandari N and Andalib AR. (2020) Effect of plasma-derived exosomes of refractory/relapsed or responsive patients with diffuse large B-cell lymphoma on natural killer cells functions. *Cell Journal,* **22**, 40–54.
169. Lou X, *et al.* (2020) MiR-7e-5p downregulation promotes transformation of low-grade follicular lymphoma to aggressive lymphoma by modulating an immunosuppressive stroma through the upregulation of FasL in M1 macrophages. *Journal of Experimental & Clinical Cancer Research,* **39**, 237.
170. Lee JY, *et al.* (2021) Exosomal miR-1305 in the oncogenic activity of hypoxic multiple myeloma cells: A biomarker for predicting prognosis. *Journal of Cancer,* **12**, 2825–2834.
171. Peng D, *et al.* (2018) miR-34c-5p promotes eradication of acute myeloid leukemia stem cells by inducing senescence through selective RAB27B targeting to inhibit exosome shedding. *Leukemia,* **32**, 1180–1188.
172. Hu Y, *et al.* (2020) MicroRNA-34a-mediated death of acute myeloid leukemia stem cells through apoptosis induction and exosome shedding inhibition via histone deacetylase 2 targeting. *IUBMB Life,* **72**, 1481–1490.
173. Farahani M, Rubbi C, Liu L, Slupsky JR and Kalakonda N. (2015) CLL exosomes modulate the transcriptome and behaviour of recipient stromal cells and are selectively enriched in miR-202-3p. *PLoS One,* **10**, e0141429.

174. Hegde GV, *et al.* (2008) Hedgehog-induced survival of B-cell chronic lymphocytic leukemia cells in a stromal cell microenvironment: A potential new therapeutic target. *Molecular Cancer Research,* **6**, 1928–1936.
175. Zhang F, *et al.* (2020) Exosomes derived from human bone marrow mesenchymal stem cells transfer miR-222-3p to suppress acute myeloid leukemia cell proliferation by targeting IRF2/INPP4B. *Molecular and Cellular Probes,* **51**, 101513.
176. Xu YC, *et al.* (2020) MicroRNAs of bone marrow mesenchymal stem cell-derived exosomes regulate acute myeloid leukemia cell proliferation and apoptosis. *Chinese Medical Journal (England)* **133**, 2829–2839.
177. Umezu T, *et al.* (2017) Replenishing exosomes from older bone marrow stromal cells with miR-340 inhibits myeloma-related angiogenesis. *Blood Advances* **1**, 812–823.
178. Wan Z, *et al.* (2019) Chronic myeloid leukemia-derived exosomes attenuate adipogenesis of adipose derived mesenchymal stem cells via transporting miR-92a-3p. *Journal of Cellular Physiology,* **234**, 21274–21283.
179. Gao X, *et al.* (2019) Chronic myelogenous leukemia cells remodel the bone marrow niche via exosome-mediated transfer of miR-320. *Theranostics,* **9**, 5642–5656.
180. Raimondo S, *et al.* (2020) Extracellular vesicle microRNAs contribute to the osteogenic inhibition of mesenchymal stem cells in multiple myeloma. *Cancers (Basel),* **12**.
181. Bitencourt R, Zalcberg I and Louro ID. (2011) Imatinib resistance: A review of alternative inhibitors in chronic myeloid leukemia. *Revista Brasileira de Hematologia,* **33**, 470–475.
182. Volpe G, Panuzzo C, Ulisciani S and Cilloni D. (2009) Imatinib resistance in CML. *Cancer Letters,* **274**, 1–9.
183. Min QH, *et al.* (2018) Exosomes derived from imatinib-resistant chronic myeloid leukemia cells mediate a horizontal transfer of drug-resistant trait by delivering miR-365. *Experimental Cell Research,* **362**, 386–393.
184. Taverna S, *et al.* (2015) Curcumin inhibits in vitro and in vivo chronic myelogenous leukemia cells growth: A possible role for exosomal disposal of miR-21. *Oncotarget,* **6**, 21918–21933.
185. Taverna S, *et al.* (2016) Curcumin modulates chronic myelogenous leukemia exosomes composition and affects angiogenic phenotype via exosomal miR-21. *Oncotarget,* **7**, 30420–30439.
186. Cheng Q, *et al.* (2018) The ceramide pathway is involved in the survival, apoptosis and exosome functions of human multiple myeloma cells in vitro. *Acta Pharmacologica Sinica,* **39**, 561–568.
187. Liu L, *et al.* (2020) C6-ceramide treatment inhibits the proangiogenic activity of multiple myeloma exosomes via the miR-29b/Akt pathway. *Journal of Translational Medicine,* **18**, 298.
188. Chinnappan M, *et al.* (2020) Exosomes as drug delivery vehicle and contributor of resistance to anticancer drugs. *Cancer Letters* **486**, 18–28.

189. Kibria G, Ramos EK, Wan Y, Gius DR and Liu H. (2018) Exosomes as a drug delivery system in cancer therapy: Potential and challenges. *Molecular Pharmaceutics,* **15**, 3625–3633.
190. Barrera-Ramirez J, *et al.* (2017) Micro-RNA profiling of exosomes from marrow-derived mesenchymal stromal cells in patients with acute myeloid leukemia: Implications in leukemogenesis. *Stem Cell Reviews and Reports,* **13**, 817–825.
191. Fang Z, Wang X, Wu J, Xiao R and Liu J. (2020) High serum extracellular vesicle miR-10b expression predicts poor prognosis in patients with acute myeloid leukemia. *Cancer Biomark,* **27**, 1–9.
192. Jiang L, Deng T, Wang D and Xiao Y. (2018) Elevated serum exosomal miR-125b level as a potential marker for poor prognosis in intermediate-risk acute myeloid leukemia. *Acta Haematology,* **140**, 183–192.
193. Lin X, *et al.* (2020) Plasma exosome-derived microRNA-532 as a novel predictor for acute myeloid leukemia. *Cancer Biomark,* **28**, 151–158.
194. Asano M, *et al.* (2017) Up-regulated exosomal miRNA-140-3p in CML patients with musculoskeletal pain associated with discontinuation of tyrosine kinase inhibitors. *International Journal of Hematology* **105**, 419–422.
195. Ohyashiki K, *et al.* (2016) Downregulation of plasma miR-215 in chronic myeloid leukemia patients with successful discontinuation of imatinib. *International Journal of Molecular Sciences,* **17**, 570.
196. Hernandez-Walias FJ, *et al.* (2020) Risk, diagnostic and predictor factors for classical hodgkin lymphoma in HIV-1-infected individuals: Role of plasma exosome-derived miR-20a and miR-21. *Journal of Clinical Medicine* **9**.
197. Feng Y, *et al.* (2019) Exosome-derived miRNAs as predictive biomarkers for diffuse large B-cell lymphoma chemotherapy resistance. *Epigenomics,* **11**, 35–51.
198. Zare N, Haghjooy Javanmard S, Mehrzad V, Eskandari N and Kefayat A. (2019) Evaluation of exosomal miR-155, let-7g and let-7i levels as a potential noninvasive biomarker among refractory/relapsed patients, responsive patients and patients receiving R-CHOP. *Leukemia & Lymphoma,* **60**, 1877–1889.
199. Ryu KJ, *et al.* (2020) Serum-derived exosomal microRNA profiles can predict poor survival outcomes in patients with extranodal natural killer/T-cell lymphoma. *Cancers (Basel),* **12**.
200. Cao D, *et al.* (2018) The value of circulating exsomal miR-451a to monitor therapy response in diffuse large B cell lymphoma. *Sichuan Da Xue Xue Bao Yi Xue Ban,* **49**, 399–403.
201. Manier S, *et al.* (2017) Prognostic role of circulating exosomal miRNAs in multiple myeloma. *Blood,* **129**, 2429–2436.
202. Zhang ZY, *et al.* (2019) Serum exosomal microRNAs as novel biomarkers for multiple myeloma. *Hematological Oncology,* **37**, 409–417.
203. Zhang ZY, Li YC, Geng CY, Wang HJ and Chen WM. (2019) Potential relationship between clinical significance and serum exosomal miRNAs in patients with multiple myeloma. *BioMed Research International,* **2019**, 1575468.

204. Peng Y, Song X, Lan J, Wang X and Wang M. (2021) Bone marrow stromal cells derived exosomal miR-10a and miR-16 may be involved in progression of patients with multiple myeloma by regulating EPHA8 or IGF1R/CCND1. *Medicine (Baltimore),* **100**, e23447.
205. Zhang L, *et al.* (2016) Potential role of exosome-associated microRNA panels and in vivo environment to predict drug resistance for patients with multiple myeloma. *Oncotarget,* **7**, 30876–30891.
206. Li WJ, *et al.* (2021) MicroRNA-34a: Potent tumor suppressor, cancer stem cell inhibitor, and potential anticancer therapeutic. *Frontiers in Cell and Developmental Biology,* **9**, 640587.
207. Hong DS, *et al.* (2020) Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. *British Journal of Cancer,* **122**, 1630–1637.
208. Liu C, *et al.* (2011) The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nature Medicine* **17**, 211–215.
209. Kelnar K, Peltier HJ, Leatherbury N, Stoudemire J and Bader AG. (2014) Quantification of therapeutic miRNA mimics in whole blood from nonhuman primates. *Analytical Chemistry,* **86**, 1534–1542.
210. Beg MS, *et al.* (2017) Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Investigational New Drugs,* **35**, 180–188.
211. Hanna J, Hossain GS and Kocerha J. (2019) The Potential for microRNA Therapeutics and Clinical Research. *Frontiers in Genetics,* **10**, 478.
212. Seto AG, *et al.* (2018) Cobomarsen, an oligonucleotide inhibitor of miR-155, coordinately regulates multiple survival pathways to reduce cellular proliferation and survival in cutaneous T-cell lymphoma. *British Journal of Haematology,* **183**, 428–444.
213. Anastasiadou E, *et al.* (2021) Cobomarsen, an oligonucleotide inhibitor of miR-155, slows DLBCL tumor cell growth in vitro and in vivo. *Clinical Cancer Research,* **27**, 1139–1149.
214. Witten L and Slack FJ. (2020) miR-155 as a novel clinical target for hematological malignancies. *Carcinogenesis,* **41**, 2–7.
215. Santolla MF, *et al.* (2018) miR-221 stimulates breast cancer cells and cancer-associated fibroblasts (CAFs) through selective interference with the A20/c-Rel/CTGF signaling. *Journal of Experimental & Clinical Cancer Research,* **37**, 94.
216. Gulla A, *et al.* (2016) A 13 mer LNA-i-miR-221 inhibitor restores drug sensitivity in melphalan-refractory multiple myeloma cells. *Clinical Cancer Research,* **22**, 1222–1233.
217. Di Martino MT, *et al.* (2014) In vitro and in vivo activity of a novel locked nucleic acid (LNA)-inhibitor-miR-221 against multiple myeloma cells. *PLoS One,* **9**, e89659.
218. Zhang S, Cheng Z, Wang Y and Han T. (2021) The risks of miRNA therapeutics: In a drug target perspective. *Drug Design, Development and Therapy,* **15**, 721–733.

219. Zhang L, Liao Y and Tang L. (2019) MicroRNA-34 family: A potential tumor suppressor and therapeutic candidate in cancer. *Journal of Experimental & Clinical Cancer Research,* **38**, 53.
220. Forterre A, Komuro H, Aminova S and Harada M. (2020) A comprehensive review of cancer microRNA therapeutic delivery strategies. *Cancers (Basel),* **12**.

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Chapter 11

Long Noncoding RNAs in Inflammation, Cancer, and in Cancer Therapy

Rachel L. Price, Avisankar Chini, Arunoday Bhan, and Subhrangsu S. Mandal*

Gene Regulation and Epigenetics Research Laboratory,
Department of Chemistry and Biochemistry,
The University of Texas at Arlington,
Arlington, Texas 76019

Abstract

The perception that large portions of the human genome lacking protein-coding capacity is "junk DNA" that produces RNA molecules regarded merely as "transcriptional noise", became obsolete in recent years with the discovery of tens of thousands of non-coding RNA (ncRNA) transcripts with clear biological functions. Genome-wide association studies of tumor samples have revealed huge numbers of long ncRNAs (lncRNAs) that are associated with numerous different cancer types. LncRNAs can act both oncogenically and as tumor suppressors in their roles as major epigenetic regulators and participants in signal transduction. Most lncRNAs are still uncharacterized, but there has been an explosion of

*Corresponding author's email: smandal@uta.edu

research into their regulatory behaviors in malignancies, including critical roles in cancer-related inflammation and immune signaling. This has led to the emergence of lncRNAs as major biological players in cancer. Since they are widely dysregulated in all common cancers and are both tumor-type specific and detectable in body fluids, lncRNAs show great promise as novel diagnostic and prognostic biomarkers, as well as potential therapeutics for this deadly disease. In this review, we discuss the mechanisms of actions of various lncRNAs through which they modulate gene regulation, and their potential therapeutic applications.

1. Introduction

The majority of the human genome's 3 billion DNA base pairs code not for proteins but for non-translated transcripts called non-coding RNAs (ncRNAs) [1]. Approximately 70% of these transcripts are >200 nucleotides in length and are known as long ncRNAs (lncRNAs) [2]. They mechanistically interact with other RNAs, DNA, lipids, and proteins within both the nucleus and the cytoplasm [3–5]. LncRNAs participate in signal transduction remodeling [6, 7], and can modulate the epigenetic machinery by acting as scaffolds, decoys, and guides to up- or downregulate the expression of specific genes [8, 9].

In addition to epigenetically regulating various protein-coding genes, cancer-related lncRNAs also modulate another important type of non-coding RNA, the highly conserved 17–25 nt microRNAs (miRNAs) [10]. These miniscule transcripts play a major regulatory role in cells by binding directly to the 3′-untranslated region (3′-UTR) of target messenger RNAs (mRNAs) and causing mRNA cleavage and/or translational repression [11]. Some miRNAs are considered proto-oncogenes while others act as tumor suppressors by inhibiting the translation of oncogenic proteins, and perturbations in the expression levels of specific miRNAs are associated with the vast majority of human malignancies [12–15]. Over half of the 140 lncRNAs analyzed in one study affected their targets in a miRNA-dependent manner [16]. LncRNAs also function as competitive endogenous RNA (ceRNA), indirectly causing the de-repression of miRNA targets [17]. For example, HOX transcript antisense intergenic RNA (HOTAIR) acts as a sink for miR-331-3p in gastric cancer cells, leading

to de-repression of this miRNA's direct target, human epithelial growth factor receptor (HER2), which oncogenically activates cellular networks involved in proliferation, motility, and angiogenesis [18].

Dysregulated lncRNAs can significantly affect cellular homeostasis pathways [19, 20], including those involving proliferation, mobility, gene expression, post-synthetic processing, and differentiation. This naturally endows lncRNAs with modulatory influence on the classic characteristics of cancer cells, such as motility, viability, and proliferation, effects that manifest themselves in the transformed phenotypes and hallmarks of malignancy, including tumorigenesis, metastasis, evasion of tumor suppression, angiogenesis, therapeutic resistance, and resistance to apoptosis [21]. LncRNAs can function not only as oncogenes but also as tumor suppressors [22]. In addition, lncRNAs are involved in several inflammatory and immune-signaling pathways, furthering implications for their involvement in malignancy since inflammation is known to promote cancer development [23]. Thousands of lncRNAs aberrantly expressed in many types of tumors, are often cancer-specific and are stable and detectible in the body fluids of cancer patients. Thus, dysregulated lncRNAs show promise in cancer as noninvasive diagnostic and prognostic biomarkers and as novel therapeutic targets [24]. In this chapter, we discuss the various action mechanisms by which lncRNAs modulate gene regulation in immune signaling, cancer, and other diseases, and how the existing knowledge of lncRNAs allows the development of lncRNA-based therapeutic targeting.

2. LncRNAs in major cancers

Figure 1 shows a partial list of various lncRNAs associated with major cancer types. This section provides an overview of specific lncRNA actions in the most common cancers.

2.1. *Breast cancer*

Breast cancer is the most common female malignancy and the second deadliest [25]. Several lncRNAs are overexpressed in breast cancer cells,

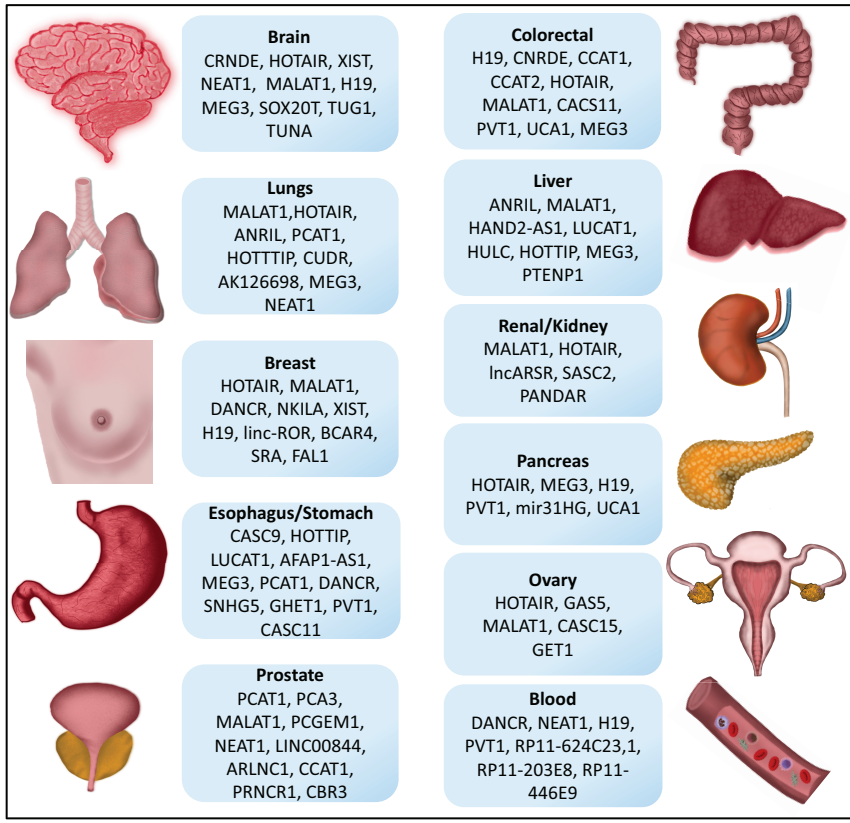

Figure 1. LncRNAs expressed in different types of cancers.

including HOTAIR, metastasis-associated lung adenocarcinoma transcript (MALAT1), differentiation antagonizing non-protein coding RNA (DANCR), and others [26–28]. HOTAIR is associated with tumorigenesis, angiogenesis, cancer stem cell differentiation, progression, invasion, drug resistance, metastasis, and poor prognosis in many types of malignancies [29–32], including breast cancer [33, 34]. HOTAIR silences genes through repressive chromatin modifications and miRNA sponging, and plays regulatory roles in protein degradation, inflammation, immune signaling, and DNA damage response [35–37]. Chromatin modification is one of the best-known mechanisms used by lncRNAs to exert epigenetic effects.

Polycomb repressive complex 2 (PRC2) is an epigenetic silencing complex that binds to approximately 20% of all lncRNAs [38]. For example, HOTAIR silences tumor-suppressor genes and thereby promotes metastasis by targeted recruitment of the EZH2 subunit of PRC2 to the promoters of these genes [39], while antisense non-coding RNA in the INK4 locus (ANRIL) does so by binding to the SUZ12 subunit [40]. In addition, HOTAIR's promoter contains multiple functional estrogen-response (ER) elements and its transcription is induced by estradiol [41] and by nanomolar concentrations of the estrogenic endocrine disruptors bisphenol-A (BPA) and diethylstilbestrol (DES) [42]. Even in breast tumors lacking ER expression, overexpressed HOTAIR is strongly correlated with lymph node metastasis [43]. The tumor suppressor BRCA1 may compete with HOTAIR for interactions with PRC2, a key gene-silencing protein complex [44].

LncRNA DANCR influences the inflammatory phenotype of breast tumor cells, via EZH2-dependent transcriptional suppression of SOCS3, promoting breast cancer progression [45]. MALAT1 is induced by hypoxia in breast cancer cells [46] and its overexpression was found in some studies to promote breast cancer relapse in postoperative patients with fever [47] and to be associated with lymph node metastasis and decreased 5-year survival [48]. Interestingly, however, other studies have shown that downregulation of MALAT1 correlated with shorter relapse-free survival [49] and its overexpression suppressed breast cancer metastasis [50]. Indeed, lncRNA involvement in cancer is not always oncogenic. X-inactive specific transcript (XIST) is known to inhibit certain cancers, including osteosarcoma and prostate cancer [51, 52]; in breast tumors, it inhibits cell growth, migration, and invasion [53]. Each of these examples involves XIST interactions with miRNAs.

2.2. *Prostate cancer*

Many dysregulated lncRNAs have been identified in prostate cancer, the most common malignancy in men in the United States and the second-leading cause of male cancer deaths [25]. Several lncRNAs are highly specific to prostate cancer. A comprehensive study identified 121

ncRNAs, termed prostate cancer-associated transcripts (PCATs), that show expression patterns distinguishing between metastatic and localized prostate cancer. The most notable of these is PCAT1, which actively promotes cell proliferation by transcriptionally regulating target genes [54]. PCAT1 post-transcriptionally upregulates C-MYC protein by interacting with the 3′ UTR of its coding mRNA, and stabilizes it by targeting its regulator, miR-34a [55]. PCAT1 also promotes progression of castration-resistant prostate cancer by activating the AKT and NF-κB signaling pathways [56].

The lncRNA prostate cancer antigen 3 (PCA3) is the most specific molecule for prostate cancer, expressed in >95% of prostate cancers and not found in any other normal human tissues [57]. Since it can be detected in the urine, PCA3 has thus been used as a diagnostic biomarker since 2012 [58]. This lncRNA modulates the gene expression of several key AR signaling molecules and transcription factors that promote tumor progression [59].

Other lncRNAs implicated in prostate cancer and remarkably specific to it include prostate-specific transcript PCGEM1, which confers a metabolic advantage on prostate cancer cells by promoting glucose uptake and cell proliferation, and modulating androgen-receptor (AR)-mediated transcription [60–62]. MALAT1, which can also be detected in the urine of prostate cancer patients, is upregulated in prostate cancer tissues, where it acts as a competing endogenous RNA to strongly downregulate miR-1, resulting in increased expression of its oncogenic GTPase protein target, KRAS [63–65]. N^6-methyladenosine (m^6A)-modified nuclear enriched abundant transcript 1 (NEAT1) promotes prostate cancer metastases to bone by facilitating assembly of a CYCLINL1/CDK19 complex, which in turn phosphorylates RNA PolII on Ser2 [66]. Many androgen-regulated lncRNAs have also been identified in prostate cancer. Long intergenic non-protein coding RNA 844 (LINC00844) is actively transcribed in AR-dependent prostate cancer cells, but rather than promoting malignancy, its upregulation prevents prostate cancer cell migration and invasion [67]. Androgen receptor regulated long noncoding RNA 1 (ARLNC1) participates in a positive feedback loop with AR to regulate cellular levels

of this receptor [68]. Colon cancer associated transcript 1 (CCAT1) is highly upregulated in castration-resistant prostate cancer (CRPC) compared to androgen-dependent prostate cancer; in the cytoplasm it blocks the tumor-suppressing activity of miR-28-5P via sponging and in the nucleus it provides scaffolding for the AR transcriptional complex, promoting CRPC progression [69].

2.3. *Lung cancer*

Although it is only the second most common malignancy in both sexes, lung cancer causes more deaths worldwide than breast, colon, brain, and prostate cancers combined [25]. Numerous dysregulated lncRNAs have been identified in lung cancer, including MALAT1, HOTAIR, ANRIL, and many others [70, 71]. MALAT1, a relatively highly conserved lncRNA, was first identified in 2003 when it was found to be three times higher in lung cancer metastatic versus non-metastatic tumor tissue, and predicted metastasis and poor prognosis [72]. This lncRNA is overexpressed in many tumors and promotes proliferation, motility, angiogenesis, and metastasis [73]. It is activated by NF-κB and other transcription factors [74], downregulated by tumor suppressor p53 [75], and targeting its expression with antisense oligonucleotides dramatically blocked lung cancer metastasis in a mouse model [76].

In several types of lung cancers, overexpressed HOTAIR contributes to chemoresistance by regulating the expression of the tumor suppressor p21, thereby enhancing cell motility and invasion [77, 78]. It promotes aggressive biological behavior and is linked to short disease-free survival in non-small cell lung cancer (NSCLC) [79]. HOTAIR can be detected in the plasma of NSCLC patients and thus has biomarker potential [80]. NSCLC tissues show increased ANRIL expression compared to adjacent normal tissues, and its overexpression was significantly correlated with tumor-node-metastasis (TNM) stage, tumor size, and poor prognosis [81]. PCAT1, mentioned above for prostate cancer, also acts in lung tumors, promoting NSCLC cell proliferation/invasion and inhibiting apoptosis via the VEGF/AKT/BCL2/CASP9 pathway [82].

2.4. *Colorectal cancer*

Colorectal malignancies are the third most common type of cancer and fourth most common cause of cancer deaths. They are associated with an array of lncRNAs, including H19, colorectal neoplasia differentially expressed (CRNDE), colon cancer associated transcript 1 and 2 (CCAT1 and CCAT2, respectively), HOTAIR, MALAT1, and others [83–85]. H19, a predictor of poor prognosis, is upregulated in numerous cancers [86] and both regulates, and is regulated by, the tumor suppressor p53 [87]. In colorectal cancer (CRC), H19 promotes EMT and accelerates tumor growth via miRNA sponging [88]. H19 also regulates the PI3K-AKT signaling pathway through a competing endogenous RNA network [89]. CRNDE promotes progression and chemoresistance in CRC by targeting miR-181a-5p expression, thus upregulating the miRNA's inhibitory targets in WNT/β-catenin signaling [90]. This lncRNA has been shown to be regulated by insulin and insulin-like growth factors (IGFs) via their downstream signaling cascades (PI3K/AKT/mTOR and RAF/MAPK pathways) and to itself regulate glucose and lipid metabolism-involved genes [91].

CCAT1 is upregulated in over 90% of colon adenocarcinomas and is found at 235-fold higher levels in malignant tissue compared to normal mucosa, where it is essentially undetectable [92]. Upregulated CCAT1 is present in all stages of CRC, from premalignant adenomas to metastatic disease [93]. CCAT2 is associated with metastatic progression of CRC [94]. HOTAIR overexpression promotes distant metastasis and poor prognosis in colon cancer [95], and MALAT1 promotes CRC cell proliferation, migration, and invasion via PRKA kinase anchor protein 9 [96] and by inhibiting miR-663a, thus preventing the degradation of most of this miRNA's colon cancer-promoting targets [97].

2.5. *Brain tumors*

RNA sequencing has revealed hundreds of lncRNAs aberrantly expressed in brain tumors, and many of these transcripts are associated with molecular subtype and can predict outcomes in glioblastomas [98, 99]. Distinct lncRNA profiles appear between different brain tumor types (diffuse versus anaplastic astrocytoma and glioma) and in comparison to normal

white matter [100]. CRNDE, which was found to be upregulated 32-fold in glioma tissue compared to normal brain tissue [101], modulates mTOR signaling in glioma to promote tumor cell growth and migration [102]. Increased HOTAIR is associated with higher grade and significantly shortened survival in glioma [103].

XIST promotes glioma tumorigenesis and angiogenesis by sponging miR-429, a transcript that suppresses the protein kinase BMK1 to inhibit glioma invasion [104, 105]. NEAT1, a lncRNA analogous to MALAT1 and regulated by the EGFR pathway, acts as a scaffold for EZH2 to target inhibitors of the WNT/β-catenin pathway and thus contributes to the progression of glioblastoma [106]. Other lncRNAs implicated in brain tumors include MALAT1, H19, maternally expressed 3 (MEG3), and others [107].

2.6. *Other cancers*

Most, if not all, of the most common deadly cancers are linked to numerous lncRNAs. In pancreatic cancer alone, a genome-wide analysis identified seven lncRNA biomarkers with regulatory roles in cell death, cell adhesion, and the cell cycle [108]; another study identified several lncRNAs as prognostic biomarkers for this malignancy [109]; and another reported a three-lncRNA signature that predicts patient survival [110]. HOTAIR silences miR-663b in pancreatic cancer by disrupting the balance between H3K27me3 and H3K4me3 at its promoter; this miRNA targets insulin-like growth factor 2, the overexpression of which is closely associated with poorer prognosis [111]. Gastric cancer is associated with MEG3, PCAT1, DANCR, small nucleolar RNA host gene 5 (SNHG5), gastric carcinoma proliferation enhancing transcript 1 (GHET1), plasmacytoma variant translocation 1 (PVT1), cancer susceptibility 11 (CASC11), and more [112–114]. LncRNAs implicated in hepatocellular cancer include ANRIL, MALAT1, heart and neural crest derivatives expressed transcript 2 antisense RNA 1 (HAND2-AS1), lung cancer associated transcript 1 (LUCAT1), highly up-regulated in liver cancer (HULC), and HOXA distal transcript antisense RNA (HOTTIP), among others [115, 116]. Bladder cancer has been linked to urothelial cancer associated 1 (UCA1), CCAT1 and CCAT2, lncRNA upregulated in bladder cancer

(UBC1), regulator of reprogramming (ROR), and others [117]. In esophageal cancer, roles for cancer susceptibility 9 (CASC9), HOTTIP, LUCAT1, actin filament associated protein 1 antisense RNA 1 (AFAP1-AS1), and other lncRNAs have been identified [118] .

LncRNA involvement is not limited to solid tumors. Investigators have identified a small subset of lncRNAs that strongly correlate with treatment response and survival in older adults with acute myeloid leukemia (AML) [119]. LncRNA in non-homologous end joining pathway 1 (LINP1) is significantly overexpressed at the time of AML diagnosis but downregulated after complete remission, and promotes the AML-cell malignant phenotype while also stimulating glucose metabolism [120]. PVT1 is transcriptionally activated by MYC protein and is correlated with cell proliferation in acute promyelocytic leukemia [121]. A transcriptome analysis identified 2,632 novel lncRNAs in diffuse large B-cell lymphoma, with half of them showing potential lymphoma-onset and/or tumor maintenance activity [122].

3. Links between cancer, immune signaling, and lncRNAs

It has been known for decades that 15–20% of all deaths from cancer may be due to underlying infection and chronic inflammation [123], and the so-called "cancer-immunity cycle" is well-established [124]. A particularly notable aspect of lncRNAs in malignancy is their involvement in many dysregulated immune-signaling pathways, including epigenetic effects on innate and trained immunity [125]. Many examples of such interactions have been highlighted in this chapter, and the link between lncRNAs and immune signaling indeed appears to be vast; one study found 1,524 lncRNAs that are expressed in cell-specific clusters during T-cell differentiation [126]. Malignancies of similar tissues tend to share perturbed lncRNA immune regulators [127].

Many different lncRNAs interfere with normal signaling pathways of the pro-inflammatory transcription factor NF-κB, a master regulator in both inflammation/immune signaling and in modulating the expression of genes involved in cell differentiation, survival, and proliferation [128].

Dysregulation of NF-κB is found in almost all malignancies, where constitutively activated NF-κB promotes tumor cell proliferation, angiogenesis, and EMT while suppressing apoptosis [129]. A number of lncRNAs are known to regulate NF-κB signaling pathways [130]. For example, HOTAIR promotes NF-κB activation by downregulating its inhibitor IκBα in LPS-treated macrophages, with one effect being the upregulation of the glucose transporter Glut1 and thus increased glucose metabolism in these cells [131] (Fig. 2). NF-κB induces expression of the lncRNA Lethe (after the "river of forgetfulness" in Greek mythology) [132], while HOXA cluster antisense RNA 3 (HOXA-AS3) positively regulates NF-κB [133]. Under LPS stimulation, functional intergenic repeating RNA element (FIRRE) responds to NF-κB signaling by using posttranscriptional mechanisms to upregulate several inflammatory genes in macrophages

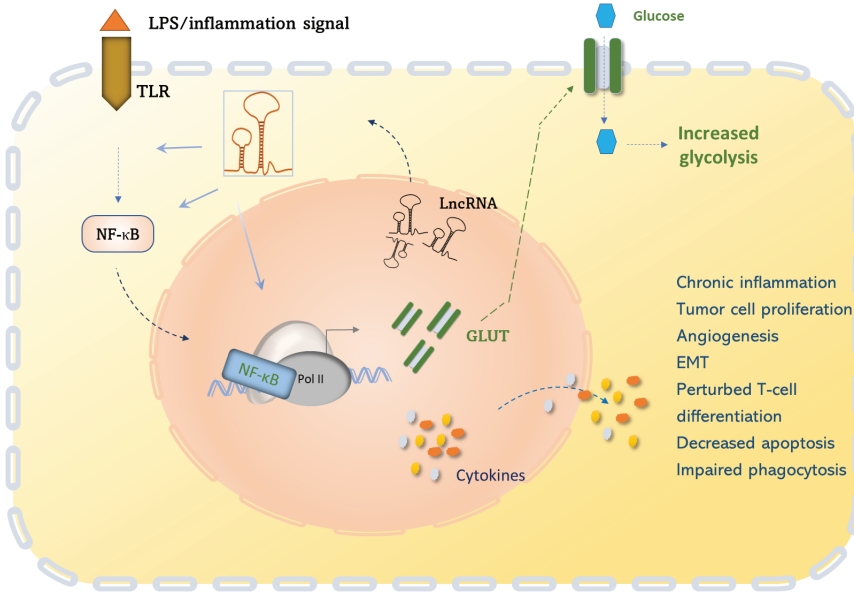

Figure 2. Potential roles for lncRNAs in inflammation, metabolism, and cancer.

Similar to cytokines, expression of lncRNAs is induced in response to inflammation. In turn, inflammatory lncRNAs may influence a variety of inflammatory signaling pathways, metabolic functions, cellular and physiological process, and contribute towards health and disease including cell proliferation, angiogenesis, and impaired cell death.

and intestinal epithelial cells [134]. PTGS2 antisense NFKB1 complex-mediated expression regulator RNA (PACERR) deregulates the cancer-associated protein COX-2 by associating with and occluding the repressive NF-κB subunit p50 [135]. Increased cytoplasmic lncRNA cardiac and apoptosis-related lncRNA (Carlr) appears to be necessary for efficient NF-κB signaling [136].

Many lncRNAs appear to play important roles in the regulation of the Toll-like receptor (TLR) signaling network, which is critical in pathophysiological inflammation [137–139]. In addition, macrophages, which are important in innate immunity and have been demonstrated to participate in the tumor microenvironment (TME), are impacted by lncRNA overexpression, with deleterious effects on M2 macrophage activation and polarization [140–142]. Hepatocellular carcinoma tumor-derived exosomal lncRNA TUC339 was shown to be transferred into macrophages in the TME, where it modifies cytokine production and phagocytosis [143]. Interleukins are critical inflammatory cytokines and lncRNAs are linked to the development of many cancers in which interleukin (IL) signaling is dysregulated [144]. For example, MALAT1 upregulates IL-6 [145] and lncRNA DILC (downregulated in liver cancer stem cells) exerts regulatory effects on liver cancer stem cells via an IL-6/STAT3 axis [146]. IL-22 induces H19 expression in intestinal epithelial cells [147], while upregulation of inflammation-related lncRNA-IL7R predicts poor clinical outcome in patients with cervical cancer [148]. LncRNAs can also be induced by interferon and regulate its signaling pathways [149]. It should be noted that inflammation-related lncRNAs are not always oncogenic; for example, by inducing inflammasome formation, lncRNA growth arrest-specific 5 (GAS5) inhibits the formation of ovarian cancer [150].

4. LncRNAs in other disorders

Unsurprisingly, lncRNAs are implicated in many disorders beyond cancer, particularly in dysmetabolic and vascular disorders. Researchers have identified links between various lncRNAs and diabetes, insulin resistance, obesity, atherosclerosis, cardiovascular disease, carbohydrate and lipid metabolism, and steatosis [151–153]. There is abnormal expression of several lncRNAs in type 2 diabetes, and genome-wide association studies

have found genetic variants in lncRNA loci in type 1 diabetes [154]. Overexpression of lncRNAs linked to vascular diseases has been demonstrated in endothelial cells, vascular smooth muscle cells, and pericytes [154]. By sponging miR-1, HOTAIR regulates hypoxia-induced cardiomyocyte apoptosis *in vitro* and is significantly downregulated in early-phase acute myocardial infarction (AMI), suggesting that HOTAIR has a protective effect in AMI [155]. HOTAIR also promotes the inflammatory response of cardiomyocytes after AMI by upregulating the receptor for advanced glycation end products (RAGEs) [156]. Another cardioprotective lncRNA is cardiomyocyte-enriched noncoding transcript (Caren), which is mostly expressed in heart tissue and localized in the cytoplasm of cardiomyocytes [157]. During pathological stress, Caren inactivates the ataxia telangiectasia mutated (ATM)-DNA damage response (DDR) pathway and activates mitochondrial bioenergetics, thus maintaining cardiomyocyte homeostasis [157]. Research has shown that lncRNAs such as NEAT1 are incorporated into vesicles and shuttled between cardiomyocytes and fibroblasts after infarction, and that in response to hypoxia/reoxygenation, numerous lncRNAs are dysregulated in cardiomyocytes [158]. In another study, after incubating human umbilical vein endothelial cells with media containing various glucose levels, MALAT1 expression was found to increase, peaking after 12 hours in high-glucose media and with a concomitant increase in the inflammatory ligand serum amyloid antigen 3 (SAA3), tumor necrosis factor-α (TNF-α), and IL-6 [159]. A diabetic cardiomyopathy (DCM) rat model showed that myocardial infarction-associated transcript (MIAT) expression is increased by high glucose levels and promotes cardiomyocyte apoptosis by acting as a competing endogenous RNA that sponges miR-22-3p, a microRNA that targets death-associated protein kinase 2 (DAPK2) [160].

In addition, many cellular processes involved in the biology of aging are impacted by lncRNAs, including differentiation, proliferation, quiescence and senescence, and telomere length [161]. For example, the lncRNAs telomerase RNA component (TERC) and telomere repeat-containing RNA (TERRA) are both part of the telomerase ribonucleoprotein complex [161]. By acting as a scaffold, TERC brings together key telomerase-protein subunits and other accessory proteins associated with the complex, thus directly participating in the maintenance of telomere length [162].

On the other hand, TERRA acts as a competitive inhibitor of telomerase reverse transcriptase (TERT), a polymerase also contained in the telomerase ribonucleoprotein complex that is essential for telomere extension, thus, TERRA suppresses telomere elongation [163].

5. LncRNAs as diagnostics and therapeutics

LncRNAs show great promise as novel, specific, and sensitive non-invasive biomarkers [28]. LncRNA genes are expressed in an exceptionally more cell- and tissue-type-specific manner than protein-coding genes and in far greater amounts [164, 165], and cell-free lncRNAs that were originally only detected in tissues have now been proven to be detectable in various bodily fluids, where they are remarkably stable [166]. For these reasons, lncRNAs could be harnessed for the development of targeted cancer diagnostics, prognostics, and therapies.

In general, most traditional tumor markers are based on proteins and feature insufficient specificity and sensitivity to serve as primary diagnostic tools [167]. For example, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), classical serum markers for CRC, are neither sensitive nor specific [168]. CCAT1 overexpression is present in 40% of peripheral blood samples from CRC patients and in none of the healthy controls; the same study showed greater than 100-fold upregulated CCAT1 in the sentinel lymph nodes of 100% of tumor-positive and 40% of otherwise histologically negative-staining CRC patients [92]. This remarkable finding suggests that CCAT1 can identify low-volume nodal metastases that standard staging techniques cannot. Plasma HOTAIR levels are not only higher in NSCLC patients, but are also associated with histology subtype and TNM stage, with greater diagnostic accuracy than CEA levels alone and even greater with a combination of HOTAIR and CEA [80]. The lncRNAs SOX2 overlapping transcript (SOX2OT) and ANRIL can be combined with tumor markers CEA, a cytokeratin 19 fragment (CYFRA21-1), and serine protease inhibitor squamous cell carcinoma antigen (SCCA) to provide a single diagnostic panel for NSCLC that is superior to that of any of these five molecules independently [169]. Some lncRNA biomarkers also escape certain confounding factors. For example, unlike the PSA test for

prostate cancer, the lncRNA PCA3 is unaffected by the patient's age, inflammatory status, surgical trauma, or certain medications [170].

LncRNAs can be detected in a variety of body fluids. AA174084, a lncRNA implicated in gastric cancer development, can be detected in gastric juice [171]. H19, HOTAIR, and MALAT1 are detectible in the plasma of gastric cancer patients [166]. Elevated MALAT1 is found in peripheral whole blood in lung cancer [172] and can be detected in the urine in prostate and bladder cancer [64, 173]. Many individual lncRNAs can also indicate tumor stage and type from biopsy tissues. For example, HOTAIR is less expressed in low-grade gliomas compared to glioblastoma multiforme (GBM), and GBM subtypes can even be suggested, with greater HOTAIR expression in classical and mesenchymal compared to neural and pro-neural subtypes [174]. LncRNAs can also be used for prognostic purposes. For example, researchers have developed a survival algorithm based on an lncRNA panel capable of separating glioma patients into distinct poor-versus-good prognostic groups [98]. Greater overexpression of NEAT1 in glioma tissues is associated with larger tumors, higher WHO grade, and recurrence, all unfavorable prognostic factors [175].

The potential of lncRNAs as therapeutic targets in cancer is a crucial area of research. Going beyond classic differential-expression analysis, state-of-the-art high-throughput functional screening methods such as CRISPR/Cas are now being used to confirm this potential [176]. In one example, a mouse breast cancer model demonstrated that metastasis is strongly suppressed by targeting lncRNA breast cancer anti-estrogen resistance 4 (BCAR4) using locked nucleic acids [177]. One of the effects of the diterpenoid Bharangin against breast cancer is the induction of tumor-suppressor lncRNAs such as MEG3 and GAS5 [178]. Another team developed a strategy using a short hairpin RNA (shRNA) cassette incorporated into a lentivirus vector, which was transduced into androgen-dependent prostate cancer cells, to stably silence lncRNA PCA3; this may be a potential *in vivo* treatment to inhibit prostate cancer growth [59].

Even when not therapeutically targeted themselves, lncRNAs can help predict whether a cancer patient will benefit from a particular treatment. A study on advanced gastric cancer provided evidence that HOTAIR may be used to identify patients who will benefit from

fluorouracil and platinum combination chemotherapy [179]. Many specific lncRNA signatures have been identified, for example in the immune cell infiltrate of NSCLC, that could be used to help identify patients who would benefit from certain oncologic immunotherapies [180]. Indeed, recent studies have highlighted the significant potential of lncRNA targets in immunotherapies for cancer. For example, lncRNA inducing MHC-I and immunogenicity of tumor (LIMIT) enhances immunotherapy efficacy by inducing the expression of major histocompatibility complex-I (MHC-I) and MHC-II, which promotes the T-cell-mediated antitumor immune response [181]. A co-expression analysis suggested that many lncRNAs are potentially involved in programmed cell death receptor 1 (PD-1) checkpoint pathways and could regulate several steps in the transcriptional and post-transcriptional modification of CD4+, CD8+, and CD14+ peripheral blood cells (PBC) in specific melanoma cell types, which could aid in the discovery of cell type-specific immunotherapies [182]. Long intergenic noncoding RNA for kinase activation (LINK-A) expression in transformed mammary gland epithelial cells causes downregulated antigenicity and thus, reduced intrinsic tumor suppression in triple-negative breast cancer, hence may serve as another immunotherapy target [183] .

Despite the abundance of published research on lncRNAs in cancer, there is much yet to be elucidated. The useful exploitation of lncRNAs in cancer diagnosis and targeted therapies will require detailed characterization of each lncRNA, including their cellular functions, SNPs, and how they themselves are regulated, for example through environmental stimuli. A crucial step will be the continued development of databases of lncRNAs with functional roles in cancer and other diseases to assist the formation of predictions about lncRNA behavior. A few such databases already exist, including LncRNA Disease [184], Lnc2Cancer [185], and the Cancer LncRNA Census [186]. Further research on therapeutic approaches is also required, including techniques using antisense oligonucleotides and/or viral vectors to target lncRNAs, genome editing with CRISPR/Cas9, and nanomedicine [187], and there is a need for clinical trials with statistically significant patient cohorts involving lncRNA diagnostics and therapeutics [188].

6. Conclusion

Initially disregarded as "junk DNA", genes transcribed into non-coding RNAs are now understood to perform critical biological roles, as key epigenetic regulators, and as actors in signal transduction in cancer, inflammation, and immune signaling pathways. LncRNAs are widely expressed in tumors, tumor-specific, and stable in body fluids. Therefore, they show great promise for diagnostic, prognostic, and therapeutic utility in cancer, including targeted immunotherapies. However, the structures and functions of most of the ~60,000 known lncRNAs are still uncharacterized, and they are poorly conserved across species, thus presenting challenges for developing *in vitro* and animal models. Despite these limitations, the emergence of lncRNAs as major biological players in immune signaling and consequently in malignancy has led to a remarkable paradigm shift in our understanding of cancer and, as a result, in our eventual capacity to more effectively diagnose and treat this deadly disease.

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References

1. Dunham I, *et al.* (2012) An integrated encyclopedia of DNA elements in the human genome. *Nature*, **489**(7414), 57–74.
2. Iyer MK, *et al.* (2015) The landscape of long noncoding RNAs in the human transcriptome. *Nature Genetics*, **47**(3), 199–208.
3. Engreitz JM, *et al.* (2016) Local regulation of gene expression by lncRNA promoters, transcription, and splicing. *Nature*, **539**(7629), 452–455.
4. Liu B, *et al.* (2015) A cytoplasmic NF-κB interacting long noncoding RNA blocks IκB phosphorylation and suppresses breast cancer metastasis. *Cancer Cell*, **27**(3), 370–381.
5. Ma Y, Zhang J, Wen L and Lin A. (2018) Membrane-lipid associated lncRNA: A new regulator in cancer signaling. *Cancer Letters*, **419**, 27–29.

6. Fu P-F, Zheng X, Fan X and Lin A-F. (2019) Role of cytoplasmic lncRNAs in regulating cancer signaling pathways. *Journal of Zhejiang University. Science. B*, **20**(1), 1.
7. Lin C and Yang L. (2018) Long noncoding RNA in cancer: Wiring signaling circuitry. *Trends in Cell biology*, **28**(4), 287–301.
8. Wang KC and Chang HY. (2011) Molecular mechanisms of long noncoding RNAs. *Molecular cell*, **43**(6), 904–914,
9. Balas MM and Johnson AM. (2018) Exploring the mechanisms behind long noncoding RNAs and cancer. *Noncoding RNA Research*, **3**(3), 108–117,
10. Cantile M, Di Bonito M, Tracey De Bellis M and Botti G. (2021) Functional interaction among lncRNA HOTAIR and MicroRNAs in cancer and other human diseases. *Cancers*, **13**(3).
11. Bartel DP. (2009) MicroRNAs: Target recognition and regulatory functions. *Cell*, **136**(2), 215–233.
12. Gozuacik D, Akkoc Y, Ozturk DG and Kocak M. (2017) Autophagy-regulating microRNAs and cancer. *Frontiers in Oncology*, **7**, 65.
13. Ling H, Girnita L, Buda O and Calin GA. (2017) Non-coding RNAs: The cancer genome dark matter that matters! *Clinical Chemistry and Laboratory Medicine (CCLM)*, **55**(5), 705–714.
14. Lu J, *et al.* (2005) MicroRNA expression profiles classify human cancers. *Nature*, **435**(7043), 834–838.
15. Volinia S, *et al.* (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. *Proceedings of the National Academy of Sciences*, **103**(7).
16. Tan JY, *et al.* (2015) Extensive microRNA-mediated crosstalk between lncRNAs and mRNAs in mouse embryonic stem cells. *Genome Research*, **25**(5), 655–666.
17. Salmena L, Poliseno L, Tay Y, Kats L and Pandolfi PP. (2011) A ceRNA hypothesis: The Rosetta stone of a hidden RNA language? *Cell*, **146**(3), 353–358.
18. Liu X, *et al.* (2014) Lnc RNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer. *Molecular Cancer*, **13**, 92.
19. Moran VA, Perera RJ and Khalil AM. (2012) Emerging functional and mechanistic paradigms of mammalian long non-coding RNAs. *Nucleic Acids Research*, **40**(14), 6391–6400.
20. Salviano-Silva A, Lobo-Alves SC, de Almeida RC, Malheiros D and Petzl-Erler ML. (2018) Besides pathology: Long non-coding RNA in cell and tissue homeostasis. *Non-Coding RNA*, **4**(1).
21. Bhan A, Soleimani M, and Mandal SS. (2017) Long noncoding RNA and cancer: A new paradigm. *Cancer Research*, **77**(15).
22. Pang B, *et al.* (2019) Landscape of tumor suppressor long noncoding RNAs in breast cancer. *Journal of Experimental & Clinical Cancer Research: CR*, **38**(1), 79.

23. Taniguchi K and Karin M. (2014) IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Seminars in Immunology*, **26**(1), 54–74.
24. Shi T, Gao G and Cao Y. (2016) Long noncoding RNAs as novel biomarkers have a promising future in cancer diagnostics. *Disease Markers*, **2016**, 9085195.
25. Siegel RL, Miller KD and Jemal A. (2020) Cancer statistics, 2020. *CA: A Cancer Journal for Clinicians*, **70**(1), 7–30.
26. Liu L, Zhang Y and Lu J. (2020) The roles of long noncoding RNAs in breast cancer metastasis. *Cell Death & Disease*, **11**(9), 1–14.
27. Liu Y, Sharma S and Watabe K. (2015) Roles of lncRNA in breast cancer. *Frontiers in bioscience (Scholar edition)*, **7**, 94–108.
28. Zhang T, *et al.* (2019) Long non-coding RNA and breast cancer. *Technology in Cancer Research & Treatment*, **18**, 1533033819843889.
29. Bhan A and Mandal SS. (2015) LncRNA HOTAIR: A master regulator of chromatin dynamics and cancer. *Biochimica et Biophysica Acta (BBA) — Reviews on Cancer*, **1856**(1), 151–164.
30. Zhang S, *et al.* (2014) Long noncoding RNA HOTAIR as an independent prognostic marker in cancer: A meta-analysis. *PLoS One*, **9**(8).
31. Zhou X, Chen J and Tang W. (2014) The molecular mechanism of HOTAIR in tumorigenesis, metastasis, and drug resistance. *Acta biochimica et biophysica Sinica*, **46**(12), 1011–1015.
32. Yuan C, Ning Y and Pan Y. (2020) Emerging roles of HOTAIR in human cancer. *Journal of Cellular Biochemistry*, **121**(5-6), 3235–3247.
33. Mozdarani H, Ezzatizadeh V and Rahbar Parvaneh R. (2020) The emerging role of the long non-coding RNA HOTAIR in breast cancer development and treatment. *Journal of Translational Medicine*, **18**, 152.
34. Gupta RA, *et al.* (2010) Long noncoding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature*, **464**(7291), 1071–1076.
35. Yoon JH, *et al.* (2013) Scaffold function of long non-coding RNA HOTAIR in protein ubiquitination. *Nature Communications*, **4**, 2939.
36. Obaid M, Udden SMN, Deb P, Shihabeddin N, Zaki MH and Mandal SS. (2018) LncRNA HOTAIR regulates lipopolysaccharide-induced cytokine expression and inflammatory response in macrophages. *Scientific Reports*, **8**(1), 15670.
37. Liu H, *et al.* (2016) Expression of long non-coding RNA-HOTAIR in oral squamous cell carcinoma Tca8113 cells and its associated biological behavior. *American Journal of Translational Research*, **8**(11), 4726–4734.
38. Rinn JL, *et al.* (2007) Functional demarcation of active and silent chromatin domains in human HOX Loci by non-coding RNAs. *Cell*, **129**(7), 1311–1323.
39. Gupta RA, *et al.* (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature*, **464**(7291), 1071–1076.
40. Kotake Y, *et al.* (2011) Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15INK4B tumor suppressor gene. *Oncogene*, **30**(16), 1956–1962.

41. Bhan A, Hussain I, Ansari KI, Kasiri S, Bashyal A and Mandal SS. (2013) Antisense-transcript long noncoding RNA (lncRNA) HOTAIR is transcriptionally induced by estradiol. *Journal of Molecular Biology,* **425**(19), 3707–3722.
42. Bhan A, Hussain I, Ansari KI, Bobzean SAM, Perrotti LI and Mandal SS. (2014) Bisphenol-A and diethylstilbestrol exposure induces the expression of breast cancer associated long noncoding RNA HOTAIR *in vitro* and *in vivo*. *The Journal of Steroid Biochemistry and Molecular Biology*, **141**, 160–170.
43. Collina F, *et al.* (2019) LncRNA HOTAIR up-regulation is strongly related with lymph nodes metastasis and LAR subtype of triple negative breast cancer. *Journal of Cancer*, **10**(9), 2018–2024.
44. Wang L, *et al.* (2013) BRCA1 is a negative modulator of the PRC2 complex. *The EMBO Journal*, **32**(11), 1584–1597.
45. Zhang KJ, Tan XL and Guo L. (2020) The long non-coding RNA DANCR regulates the inflammatory phenotype of breast cancer cells and promotes breast cancer progression via EZH2-dependent suppression of SOCS3 transcription. *Molecular Oncology*, **14**(2), 309–328.
46. Stone JK *et al.* (2019) Hypoxia induces cancer cell-specific chromatin interactions and increases MALAT1 expression in breast cancer cells. *The Journal of Biological Chemistry*, **294**(29), 11213–11224.
47. Li Z, *et al.* (2018) LncRNA MALAT1 promotes relapse of breast cancer patients with postoperative fever. *American Journal of Translational Research*, **10**(10), 3186–3197.
48. Miao Y, Fan R, Chen L and Qian H. (2016) Clinical significance of long noncoding RNA MALAT1 expression in tissue and serum of breast cancer. *Annals of Clinical & Laboratory Science*, **46**(4), 418–424.
49. Xu S, *et al.* (2015) Downregulation of long noncoding RNA MALAT1 induces epithelial-to-mesenchymal transition via the PI3K-AKT pathway in breast cancer. *International Journal of Clinical and Experimental Pathology*, **8**(5), 4881–4891.
50. Kim J, *et al.* (2018) Long non-coding RNA MALAT1 suppresses breast cancer metastasis. *Nature genetics*, **50**(12), 1705–1715.
51. Du Y, *et al.* (2017) LncRNA XIST acts as a tumor suppressor in prostate cancer through sponging miR-23a to modulate RKIP expression. *Oncotarget*, **8**(55), 94358–94370.
52. Zhang R, and Xia T. (2017) Long non-coding RNA XIST regulates PDCD4 expression by interacting with miR-21-5p and inhibits osteosarcoma cell growth and metastasis. *International Journal of Oncology*, **51**(5), 1460–1470.
53. Zheng R, *et al.* (2018) Long non-coding RNA XIST inhibited breast cancer cell growth, migration, and invasion via miR-155/CDX1 axis. *Biochemical and Biophysical Research Communications*, **498**(4), 1002–1008.
54. Prensner JR, *et al.* (2011) Transcriptome sequencing identifies PCAT-1, a novel lincRNA implicated in prostate cancer progression. *Nature biotechnology*, **29**(8), 742–749.

55. Prensner JR, *et al.* (2014) The long non-coding RNA *PCAT-1* promotes prostate cancer cell proliferation through cMyc. *Neoplasia (New York, NY)*, **16**(11), 900–908.
56. Shang Z, *et al.* (2019) LncRNA PCAT1 activates AKT and NF-κB signaling in castration-resistant prostate cancer by regulating the PHLPP/FKBP51/IKKα complex. *Nucleic Acids Research*, **47**(8), 4211–4225.
57. Schalken JA, Hessels D and Verhaegh G. (2003) New targets for therapy in prostate cancer: Differential display code 3 (DD3PCA3), a highly prostate cancer–specific gene. *Urology*, **62**(5, Supplement 1), 34–43.
58. Lemos AEG, Matos ADR, Ferreira LB and Gimba ERP. (2019) The long noncoding RNA PCA3: An update of its functions and clinical applications as a biomarker in prostate cancer. *Oncotarget*, **10**(61), 6589–6603.
59. Lemos AEG, Ferreira LB, Batoreu NM, de Freitas PP, Bonamino MH and Gimba ERP. (2016) PCA3 long noncoding RNA modulates the expression of key cancer-related genes in LNCaP prostate cancer cells. *Tumor Biology*, **37**(8), 11339–11348.
60. Hung C-L, *et al.* (2014) A long noncoding RNA connects c-Myc to tumor metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, **111**(52), 18697–18702.
61. Walsh AL, Tuzova AV, Bolton EM, Lynch TH and Perry AS. (2014) Long noncoding RNAs and prostate carcinogenesis: The missing 'linc'? *Trends in Molecular Medicine*, **20**(8), 428-436.
62. Yang L, *et al.* (2013) lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. *Nature*, **500**(7464), 598–602.
63. Ren S, *et al.* (2012) RNA-seq analysis of prostate cancer in the Chinese population identifies recurrent gene fusions, cancer-associated long noncoding RNAs and aberrant alternative splicings. *Cell Research*, **22**(5), 806–821.
64. Wang F, *et al.* (2014) Development and prospective multicenter evaluation of the long noncoding RNA MALAT-1 as a diagnostic urinary biomarker for prostate cancer. *Oncotarget*, **5**(22), 11091–11102.
65. Chang J, Xu W, Du X and Hou J. (2018) MALAT1 silencing suppresses prostate cancer progression by upregulating miR-1 and downregulating KRAS. *OncoTargets and Therapy*, **11**, 3461–3473.
66. Wen S, Wei Y, Zen C, Xiong W, Niu Y and Zhao Y. (2020) Long non-coding RNA NEAT1 promotes bone metastasis of prostate cancer through N6-methyladenosine. *Molecular Cancer*, **19**(1), 171.
67. Lingadahalli S, *et al.* (2018) Novel lncRNA LINC00844 regulates prostate cancer cell migration and invasion through AR signaling. *Molecular Cancer Research*, **16**(12), 1865–1878.
68. Zhang Y, *et al.* (2018) Analysis of the androgen receptor-regulated lncRNA landscape identifies a role for ARLNC1 in prostate cancer progression. *Nature Genetics*, **50**(6), 814–824.

69. You Z, *et al.* (2019) LncRNA CCAT1 promotes prostate cancer cell proliferation by interacting with DDX5 and MIR-28-5P. *Molecular Cancer Therapeutics*, **18**(12), 2469–2479.
70. Jin D, Song Y, Chen Y and Zhang P. (2020) Identification of three lncRNAs as potential predictive biomarkers of lung adenocarcinoma. *BioMed Research International*, **2020**.
71. Herrera-Solorio AM, Armas-López L, Arrieta O, Zúñiga J, Piña-Sánchez P and Ávila-Moreno F. (2017) Histone code and long non-coding RNAs (lncRNAs) aberrations in lung cancer: Implications in the therapy response. *Clinical Epigenetics*, **9**.
72. Ji P, *et al.* (2003) MALAT-1, a novel noncoding RNA, and thymosin β 4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene*, **22**(39), 8031–8041.
73. Yoshimoto R, Mayeda A, Yoshida M and Nakagawa S. (2016) MALAT1 long non-coding RNA in cancer. *Biochimica et Biophysica Acta (BBA) — Gene Regulatory Mechanisms*, **1859**(1), 192–199.
74. Zhao G, Su Z, Song D, Mao Y and Mao X. (2016) The long noncoding RNA MALAT1 regulates the lipopolysaccharide-induced inflammatory response through its interaction with NF-κB. *FEBS Letters*, **590**(17), 2884–2895.
75. Jeffers LK, *et al.* (2013) Correlation of transcription of MALAT-1, a novel noncoding RNA, with deregulated expression of tumor suppressor p53 in small DNA tumor virus models. *Journal of Cancer Therapy*, **4**(3).
76. Gutschner T, *et al.* (2013) The noncoding RNA *MALAT1* is a critical regulator of the metastasis phenotype of lung cancer cells. *Cancer Research*, **73**(3), 1180–1189.
77. Fang S, *et al.* (2018) H3K27me3 induces multidrug resistance in small cell lung cancer by affecting HOXA1 DNA methylation via regulation of the lncRNA HOTAIR. *Annals of Translational Medicine*, **6**(22).
78. Liu Z, *et al.* (2013) The long noncoding RNA HOTAIR contributes to cisplatin resistance of human lung adenocarcinoma cells via downregualtion of p21WAF1/CIP1 expression. *PLoS One*, **8**(10).
79. Nakagawa T, *et al.* (2013) Large noncoding RNA HOTAIR enhances aggressive biological behavior and is associated with short disease-free survival in human non-small cell lung cancer. *Biochemical and Biophysical Research Communications*, **436**(2), 319–324.
80. Li N, *et al.* (2017) Identification of circulating long noncoding RNA HOTAIR as a novel biomarker for diagnosis and monitoring of non–small cell lung cancer. *Technology in Cancer Research & Treatment*, **16**(6), 1060–1066.
81. Nie F-Q, *et al.* (2015) Long noncoding RNA ANRIL promotes non–small cell lung cancer cell proliferation and inhibits apoptosis by silencing KLF2 and P21 expression. *Molecular Cancer Therapeutics*, **14**(1), 268–277.

82. Liu S-Y, Zhao Z-Y, Qiao Z, Li S-M and Zhang W-N. (2021) LncRNA PCAT1 interacts with DKC1 to regulate proliferation, invasion and apoptosis in NSCLC cells via the VEGF/AKT/Bcl2/Caspase9 pathway. *Cell Transplantation*, **30**.
83. Wang L, Cho KB, Li Y, Tao G, Xie Z and Guo B. (2019) Long noncoding RNA (lncRNA)-mediated competing endogenous RNA networks provide novel potential biomarkers and therapeutic targets for colorectal cancer. *International Journal of Molecular Sciences,* **20**(22).
84. Ye L-C, Zhu D-X, Qiu J-J, Xu J and Wei YE. (2015) Involvement of long noncoding RNA in colorectal cancer: From benchtop to bedside (Review). *Oncology Letters*, **9**(3), 1039–1045.
85. Bian Z, *et al.* (2018) LncRNA–FEZF1-AS1 promotes tumor proliferation and metastasis in colorectal cancer by regulating PKM2 signaling. *Clinical Cancer Research*, **24**(19), 4808–4819.
86. Ghafouri-Fard S, Esmaeili M and Taheri M. (2020) H19 lncRNA: Roles in tumorigenesis. *Biomedicine & Pharmacotherapy*, **123**, 109774.
87. Raveh E, Matouk IJ, Gilon M and Hochberg A. (2015) The H19 Long non-coding RNA in cancer initiation, progression and metastasis — A proposed unifying theory. *Molecular Cancer*, **14**(1), 184.
88. Liang W-C, *et al.* (2015) The lncRNA H19 promotes epithelial to mesenchymal transition by functioning as miRNA sponges in colorectal cancer. *Oncotarget*, **6**(26), 22513–22525.
89. Zhong M-E, Chen Y, Zhang G, Xu L, Ge W and Wu B. (2019) LncRNA H19 regulates PI3K–Akt signal pathway by functioning as a ceRNA and predicts poor prognosis in colorectal cancer: Integrative analysis of dysregulated ncRNA-associated ceRNA network. *Cancer Cell International*, **19**.
90. Han P, *et al.* (2017) The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/β-catenin signaling. *Molecular Cancer*, **16**.
91. Ellis BC, Graham LD and Molloy PL. (2014) CRNDE, a long non-coding RNA responsive to insulin/IGF signaling, regulates genes involved in central metabolism. *Biochimica et Biophysica Acta (BBA) — Molecular Cell Research*, **1843**(2), 372–386.
92. Nissan A, *et al.* (2012) Colon cancer associated transcript-1: A novel RNA expressed in malignant and pre-malignant human tissues. *International Journal of Cancer*, **130**(7), 1598–1606.
93. Alaiyan B, *et al.* (2013) Differential expression of colon cancer associated transcript1 (CCAT1) along the colonic adenoma-carcinoma sequence. *BMC Cancer*, **13**, 196.
94. Ling H, *et al.* (2013) CCAT2, a novel noncoding RNA mapping to 8q24, underlies metastatic progression and chromosomal instability in colon cancer. *Genome Research*, **23**(9), 1446–1461.

95. Peng CL, Zhao XJ, Wei CC and Wu JW. (2019) LncRNA HOTAIR promotes colon cancer development by down-regulating miRNA-34a. *European Review for Medical and Pharmacological Sciences*, **23**(13), 5752–5761.
96. Yang M-H, *et al.* (2015) MALAT1 promotes colorectal cancer cell proliferation/migration/invasion via PRKA kinase anchor protein 9. *Biochimica et Biophysica Acta*, **1852**(1), 166–174.
97. Tian W, Du Y, Ma Y, Gu L, Zhou J and Deng D. (2018) MALAT1 — miR663a negative feedback loop in colon cancer cell functions through direct miRNA–lncRNA binding. *Cell Death & Disease*, **9**(9), 1–12.
98. Reon BJ, *et al.* (2016) Expression of lncRNAs in low-grade gliomas and glioblastoma multiforme: An in silico analysis. *PLoS Medicine*, **13**(12).
99. Pop S, Enciu A-M, Necula LG and Tanase C. (2018) Long non-coding RNAs in brain tumours: Focus on recent epigenetic findings in glioma. *Journal of Cellular and Molecular Medicine*, **22**(10), 4597.
100. Kraus TFJ, Greiner A, Guibourt V, Lisec K and Kretzschmar HA. (2015) Identification of stably expressed lncRNAs as valid endogenous controls for profiling of human glioma. *Journal of Cancer*, **6**(2), 111–119.
101. Zhang X, *et al.* (2012) Long non-coding RNA expression profiles predict clinical phenotypes in glioma. *Neurobiology of Disease*, **48**(1), 1–8.
102. Wang Y, Wang Y, Li J, Zhang Y, Yin H and Han B. (2015) CRNDE, a long-noncoding RNA, promotes glioma cell growth and invasion through mTOR signaling. *Cancer Letters*, **367**(2), 122–128.
103. Xavier-Magalhães A, *et al.* (2018) The long non-coding RNA HOTAIR is transcriptionally activated by HOXA9 and is an independent prognostic marker in patients with malignant glioma. *Oncotarget*, **9**(21), 15740–15756.
104. Cheng Z, *et al.* (2017) Long non-coding RNA XIST promotes glioma tumorigenicity and angiogenesis by acting as a molecular sponge of miR-429. *Journal of Cancer*, **8**(19), 4106–4116.
105. Chen W, *et al.* (2015) miR-429 inhibits glioma invasion through BMK1 suppression. *Journal of Neuro-Oncology*, **125**(1), 43–54.
106. Chen Q, *et al.* (2018) Long noncoding RNA NEAT1, regulated by the EGFR pathway, contributes to glioblastoma progression through the WNT/β-Catenin pathway by scaffolding EZH2. *Clinical Cancer Research*, **24**(3), 684–695.
107. Zeng T, Li L, Zhou Y and Gao L. (2018) Exploring long noncoding RNAs in glioblastoma: Regulatory mechanisms and clinical potentials. *International Journal of Genomics*.
108. Zhou M, *et al.* (2016) Construction and analysis of dysregulated lncRNA-associated ceRNA network identified novel lncRNA biomarkers for early diagnosis of human pancreatic cancer. *Oncotarget*, **7**(35), 56383–56394.
109. Giulietti M, Righetti A, Principato G and Piva F. (2018) LncRNA co-expression network analysis reveals novel biomarkers for pancreatic cancer. *Carcinogenesis*, **39**(8), 1016–1025.

110. Wu B, *et al.* (2018) Novel three-lncRNA signature predicts survival in patients with pancreatic cancer. *Oncology Reports*, **40**(6), 3427–3437.
111. Cai H, *et al.* (2016) Epigenetic inhibition of miR-663b by long non-coding RNA HOTAIR promotes pancreatic cancer cell proliferation via up-regulation of insulin-like growth factor 2. *Oncotarget*, **7**(52), 86857–86870.
112. Wei GH and Wang X. (2017) lncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway. *European Review for Medical and Pharmacological Sciences*, **21**(17), 3850–3856.
113. Zhang L, Kang W, Lu X, Ma S, Dong L and Zou B. (2018) LncRNA CASC11 promoted gastric cancer cell proliferation, migration and invasion in vitro by regulating cell cycle pathway. *Cell Cycle*, **17**(15), 1886–1900.
114. Wei L, *et al.* (2020) Noncoding RNAs in gastric cancer: Implications for drug resistance. *Molecular Cancer*, **19**(1), 62.
115. Huang Z, Zhou J-K, Peng Y, He W and Huang C. (2020) The role of long noncoding RNAs in hepatocellular carcinoma. *Molecular Cancer*, **19**(1), 77.
116. Lim LJ. *et al.* (2019) Roles and regulation of long noncoding RNAs in hepatocellular carcinoma. *Cancer Research*, **79**(20), 5131–5139.
117. Quan J, *et al.* (2018) LncRNA as a diagnostic and prognostic biomarker in bladder cancer: A systematic review and meta-analysis. *OncoTargets and Therapy*, **11**, 6415–6424.
118. Liu W, Zhang Y, Chen M, Shi L, Xu L and Zou X. (2018) A genome-wide analysis of long noncoding RNA profile identifies differentially expressed lncRNAs associated with Esophageal cancer. *Cancer Medicine*, **7**(8), 4181–4189.
119. Garzon R, *et al.* (2014) Expression and prognostic impact of lncRNAs in acute myeloid leukemia. *Proceedings of the National Academy of Sciences of the United States*, **111**(52), 18679–18684.
120. Shi J, Dai R, Chen Y, Guo H, Han Y and Zhang Y. (2019) LncRNA LINP1 regulates acute myeloid leukemia progression via HNF4α/AMPK/WNT5A signaling pathway. *Hematology & Oncology*, **37**(4), 474–482.
121. Zeng C, Yu X, Lai J, Yang L, Chen S and Li Y. (2015) Overexpression of the long non-coding RNA PVT1 is correlated with leukemic cell proliferation in acute promyelocytic leukemia. *Journal of Hematology & Oncology*, **8**, 126.
122. Verma A, Jiang Y, Du W, Fairchild L, Melnick A and Elemento O. (2015) Transcriptome sequencing reveals thousands of novel long non-coding RNAs in B cell lymphoma. *Genome Medicine,* **7**, 110.
123. Kuper H, Adami HO and Trichopoulos D. (2000) Infections as a major preventable cause of human cancer. *Journal of Internal Medicine*, **248**(3), 171–183.
124. Chen DS and Mellman I. (2013) Oncology meets immunology: The cancer-immunity cycle. *Immunity*, **39**(1), 1–10.
125. Fok ET, Davignon L, Fanucchi S and Mhlanga MM. (2018) The lncRNA connection between cellular metabolism and epigenetics in trained immunity. *Frontiers in Immunology*, **9**, 3184.

126. Hu G, *et al.* (2013) Expression and regulation of lincRNAs during T cell development and differentiation. *Nature Immunology*, **14**(11), 1190–1198.
127. Li Y, *et al.* (2020) Pan-cancer characterization of immune-related lncRNAs identifies potential oncogenic biomarkers. *Nature Communications*, **11**(1), 1000.
128. Hayden MS and Ghosh S. (2012) NF-κB, the first quarter-century: Remarkable progress and outstanding questions. *Genes & Development*, **26**(3), 203–234.
129. Xia Y, Shen S and Verma IM. (2014) NF-κB, an active player in human cancers. *Cancer Immunology Research*, **2**(9), 823–30.
130. Mao X, Su Z and Mookhtiar AK. (2017) Long non-coding RNA: A versatile regulator of the nuclear factor-κB signalling circuit. *Immunology*, **150**(4), 379–388.
131. Obaid M, Udden SMN, Alluri P and Mandal SS. (2021) LncRNA HOTAIR regulates glucose transporter Glut1 expression and glucose uptake in macrophages during inflammation. *Scientific Reports*, **11**(1), 232.
132. Rapicavoli NA, Qu K, Zhang J, Mikhail M, Laberge RM and Chang HY. (2013) A mammalian pseudogene lncRNA at the interface of inflammation and anti-inflammatory therapeutics. *eLife*, **2**, e00762.
133. Zhu X, *et al.* (2019) Long noncoding RNA HOXA-AS3 integrates NF-κB signaling to regulate endothelium inflammation. *Molecular and Cellular Biology*, **39**(19).
134. Lu Y, *et al.* (2017) The NF-κB–responsive long noncoding RNA FIRRE regulates posttranscriptional regulation of inflammatory gene expression through interacting with hnRNPU. *The Journal of Immunology*, **199**(10), 3571–3582.
135. Krawczyk M and Emerson BM. (2014) p50-associated COX-2 extragenic RNA (PACER) activates COX-2 gene expression by occluding repressive NF-κB complexes. *eLife*, **3**, e01776.
136. Castellanos-Rubio A, *et al.* (2017) Cytoplasmic form of Carlr lncRNA facilitates inflammatory gene expression upon NF-κB activation. *The Journal of Immunology*, **199**(2), 581–588.
137. Chu Y, Liu Z, Liu J, Yu L, Zhang D and Pei F. (2020) Characterization of lncRNA-perturbed TLR-signaling network identifies novel lncRNA prognostic biomarkers in colorectal cancer. *Frontiers in Cell and Developmental Biology*, **8**, 503.
138. Fan H, *et al.* (2019) A novel lncRNA regulates the toll-like receptor signaling pathway and related immune function by stabilizing FOS mRNA as a competitive endogenous RNA. *Frontiers in Immunology*, **10**, 838.
139. Murphy MB and Medvedev AE. (2016) Long noncoding RNAs as regulators of Toll-like receptor signaling and innate immunity. *Journal of Leukocyte Biology*, **99**(6), 839–850.
140. Han X, *et al.* (2019) LncRNA PTPRE-AS1 modulates M2 macrophage activation and inflammatory diseases by epigenetic promotion of PTPRE. *Science Advances*, **5**(12), eaax9230.
141. Hu G, *et al.* (2016) LincRNA-Cox2 promotes late inflammatory gene transcription in macrophages through modulating SWI/SNF-mediated chromatin remodeling. *The Journal of Immunology*, **196**(6), 2799–2808.

142. Huang Z, *et al.* (2016) Identification of differentially expressed long non-coding RNAs in polarized macrophages. *Scientific Reports*, **6**(1), 19705.
143. Li X, Lei Y, Wu M and Li N. (2018) Regulation of macrophage activation and polarization by HCC-derived exosomal lncRNA TUC339. *International Journal of Molecular Sciences*, **19**(10), 2958.
144. Zhang J and Chu M. (2019) Targeting of IL-6-relevant long noncoding RNA profiles in inflammatory and tumorous disease. *Inflammation*, **42**(4), 1139–1146.
145. Puthanveetil P, Chen S, Feng B, Gautam A and Chakrabarti S. (2015) Long non-coding RNA MALAT1 regulates hyperglycaemia induced inflammatory process in the endothelial cells. *Journal of Cellular and Molecular Medicine*, **19**(6), 1418–1425.
146. Wang X, *et al.* (2016) Long non-coding RNA DILC regulates liver cancer stem cells via IL-6/STAT3 axis. *Journal of Hepatology*, **64**(6), 1283–1294.
147. Geng H, *et al.* (2018) In inflamed intestinal tissues and epithelial cells, interleukin 22 signaling increases expression of H19 long noncoding RNA, which promotes mucosal regeneration. *Gastroenterology*, **155**(1), 144–155.
148. Fan Y, Nan Y, Huang J, Zhong H and Zhou W. (2018) Up-regulation of inflammation-related LncRNA-IL7R predicts poor clinical outcome in patients with cervical cancer. *Bioscience Reports*, **38**(3).
149. Xue Z, *et al.* (2018) Identification of LncRNA Linc00513 containing lupus-associated genetic variants as a novel regulator of interferon signaling pathway. *Frontiers in Immunology*, **9**, 2967.
150. Li J, Yang C, Li Y, Chen A, Li L and You Z. (2018) LncRNA GAS5 suppresses ovarian cancer by inducing inflammasome formation. *Bioscience Reports*, **38**(2), BSR20171150.
151. Mongelli A, Martelli F, Farsetti A and Gaetano C. (2019) The dark that matters: Long non-coding RNAs as master regulators of cellular metabolism in non-communicable diseases. *Frontiers in Physiology*, **10**, 369.
152. Taheri M, Shoorei H, Dinger ME and Ghafouri-Fard S. (2020) Perspectives on the role of non-coding RNAs in the regulation of expression and function of the estrogen receptor. *Cancers (Basel)*, **12**(8), 2162.
153. Fu X, Zhu J, Zhang L and Shu J. (2019) Long non-coding RNA NEAT1 promotes steatosis via enhancement of estrogen receptor alpha-mediated AQP7 expression in HepG2 cells. *Artificial Cells*, *Nanomedicine*, *and Biotechnology*, **47**(1), 1782–1787.
154. Leung A, Amaram V and Natarajan R. (2019) Linking diabetic vascular complications with LncRNAs. *Vascular Pharmacology*, **114**, 139–144.
155. Gao L, *et al.* (2017) Circulating long noncoding RNA HOTAIR is an essential mediator of acute myocardial infarction. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology*, *Biochemistry*, *and Pharmacology*, **44**(4), 1497–1508.
156. Lu W, Zhu L, Ruan Z-B, Wang M-X, Ren Y and Li W. (2018) HOTAIR promotes inflammatory response after acute myocardium infarction by upregulating RAGE. *European Review for Medical and Pharmacological Sciences*, **22**(21), 7423–7430.

157. Sato M, *et al.* (2021) The lncRNA Caren antagonizes heart failure by inactivating DNA damage response and activating mitochondrial biogenesis. *Nature Communications*, **12**(1), 2529.
158. Kenneweg F, *et al.* (2019) Long noncoding RNA-enriched vesicles secreted by hypoxic cardiomyocytes drive cardiac fibrosis. *Molecular Therapy — Nucleic Acids*, **18**, 363–374.
159. Puthanveetil P, Chen S, Feng B, Gautam A and Chakrabarti S. (2015) Long non-coding RNA MALAT1 regulates hyperglycaemia induced inflammatory process in the endothelial cells. *Journal of Cellular and Molecular Medicine*, **19**(6), 1418–14125.
160. Zhou X, Zhang W, Jin M, Chen J, Xu W and Kong X. (2017) lncRNA MIAT functions as a competing endogenous RNA to upregulate DAPK2 by sponging miR-22-3p in diabetic cardiomyopathy. *Cell Death & Disease*, **8**(7), e2929.
161. Grammatikakis I, Panda AC, Abdelmohsen K and Gorospe M. (2014) Long noncoding RNAs(lncRNAs) and the molecular hallmarks of aging. *Aging (Albany NY)*, **6**(12), 992–1009.
162. Samper E, Flores JM and Blasco MA. (2001) Restoration of telomerase activity rescues chromosomal instability and premature aging in Terc-/- mice with short telomeres. *EMBO Reports*, **2**(9), 800–807,
163. Redon S, Reichenbach P and Lingner J. (2010) The non-coding RNA TERRA is a natural ligand and direct inhibitor of human telomerase. *Nucleic Acids Research*, **38**(17), 5797–5806.
164. Cabili MN, *et al.* (2011) Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes & Development*, **25**(18), 1915–1927.
165. Mercer TR, Dinger ME and Mattick JS. (2009) Long non-coding RNAs: Insights into functions. *Nature Reviews Genetics*, **10**(3), 155–159.
166. Arita T, *et al.* (2013) Circulating long non-coding RNAs in plasma of patients with gastric cancer. *Anticancer Research*, **33**(8), 3185–3193.
167. Sharma S. (2009) Tumor markers in clinical practice: General principles and guidelines. *Indian Journal of Medical and Paediatric Oncology*, **30**(1), 1–8.
168. Herszényi L, *et al.* (2008) Tumor marker utility and prognostic relevance of cathepsin B, cathepsin L, urokinase-type plasminogen activator, plasminogen activator inhibitor type-1, CEA and CA 19-9 in colorectal cancer. *BMC Cancer*, **8**, 194.
169. Xie Y, *et al.* (2018) Circulating long noncoding RNA act as potential novel biomarkers for diagnosis and prognosis of non-small cell lung cancer. *Molecular Oncology*, **12**(5), 648–658.
170. Truong M, Yang B and Jarrard D. (2013) Towards the detection of prostate cancer in urine: A critical analysis. *The Journal of Urology*, **189**(2), 422–429.
171. Shao Y, *et al.* (2014) Gastric juice long noncoding RNA used as a tumor marker for screening gastric cancer. *Cancer*, **120**(21), 3320–3328.

172. Guo F, *et al.* (2015) Expression of MALAT1 in the peripheral whole blood of patients with lung cancer. *Biomedical Reports*, **3**(3), 309–312.
173. Wang X-S, *et al.* (2006) Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. *Clinical Cancer Research*, **12**(16), 4851–4858.
174. Zhang J-X, *et al.* (2013) HOTAIR, a cell cycle–associated long noncoding RNA and a strong predictor of survival, is preferentially expressed in classical and mesenchymal glioma. *Neuro-Oncology*, **15**(12), 1595–1603.
175. He C, Jiang B, Ma J and Li Q. (2016) Aberrant NEAT1 expression is associated with clinical outcome in high grade glioma patients. *APMIS*, **124**(3), 169–174.
176. Lucere KM, O'Malley MMR and Diermeier SD. (2020) Functional screening techniques to identify long non-coding RNAs as therapeutic targets in cancer. *Cancers*, **12**(12), 3695.
177. Xing Z, *et al.* (2014) LncRNA directs cooperative epigenetic regulation downstream of chemokine signals. *Cell*, **159**(5), 1110–1125.
178. Awasthee N, Rai V, Verma SS, Sajin Francis K, Nair MS and Gupta SC. (2018) Anti-cancer activities of Bharangin against breast cancer: Evidence for the role of NF-κB and lncRNAs. *Biochimica et Biophysica Acta (BBA) — General Subjects*, **1862**(12), 2738–2749.
179. Zhao W, *et al.* (2015) HOTAIR is a predictive and prognostic biomarker for patients with advanced gastric adenocarcinoma receiving fluorouracil and platinum combination chemotherapy. *American Journal of Translational Research*, **7**(7), 1295–1302.
180. Sun J, *et al.* (2020) Identification of tumor immune infiltration-associated lncRNAs for improving prognosis and immunotherapy response of patients with non-small cell lung cancer. *The Journal for ImmunoTherapy of Cancer*, **8**(1).
181. Li G, *et al.* (2021) LIMIT is an immunogenic lncRNA in cancer immunity and immunotherapy. *Nature Cell Biology*, **3**(5), 526–537.
182. Wang L, *et al.* (2018) Integrative genome-wide analysis of long noncoding RNAs in diverse immune cell types of melanoma patients. *Cancer Research*, **78**(15), 4411–4423,
183. Hu Q, *et al.* (2019) Oncogenic lncRNA downregulates cancer cell antigen presentation and intrinsic tumor suppression. *Nature Immunology*, **20**(7), 835–851.
184. Chen G, *et al.* (2013) LncRNADisease: A database for long-non-coding RNA-associated diseases. *Nucleic Acids Research*, **41**(D1), D983–D986.
185. Ning S, *et al.* (2016) Lnc2Cancer: A manually curated database of experimentally supported lncRNAs associated with various human cancers. *Nucleic Acids Research*, **44**(D1), D980–D985.
186. Carlevaro-Fita J, *et al.* (2020) Cancer LncRNA census reveals evidence for deep functional conservation of long noncoding RNAs in tumorigenesis. *Communications Biology*, **3**(1), 56.

187. Jiang M-C, Ni J-J, Cui W-Y, Wang B-Y and Zhuo W. (2019) Emerging roles of lncRNA in cancer and therapeutic opportunities. *American Journal of Cancer Research*, **9**(7), 1354–1366.
188. Qian Y, Shi L and Luo Z. (2020) Long non-coding RNAs in cancer: Implications for diagnosis, prognosis, and therapy. *Frontiers in Medicine*, **7**, 612393.

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Chapter 12

The Epitranscriptome and Cancer

J. David Rawn[1,*] and Gerald M. Wilson[2,**]

[1]*Department of Chemistry, Towson University, Towson, MD 21252, USA, and*
[2]*Department of Biochemistry and Molecular Biology and Marlene and Stewart Greenebaum NCI Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA*

Abstract

With the exception of some RNA viruses, genomic DNA provides the template for synthesis of all RNA molecules needed for life, based on a four-letter code that is transcribed into nascent RNA with high fidelity. However, chemical analyses of cellular RNA molecules have revealed over 170 distinct modified nucleobase forms that are found with varying frequencies in all major types of RNA. This heterogeneous and dynamic population of RNA modifications has become known as the "epitranscriptome." In ribosomal (rRNA) and transfer RNA (tRNA), many of these modifications are critical for translation. In messenger RNA (mRNA), post-transcriptional chemical modifications of select nucleobases have important regulatory roles, modulating pre-mRNA processing, nucleocytoplasmic export, mRNA decay, and translational efficiency. Newly discovered modifications in long non-coding RNA (lncRNA) and

*Corresponding author's email: jdrawn@towson.edu
**Corresponding author's email: gwilson@som.umaryland.edu

microRNA (miRNA) can modulate their biogenesis and *trans*-acting gene regulatory functions. In this chapter, we describe the major types of RNA modifications found in coding and non-coding RNAs. We also discuss the cellular machinery responsible for generating and removing these modifications ("writers" and "erasers," respectively), and factors that mediate their regulatory functions ("readers"). Since RNA modifications influence many aspects of gene expression, disruption of these regulatory processes can impact the development and progression of cancer in diverse ways. Recent discoveries in this area have defined mechanistic links between the epitranscriptome and cancer progression, the potential utility of modified RNAs as cancer biomarkers, and a palette of RNA-modifying enzymes that may serve as targets for development of novel anti-cancer therapeutics.

1. Structural modifications of RNA molecules

In all living things, RNA is required for every stage of gene expression. Several forms play key mechanical roles, including messenger RNA (mRNA), which carries information encoded within genomic DNA to the ribosomes where that information may serve as a template for protein synthesis. The ribosomes contain both ribosomal RNA (rRNA) and proteins, with the RNA moiety responsible for critical structural and catalytic functions, while transfer RNA (tRNA) molecules deliver specific amino acids to translating ribosomes. In eukaryotes, some additional small RNAs are essential for RNA-processing mechanisms including the small nuclear RNAs (snRNAs) that organize assembly of the small nuclear ribonucleoprotein (snRNP) complexes required for pre-mRNA splicing and the small nucleolar RNAs (snoRNAs) involved in posttranscriptional maturation of rRNAs. Additional classes of RNAs resident in higher eukaryotes play regulatory roles, including microRNA (miRNA) and long noncoding RNA (lncRNA).

Since RNA molecules are encoded by genomic DNA, the information content of the initial RNA copy, called the primary transcript, is limited to a linear polymer composed of the four canonical nucleobases of adenine (A), cytosine (C), guanine (G), and uracil (U) linked through a ribose-phosphate backbone. However, over the past six decades, many covalently

modified nucleobases have been identified in cellular RNA, first in rRNA and tRNA molecules, but now expanding into other forms of RNA. Research over the last decade, in particular, has revealed an explosion of chemical diversification in RNA. This now greatly-expanded palette of nucleobases, together with exciting developments in understanding the function and regulation of RNA modifications, has led to their collective designation as the "epitranscriptome" [1]. Growing evidence also demonstrates that many of these modifications are reversible, indicating that they can be dynamically regulated in response to cellular conditions [2–5]. Structural characterization of this plethora of modified nucleobases has been facilitated by a variety of experimental methods, with nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry now playing dominant roles [6, 7]. While the positions of many modified bases in rRNA and tRNA have long been established, more comprehensive surveys of modification sites in mRNA, lncRNA, and miRNA are benefitting from next-generation and now single-molecule sequencing approaches, analyzed using sophisticated computational methods including applications of artificial intelligence [7–9].

In this chapter, we will focus on chemical modifications of RNAs that generate non-canonical nucleobases or, in the case of rRNA, methylate the ribose 2′-OH group. This will exclude the 7-methylguanosine cap appended to the 5′-end of the vast majority of mRNAs, as well as RNA editing mechanisms that convert individual RNA nucleobases to distinct canonical forms such as the A-to-inosine (I) editing mediated by ADAR (adenosine deaminase, RNA-specific) proteins and the C-to-U editing performed by the APOBEC (apolipoprotein B mRNA editing catalytic polypeptide-like) family of cytosine deaminases. Dysregulation of these latter mechanisms can also contribute to cancer and is reviewed extensively elsewhere [10–12].

2. Major modifications of mRNA, lncRNA, and miRNA

Among the myriad modified nucleobases that exist in cellular RNA, five of the most abundant are shown in Figure 1. They are among the most

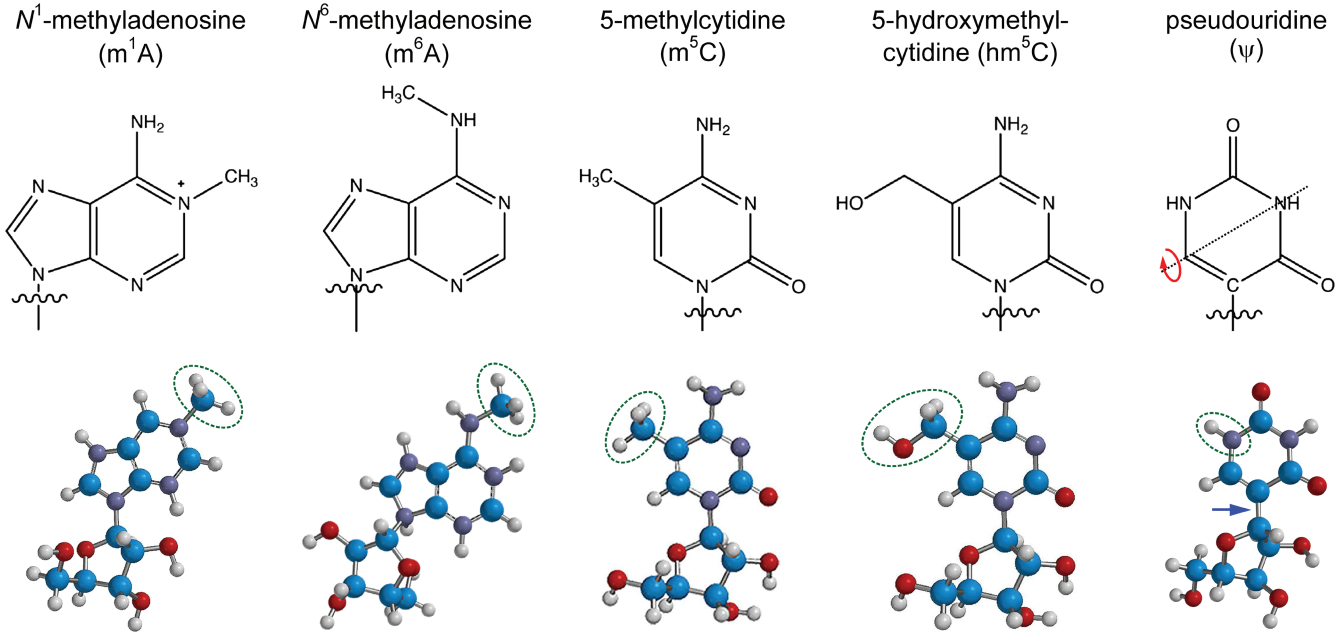

Figure 1. Names and abbreviations of five of the most common base-modified nucleosides in RNA (top) with bond line diagrams of associated nucleobases (middle).

Molecular models of modified ribonucleosides (bottom) highlighting adducts on m^1A, m^6A, m^5C, and hm^5C (dashed ellipses). ψ results from rotation of uridine 180° about the N3-C6 axis (red arrow and dotted line); this replaces the normal N1-C1′ glycosidic bond by a C-C linkage (blue arrow) while adding a new H-bond donor at N5 (dashed ellipse).

common base modifications found in mRNA and lncRNA, with some also identified in miRNAs. Additionally, these modifications are found in varying extents in rRNA and tRNA, which will be discussed in later sections.

From a structural standpoint, these chemical modifications of canonical nucleobases might seem modest. However, they can modulate the biochemical properties and functions of RNA molecules in two principal ways. First, most of these modifications alter RNA base pair potential. For example, the positive charge resulting from adenosine methylation at N1 in m^1A strongly disrupts A-form RNA helices, leading to local duplex melting [13]. Conversely, methylation of cytidine at C5 does not obstruct RNA base pair interactions, rather, the increased hydrophobicity of m^5C relative to unmodified C stabilizes RNA duplexes by enhancing base stacking and the strength of H-bonds to guanine [14]. The isomerization of uridine to ψ yields yet another consequence on RNA structure since, given some conformational flexibility, ψ can stabilize RNA duplexes when paired with any base [15]. The second method by which RNA modifications impact RNA is by providing unique molecular determinants that serve as docking sites for selective recruitment of regulatory factors. These concepts are discussed in detail below.

The biogenesis, function, and removal of these modified nucleobases involves the activity of three classes of cellular machinery, which can range in complexity from single enzymes to large multienzyme complexes. Enzymes that generate each base modification are called "writers" while those that remove modifications are called "erasers." The principal writers and erasers of m^1A, m^6A, m^5C, hm^5C, and ψ are shown in Figure 2. mRNA is modified by the same enzymatic machinery that targets applicable sites on other RNA molecules, particularly tRNA [9]. Accordingly, many mRNA modifications are catalyzed at sites mimicking the sequences and/or structural features present at tRNA modification sites. For example, synthesis of ψ on mRNA by pseudouridine synthase 4 (PUS4, also known as TruB pseudouridine synthase family member 1 (TRUB1)) requires a GUUCNANNC sequence and stem-loop structure analogous to that located within tRNA TψC arm [16]. The same motif is required for methylating adenosine to m^1A by the tRNA methyltransferase (TRMT)6/TRMT61A complex on both mRNA and tRNA targets [17]. Another example is the conversion of U to ψ by PUS7, which targets sequences

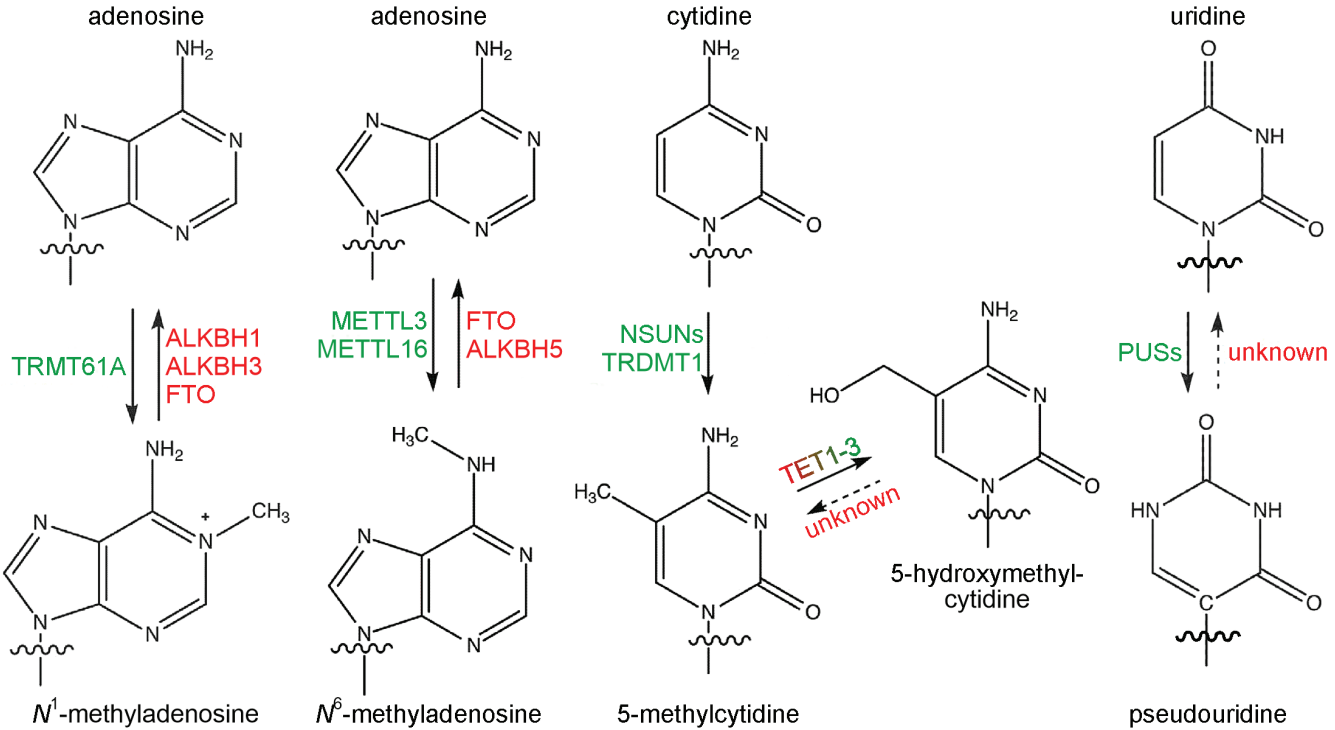

Figure 2. Writers and erasers of select modified nucleosides in mRNA, lncRNA, and tRNA.

Associated ribose sugars are not shown. Key writer enzymes are listed in green. For multi-subunit writers, only catalytic subunits are included. Erasers are listed in red. TET1-3 is shown in a color gradient because these proteins are erasers for 5-methylcytidine but writers for 5-hydroxymethylcytidine. Abbreviations: TRMT: tRNA methyltransferase; ALKBH: AlkB homolog; FTO: fat mass and obesity-associated protein; METTL: methyltransferase-like; NSUN: NOP2/Sun RNA methyltransferase; TRDMT: tRNA aspartic acid methyltransferase; TET: ten-eleven translocation; PUS: pseudouridine synthase.

within the context UGUAG (modified U is underlined) in mRNA, tRNA, and snRNA substrates [16, 18]. Proteins that bind modified bases and mediate their regulatory functions are called "readers." In this section, we discuss writers, readers, and erasers of some of the more common modifications in mRNA.

2.1. *N^6-methyladenosine (m^6A)*

m^6A is the most abundant modified nucleobase in eukaryotic mRNA, representing 0.1–0.4% of all adenosines in mRNA and lncRNAs, or 3–5 m^6A residues per molecule on average [19]. In mRNA, this modification is added co-transcriptionally in the nucleus, most frequently in coding regions and 3′-untranslated regions (UTR) (Fig. 3), and particularly in sequences encoded by long internal exons [20]. Deposition of m^6A impacts many aspects of mRNA metabolism including pre-mRNA processing, degradation, and translation efficiency [21–23]. While relatively rare, m^6A modifications in some mRNA 5′UTRs can also promote cap-independent translation [24]. The ability of m^6A residues to modulate most of these processes depends on their recognition by different reader proteins. However, deposition of m^6A in mRNA coding sequences can slow translation directly by decelerating codon recognition by cognate tRNAs [25].

m^6A is also found in miRNAs, snRNAs, snoRNAs, tRNAs, and rRNAs [26, 27]. In miRNAs, m^6A is normally enriched in unprocessed primary transcripts (pri-miRNAs) and aids in recruiting the nuclear Microprocessor complex to convert pri-miRNAs to pre-miRNAs early in the miRNA processing pathway [28]. However, in a growing number of cases, m^6A has also been found in mature, fully processed miRNAs, where

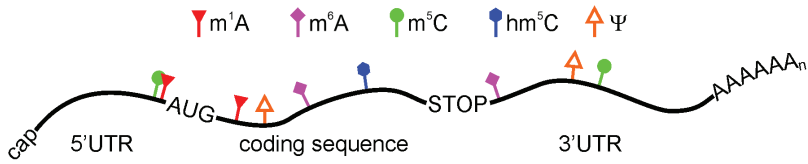

Figure 3. Distribution of major RNA base modifications in mRNA.

these residues change the ability of miRNA-loaded RNA-induced silencing complexes (RISC) to recognize and silence mRNA substrates [29, 30].

2.1.1. *m^{6}A writers and erasers*

m^{6}A modifications are added ("written") by a nuclear multi-subunit complex containing a heterodimer of methyltransferase-like 3 (METTL3) and METTL14, together with adapter proteins required for complex assembly [5]. METTL3 is the catalytic subunit that transfers a methyl group from *S*-adenosyl methionine (SAM) to the recipient adenosine residue, most frequently within G(m^{6}A)C and A(m^{6}A)C motifs [31], while METTL14 is an RNA-binding protein that recognizes cognate RNA substrates. An independent single-enzyme m^{6}A writer, METTL16, generates m^{6}A modifications at UAC(m^{6}A)GAGAA sequences in a subset of snRNAs and intronic sites in some pre-mRNAs [32, 33], while zinc finger CCHC-type containing 4 (ZCCHC4) and METTL5 catalyze N^6-methylation of specific adenosine residues in rRNAs [31].

Removal of ("erasing") the methyl group from m^{6}A to regenerate adenosine is principally performed by one of two specific demethylase enzymes: fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5), both members of the nonheme Fe(II)/α-ketoglutarate-dependent family of dioxygenases [5]. FTO demethylates a number of different methylated nucleobases in addition to m^{6}A and is active in both nuclear and cytoplasmic compartments [34]. Recent data suggest that the RNA substrate preferences of FTO may be influenced by interactions with splicing factor proline and glutamine-rich (SFPQ), by preferentially recruiting the demethylase to RNA regions directly targeted by SFPQ [35]. AlkB homolog 3 (ALKBH3) is another member of this dioxygenase family, but preferentially removes m^{6}A from tRNA rather than mRNA [27].

2.1.2. *m^{6}A readers and functions*

A variety of proteins have been identified that preferentially bind to m^{6}A-containing transcripts and mediate diverse functional consequences on targeted RNAs (Table 1). Recruitment of these proteins to or near sites of

Table 1. Select m^{6}A readers and their biological functions.

Protein(s)	Function	Refs.
Direct m^{6}A readers		
YTHDC1	Binds m^{6}A in nuclear ncRNA and mRNA; can modulate splicing and export to the cytoplasm	[21, 37]
YTHDF1	Binds m^{6}A in cytoplasmic mRNA and promotes translation	[23]
YTHDF2	Binds m^{6}A in cytoplasmic mRNA and promotes mRNA degradation	[20]
YTHDF3	Binds m^{6}A in cytoplasmic mRNA and promotes translation and mRNA degradation	[43]
eIF3	Binds m^{6}A sites in the 5′UTRs of cytoplasmic mRNAs and promotes cap-independent translation	[24]
Indirect m^{6}A readers		
hnRNP C	Principally binds intronic sequences in nuclear pre-mRNA and regulates splicing	[44]
hnRNP A2/B1	Binds pre-mRNA and miRNA precursors and regulates nuclear processing	[40, 41]
FMR1	May weakly bind m^{6}A; binds YTHDF2 directly and stabilizes m^{6}A-containing mRNAs	[20, 42]

Abbreviations: eIF3: eukaryotic translation initiation factor 3; FMR1: fragile X messenger ribonucleoprotein 1; hnRNP: heterogeneous nuclear ribonucleoprotein; ncRNA: noncoding RNA; YTHDC: YTH domain-containing protein; YTHDF: YTH domain family protein.

m^{6}A modification is achieved through one of two principal mechanisms [20]. First, some m^{6}A reader proteins recognize this modified base directly. These include a family of proteins containing a common RNA-binding module termed the YT521-B homology (YTH) domain that sandwiches m^{6}A residues between tyrosine and tryptophan side chains to form an "aromatic cage" [36]. One reader that employs this mechanism is YTH domain-containing protein 1 (YTHDC1), a nuclear protein that binds m^{6}A sites in pre-mRNA to promote recruitment of serine and arginine rich splicing factor 3 (SRSF3), resulting in preferential inclusion of nearby exons and enhanced nuclear export of processed mRNAs [21, 37]. A second example is YTH domain family protein 2 (YTHDF2), which recognizes m^{6}A residues in cytoplasmic mRNAs. In addition to their YTH m^{6}A-binding domains, YTHDF2 and its paralogs also contain extended low-complexity

regions rich in prion-like sequences that facilitate liquid-liquid phase separation of the proteins and their associated mRNAs [38]. In the case of YTHDF2, this principally targets m^6A-containing mRNAs to P bodies, subcellular assemblies of mRNA degradation factors [20].

The second class of m^6A readers associates with RNA substrates as an indirect consequence of m^6A modification. In some cases, these are recruited to RNA sequences where local RNA structural potential is weakened by the m^6A modification. In RNA duplexes, m^6A remains capable of forming base pairs with U, but does so at a 0.5–1.7 kcal/mol penalty relative to an A=U base pair [39]. This effectively lowers the melting temperature of local RNA structures and promotes presentation of proximal protein-binding sites as single-stranded RNA. An example of this mechanism is recruitment of heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1, which recognizes an unpaired RNA sequence motif that can be exposed by nearby m^6A modifications [40]. In pre-mRNAs, hnRNP A2/B1 binding can induce alternative splicing, while on primary miRNA transcripts, this factor recruits the Microprocessor complex by directly interacting with the DiGeorge syndrome critical region 8 (DGCR8) subunit, enhancing pri-mRNA processing to generate pre-miRNA intermediates [41]. Another example is fragile X messenger ribonucleoprotein 1 (FMR1), which may associate weakly with m^6A residues, but is more robustly recruited to modified RNA substrates by binding the direct m^6A reader protein YTHDF2 and stabilizing RNA targets by counteracting YTHDF2 function [42].

2.2. *N^1-methyladenosine (m^1A)*

The incidence of m^1A in mRNA is roughly 10-fold lower than m^6A, and is preferentially found in 5′UTRs and coding sequences, but underrepresented in 3′UTRs (Fig. 3). m^1A is particularly enriched in structured RNA regions within the mRNA 5′UTR and near the translation initiation site [45, 46]. The m^1A modification is typically present at only a single site on each affected mRNA, with ~20% of all mRNAs so modified in human cells [45]. m^1A is also present in other classes of RNA molecules including tRNA and rRNA [47, 48].

2.2.1. *m^1A writers and erasers*

To date, only one enzyme complex has been identified that methylates adenosine on N1. It consists of the proteins TRMT6, which binds target RNAs, and TRMT61A, which binds the SAM methyl donor and catalyzes the methyl transfer reaction [49]. This same complex generates m^1A in both mRNA and tRNA substrates, selecting target sites based on sequence and structural features similar to the tRNA TψC arm and loop [17]. Distinct but related enzymes TRMT61B and TRMT10C generate m^1A on mitochondrial RNAs [49].

The m^1A modification can be removed from mRNA, tRNA, and rRNA substrates by the dioxygenase enzyme ALKBH3 [27, 46]. For many coding and noncoding transcripts, methylation of specific adenosines at N1 is enhanced by cellular stresses including serum starvation and peroxide treatment [46], although it is currently unclear whether this results from activation of m^1A writers or inhibition of corresponding eraser enzymes. In tRNAs, distinct subsets of m^1A modifications are reversed by ALKBH3, FTO, and ALKBH1 [27, 34, 50]. Similar to mRNA, m^1A levels in tRNA can also be changed by cellular stress. For example, glucose deprivation reduces the m^1A-modified fractions of some tRNAs in HeLa cervical carcinoma cells by increasing expression of the m^1A eraser ALKBH1 [50].

2.2.2. *m^1A readers and functions*

In mRNA, the presence of m^1A residues is generally associated with increased translation efficiency [45] but the roles of specific reader proteins in enhancing protein synthesis are less well defined than for m^6A. The m^6A reader proteins YTHDF1-3 and YTHDC1, but not YTHDC2, can bind directly to m^1A in RNA [51], suggesting some similarities between the regulatory consequences of both epitranscriptomic marks. For example, YTHDF2 binding to m^1A-modified mRNAs accelerates their degradation, analogous to its role in destabilizing m^6A-containing mRNAs [52]. However, unlike m^6A, adenosine methylation at N1 generates a positive charge that has significantly stronger effects on local

RNA duplex stability than m^6A [13]. This suggests that m^1A might trigger more dramatic indirect or even reader-independent consequences on labeled RNAs than observed for m^6A. For example, the ability of m^1A to strongly disrupt local structure within mRNA 5′UTRs could enhance recruitment of 40S ribosome subunits downstream of the 5′-cap, or could accelerate scanning by ribosomal 43S preinitiation complexes through the 5′UTR.

For tRNAs, however, current data on the translational regulatory roles of m^1A are more complicated. While m^1A appears to enhance translation when deposited in mRNA, some m^1A modifications in tRNA are associated with suppression of protein synthesis. In particular, global translation rates are increased when tRNAs are demethylated by ALKBH3 and ALKBH1 [27, 50]. ALKBH3-demethylated tRNA also accelerates protein synthesis *in vitro*, indicating that the m^1A-inhibitory effect regulated by this m^1A eraser enzyme is not the result of tRNA depletion or fragmentation [27]. Enhanced demethylation of adenosine at N1 by ALKBH1 also inhibits protein synthesis in HeLa cells, but is accompanied by depletion of select tRNAs, including the translation initiator $tRNA^{iMet}$ [50]. By contrast, demethylation of m^1A on tRNA by the eraser FTO represses translation, indicating that this subset of m^1A modifications contributes positively to the efficiency of protein synthesis [34]. While mechanisms explaining this diverse array of m^1A effects on tRNA function remain unknown, part of the explanation will likely include the differing identities of tRNAs demethylated by each eraser protein, and the specific m^1A residues that they recognize.

2.3. *5-methylcytidine (m^5C)*

m^5C is best known as an epigenetic DNA modification that silences transcription [53]. However, m^5C is also found in every major form of RNA including tRNA, rRNA, mRNA, lncRNA, and more recently even in mature miRNAs [47, 48, 54–56]. Its incidence in mRNA is 3- to 10-fold lower than m^6A, ranging from 0.03–0.1% of all cytidine residues with as many as 10,000 m^5C sites identified across the human mRNA and lncRNA fractions [57, 58]. In mRNA, m^5C is most frequently found in the 5′ and 3′ untranslated regions [59].

2.3.1. *m^5C writers and erasers*

Methylation of cytidine on C5 is performed by members of the NOP2/Sun (NSUN) family of RNA methyltransferases and tRNA aspartic acid methyltransferase 1 (TRDMT1, also known as DNA methyltransferase member 2 or DNMT2) (Fig. 2). While several of these enzymes (NSUN2, NSUN3, NSUN6, and TRDMT1) generate m^5C at distinct locations on cytoplasmic tRNA targets [5], NSUN2 and NSUN6 are the major m^5C writers on mRNA. Cytidine residues methylated by NSUN2 are typically located within CNGGG (modified C is underlined) sequences that tend to be positioned at the 5′-end of a stem-loop structure, closely mirroring the sequence and structural features of known m^5C sites in tRNAs [59]. NSUN2 is also the principal m^5C writer for lncRNAs and miRNAs [5]. By contrast, a CTCCA motif capping a hairpin structure within the 3′UTR is the preferential mRNA sequence context for m^5C deposition by NSUN6 [60]. Other NSUN family members NSUN3 and NSUN4 modify mitochondrial tRNAs and rRNA, respectively, while NSUN1 and NSUN5 modify 28S rRNA during rRNA processing in the nucleolus [5].

Unlike m^6A and m^1A, methylation of cytidine at C5 does not appear to be directly reversible. Instead, currently known erasers of m^5C convert the residue to different modified forms. In nuclear and cytoplasmic RNAs, m^5C is oxidized to hm^5C by members of the ten-eleven translocation (TET) family of Fe(II)/α-ketoglutarate-dependent dioxygenases TET1, 2, and 3 (Fig. 2) [61]. These are the same enzymes responsible for erasing m^5C from DNA, but with key differences. In DNA, TET enzymes sequentially oxidize hm^5C into 5-formyl-2′-deoxycytidine and 5-carboxyl-2′-deoxycytidine, nucleosides that are recognized by thymine DNA glycosylase, permitting unmodified 2′-deoxycytidine to be regenerated via the DNA base excision repair pathway [62, 63]. In RNA however, TET enzymes do not appreciably oxidize hm^5C further [61]. m^5C can be oxidized to 5-formylcytidine (f^5C) by ALKBH1, but current data suggest that this modification is specific for the wobble position of mitochondrial tRNAs and functions to expand tRNA decoding potential in this organelle [64].

2.3.2. *m^5C readers and functions*

The consequences of m^5C on mRNA substrates vary according to the location of the modified nucleoside. In coding sequences, m^5C is associated with decreased translational efficiency [59]. Conversely, cytidine methylation by NSUN6, occurring principally in mRNA 3′UTRs, increases both the levels and translational efficiency of modified mRNAs, while also enhancing translation fidelity by suppressing readthrough at termination codons [60]. Mechanisms through which m^5C directs these effects on mRNA abundance and function remain unclear but may, in part, be mediated by newly-discovered m^5C reader proteins. One example is the Aly/REF export factor (ALYREF). ALYREF binding to mRNA is significantly enhanced by NSUN2-directed methylation of cytidine, and functions to enhance export of associated transcripts from the nucleus [65]. A second m^5C reader protein is Y-box binding protein 1 (YBX1), which slows turnover of some m^5C-modified mRNAs by recruiting the mRNA-stabilizing factor Hu antigen R (HuR) [66]. Roles of m^5C in rRNA and tRNA functions are discussed in sections 3 and 4, respectively.

2.4. *5-hydroxymethylcytidine (hm^5C)*

hm^5C is an oxidation product of m^5C (Fig. 2). Its abundance is very low in RNA, occurring approximately 5000-fold less frequently than m^5C [61]. In *Drosophila* and human cell lines, hm^5C is preferentially recovered in polyadenylated RNAs, with disproportionately higher representation in coding sequences versus UTRs [67, 68]. Some data indicate that hm^5C is enriched in the central nervous system, but comprehensive analysis of tissue distribution has not yet been reported [67, 69]. In *Drosophila*, this RNA modification is detected most abundantly in the brain, and is required for normal neural development [67].

2.4.1. *hm^5C writers and erasers*

In RNA, oxidation of m^5C to generate hm^5C is performed by the TET dioxygenase enzymes TET1, TET2, and TET3 as described in section

2.3.1. [61, 68]. However, some hm^5C is still recovered in RNA from cells lacking all of these enzymes [70], indicating that additional hm^5C writer proteins likely exist. No erasers of hm^5C are currently known.

2.4.2. *hm^5C readers and functions*

Relatively little is presently known about the function of hm^5C in RNA. In *Drosophila*, hm^5C-modified mRNAs are typically translated more efficiently than transcripts lacking this nucleoside [67]. Conversely, hm^5C reduces the stability of select transcripts encoding pluripotency factors in mouse embryonic stem cells [70]. While a few nuclear proteins have been identified that preferentially bind hm^5A-modified DNA substrates [71], no readers of hm^5C in RNA have been reported to date.

2.5. *Pseudouridine (ψ)*

ψ is an isomer of uridine where the nucleobase is flipped so that C5 is bonded directly to the 1′-carbon of ribose (Fig. 1). It is the most frequently modified base in rRNA and tRNA, and is incorporated at lower frequencies in all other major forms of RNA. Bulk ψ concentrations approach 0.3–0.4% of all uridines [72], similar to levels of m^6A.

ψ has been identified in hundreds of different mRNAs and lncRNAs [73, 74]. While this modification has been found at many locations throughout these transcripts, it is most frequently deposited within coding sequences [72, 74]. A recent study elegantly demonstrated that ψ modifications can be installed co-transcriptionally, and that within pre-mRNAs, they are roughly proportionately distributed between exons and introns [75]. Many consequences have been ascribed to ψ deposition in mRNA, including inhibition of mRNA decay and translation, as well as regulation of pre-mRNA splicing and 3′-processing [5, 75].

2.5.1. *ψ writers*

Two different mechanistic schemes are used to convert U to ψ. The first is RNA-dependent pseudouridylation, where an RNA scaffold is used to both assemble the catalytic machinery and target its delivery to RNA

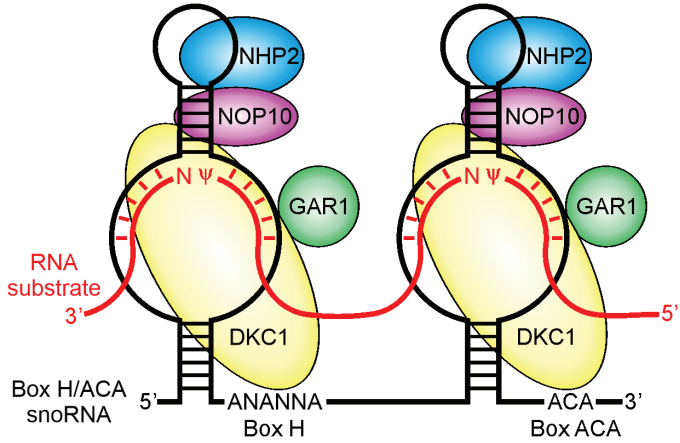

Figure 4. Architecture of H/ACA snoRNPs and recognition of RNA substrates for pseudouridylation.

Abbreviations: NHP2: non-histone protein 2; NOP10: nucleolar protein 10; GAR1: glycine-arginine-rich protein 1.

substrates. These complexes are known as box H/ACA small nucleolar ribonucleoproteins (snoRNPs) and consist of a box H/ACA snoRNA bound to the enzyme dyskerin pseudouridine synthase 1 (DKC1, also known as NAP57 and CBF5) and three ancillary protein factors [76, 77]. Recruitment of H/ACA snoRNPs to specific RNA targets is mediated by base-pair complementarity between the H/ACA snoRNA and sequences flanking the modification site (Fig. 4), and is the principal mechanism used to convert U to ψ in rRNAs and snRNAs [5].

The second major pseudouridylation mechanism is RNA-independent, mediated by pseudouridine synthase (PUS) enzymes that recognize and modify RNA substrates directly. At least 14 different PUS enzymes are known to exist in eukaryotes, 10 of which constitute the PUS gene family (PUS1-10) [5, 78]. Substrate recognition by PUS enzymes is based on RNA sequences and/or secondary structures near the target uridine [79]. These binding determinants vary between different PUS enzymes resulting in distinct but occasionally overlapping substrate populations. For example, PUS4 recognizes a GU<u>U</u>CNANNC sequence (modified U is underlined) within a specific stem-loop structure. This motif is present in the tRNA TψC arm and is responsible for PUS4-dependent pseudouridylation at U55, which is very highly conserved among tRNA molecules

[79]. PUS4 does not modify tRNA at any other position, and only generates ψ within mRNAs where this specific sequence and structural motif is present [16]. By contrast, some other PUS enzymes have much broader selectivity for RNA substrates. Two examples are PUS7, which modifies uridines within the context UGUAG (modified U is underlined) in a variety of RNA substrates and may be induced by cellular stress [73], and PUS1, which modifies tRNA in at least nine different positions in addition to depositing ψ at multiple sites within mRNAs, pre-mRNAs, snRNAs, and other noncoding transcripts [16, 18, 75].

Unlike other RNA modifications, ψ is linked to its associated ribose sugar by a C-C bond rather than the canonical C-N glycosidic bond, suggesting that this modification is unlikely to be readily reversible [80]. Supporting this hypothesis, no ψ eraser activities have been reported to date.

2.5.2. *ψ functions*

No proteins that specifically recognize the ψ nucleobase have been identified. Accordingly, its functions on RNA biochemistry are likely mediated by its effects on local RNA structure, conformational dynamics, base pairing potential, and by modifying recognition sites for cellular RNA-binding proteins. The three-dimensional structures of RNA molecules are stabilized by ψ because: 1) ψ can form base pairs with any canonical base given modest conformational freedom [15], and 2) RNA duplexes containing ψ are stronger than analogous hybrids containing U. The stability of RNA base pairs containing ψ benefits from enhanced stacking between adjacent nucleobases, strengthening local RNA structure in double-stranded regions but also limiting conformational heterogeneity in single-stranded domains [81, 82]. In some contexts, ψ-containing duplexes are also stabilized by water molecules coordinated between the unique N5 H-bond donor of ψ (Fig. 1) and the C5′-linked phosphate group [83].

ψ can exert many regulatory consequences on RNA molecules, including effects on nuclear pre-mRNA processing events. In eukaryotes, most protein-coding genes are expressed as extended pre-mRNAs that contain both exon and intron sequences. Transcript maturation involves three key steps: 1) co-transcriptional addition of a 7-methylguanosine (m^7G) cap structure to the 5′-end, 2) removal of introns and ligation of flanking exons by nuclear splicing, and 3) 3′-cleavage of the pre-mRNA and addition of a poly(A) tail [84].

Nuclear splicing is performed by the spliceosome, a dynamic multi-megadalton complex that in most cases includes the small nuclear ribonucleoproteins (snRNPs) U1, U2, U4, U5, and U6, each consisting of a cluster of proteins assembled about an eponymous snRNA [85]. ψ impacts splicing from two perspectives since these modifications occur in both the spliceosomal RNA components and their pre-mRNA substrates. For example, all major snRNAs contain ψ, although it is most abundant in U2 snRNA, responsible for assembling the U2 snRNP and delivering it to the intron branch site [86]. Generation of ψ35 in U2 snRNA by PUS7 alters the structure of the U2 snRNA:intron branch site duplex such that a single unpaired A residue is excluded from the helix [87]. The enhanced reactivity of this nucleotide, called the "branch-point A", is critical for the first catalytic step in splicing, a nucleophilic attack by its 2′-OH on the 5′-splice site. Conversely, ψ incorporation in pre-mRNA can induce varied outcomes depending on location, including selective inclusion or exclusion of alternatively spliced exons [75]. ψ probably mediates these effects by modulating access for select splicing regulators since these residues are enriched at or near pre-mRNA sequences that are targeted by splicing and other processing factors. The same rationale likely accounts for differences in the sites of pre-mRNA 3′-cleavage and polyadenylation observed as a result of ψ modifications [75].

In mature mRNAs, ψ modifications within coding sequences can slow translation elongation and permit amino acid substitutions at modified sites owing to the plurality of potential tRNAs capable of recognizing ψ-containing codons [88]. Additionally, stress-induced pseudouridylation may stabilize modified mRNAs. In yeast, heat shock induces PUS7-dependent ψ deposition in mRNAs at over 200 sites and significantly increases levels of modified transcripts relative to cells lacking PUS7 [73]. Similarly, ψ-modified reporter mRNAs are significantly stabilized relative to unmodified mRNAs when transfected into cultured human cell models or mouse tissues [89].

3. Modifications of rRNA

Ribosomes are the engines of protein synthesis in all living things and are organized into two major subunits containing both RNA (rRNA) and protein components. In higher eukaryotes, the large subunit (60S) is assembled from three rRNAs (28S, 5.8S, and 5S) and 47 proteins, while the small subunit consists of a single rRNA (18S) complexed with 33 proteins

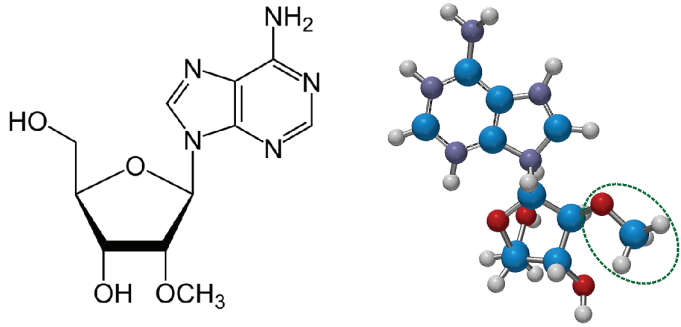

Figure 5. Chemical structure (left) and molecular model (right) of 2′-*O*-methyladenosine with the 2′-*O*-methyl group highlighted (dashed ellipse).

[90]. The RNA moieties of each subunit are heavily modified, affecting approximately 2% of all nucleotides in rRNA [2]. The most abundant modifications are 2′-*O*-methylation of ribose sugars (2′-*O*-me; Fig. 5) and ψ, each occurring approximately 100 times in human rRNA [76]. The vast majority of 2′-*O*-me groups are added by the methyltransferase fibrillarin (FBL), guided to specific target sites by base pair complementarity to C/D box snoRNAs. Pseudouridylation of rRNAs is almost exclusively performed by DKC1 targeted by H/ACA box snoRNAs [77] as discussed in section 2.5.1. Interestingly, both snoRNA-guided (2′-*O*-me, ψ) and a subset of other nucleotide modifications are clustered in rRNA functional domains including the mRNA decoding site, peptidyltransferase center, and the intersubunit interface [2], supporting key roles for these modifications in ribosome structure and function.

Mutagenesis studies showed that eliminating 2′-*O*-methylation or pseudouridylation at individual sites on rRNAs generally has little effect on ribosome biogenesis or function, but that inhibiting modifications at multiple positions cumulatively impairs ribosome production and activity [91, 92]. Ribosomal RNAs are also subject to other base-specific modifications including m^6A, m^1A, and m^5C, but at significantly lower frequencies [93]. Deposition of m^1A on 28S rRNA by the nucleolar protein nucleomethylin (NML) is required for formation of mature 60S subunits [94]. Conversely, inhibition of select m^6A and m^5C modifications does not appear to inhibit biogenesis of ribosomes but can impair their activity [95, 96]. Contributions of dysregulated rRNA modifications to cancer are discussed in section 5.

4. Modifications of tRNA

tRNA molecules are the physical link between the sequences of mRNAs and their encoded polypeptides, delivering individual amino acids to the ribosome according to base pair complementarity between the mRNA codons and tRNA anticodon sequences. Mature tRNAs are short, varying between 73 and 94 nucleotides in length, but are the most chemically diverse RNA molecules with covalent modifications to approximately 17% of all residues [97, 98]. Some of the most common base and sugar modifications in human tRNAs are shown in the "cloverleaf" presentation of tRNA secondary structure in Figure 6. Mitochondrial tRNAs exhibit many similar modifications although with some distinct features; these are reviewed at length elsewhere [47].

Modification of canonical ribonucleosides does not appear to be essential for adoption of overall tRNA structure [99], but rather plays key roles in optimizing tRNA abundance and function. For example, experiments in yeast demonstrated that 7-methylguanosine (m^7G) and m^5C protect mature tRNAs from degradation by the rapid tRNA decay (RTD) pathway, a surveillance mechanism that primarily targets hypomodified tRNAs [100]. RNA modifications also optimize the balance between tRNA flexibility and stability, influencing aminoacylation kinetics and accuracy while also permitting conformational transitions during the accommodation and translocation steps of translational elongation [101–103].

Base modifications within the anticodon loop play two key roles: 1) stabilizing codon:anticodon recognition to improve translation fidelity by preventing substitution and frameshift errors [104, 105], and 2) expanding codon recognition by re-programming the 5′-anticodon "wobble" position (position 34 in Fig. 6a) to permit decoding of synonymous mRNA codons [4, 106]. In this manner, alterations in tRNA modifications can "fine-tune" the kinetics of protein synthesis by preferentially translating mRNAs containing select codons. An example of this functionality is given by the incorporation of queuosine (Q, Fig. 7). The Q modification is found in the wobble (first) position of tRNAs containing the anticodon sequence $G_{34}U_{35}N_{36}$, where N is any base [107]. These anticodons are complementary to codons 5′-NA(C/U)-3′, encoding the amino acids asparagine, aspartic acid, histidine, and tyrosine. Using $tRNA^{Asp}$ as a model, computational modeling suggests that Q in position 34 makes

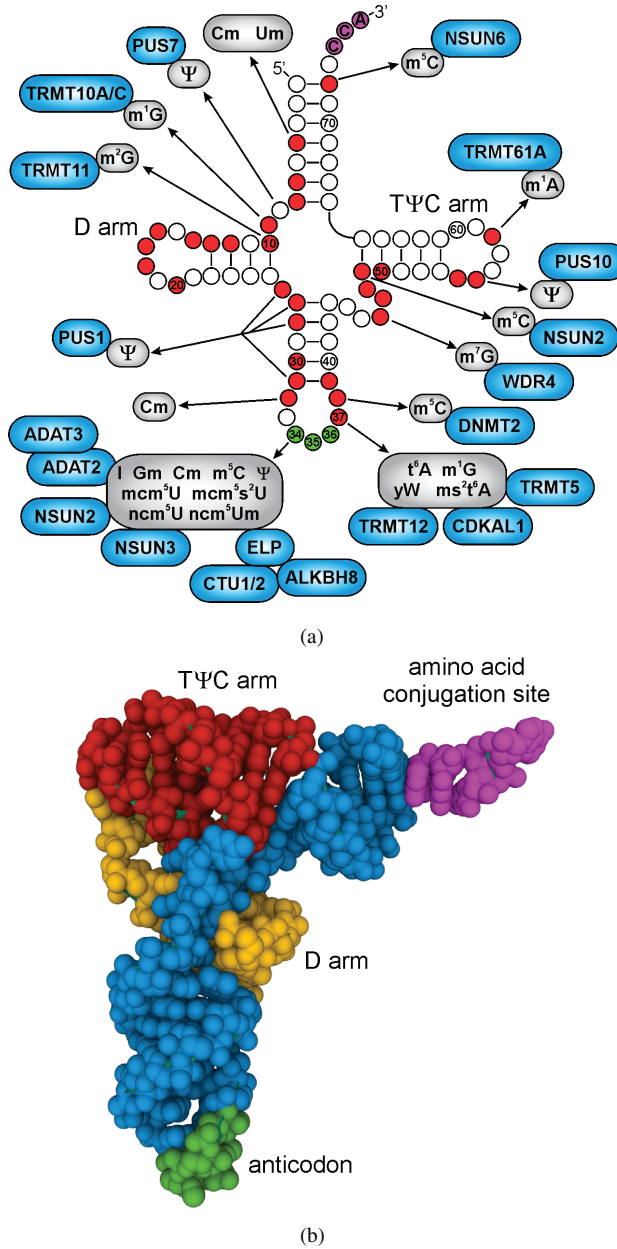

Figure 6. Modifications and structure of tRNA.

(a) Secondary structure map of a tRNA highlighting nucleotides subject to modification (red) and the identities of a subset of commonly modified bases (gray) along with the

Figure 6. (*Continued*) modifying enzymes ("writers") involved in many cases (blue). Nucleotides comprising the anticodon and amino acid conjugation sites are highlighted in green and magenta, respectively. Figure adapted from [97]. (b) Space filling model of yeast $tRNA^{Phe}$ from PDB ID 4TRA. The anticodon loop is in green and the amino acid conjugation site is in magenta. Base pair contacts between the exposed loops of the D (yellow) and TψC (red) arms contribute to the overall three-dimensional structure of tRNA. Abbreviations: m^1A: N^1-methyladenosine; ψ: pseudouridine; m^5C: 5-methylcytosine; m^7G: 7-methylguanosine; ms^2t^6A: 2-methylthio-N^6-threonylcarbamoyladenosine; m^1G: 1-methylguanosine; yW: wybutosine; I: inosine; Gm: 2′-*O*-methylguanosine; Cm: 2′-*O*-methylcytidine; mcm^5U: 5-methoxycarbonylmethyluridine; mcm^5s^2U: 5-methoxycarbonylmethyl-2-thiouridine; ncm^5U: 5-carbamoylmethyluridine; ncm^5Um: 5-carbamoylmethyl-2′-*O*-methyluridine; m^2G: N^2-methylguanosine; Um: 2′-*O*-methyluridine; PUS: pseudouridylate synthase; NSUN: NOP2/Sun RNA methyltransferase family member; WDR4: WD repeat domain 4; DNMT2: DNA methyltransferase 2; TRMT: tRNA methyltransferase; CDKAL1: Cdk5 regulatory associated protein 1-like 1; ELP: elongator protein homolog; CTU: cytosolic thiouridylase; ALKBH8: AlkB homolog 8 tRNA methyltransferase; ADAT: adenosine deaminase acting on tRNA.

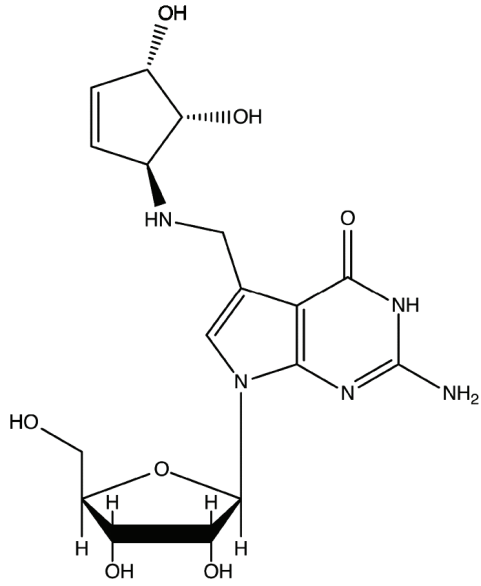

Figure 7. Chemical structure of queuosine.

hydrogen bond contacts with U33 that decrease anticodon flexibility, altering bias for specific codons [108]. In the case of $tRNA^{Asp}$, the unmodified anticodon (with G in position 34) forms stronger interactions with GAC versus GAU codons. By contrast, $tRNA^{Asp}$ containing Q in position 34 binds either codon with similar energetics [108]. In this way, Q can alter translation efficiency for specific subsets of mRNAs based on codon preferences. Interestingly, eukaryotic organisms cannot synthesize Q *de novo*, rather, it must be salvaged from its cognate nucleobase queunine, which is generated exclusively in prokaryotes [109]. This system thus constitutes a mechanism by which the specificity of cellular protein synthesis can be altered by inputs from the diet and/or gut microbiota.

A final contribution of RNA modifications to tRNA function is by controlling the cleavage of select tRNAs into populations of RNA segments collectively called tRNA-derived small RNA fragments (tRFs). tRFs vary in length from 18–40 nt and most frequently include sequences from the extreme 5′- or 3′-ends of tRNA progenitors, although fragments spanning internal domains have also been detected [110]. The largest forms, called tRNA halves, result from cleavage within the anticodon loop. In mammals, the best-characterized tRNA endonuclease is the RNase A family member angiogenin (ANG), although ribonuclease L (RNase L) and Schlafen family members 11 (SNFL11) and 13 (SNFL13) have also been identified as tRNA-cleaving enzymes [111–114]. Formation of the larger tRFs is activated by stress in many contexts, including oxidative and genotoxic damage [113, 115], while some other forms are constitutively expressed in select cell types, particularly those associated with hematopoiesis and some developing tissues [116]. tRF formation does not significantly deplete cellular tRNA pools, rather, tRFs function as signaling molecules to regulate gene expression. Some suppress global protein translation while others silence specific transcripts by recruiting components of the RNA-induced silencing complex (RISC) via complementary base-pairing to mRNA targets (à la miRNAs) or modulate posttranscriptional gene regulatory networks by sequestering specific RNA-binding proteins [110]. The generation of many tRFs is closely linked to tRNA modifications. For example, both m^5C and Q modifications inhibit formation of tRNA halves. Methylation of C38 on $tRNA^{Gly}$ by DNMT2 inhibits production of tRFs following heat shock, while NSUN2-directed methylation at C48 and C49 prevent tRF

synthesis in neurons, which in turn protects cells from pro-apoptotic stimuli [117, 118]. Similarly, suppressing expression of the demethylase ALKBH3, required for reversing m^1A and 3-methylcytidine (m^3C) modifications, also decreases production of these large tRFs [110].

The examples above demonstrate that RNA modifications control many critical aspects of tRNA function, impacting the efficiency and accuracy of protein synthesis, both globally and at the level of specific mRNAs. As such, alterations in tRNA modifications have significant power to disrupt cellular homeostasis and trigger pathophysiological states, including cancer. These consequences of aberrant nucleotide modification in tRNAs are discussed below in section 5.

5. Roles of RNA modifications in cancer

The development and progression of cancer are facilitated by dramatic reprogramming of cellular gene expression networks. Given the central role of RNA molecules in almost every stage of gene expression and regulation, it is not surprising that RNA modifications can have major effects on cancer cell phenotypes. In this rapidly developing field, new studies revealing that altered levels or functions of epitranscriptomic writers, erasers, and readers promote or inhibit tumor cell functions are reported weekly. Though RNA-modifying enzymes and readers are generally not considered to be drivers of oncogenesis, many have been functionally linked to survival, proliferation, growth, and differentiation of tumor cells [5, 97, 119, 120]. The RNA targets through which these chemical modifications influence cancer cell physiology include all major forms, giving many of the associated factors the ability to manipulate expression of multiple genes simultaneously, potentially using several regulatory mechanisms. In this section, we describe how altered deposition, removal, or recognition of select RNA modifications control cancer cell phenotypes.

5.1. *Roles of m^6A in cancer*

N^6-methylation of adenosines in RNA plays a critical role in many physiological processes including regulation of circadian rhythms, spermatogenesis, embryogenesis, DNA damage and stress responses, pluripotency, and cell reprogramming [121]. Factors that regulate m^6A formation,

removal, or function are also closely associated with diverse oncogenic or tumor suppressive functions, including proliferation, differentiation, invasion, metastasis, and immune system evasion. Representative roles of m^6A writers, erasers and readers in cancers are summarized in Table 2.

Table 2. Roles of select m^6A writers, erasers, and readers in cancer.

Protein	Cancer	Function	Alteration in cancer[a]	Refs.
Writers				
METTL3	GBM	Oncogenic — increases production and radioresistance of CSCs by stabilizing SOX2 mRNA	↑	[122]
	AML	Oncogenic — increases proliferation and apoptotic resistance by accelerating translation of c-*myc*, BCL2, and PTEN mRNAs	↑	[123]
	HCC	Oncogenic — promotes EMT by increasing translation of SNAI1 mRNA	↑	[124]
METTL14	HCC	Tumor suppressive — enhances processing of m^6A-labeled pri-miRNAs	↓	[125]
Erasers				
FTO	melanoma	Oncogenic — accelerates proliferation and decreases response to immunotherapy by stabilizing select mRNAs including PDCD1, CXCR4, and SOX10	↑	[126]
	AML	Oncogenic — enhances leukemogenesis and blocks efficacy of ATRA differentiation therapy by stabilizing ASB2 and RARA mRNAs	↑	[127]
ALKBH5	breast	Oncogenic — enhances CSC phenotype induced by hypoxia by stabilizing NANOG mRNA	—	[128]
	GBM	Oncogenic — enhances proliferation and stemness by posttranscription induction of FOXM1 expression	↑	[129]

(*Continued*)

Table 2. (*Continued*)

Protein	Cancer	Function	Alteration in cancer[a]	Refs.
Readers				
YTHDC2	CRC	Oncogenic — promotes metastasis by enhancing translation of metastasis-associated mRNAs including HIF-1α and TWIST1	↑[b]	[130]
YTHDF2	AML	Oncogenic — suppresses differentiation and inhibits apoptosis by destabilizing TNFRSF1B mRNA	↑	[131]
	pancreatic	Mixed — promotes proliferation by activating AKT1/GSK3β/cyclin D1 but suppresses metastasis by destabilizing YAP mRNA	↑	[132]
IGF2BP1	HCC	Oncogenic — promotes tumor growth by binding and stabilizing m^{6}A-modified SRF mRNA	—	[133]

Abbreviations: AKT1: AKT serine/threonine kinase 1; AML: acute myelocytic leukemia; ASB2: ankyrin repeat and SOCS box containing 2; BCL2: B cell lymphoma 2; CRC: colorectal cancer; CSC: cancer stem cell; CXCR4: C-X-C motif chemokine receptor 4; EMT: epithelial-mesenchymal transition; FOXM1: forkhead box M1; GBM: glioblastoma multiforme; GSK3β: glycogen synthase kinase 3β; HCC: hepatocellular carcinoma; HIF1A: hypoxia inducible factor 1α; IGF2BP1: insulin-like growth factor 2 mRNA binding protein 1; NANOG: Nanog homeobox; PDCD1: programmed cell death 1; PTEN: phosphatase and tensin homolog; RARA: retinoic acid receptor α; SOX: SRY-box transcription factor; SNAI1: snail family transcriptional repressor 1; SRF: serum response factor; TNFRSF1B: TNF receptor superfamily member 1B; TWIST1: twist family bHLH transcription factor 1; YAP: Yes-associated protein

[a]Change in expression of listed m^{6}A writer, eraser, or reader in cancer versus non-transformed tissue

[b]Expression positively correlated with tumor stage

5.1.1. *m^{6}A writers in cancer*

The most prolific writer of m^{6}A on RNA substrates is METTL3, whose overexpression in many cancer types contributes to pro-tumorigenic phenotypes, although this association is not universal [5]. For example, elevated levels of METTL3 in glioblastoma multiforme (GBM) increases m^{6}A modifications in RNA, including the mRNA encoding the pluripotency factor SRY-box transcription factor 2 (SOX2) [122].

METTL3-induced modification stabilizes SOX2 mRNA, promoting the maintenance of cancer stem cell (CSC) populations, resistance to ionizing radiation, and accelerated tumor growth and mortality in xenografted mouse models. However, an independent study suggested a tumor suppressive role for METTL3 in GBM, whereby increased m^6A levels suppressed CSC maintenance and tumor development [134]. While this paradox has not yet been reconciled, it is possible that the patient-derived GBM cell models used in each study represented distinct and as-yet undefined glioblastoma subtypes [120]. METTL3 expression is also enhanced in hepatocellular carcinoma (HCC), where resulting increases in m^6A deposition increase translation of snail family transcriptional repressor 1 (SNAI1) mRNA [124]. The resulting increase in SNAI1 protein levels induces expression of genes that promote epithelial-mesenchymal transition (EMT), a process that facilitates tumor cell invasion and metastasis in many types of cancer [135].

METTL14 is an m^6A writer that can exhibit tumor suppressor function. While METTL14 is an RNA-binding protein that aids in site-specific recruitment of the m^6A writer METTL3 (section 2.1.1., above), it may also perform an analogous function in loading the Microprocessor complex onto m^6A-modified pri-miRNAs to promote miRNA maturation, although it is unclear whether this mechanism requires the participation of specific m^6A reader proteins. However, downregulation of METTL14 in advanced HCC is associated with decreased production of miRNAs including miR-126, a known inhibitor of metastasis, while forced overexpression of METTL14 was sufficient to diminish tumor cell aggressiveness [125].

5.1.2. *m^6A erasers in cancer*

Most studies reported to date support pro-tumorigenic roles for m^6A erasers. For example, FTO is upregulated in melanoma, where resulting demethylation of RNA substrates leads to stabilization of select mRNAs including programmed cell death 1 (PDCD1, also known as PD-1), C-X-C motif chemokine receptor 4 (CXCR4), and SRY-box transcription factor 10 (SOX10) [126]. Key consequences of FTO-directed upregulation of

these factors are acceleration of cell proliferation, enhanced migration and invasiveness, and resistance to anti-PD-1 immunotherapy. Overexpression of FTO in acute myelocytic leukemia (AML) yields similar effects, although involving different factors. In AML, depletion of m^6A by FTO stabilizes distinct mRNA targets including ankyrin repeat and SOCS box containing 2 (ASB2) and retinoic acid receptor α (RARA), accelerating disease progression in transplanted mouse models, while also suppressing efficacy of all-*trans* retinoic acid (ATRA) differentiation therapy [127], a standard approach for treating this disease [136].

5.1.3. *m^6A readers in cancer*

Levels of the m^6A reader protein YTHDC2 are significantly elevated in stage 3 and 4 colorectal cancer (CRC) relative to less advanced tumors, and in tumors from patients with lymph node metastases versus non-metastatic disease [130]. Suppressing YTHDC2 in CRC cell models significantly attenuates migration of cultured cells as well as tumor growth and metastatic potential in mouse models. An important gene regulatory function of YTHDC2 in these cells is to enhance translation of hypoxia inducible factor 1α (HIF1A, also known as HIF-1α) and twist family bHLH transcription factor 1 (TWIST1) mRNAs [130]. HIF1A and TWIST1 proteins are potent activators of angiogenesis, cell motility, and EMT, all key properties of metastatic tumor cells [137, 138].

YTHDF2 is upregulated in AML and pancreatic tumors, but exerts different effects on each cell type. In AML, YTHDF2 maintains the leukemic stem cells responsible for disease progression by inhibiting differentiation and sensitivity to pro-apoptotic stimuli [131]. YTHDF2 directs the latter function by destabilizing TNF receptor superfamily member 1B (TNFRSF1B) mRNA, whose encoded protein normally primes cells for apoptosis induced by its ligand, tumor necrosis factor α (TNFα). By contrast, elevated YTHDF2 enhances proliferation of pancreatic cancer cells by activating signaling through the AKT serine/threonine kinase 1 (AKT1) — glycogen synthase kinase 3β (GSK3β) — cyclin D1 axis [132]. Pathway activation includes accumulation of phosphorylated AKT1 via an unknown mechanism that is independent of changes in AKT1 expression. However, concomitant with accelerating proliferation of pancreatic

cancer cells, YTHDF2 suppresses their metastatic potential by decreasing expression of the EMT-promoting transcriptional co-regulator Yes-associated protein (YAP) [132]. While the mechanism by which YTHDF2 regulates YAP has not been resolved in pancreatic cells, a recent study in an HCC model identifies m^6A-modified circular cleavage and polyadenylation specific factor 6 (circCPSF6) as a novel circular RNA (circRNA) substrate of YTHDF2 [139]. In the HCC cell model, circCPSF6 normally stabilizes YAP mRNA by competing with it for the mRNA-destabilizing factor poly(rC)-binding protein 2 (PCBP2, also known as hnRNP E2). YTHDF2 binding to circCPSF6 inhibits this circuit by destabilizing the circRNA, thus abrogating its ability to stabilize YAP mRNA and leading to the depletion of its encoded pro-EMT transcriptional regulator.

A third example of an m^6A reader implicated in cancer is insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1). All three members of the IGF2BP family (1/2/3) bind m^6A in mRNA and stabilize associated transcripts [140]. In HCC cells, one target of IGF2BP1 is the mRNA encoding serum response factor (SRF), a transcription factor that activates expression of a suite of proteins that promote tumor growth and invasiveness [133]. Upon binding to the m^6A-modified 3′UTR of SRF mRNA, IGF2BP1 stabilizes the transcript by inhibiting recruitment of microRNAs miR-23 and miR-125, which normally function to suppress SRF expression.

5.1.4. *m^6A impacts on miRNA in cancer*

Some oncogenic and tumor suppressive roles of m^6A are mediated by deposition of this modification in unprocessed pri-miRNAs and mature miRNAs. Select examples are listed in Table 3. To date, the best characterized cases are those where m^6A modifications modulate cellular processing of pri-miRNAs into mature, functional miRNAs. Depending on the pri-miRNA target and mRNA subpopulation regulated by its encoded miRNA, the consequences of m^6A in this context can be pro- or anti-oncogenic. For example, elevated expression of the m^6A writer METTL3 in aggressive CRC cells leads to methylation of pri-miR-1246. This increases production of its mature miRNA product, which in turn suppresses levels of and/or translation from miR-1246 substrate transcripts, including the mRNA encoding sprouty related EVH1 domain

Table 3. Select oncogenic and tumor suppressive roles of m^{6}A deposition in miRNA.

Cancer	modified miRNA(s)	Alteration in cancer[a]	Impact on miRNA	Functional consequences	Refs.
CRC	miR-1246	↑	processing	Suppresses SPRED2, which activates the RAF/MEK/ERK pathway	[141]
	miR-483 miR-676 miR-877	n.d.	processing	Suppress expression of electron transport chain factors; modulates mitochondrial metabolism	[144]
	miR-17-5p let-7a-5p	↑	target binding	Unknown	[30]
pancreatic	miR-25-3p	↑	processing	Suppresses expression of PHLPP2; results in activation of oncogenic AKT signaling	[145]
	miR-17-5p let-7a-5p	↑	target binding	Unknown	[30]
HCC	miR-126	↓	processing	Enhances expression of several factors that promote proliferation, stemness, and angiogenesis	[125]
bladder	miR-221 miR-222	↑	processing	Suppresses expression of PTEN; promotes proliferation	[146]
lung	miR-143-3p	↑	processing	Suppresses expression of VASH1; promotes angiogenesis and metastatic potential	[147]

Abbreviations: AKT: AKT serine/threonine kinase 1; ERK: extracellular-regulated kinase; MEK: mitogen-activated protein kinase kinase 1; PHLPP2: PH domain leucine-rich repeat protein phosphatase 2; PTEN: phosphatase and tensin homolog; RAF: rapidly accelerated fibrosarcoma; SPRED2: sprouty related EVH1 domain containing 2; VASH1: vasohibin-1

[a]Change in abundance of m^{6}A modification in cancer versus non-transformed tissue

containing 2 (SPRED2) [141]. SPRED2 is an inhibitor of mitogen-activated protein kinase (MAPK) signaling, so its repression by miR-1246 exacerbates tumorigenic phenotypes by activating the rapidly accelerated fibrosarcoma-1 (Raf-1)/mitogen-activated protein kinase kinase 1 (MAP2K1, also known as MEK)/extracellular-regulated kinase (ERK)

pathway [142]. By contrast, m^6A modifications are decreased in HCC, in part by downregulation of the m^6A writer METTL14 (Table 2). One target of METTL14 is pri-miR-126, whose processing is accelerated following methylation [125]. In many cancer types, miR-126 functions as a tumor suppressor by inhibiting the production of several proteins that enhance cell proliferation, stemness, and angiogenesis [143]. Repression of METTL14 in HCC decreases miR-126 production, thus removing this impediment to development of aggressive oncogenic phenotypes [125].

The second principal mechanism by which m^6A can alter miRNA functions in cancer is by direct modification within the mature miRNA sequence. On select miRNAs, the abundance of m^6A is significantly elevated in tumors compared to paired non-transformed tissues, and in the serum of cancer patients relative to healthy controls, suggesting potential utility as an oncogenic biomarker [30]. To date, the effect of m^6A modifications on regulation of mRNA targets remains largely unknown. Molecular dynamics simulations suggest that m^6A modifications within mature miRNAs can cause dramatic structural changes in miRISC complexes, which conceivably could impair recognition or post-transcriptional silencing of their mRNA substrates [30].

5.2. *Roles of m^5C in cancer*

Contributions of RNA m^5C modifications to cancer are less well-defined than for m^6A, in part because some m^5C writers (e.g., DMNT2) and erasers (e.g., TET family of dioxygenase enzymes) also modify DNA substrates, thus complicating dissection of their RNA-specific contributions to cancer biology. However, correlative studies have revealed that several m^5C writers, erasers, and readers are dysregulated in many cancer types. Furthermore, alterations in m^5C deposition affect a broader distribution of RNA substrates than observed for m^6A, thus inducing more prominent roles for modifications of tRNA and rRNA in addition to protein coding transcripts. Some representative examples are described below.

5.2.1. *m^5C writers in cancer*

Dysregulated expression of cytidine methyltransferases occurs in a many cancer types including leukemias as well as tumors of the breast, lung,

prostate, bladder, skin, and gastrointestinal tract, with most cases presenting elevated levels of one or more m^5C writers [5, 148]. However, despite this commonality, the molecular mechanisms by which altered m^5C writer activity impacts tumor cell phenotypes vary widely among different cancers. For example, enhanced expression of NSUN2 occurs in many bladder cancers and is associated with poor patient prognosis [66]. Elevated NSUN2 in these tumors results in hypermethylation and stabilization of mRNAs encoding several oncogenic proteins including heparin binding growth factor (HDGF), F11 receptor (F11R), and RAB11 family interacting protein 4 (RAB11FIP4). In the bone osteosarcoma cell line U2OS, NSUN2 accelerates cell proliferation by methylating a specific cytosine residue in the 3′UTR of cyclin-dependent kinase 1 (CDK1) mRNA resulting in increased translation and activity of this cell cycle regulatory kinase [149]. However, the oncogenic consequences of m^5C deposition are not limited to mRNA substrates. NSUN2 is also upregulated in esophageal squamous cell carcinoma (ESCC), where it methylates a long noncoding transcript termed NSUN2 methylated lncRNA (NMR, also known as LINC01672) that contributes to tumor metastasis and drug resistance [150].

In other cases, activity of m^5C writers has been associated with tumor suppressive functions. For example, NSUN2 levels are inversely correlated with tumor progression in human skin cancers, while deletion of the NSUN2 gene enhances tumor formation in a transgenic mouse model of skin cancer [151]. In these tumors, loss of NSUN2 dramatically decreases m^5C modification on tRNA, leading to increased production of tRFs as described in section 4. Accumulation of tRFs imposes a general inhibition of cap-dependent protein synthesis, but promotes a pro-oncogenic cap-independent translational program in these cells [151]. A second example is observed in glioma, where epigenetic silencing of NSUN5 is associated with more advanced disease and poor patient outcomes [152]. A key methylation target of NSUN5 is 28S rRNA at C3782; silencing NSUN5 precludes this modification, resulting in a decrease in overall protein synthesis but preferential translation of a subset of proteins involved in cellular stress responses.

5.2.2. *m^5C erasers in cancer*

Altered regulation or activity of several members of the TET family of m^5C erasers is associated with cancer. For example, glioblastoma is

associated with upregulation of TET1, but suppression of TET2 and TET3 [5]. Suppression of TET2 also contributes to some myeloid leukemias by enhancing self-renewal of hematopoietic stem cells and proliferation of myeloid cell populations [153]. However, since TET family enzymes erase m^5C from both RNA and DNA substrates, it is currently unknown whether alterations of these eraser activities modulate disease states via their RNA- or DNA-targeted functions.

5.2.3. *m^5C readers in cancer*

The best characterized m^5C reader protein is YBX1, which is upregulated and associated with poor prognosis in a variety of cancers including tumors of the kidney, bladder, breast, and lung [66, 154–156]. Mechanisms by which YBX1 exacerbates tumorigenic phenotypes are also varied. For example, in renal cell carcinoma, elevated YBX1 promotes cell adhesion, migration, and invasion, while suppressing sensitivity to pro-apoptotic stimuli [74, 157]. In bladder and breast cancers, elevated YBX1 also induces multidrug resistance by increasing expression of the multidrug resistance 1 (MDR1) drug efflux pump [158, 159]. In cells, YBX1 is a multifunctional protein with a broad range of functions. Since it can regulate diverse processes including DNA repair, transcription, pre-mRNA splicing, RNA turnover, and translation [160], in most cases it has been difficult to discern oncogenic roles resulting explicitly from its m^5C reader activity.

Like YBX1, the m^5C reader protein ALYREF is also overexpressed in many tumor types [5]. Elevated ALYREF levels are also poor prognostic indicators in many cancers, including breast cancer, colon cancer, HCC, and neuroblastoma [161–164]. Some oncogenic activities of ALYREF appear to be independent of its RNA-targeted functions, including a role as a transcriptional coactivator complex with N-*myc* in neuroblastoma [164]. However, other cases are clearly RNA-centric. One example is in glioma cell models, where ALYREF enhances c-*myc* expression via a feed-forward loop whereby c-Myc protein transcriptionally activates ALYREF expression, increasing levels of ALYREF protein, which then binds and stabilizes c-*myc* mRNA [165]. While this regulatory loop clearly utilizes ALYREF's RNA-binding function, it is not yet known whether ALYREF recruitment is specific to m^5C-modified c-*myc*

mRNA. By contrast, in the glioma cell line U87, an m^5C-dependent oncogenic role for ALYREF has been verified. In this cell model, NSUN2 methylates C2756 within the 3′UTR of autotaxin (ATX) mRNA. This modified nucleotide is recognized by ALYREF, which accelerates transport of the modified ATX mRNA to the cytoplasm and enhances its translation, elevating ATX protein levels which in turn increase cell migratory potential [166].

5.2.4. *m^5C impacts on miRNA in cancer*

To date, very few studies have assessed the role of m^5C modifications on miRNA expression or function. A recent transcriptome-wide survey indicates that as many as 40% of all mature miRNAs in human cells contain at least one modified C residue, and that a significant subset of those are m^5C residues that have been converted to hm^5C [56]. In some cases, the extent of miRNA m^5C modification may vary as a function of cell type [56] or disease state. An example of the latter was measured in pancreatic and colorectal cancer tissues where m^5C methylation, but not abundance of miR-200c-3p and miR-21-3p, was increased relative to healthy controls [30]. Similar enrichment of these m^5C-modified miRNAs was observed in serum samples from these patients relative to healthy donors, suggesting potential utility as a biomarker of early pancreatic and colorectal cancer. However, the significance of m^5C residues in miRNA is unclear. For example, molecular dynamics simulations suggest that m^5C has relatively modest effects on the structure of the miR-200c-3p-loaded RISC complex, while RNA-Seq experiments revealed no significant alteration in the RNA silencing activity of this miRNA [30]. By contrast, m^5C modification of miR-181a-5p in the glioblastoma cell line U87 by a complex of DNA methyltransferase member 3A (DNMT3A) and argonaute RISC component 4 (AGO4) inhibits recognition of its mRNA substrates, including that encoding the proapoptotic factor B-cell lymphoma 2 (BCL2)-like 11 (BCL2L11, also known as BIM) [167]. While broader conclusions regarding the consequences of m^5C modifications in miRNA await characterization of additional examples, it is likely that they will vary with the identity of the miRNA, the position of the modification, and the substrate mRNA(s) targeted.

5.3. *Roles of ψ in cancer*

ψ was the first modified nucleotide proposed to be associated with cancer, based on decades-old observations that ψ levels were often elevated in the urine of cancer patients, but would regress following chemotherapy [168, 169]. Subsequently, quantitation of ψ in biological fluids was proposed as a potential cancer biomarker, but has not yet been robustly incorporated as a diagnostic tool [119, 170]. Contributions of ψ to cancer are currently limited to altered expression or activity of ψ writers, since corresponding eraser or reader proteins have not yet been discovered. Dysregulation of both RNA-dependent and RNA-independent pseudouridylation activities can contribute to oncogenic cell phenotypes.

5.3.1. *RNA-dependent ψ writers in cancer*

The most commonly dysregulated ψ writer in cancer is DKC1, the enzymatic subunit of the H/ACA snoRNPs (Fig. 4). As described in section 2.5.1., rRNAs and snRNAs are the major substrates of DKC1, but this enzyme also plays a key role in the telomerase complexes that stabilize and maintain telomere sequences at the termini of chromosomes [171]. It is this last function for which DKC1 is best known, as select mutations in the DKC1 gene cause X-linked dyskeratosis congenita (X-DC), an incurable premature aging disorder with complex symptomology that normally causes death from bone marrow failure [172].

DKC1 expression is suppressed in breast cancer and chronic lymphocytic leukemia (CLL), but upregulated in many other cancer types including CRC, GBM, HCC, and tumors of the lung and prostate, while sporadic mutations within the DKC1 gene have been observed in some other cancer types [5]. The opposing changes in DKC1 expression and/or function observed in different tumors, coupled with the variety of gene and/or telomere regulatory consequences induced, suggest that DKC1 can play tumor suppressive or oncogenic roles in different cellular contexts. For example, DKC1 can act as an inhibitor of tumor development by enhancing synthesis of select tumor suppressor proteins. ψ deposition in rRNA is required for initiating translation from some internal ribosome entry sites (IRESes), including those responsible for production of the tumor suppressor proteins p53 and cyclin dependent kinase inhibitor 1B

(CDKN1B, also known as p27) [173, 174]. By inhibiting DKC1 function, ribosomes that lack ψ modifications accumulate, which are incapable of translating these tumor suppressor proteins. However, some other IRES-containing mRNAs encode pro-oncogenic factors. One example is the mRNA encoding angiogenesis-promoting protein vascular endothelial growth factor (VEGF), whose translation is enhanced when DKC1 function is lost [175].

Conversely, significantly less is known about the pro-oncogenic mechanisms activated by DKC1. One logical pathway is by promoting telomerase activity to enable replicative immortality of tumor cells. An example of this mechanism was demonstrated in the human breast carcinoma cell line MCF-7, where silencing DKC1 reduces telomerase activity [176]. Consistent with this principle, low DKC1 expression is associated with improved clinical outcomes in breast cancer patients [176]. In gliomas, overexpression of DKC1 is also frequently observed, and is associated with advanced tumor stages. Suppression of DKC1 in glioma cell lines U251 and U87 slows cell proliferation, migration, and invasiveness, accompanied by decreased expression of several EMT markers [177], although specific mechanisms by which DKC1 would normally exacerbate these processes remain unknown.

5.3.2. *RNA-independent ψ writers in cancer*

Much less is currently known about the contributions of RNA-independent ψ writers to cancer. PUS1 and PUS10 have roles in cancer-associated pathways but there is no evidence that their expression or activity is significantly modulated in tumors. For example, PUS1 modifies select U residues in a noncoding RNA called steroid receptor RNA activator (SRA), activating its function as a transcriptional coactivator of retinoic acid receptor γ (RARγ) and estrogen receptor [178]. By contrast, during apoptosis stimulated by TNF-related apoptosis-inducing ligand (TRAIL), PUS10 translocates from the nucleus to the mitochondrion in a caspase-3-dependent manner leading to increased release of cytochrome c and the inhibitor of apoptosis protein (IAP)-binding mitochondrial protein (DIABLO) [179]. Release of these proteins further activates caspase-3 in a feedback amplification loop, but it is unclear whether this requires PUS10's pseudouridylase activity.

An RNA-independent ψ writer that has been more closely linked to cancer is PUS7. High PUS7 expression is associated with poor outcomes in both glioblastoma and CRC [180, 181]. In colon cancer cell models, PUS7 levels correlate with activation of cell proliferation and invasiveness, while inhibiting apoptotic sensitivity although involvement of its ψ writer function in these processes remains unknown [181]. However, in glioblastoma, PUS7 drives tumor formation by promoting growth and renewal of glioblastoma stem cells (GSCs). This process is mediated by PUS7-directed ψ modifications in tRNAs that fine-tune translation at a codon-specific level, favoring synthesis of proteins that enhance GSC growth including multiple members of interferon signaling pathways [180].

5.4. *Selected roles of other RNA modifications in cancer*

Recent literature reports several correlations and mechanisms linking specific RNA modifications to cancer beyond those described in the subsections above. Two examples presented below highlight additional methods by which the epitranscriptome can manipulate oncogenic processes.

Several examples have already illustrated how alterations in rRNA modifications can manipulate translation fidelity and initiation from internal ribosome entry sites (IRESes). This control extends to 2′-*O*-methylation of rRNAs catalyzed by the C/D box snoRNA-associated methyltransferase FBL described in section 3. In breast cancer patients, elevated FBL levels are associated with poor survival [182]. FBL expression is normally limited at the transcriptional level by the tumor suppressor protein p53; accordingly, in tumor cells lacking functional p53, FBL levels accumulate. In immortalized human mammary epithelial (HME) cells, suppressing p53 enhances FBL production and rRNA 2′-*O*-methylation, leading to increased IRES-initiated translation of pro-proliferative factors, including insulin like growth factor 1 receptor (IGF1R) and c-Myc [182]. FBL is also overexpressed in prostate cancer, but in this case, is transcriptionally induced by c-Myc [183], raising the possibility of a feed-forward loop between these pro-oncogenic proteins.

The second example involves ALKBH3, which functions as an eraser for m^1A, m^6A, and m^3C on some modified RNA substrates [27] and enhances tumorigenic phenotypes by multiple mechanisms. In breast and ovarian cancer cell models, elevated ALKBH3 levels demethylate an m^1A

nucleobase within colony stimulating factor 1 (CSF1) mRNA, stabilizing this transcript and enhancing CSF1 production, which leads to increased cell invasiveness [184]. In the pancreatic cancer cell lines PANC-1 and MIAPaCa-2, elevated ALKBH3 promotes angiogenesis and inhibits apoptosis. While the specific molecular targets of this enzyme are unknown in this tissue model, high ALKBH3 expression is a poor prognostic indicator in pancreatic cancer [185]. However, in a variety of cancer cell types, ALKBH3 promotes tumor development by demethylating tRNAs, thus inducing production of specific subsets of tRFs. Interestingly, the tRFs generated by this pathway promote a generalized increase in translational efficiency that enhances cell proliferation, migration, and invasiveness as well as tumor growth in xenograft models [186].

Covalent modifications of RNA and their roles as manipulators and biomarkers of tumorigenesis are among the most active areas of research in molecular oncology today. Extensive pre-clinical studies are now focusing on development of small molecules to inhibit the activities of individual epitranscriptomic writers, erasers, and readers in a wide array of cancer models [5, 119, 187, 188]. In the coming years, we anticipate that a diverse subset of these drug candidates will enter and advance through clinical trials, potentially making fundamental changes to the way that cancer is treated.

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References

1. Saletore Y, Meyer K, Korlach J, Vilfan ID, Jaffrey S and Mason CE. (2012) The birth of the Epitranscriptome: Deciphering the function of RNA modifications. *Genome Biology*, **13**, 175.
2. Sloan KE, Warda AS, Sharma S, Entian KD, Lafontaine DLJ and Bohnsack MT. (2017) Tuning the ribosome: The influence of rRNA modification on eukaryotic ribosome biogenesis and function. *RNA Biology*, **14**, 1138–1152.

3. Bohnsack MT and Sloan KE. (2018) Modifications in small nuclear RNAs and their roles in spliceosome assembly and function. *Biological Chemistry*, **399**, 1265–1276.
4. Endres L, Dedon PC and Begley TJ. (2015) Codon-biased translation can be regulated by wobble-base tRNA modification systems during cellular stress responses. *RNA Biology*, **12**, 603–614.
5. Nombela P, Miguel-López B and Blanco S. (2021) The role of m^6A, m^5C and y RNA modifications in cancer: Novel therapeutic opportunities. *Molecular Cancer*, **20**, 18.
6. Lauman R and Garcia BA. (2020) Unraveling the RNA modification code with mass spectrometry. *Molecular Omics*, **16**, 305–315.
7. Yoluç Y, Ammann G, Barraud P, Jora M, Limbach PA, Motorin Y, Marchand V, Tisné C, Borland K and Kellner S. (2021) Instrumental analysis of RNA modifications. *Critical Reviews in Biochemistry and Molecular Biology*, **56**, 178–204.
8. El Allali A, Elhamraoui Z and Daoud R. (2021) Machine learning applications in RNA modification sites prediction. *Computational and Structural Biotechnology Journal*, **19**, 5510–5524.
9. Wiener D and Schwartz S. (2021) The epitranscriptome beyond m^6A. *Nature Reviews Genetics*, **22**, 119–131.
10. Witkin KL, Hanlon SE, Strasburger JA, Coffin JM, Jaffrey SR, Howcroft TK, Dedon PC, Steitz JA, Daschner PJ and Read-Connole E. (2015) RNA editing, epitranscriptomics, and processing in cancer progression. *Cancer Biology & Therapy*, **16**, 21–27.
11. Baker AR and Slack FJ. (2022) *ADAR1* and its implications in cancer development and treatment. *Trends in Genetics*, **38**, 821–830.
12. Petljak M and Maciejowski J. (2020) Molecular origins of APOBEC-associated mutations in cancer. *DNA Repair*, **94**, 102905.
13. Zhou H, Kimsey IJ, Nikolova EN, Sathyamoorthy B, Grazioli G, McSally J, Bai T, Wunderlich CH, Kreutz C, Andricioaei I, *et al.* (2016) m1A and m1G disrupt A-RNA structure through the intrinsic instability of Hoogsteen base pairs. *Nature Structural & Molecular Biology*, **23**, 803–810.
14. Harcourt EM, Kietrys AM and Kool ET. (2017) Chemical and structural effects of base modifications in messenger RNA. *Nature*, **541**, 339–346.
15. Kierzek E, Malgowska M, Lisowiec J, Turner DH, Gdaniec Z and Kierzek R. (2014) The contribution of pseudouridine to stabilities and structure of RNAs. *Nucleic Acids Research*, **42**, 3492–3501.
16. Safra M, Nir R, Farouq D, Vainberg Slutskin I and Schwartz S. (2017) TRUB1 is the predominant pseudouridine synthase acting on mammalian mRNA via a predictable and conserved code. *Genome Research*, **27**, 393–406.
17. Safra M, Sas-Chen A, Nir R, Winkler R, Nachshon A, Bar-Yaacov D, Erlacher M, Rossmanith W, Stern-Ginossar N and Schwartz S. (2017) The m^1A landscape on cytosolic and mitochondrial mRNA at single-base resolution. *Nature*, **551**, 251–255.

18. Behm-Ansmant I, Urban A, Ma X, Yu Y-T, Motorin Y and Branlant C. (2003) The *Saccharomyces cerevisiae* U2 snRNA:pseudouridine-synthase Pus7p is a novel multisite-multisubstrate RNA:y-synthase also acting on tRNAs. *RNA*, **9**, 1371–1382.
19. Schibler U, Kelley DE and Perry RP. (1977) Comparison of methylated sequences in messenger RNA and heterogeneous nuclear RNA from mouse L cells. *Journal of Molecular Biology*, **115**, 695–714.
20. Zaccara S, Ries RJ and Jaffrey SR. (2019) Reading, writing and erasing mRNA methylation. *Nature Reviews Molecular Cell Biology*, **20**, 608–624.
21. Xiao W, Adhikari S, Dahal U, Chen YS, Hao YJ, Sun BF, Sun HY, Li A, Ping XL, Lai WY, *et al.* (2016) Nuclear m6A reader YTHDC1 regulates mRNA splicing. *Molecular Cell*, **61**, 507–519.
22. Ke S, Pandya-Jones A, Saito Y, Fak JJ, Vågbø CB, Geula S, Hanna JH, Black DL, Darnell JE and Darnell RB. (2017) m6A mRNA modifications are deposited in nascent pre-mRNA and are not required for splicing but do specify cytoplasmic turnover. *Genes & Development*, **31**, 990–1006.
23. Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, Weng X, Chen K, Shi H and He C. (2015) N^6-methyladenosine modulates messenger RNA translation efficiency. *Cell*, **161**, 1388–1399.
24. Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, Pestova TV, Qian SB and Jaffrey SR. (2015) 5′UTR m^6A promotes cap-independent translation. *Cell*, **163**, 999–1010.
25. Choi J, Ieong KW, Demirci H, Chen J, Petrov A, Prabhakar A, O'Leary SE, Dominissini D, Rechavi G, Soltis SM, *et al.* (2016) N^6-methyladenosine in mRNA disrupts tRNA selection and translation-elongation dynamics. *Nature Structural & Molecular Biology*, **23**, 110–115.
26. Huang W, Chen TQ, Fang K, Zeng ZC, Ye H and Chen YQ. (2021) N^6-methyladenosine methyltransferases: Functions, regulation, and clinical potential. *Journal of Hematology & Oncology*, **14**, 117.
27. Ueda Y, Ooshio I, Fusamae Y, Kitae K, Kawaguchi M, Jingushi K, Hase H, Harada K, Hirata K and Tsujikawa K. (2017) AlkB homolog 3-mediated tRNA demethylation promotes protein synthesis in cancer cells. *Scientific Reports*, **7**, 42271.
28. Alarcón CR, Lee H, Goodarzi H, Halberg N and Tavazoie SF. (2015) N^6-methyladenosine marks primary microRNAs for processing. *Nature*, **519**, 482–485.
29. De Paolis V, Lorefice E, Orecchini E, Carissimi C, Laudadio I and Fulci V. (2021) Epitranscriptomics: A new layer of microRNA regulation in cancer. *Cancers*, **13**, 3372.
30. Konno M, Koseki J, Asai A, Yamagata A, Shimamura T, Motooka D, Okuzaki D, Kawamoto K, Mizushima T, Eguchi H, *et al.* (2019) Distinct methylation levels of mature microRNAs in gastrointestinal cancers. *Nature Communications*, **10**, 3888.

31. Zhang W, Qian Y and Jia G. (2021) The detection and functions of RNA modification m^6A based on m^6A writers and erasers. *Journal of Biological Chemistry*, **297**, 100973.
32. Warda AS, Kretschmer J, Hackert P, Lenz C, Urlaub H, Höbartner C, Sloan KE and Bohnsack MT. (2017) Human METTL16 is a N^6-methyladenosine (m^6A) methyltransferase that targets pre-mRNAs and various non-coding RNAs. *EMBO Reports*, **18**, 2004–2014.
33. Pendleton KE, Chen B, Liu K, Hunter OV, Xie Y, Tu BP and Conrad NK. (2017) The U6 snRNA m^6A methyltransferase METTL16 regulates SAM synthetase intron retention. *Cell*, **169**, 824–835.
34. Wei J, Liu F, Lu Z, Fei Q, Ai Y, He PC, Shi H, Cui X, Su R, Klungland A, *et al.* (2018) Differential m^6A, m^6A_m, and m^1A demethylation mediated by FTO in the cell nucleus and cytoplasm. *Molecular Cell*, **71**, 973–985.
35. Song H, Wang Y, Wang R, Zhang X, Liu Y, Jia G and Chen PR. (2020) SFPQ is an FTO-binding protein that facilitates the demethylation substrate preference. *Cell Chemical Biology*, **27**, 283–291.
36. Luo S and Tong L. (2014) Molecular basis for the recognition of methylated adenines in RNA by the eukaryotic YTH domain. *Proceedings of the National Academy of Sciences of the United States*, **111**, 13834–13839.
37. Roundtree IA, Luo GZ, Zhang Z, Wang X, Zhou T, Cui Y, Sha J, Huang X, Guerrero L, Xie P, *et al.* (2017) YTHDC1 mediates nuclear export of N^6-methyladenosine methylated mRNAs. *eLife Sciences*, **6**, e31311.
38. Ries RJ, Zaccara S, Klein P, Olarerin-George A, Namkoong S, Pickering BF, Patil DP, Kwak H, Lee JH and Jaffrey SR. (2019) m^6A enhances the phase separation potential of mRNA. *Nature*, **571**, 424–428.
39. Roost C , Lynch SR, Batista PJ, Qu K, Chang HY and Kool ET. (2015) Structure and thermodynamics of N^6-methyladenosine in RNA: A spring-loaded base modification. *Journal of the American Chemical Society*, **137**, 2107–2115.
40. Wu B, Su S, Patil DP, Liu H, Gan J, Jaffrey SR and Ma J. (2018) Molecular basis for the specific and multivariant recognitions of RNA substrates by human hnRNP A2/B1. *Nature Communications*, **9**, 420.
41. Alarcón CR, Goodarzi H, Lee H, Liu X, Tavazoie S and Tavazoie SF. (2015) HNRNPA2B1 is a mediator of m^6A-dependent nuclear RNA processing events. *Cell*, **162**, 1299–1308.
42. Zhang F, Kang Y, Wang M, Li Y, Xu T, Yang W, Song H, Wu H, Shu Q and Jin P. (2018) Fragile X mental retardation protein modulates the stability of its m^6A-marked messenger RNA targets. *Human Molecular Genetics*, **27**, 3936–3950.
43. Shi H, Wang X, Lu Z, Zhao BS, Ma H, Hsu PJ, Liu C and He C. (2017) YTHDF3 facilitates translation and decay of N^6-methyladenosine-modified RNA. *Cell Research*, **27**, 315–328.
44. Liu N, Dai Q, Zheng G, He C, Parisien M and Pan T. (2015) N^6-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions. *Nature*, **518**, 560–564.

45. Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S, Peer E, Kol N, Ben-Haim MS, Dai Q, Di Segni A, Salmon-Divon M, Clark WC, *et al.* (2016) The dynamic N^1-methyladenosine methylome in eukaryotic messenger RNA. *Nature*, **530**, 441–446.
46. Li X, Xiong X, Wang K, Wang L, Shu X, Ma S and Yi C. (2016) Transcriptome-wide mapping reveals reversible and dynamic N^1-methyladenosine methylome. *Nature Chemical Biology*, **12**, 311–316.
47. Duechler M, Leszczynska G, Sochacka E and Nawrot B. (2016) Nucleoside modifications in the regulation of gene expression: Focus on tRNA. *Cellular and Molecular Life Sciences*, **73**, 3075–3095.
48. Natchiar SK, Myasnikov AG, Kratzat H, Hazemann I and Klaholz BP. (2017) Visualization of chemical modifications in the human 80S ribosome structure. *Nature*, **551**, 472–477.
49. Zhang C and Jia G. (2018) Reversible RNA modification N^1-methyladenosine (m^1A) in mRNA and tRNA. *Genomics, Proteomics & Bioinformatics*, **16**, 155–161.
50. Liu F, Clark W, Luo G, Wang X, Fu Y, Wei J, Wang X, Hao Z, Dai Q, Zheng G, *et al.* (2016) ALKBH1-mediated tRNA demethylation regulates translation. *Cell*, **167**, 816–828.
51. Dai X, Wang T, Gonzalez G and Wang Y. (2018) Identification of YTH domain-containing proteins as the readers for *N*1-methyladenosine in RNA. *Analytical Chemistry*, **90**, 6380–6384.
52. Seo KW and Kleiner RE. (2020) YTHDF2 recognition of N^1-methyladenosine (m^1A)-modified RNA is associated with transcript destabilization. *ACS Chemical Biology*, **15**, 132–139.
53. Moore LD, Le T and Fan G. (2013) DNA methylation and its basic function. *Neuropsychopharmacology*, **38**, 23–38.
54. García-Vílchez R, Sevilla A and Blanco S. (2019) Post-transcriptional regulation by cytosine-5 methylation of RNA. *Biochimica et Biophysica Acta — Gene Regulatory Mechanisms*, **1862**, 240–252.
55. Amort T, Soulière MF, Wille A, Jia XY, Fiegl H, Wörle H, Micura R and Lusser A. (2013) Long non-coding RNAs as targets for cytosine methylation. *RNA Biology*, **10**, 1002–1008.
56. Carissimi C, Laudadio I, Lorefice E, Azzalin G, De Paolis V and Fulci V. (2021) Bisulphite miRNA-seq reveals widespread CpG and non-CpG 5-(hydroxy)methylcytosine in human microRNAs. *RNA Biology*, **18**, 2226–2235.
57. Trixl L and Lusser A. (2019) The dynamic RNA modification 5-methylcytosine and its emerging role as an epitranscriptomic mark. *Wiley Interdisciplinary Reviews: RNA,* **10**, e1510.
58. Squires JE, Patel HR, Nousch M, Sibbritt T, Humphreys DT, Parker BJ, Suter CM and Preiss T. (2012) Widespread occurrence of 5-methylcytosine in human coding and non-coding RNA. *Nucleic Acids Research*, **40**, 5023–5033.

59. Huang T, Chen W, Liu J, Gu N and Zhang R. (2019) Genome-wide identification of mRNA 5-methylcytosine in mammals. *Nature Structural & Molecular Biology*, **26**, 380–388.
60. Selmi T, Hussain S, Dietmann S, Heib M, Borland K, Flad S, Carter JM, Dennison R, Huang YL, Kellner S, *et al.* (2021) Sequence- and structure-specific cytosine-5 mRNA methylation by NSUN6. *Nucleic Acids Research*, **49**, 1006–1022.
61. Fu L, Guerrero CR, Zhong N, Amato NJ, Liu Y, Liu S, Cai Q, Ji D, Jin SG, Niedernhofer LJ, *et al.* (2014) Tet-mediated formation of 5-hydroxymethylcytosine in RNA. *Journal of the American Chemical Society*, **136**, 11582–11585.
62. Wu H and Zhang Y. (2011) Mechanisms and functions of Tet protein-mediated 5-methylcytosine oxidation. *Genes & Development*, **25**, 2436–2452.
63. Maiti A and Drohat AC. (2011) Thymine DNA glycosylase can rapidly excise 5-formylcytosine and 5-carboxylcytosine: Potential implications for active demethylation of CpG sites. *Journal of Biological Chemistry*, **286**, 35334–35338.
64. Haag S, Sloan KE, Ranjan N, Warda AS, Kretschmer J, Blessing C, Hübner B, Seikowski J, Dennerlein S, Rehling P, *et al.* (2016) NSUN3 and ABH1 modify the wobble position of mt-tRNA^{Met} to expand codon recognition in mitochondrial translation. *EMBO Journal*, **35**, 2104–2119.
65. Yang X, Yang Y, Sun BF, Chen YS, Xu JW, Lai WY, Li A, Wang X, Bhattarai DP, Xiao W, *et al.* (2017) 5-methylcytosine promotes mRNA export — NSUN2 as the methyltransferase and ALYREF as an m^{5}C reader. *Cell Research*, **27**, 606–625.
66. Chen X, Li A, Sun BF, Yang Y, Han YN, Yuan X, Chen RX, Wei WS, Liu Y, Gao CC, *et al.* (2019) 5-methylcytosine promotes pathogenesis of bladder cancer through stabilizing mRNAs. *Nature Cell Biology*, **21**, 978–990.
67. Delatte B, Wang F, Ngoc LV, Collignon E, Bonvin E, Deplus R, Calonne E, Hassabi B, Putmans P, Awe S, *et al.* (2016) Transcriptome-wide distribution and function of RNA hydroxymethylcytosine. *Science*, **351**, 282–285.
68. Huber SM, van Delft P, Mendil L, Bachman M, Smollett K, Werner F, Miska EA and Balasubramanian S. (2015) Formation and abundance of 5-hydroxymethylcytosine in RNA. *Chembiochem*, **16**, 752–755.
69. Miao Z, Xin N, Wei B, Hua X, Zhang G, Leng C, Zhao C, Wu D, Li J, Ge W, *et al.* (2016) 5-hydroxymethylcytosine is detected in RNA from mouse brain tissues. *Brain Research*, **1642**, 546–552.
70. Lan J, Rajan N, Bizet M, Penning A, Singh NK, Guallar D, Calonne E, Li Greci A, Bonvin E, Deplus R, *et al.* (2020) Functional role of Tet-mediated RNA hydroxymethylcytosine in mouse ES cells and during differentiation. *Nature Communications*, **11**, 4956.
71. Iurlaro M, Ficz G, Oxley D, Raiber E, Bachman M, Booth MJ, Andrews S, Balasubramanian S and Reik W. (2013) A screen for hydroxymethylcytosine and formylcytosine binding proteins suggests functions in transcription and chromatin regulation. *Genome Biology*, **14**, R119.

72. Khoddami V, Yerra A, Mosbruger TL, Fleming AM, Burrows CJ and Cairns BR. (2019) Transcriptome-wide profiling of multiple RNA modifications simultaneously at single-base resolution. *Proceedings of the National Academy of Sciences of the United States*, **116**, 6784–6789.
73. Schwartz S, Bernstein DA, Mumbach MR, Jovanovic M, Herbst RH, León-Ricardo BX, Engreitz JM, Guttman M, Satija R, Lander ES, *et al.* (2014) Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. *Cell*, **159**, 148–162.
74. Carlile TM, Rojas-Duran MF, Zinshteyn B, Shin H, Bartoli KM and Gilbert WV. (2014) Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. *Nature*, **515**, 143–146.
75. Martinez NM, Su A, Burns MC, Nussbacher JK, Schaening C, Sathe S, Yeo GW and Gilbert WV. (2022) Pseudouridine synthases modify human pre-mRNA co-transcriptionally and affect pre-mRNA processing. *Molecular Cell*, **82**, 645–659.
76. Watkins NJ and Bohnsack MT. (2012) The box C/D and H/ACA snoRNPs: Key players in the modification, processing and the dynamic folding of ribosomal RNA. *Wiley Interdisciplinary Reviews: RNA,* **3**, 397–414.
77. Babler J and Hurt E. (2019) Eukaryotic ribosome assembly. *Annual Review of Biochemistry*, **88**, 281–306.
78. Song J and Yi C. (2020) Reading chemical modifications in the transcriptome. *Journal of Molecular Biology*, **432**, 1824–1839.
79. Rintala-Dempsey AC and Kothe U. (2017) Eukaryotic stand-alone pseudouridine synthases — RNA modifying enzymes and emerging regulators of gene expression? *RNA Biology*, **14**, 1185–1196.
80. Roundtree IA, Evans ME, Pan T and He C. (2017) Dynamic RNA modifications in gene expression regulation. *Cell*, **169**, 1187–1200.
81. Hudson GA, Bloomingdale RJ and Znosko BM. (2013) Thermodynamic contribution and nearest-neighbor parameters of pseudouridine-adenosine base pairs in oligoribonucleotides. *RNA*, **19**, 1474–1482.
82. Davis DR, Veltri CA and Nielsen L. (1998) An RNA model system for investigation of pseudouridine stabilization of the codon-anticodon interaction in $tRNA^{Lys}$, $tRNA^{His}$ and $tRNA^{Tyr}$. *Journal of Biomolecular Structure and Dynamics*, **15**, 1121–1132.
83. Arnez JG and Steitz TA. (1994) Crystal structure of unmodified $tRNA^{Gln}$ complexed with glutaminyl-tRNA synthetase and ATP suggests a possible role for pseudo-uridines in stabilization of RNA structure. *Biochemistry*, **33**, 7560–7567.
84. Moore MJ and Proudfoot NJ. (2009) Pre-mRNA processing reaches back to transcription and ahead to translation. *Cell*, **136**, 688–700.
85. Matera AG and Wang Z. (2014) A day in the life of the spliceosome. *Nature Reviews Molecular Cell Biology*, **15**, 108–121.
86. Zhao Y, Dunker W, Yu Y-T and Karijolich J. (2018) The role of noncoding RNA pseudouridylation in nuclear gene expression events. *Frontiers in Bioengineering and Biotechnology*, **6**, 8.

87. Newby MI and Greenbaum NL. (2002) Sculpting of the spliceosomal branch site recognition motif by a conserved pseudouridine. *Nature Structural & Molecular Biology*, **9**, 958–965.
88. Eyler DE, Franco MK, Batool Z, Wu MZ, Dubuke ML, Dobosz-Bartoszek M, Jones JD, Polikanov YS, Roy B and Koutmou KS. (2019) Pseudouridinylation of mRNA coding sequences alters translation. *Proceedings of the National Academy of Sciences of the United States*, **116**, 23068–23074.
89. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S and Weissman D. (2008) Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Molecular Therapy*, **16**, 1833–1840.
90. Melnikov S, Ben-Shem A, Garreau de Loubresse N, Jenner L, Yusupova G and Yusupov M. (2012) One core, two shells: Bacterial and eukaryotic ribosomes. *Nature Structural & Molecular Biology*, **19**, 560–567.
91. Liang X, Liu Q and Fournier MJ. (2007) rRNA modifications in an intersubunit bridge of the ribosome strongly affect both ribosome biogenesis and activity. *Molecular Cell*, **28**, 965–977.
92. Baxter-Roshek JL, Petrov AN and Dinman JD. (2007) Optimization of ribosome structure and function by rRNA base modification. *PLoS ONE*, **2**, e174.
93. Fazi F and Fatica A. (2021) Regulation of ribosome function by RNA modifications in hematopoietic development and leukemia: It is not only a matter of m^6A. *International Journal of Molecular Sciences*, **22**, 4755.
94. Waku T, Nakajima Y, Yokoyama W, Nomura N, Kako K, Kobayashi A, Shimizu T and Fukamizu A. (2016) NML-mediated rRNA base methylation links ribosomal subunit formation to cell proliferation in a p53-dependent manner. *Journal of Cell Science*, **129**, 2382–2393.
95. Ignatova VV, Stolz P, Kaiser S, Gustafsson TH, Lastres PR, Sanz-Moreno A, Cho YL, Amarie OV, Aguilar-Pimentel A, Klein-Rodewald T, *et al.* (2020) The rRNA m6A methyltransferase METTL5 is involved in pluripotency and developmental programs. *Genes & Development*, **34**, 715–729.
96. Heissenberger C, Liendl L, Nagelreiter F, Gonskikh Y, Yang G, Stelzer EM, Krammer TL, Micutkova L, Vogt S, Kreil DP, *et al.* (2019) Loss of the ribosomal RNA methyltransferase NSUN5 impairs global protein synthesis and normal growth. *Nucleic Acids Research*, **47**, 11807–11825.
97. Delaunay S and Frye M. (2019) RNA modifications regulating cell fate in cancer. *Nature Cell Biology*, **21**, 552–559.
98. Jackman JE and Alfonzo JD. (2013) Transfer RNA modifications: Nature's combinatorial chemistry playground. *Wiley Interdisciplinary Reviews: RNA,* **4**, 35–48.
99. Byrne RT, Konevega AL, Rodnina MV and Antson AA. (2010) The crystal structure of unmodified $tRNA^{Phe}$ from *Escherichia coli*. *Nucleic Acids Research*, **38**, 4154–4162.

100. Alexandrov A, Chernyakov I, Gu W, Hiley SL, Hughes TR, Grayhack EJ and Phizicky EM. (2006) Rapid tRNA decay can result from lack of nonessential modifications. *Molecular Cell*, **21**, 87–96.
101. Motorin Y and Helm M. (2010) tRNA stabilization by modified nucleotides. *Biochemistry*, **49**, 4934–4944.
102. Pütz J, Florentz C, Benseler F and Giegé R. (1994) A single methyl group prevents the mischarging of a tRNA. *Nature Structural & Molecular Biology*, **1**, 580–582.
103. Voorhees RM and Ramakrishnan V. (2013) Structural basis of the translational elongation cycle. *Annual Review of Biochemistry*, **82**, 203–236.
104. Agris PF, Narendran A, Sarachan K, Väre VYP and Eruysal E. (2017) The importance of being modified: The role of RNA modifications in translational fidelity. *Emzymes*, **41**, 1–50.
105. Hori H. (2014) Methylated nucleosides in tRNA and tRNA methyltransferases. *Frontiers in Genetics*, **5**, 144.
106. Agris PF, Vendeix FAP and Graham WD. (2007) tRNA's wobble decoding of the genome: 40 years of modification. *Journal of Molecular Biology*, **366**, 1–13.
107. Tuorto F and Lyko F. (2016) Genome recoding by tRNA modifications. *Open Biology*, **6**, 160287.
108. Morris RC, Brown KG and Elliott MS. (1999) The effect of queuosine on tRNA structure and function. *Journal of Biomolecular Structure and Dynamics*, **16**, 757–774.
109. Fergus C, Barnes D, Alqasem MA and Kelly VP. (2015) The queuine micronutrient: Charting a course from microbe to man. *Nurients*, **7**, 2897–2929.
110. Su Z, Wilson B, Kumar P and Dutta A. (2020) Noncanonical roles of tRNAs: tRNA fragments and beyond. *Annual Review of Genetics*, **54**, 47–69.
111. Lyons SM, Fay MM, Akiyama Y, Anderson PJ and Ivanov P. (2017) RNA biology of angiogenin: Current state and perspectives. *RNA Biology*, **14**, 171–178.
112. Donovan J, Rath S, Kolet-Mandrikov D and Korennykh A. (2017) Rapid RNase L-driven arrest of protein synthesis in the dsRNA response without degradation of translation machinery. *RNA*, **23**, 1660–1671.
113. Li M, Kao E, Malone D, Gao X, Wang JYJ and David M. (2018) DNA damage-induced cell death relies on SLFN11-dependent cleavage of distinct type II tRNAs. *Nature Structural & Molecular Biology*, **25**, 1047–1058.
114. Yang JY, Deng XY, Li YS, Ma XC, Feng JX, Yu B, Chen Y, Luo YL, Wang X, Chen ML, *et al.* (2018) Structure of Schlafen13 reveals a new class of tRNA/rRNA-targeting RNase engaged in translational control. *Nature Communications*, **9**, 1165.
115. Ivanov P, Emara MM, Villen J, Gygi SP and Anderson P. (2011) Angiogenin-induced tRNA fragments inhibit translation initiation. *Molecular Cell*, **43**, 613–623.
116. Dhahbi JM. (2015) 5′ tRNA halves: The next generation of immune signaling molecules. *Frontiers in Immunology*, **6**, 74.
117. Schaefer M, Pollex T, Hanna K, Tuorto F, Meusburger M, Helm M and Lyko F. (2010) RNA methylation by Dnmt2 protects transfer RNAs against stress-induced cleavage. *Genes & Development*, **24**, 1590–1595.

118. Blanco S, Dietmann S, Flores JV, Hussain S, Kutter C, Humphreys P, Lukk M, Lombard P, Treps L, Popis M, *et al.* (2014) Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. *EMBO Journal*, **33**, 2020–2039.
119. Huang H, Weng H, Deng X and Chen J. (2020) RNA modifications in cancer: Functions, mechanisms, and therapeutic implications. *Annual Review of Cancer Biology*, **4**, 221–240.
120. Barbieri I and Kouzarides T. (2020) Role of RNA modifications in cancer. *Nature Reviews Cancer*, **20**, 303–322.
121. Sun T, Wu R and Ming L. (2019) The role of m6A RNA methylation in cancer. *Biomedicine & Pharmacotherapy*, **112**, 108613.
122. Visvanathan A, Patil V, Arora A, Hegde AS, Arivazhagan A, Santosh V and Somasundaram K. (2018) Essential role of METTL3-mediated m^6A modification in glioma stem-like cells maintenance and radioresistance. *Oncogene*, **37**, 522–533.
123. Vu LP, Pickering BF, Cheng Y, Zaccara S, Nguyen D, Minuesa G, Chou T, Chow A, Saletore Y, MacKay M, *et al.* (2017) The N^6-methyladenosine (m^6A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. *Nature Medicine*, **23**, 1369–1376.
124. Lin X, Chai G, Wu Y, Li J, Chen F, Liu J, Luo G, Tauler J, Du J, Lin S, *et al.* (2019) RNA m^6A methylation regulates the epithelial mesenchymal transition of cancer cells and translation of Snail. *Nature Communications*, **10**, 2065.
125. Ma JZ, Yang F, Zhou CC, Liu F, Yuan JH, Wang F, Wang TT, Xu QG, Zhou WP and Sun SH. (2017) METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N^6-methyladenosine-dependent primary MicroRNA processing. *Hepatology*, **65**, 529–543.
126. Yang S, Wei J, Cui YH, Park G, Shah P, Deng Y, Aplin AE, Lu Z, Hwang S, He C, *et al.* (2019) m^6A mRNA demethylase FTO regulates melanoma tumorigenicity and response to anti-PD-1 blockade. *Nature Communications*, **10**, 2782.
127. Li Z, Weng H, Su R, Weng X, Zuo Z, Li C, Huang H, Nachtergaele S, Dong L, Hu C, *et al.* (2017) FTO plays an oncogenic role in acute myeloid leukemia as a N^6-methyladenosine RNA demethylase. *Cancer Cell*, **31**, 127–141.
128. Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, Chen I, He X and Semenza GL. (2016) Hypoxia induces the breast cancer stem cell phenotype by HIF-dependent and ALKBH5-mediated m^6A-demethylation of NANOG mRNA. *Proceedings of the National Academy of Sciences of the United States*, **113**, E2047–E2056.
129. Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z, Chen Y, Sulman EP, Xie K, Bögler O, *et al.* (2017) m^6A demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXM1 expression and cell proliferation program. *Cancer Cell*, **31**, 591–606.
130. Tanabe A, Tanikawa K, Tsunetomi M, Takai K, Ikeda H, Konno J, Torigoe T, Maeda H, Kutomi G, Okita K, *et al.* (2016) RNA helicase YTHDC2 promotes cancer metastasis via the enhancement of the efficiency by which *HIF-1a* mRNA is translated. *Cancer Letters*, **376**, 34–42.

131. Paris J, Morgan M, Campos J, Spencer GJ, Shmakova A, Ivanova I, Mapperley C, Lawson H, Wotherspoon DA, Sepulveda C, *et al.* (2019) Targeting the RNA m^{6}A reader YTHDF2 selectively compromises cancer stem cells in acute myeloid leukemia. *Cell Stem Cell*, **25**, 137–148.
132. Chen J, Sun Y, Xu X, Wang D, He J, Zhou H, Lu Y, Zeng J, Du F, Gong A, *et al.* (2017) YTH domain family 2 orchestrates epithelial-mesenchymal transition/proliferation dichotomy in pancreatic cancer cells. *Cell Cycle*, **16**, 2259–2271.
133. Müller S, Glaß M, Singh AK, Haase J, Bley N, Fuchs T, Lederer M, Dahl A, Huang H, Chen J, *et al.* (2019) IGF2BP1 promotes SRF-dependent transcription in cancer in a m^{6}A- and miRNA-dependent manner. *Nucleic Acids Research*, **47**, 375–390.
134. Cui Q, Shi H, Ye P, Li L, Qu Q, Sun G, Sun G, Lu Z, Huang Y, Yang C, *et al.* (2017) m^{6}A RNA methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells. *Cell Reports*, **18**, 2622–2634.
135. Kalluri R and Weinberg RA. (2009) The basics of epithelial-mesenchymal transition. *Journal of Clinical Investigation*, **119**, 1420–1428.
136. Wang Z and Chen Z. (2008) Acute promyelocytic leukemia: From highly fatal to highly curable. *Blood*, **111**, 2505–2515.
137. Maynard MA and Ohh M. (2007) The role of hypoxia-inducible factors in cancer. *Cellular and Molecular Life Sciences*, **64**, 2170–2180.
138. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A and Weinberg RA. (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell*, **117**, 927–939.
139. Chen Y, Ling Z, Cai X, Xu Y, Lv Z, Man D, Ge J, Yu C, Zhang D, Zhang Y, *et al.* (2021) Activation of YAP1 by N^6-methyladenosine-modified circCPSF6 drives malignancy in hepatocellular carcinoma. *Cancer Research*, **82**, 599–614.
140. Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, Zhao BS, Mesquita A, Liu C, Yuan CL, *et al.* (2018) Recognition of RNA *N*^{6}-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. *Nature Cell Biology*, **20**, 285–295.
141. Peng W, Li J, Chen R, Gu Q, Yang P, Qian W, Ji D, Wang Q, Zhang Z, Tang J, *et al.* (2019) Upregulated METTL3 promotes metastasis of colorectal cancer via miR-1246/SPRED2/MAPK signaling pathway. *Journal of Experimental & Clinical Cancer Research*, **38**, 393.
142. Wang H, Liu S, Kong F, Xiao F, Li Y, Wang H, Zhang S, Huang D, Wang L and Yang Y. (2020) Spred2 inhibits epithelial-mesenchymal transition of colorectal cancer cells by impairing ERK signaling. *Oncology Reports*, **44**, 174–184.
143. Ebrahimi F, Gopalan V, Smith RA and Lam AK. (2014) miR-126 in human cancers: Clinical roles and current perspectives. *Experimental and Molecular Pathology*, **96**, 98–107.
144. Sun L, Wan A, Zhou Z, Chen D, Liang H, Liu C, Yan S, Niu Y, Lin Z, Zhan S, *et al.* (2021) RNA-binding protein RALY reprogrammes mitochondrial metabolism via mediating miRNA processing in colorectal cancer. *Gut*, **70**, 1698–1712.

145. Zhang J, Bai R, Li M, Ye H, Wu C, Wang C, Li S, Tan L, Mai D, Li G, *et al.* (2019) Excessive miR-25-3p maturation via N^6-methyladenosine stimulated by cigarette smoke promotes pancreatic cancer progression. *Nature Communications*, **10**, 1858.
146. Han J, Wang J, Yang X, Yu H, Zhou R, Lu H, Yuan W, Lu J, Zhou Z, Lu Q, *et al.* (2019) METTL3 promote tumor proliferation of bladder cancer by accelerating pri-miR221/222 maturation in m6A-dependent manner. *Molecular Cancer*, **18**, 110.
147. Wang H, Deng Q, Lv Z, Ling Y, Hou X, Chen Z, Dinglin X, Ma S, Li D, Wu Y, *et al.* (2019) *N6*-methyladenosine induced miR-143-3p promotes the brain metastasis of lung cancer via regulation of VASH1. *Molecular Cancer*, **18**, 181.
148. Janin M, Coll-SanMartin L and Esteller M. (2020) Disruption of the RNA modifications that target the ribosome translation machinery in human cancer. *Molecular Cancer*, **19**, 70.
149. Xing J, Yi J, Cai X, Tang H, Liu Z, Zhang X, Martindale JL, Yang X, Jiang B, Gorospe M, *et al.* (2015) NSun2 promotes cell growth via elevating cyclin-dependent kinase 1 translation. *Molecular Cell Biology*, **35**, 4043–4052.
150. Li Y, Li J, Luo M, Zhou C, Shi X, Yang W, Lu Z, Chen Z, Sun N and He J. (2018) Novel long noncoding RNA NMR promotes tumor progression via NSUN2 and BPTF in esophageal squamous cell carcinoma. *Cancer Letters*, **430**, 57–66.
151. Blanco S, Bandiera R, Popis M, Hussain S, Lombard P, Aleksic J, Sajini A, Tanna H, Cortés-Garrido R, Gkatza N, *et al.* (2016) Stem cell function and stress response are controlled by protein synthesis. *Nature*, **534**, 335–340.
152. Janin M, Ortiz-Barahona V, Castro de Moura M, Martínez-Cardús A, Llinàs-Arias P, Soler M, Nachmani D, Pelletier J, Schumann U, Calleja-Cervantes ME, *et al.* (2019) Epigenetic loss of RNA-methyltransferase NSUN5 in glioma targets ribosomes to drive a stress adaptive translational program. *Acta Neuropathology*, **138**, 1053–1074.
153. Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, Lobry C, Figueroa ME, Vasanthakumar A, Patel J, Zhao X, *et al.* (2011) *Tet2* loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell*, **20**, 11–24.
154. Cui Q, Wang C, Liu S, Du R, Tian S, Chen R, Geng H, Subramanian S, Niu Y, Wang Y, *et al.* (2021) YBX1 knockdown induces renal cell carcinoma cell apoptosis via Kindlin-2. *Cell Cycle*, **20**, 2413–2427.
155. Habibi G, Leung S, Law JH, Gelmon K, Masoudi H, Turbin D, Pollak M, Nielsen TO, Huntsman D and Dunn S.E. (2008) Redefining prognostic factors for breast cancer: YB-1 is a stronger predictor of relapse and disease-specific survival than estrogen receptor or HER-2 across all tumor subtypes. *Breast Cancer Research*, **10**, R86.
156. Zhao S, Guo W, Li J, Yu W, Guo T, Deng W and Gu C. (2016) High expression of Y-box-binding protein 1 correlates with poor prognosis and early recurrence in patients with small invasive lung adenocarcinoma. *OncoTargets and Therapy*, **9**, 2683–2692.

157. Wang Y, Su J, Wang Y, Fu D, Ideozu JE, Geng H, Cui Q, Wang C, Chen R, Yu Y, (2019) The interaction of YBX1 with G3BP1 promotes renal cell carcinoma cell metastasis via YBX1/G3BP1-SPP1-NF-kB signaling axis. *Journal of Experimental & Clinical Cancer Research*, **38**, 386.
158. Yamashita T, Higashi M, Momose S, Morozumi M and Tamaru J. (2017) Nuclear expression of Y box binding-1 is important for resistance to chemotherapy including gemcitabine in TP53-mutated bladder cancer. *International Journal of Oncology*, **51**, 579–586.
159. Bargou RC, Jürchott K, Wagener C, Bergmann S, Metzner S, Bommert K, Mapara MY, Winzer K, Dietel M, Dörken B, *et al.* (1997) Nuclear localization and increased levels of transcription factor YB-1 in primary human breast cancers are associated with intrinsic MDR1 gene expression. *Nature Medicine*, **3**, 447–450.
160. Lyabin DN, Eliseeva IA and Ovchinnikov LP. (2014) YB-1 protein: Functions and regulation. *Wiley Interdisciplinary Reviews: RNA,* **5**, 95–110.
161. Liu J, Xiao S, Chen J, Lou W and Chen X. (2022) A comprehensive analysis for expression, diagnosis, and prognosis of m^5C regulator in breast cancer and its ncRNA-mRNA regulatory mechanism. *Frontiers in Genetics*, **13**, 822721.
162. Huang Y, Huang C, Jiang X, Yan Y, Zhuang K, Liu F, Li P and Wen Y. (2022) Exploration of potential roles of m5C-related regulators in colon adenocarcinoma prognosis. *Frontiers in Genetics*, **13**, 816173.
163. Xue C, Zhao Y, Li G and Li L. (2021) Multi-omic analyses of the m^5C regulator ALYREF reveal its essential roles in hepatocellular carcinoma. *Frontiers in Oncology*, **11**.
164. Nagy Z, Seneviratne JA, Kanikevich M, Chang W, Mayoh C, Venkat P, Du Y, Jiang C, Salib A, Koach J, *et al.* (2021) An ALYREF-MYCN coactivator complex drives neuroblastoma tumorigenesis through effects on USP3 and MYCN stability. *Nature Communications*, **12**, 1881.
165. Wang J, Li Y, Xu B, Dong J, Zhao H, Zhao D and Wu Y. (2021) ALYREF drives cancer cell proliferation through an ALYREF-MYC positive feedback loop in glioblastoma. *OncoTargets and Therapy*, **14**, 145–155.
166. Xu X, Zhang Y, Zhang J and Zhang X. (2020) NSun2 promotes cell migration through methylating autotaxin mRNA. *Journal of Biological Chemistry*, **295**, 18134–18147.
167. Cheray M, Etcheverry A, Jacques C, Pacaud R, Bougras-Cartron G, Aubry M, Denoual F, Peterlongo P, Nadaradjane A, Briand J, *et al.* (2020) Cytosine methylation of mature microRNAs inhibits their functions and is associated with poor prognosis in glioblastoma multiforme. *Molecular Cancer*, **19**, 36.
168. Weissman SM, Lewis M and Karon M. (1963) Pseudouridine metabolism. IV. Excretion of pseudouridine and other nitrogenous metabolites in chronic leukemia. *Blood*, **22**, 657–663.
169. Gehrke CW, Kuo KC, Waalkes TP and Borek E. (1979) Patterns of urinary excretion of modified nucleosides. *Cancer Research*, **39**, 1150–1153.

170. Penzo M, Guerrieri AN, Zacchini F, Treré D and Montanaro L. (2017) RNA pseudouridylation in physiology and medicine: For better and for worse. *Genes*, **8**, 301.
171. Hamma T and Ferré D'Amaré AR. (2006) Pseudouridine synthases. *Chemistry & Biology*, **13**, 1125–1135.
172. Garus A and Autexier C. (2021) Dyskerin: An essential pseudouridine synthase with multifaceted roles in ribosome biogenesis, splicing, and telomere maintenance. *RNA*, **27**, 1441–1458.
173. Bellodi C, Kopmar N and Ruggero D. (2010) Deregulation of oncogene-induced senescence and p53 translational control in X-linked dyskeratosis congenita. *EMBO Journal*, **29**, 1865–1876.
174. Bellodi C, Krasnykh O, Haynes N, Theodoropoulou M, Peng G, Montanaro L and Ruggero D. (2010) Loss of function of the tumor suppressor DKC1 perturbs p27 translation control and contributes to pituitary tumorigenesis. *Cancer Research*, **70**, 6026–6035.
175. Rocchi L, Pacilli A, Sethi R, Penzo M, Schneider RJ, Treré D, Brigotti M and Montanaro L. (2013) Dyskerin depletion increases VEGF mRNA internal ribosome entry site-mediated translation. *Nucleic Acids Research*, **41**, 8308–8318.
176. Montanaro L, Brigotti M, Clohessy J, Barbieri S, Ceccarelli C, Santini D, Taffurelli M, Calienni M, Teruya-Feldstein J, Treré D, *et al.* (2006) Dyskerin expression influences the level of ribosomal RNA pseudo-uridylation and telomerase RNA component in human breast cancer. *Journal of Pathology*, **210**, 10–18.
177. Miao F, Chu K, Chen H, Zhang M, Shi P, Bai J and You Y. (2019) Increased DKC1 expression in glioma and its significance in tumor cell proliferation, migration and invasion. *Investigational New Drugs*, **37**, 1177–1186.
178. Zhao X, Patton JR, Davis SL, Florence B, Ames SJ and Spanjaard RA. (2004) Regulation of nuclear receptor activity by a pseudouridine synthase through post-transcriptional modification of steroid receptor RNA activator. *Molecular Cell*, **15**, 549–558.
179. Jana S, Hsieh AC and Gupta R. (2017) Reciprocal amplification of caspase-3 activity by nuclear export of a putative human RNA-modifying protein, PUS10 during TRAIL-induced apoptosis. *Cell Death & Disease*, **8**, e3093.
180. Cui Q, Yin K, Zhang X, Ye P, Chen X, Chao J, Meng H, Wei J, Roeth D, Li L, *et al.* (2021) Targeting PUS7 suppresses tRNA pseudouridylation and glioblastoma tumorigenesis. *Nature Cancer*, **2**, 932–949.
181. Du J, Gong A, Zhao X and Wang G. (2022) Pseudouridylate synthase 7 promotes cell proliferation and invasion in colon cancer through activating PI3K/AKT/mTOR signaling pathway. *Digestive Diseases and Sciences*, **67**, 1260–1270.
182. Marcel V, Ghayad SE, Belin S, Therizols G, Morel AP, Solano-Gonzàlez E, Vendrell JA, Hacot S, Mertani HC, Albaret MA, *et al.* (2013) p53 acts as a safeguard of translational control by regulating fibrillarin and rRNA methylation in cancer. *Cancer Cell*, **24**, 318–330.

183. Koh CM, Gurel B, Sutcliffe S, Aryee MJ, Schultz D, Iwata T, Uemura M, Zeller KI, Anele U, Zheng Q, *et al.* (2011) Alterations in nucleolar structure and gene expression programs in prostatic neoplasia are driven by the *MYC* oncogene. *American Journal of Pathology*, **178**, 1824–1834.
184. Woo H and Chambers SK. (2019) Human ALKBH3-induced m^1A demethylation increases the CSF-1 mRNA stability in breast and ovarian cancer cells. *Biochimica et Biophysica Acta — Gene Regulatory Mechanisms*, **1862**, 35–46.
185. Yamato I, Sho M, Shimada K, Hotta K, Ueda Y, Yasuda S, Shigi N, Konishi N, Tsujikawa K and Nakajima Y. (2012) PCA-1/ALKBH3 contributes to pancreatic cancer by supporting apoptotic resistance and angiogenesis. *Cancer Research*, **72**, 4829–4839.
186. Chen Z, Qi M, Shen B, Luo G, Wu Y, Li J, Lu Z, Zheng Z, Dai Q and Wang H. (2019) Transfer RNA demethylase ALKBH3 promotes cancer progression via induction of tRNA-derived small RNAs. *Nucleic Acids Research*, **47**, 2533–2545.
187. Liu S, Chen S, Tang C, Zhao Y, Cui W, Jia L and Wang L. (2022) The emerging therapeutic target of dynamic and reversible N6-methyladenosine modification during cancer development. *Frontiers in Oncology*, **12**, 970833.
188. Yang B, Wang J, Tan Y, Yuan R, Chen Z and Zou C. (2021) RNA methylation and cancer treatment. *Pharmacological Research*, **174**, 105937.

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Chapter 13

miRNA-based Therapeutic Strategies

Sumit Biswas* and Lakshmi Menon

ViStA Lab, BITS, Pilani — KK Birla Goa Campus, India

Abstract

The discovery of the unique group of small non-coding RNAs named microRNAs in the 1990's opened up possibilities of a novel method of intervention in cancer therapy. Mature microRNAs, which are processed through an intricate biogenesis pathway, have been known to bind target mRNAs and behave as either oncogenes or tumor suppressors. The stable yield of microRNAs in the body fluid coupled with tissue specificity, led to the use of microRNAs as new theranostic biomarkers, as they could ease the pain of invasive detection methods. With the elucidation of their roles in the cancer pathway, the last couple of decades have seen a lot of work being dedicated to designing molecules aimed at the prevention of microRNA binding to target RNAs. Currently, these microRNA "inhibitors" are in advanced stages of deployment as therapeutic molecules. A number of different approaches, both viral and non-viral, have also been devised and tested to successfully deliver these molecules to their targets. This discussion is a comprehensive treatise on the discovery and characterization of microRNAs in cancer types,

*Corresponding author's email: sumit@goa.bits-pilani.ac.in

the repertoire of anti-microRNA compounds that we currently possess, and their uses in deriving new strategies to combat cancer.

1. miRNAs in cancer

Since the period in the 1990s when Victor Ambros' pioneering work revealed the wonderful realm of microRNAs [1], this intriguing group of small RNAs has added new dimensions and perspectives to our understanding of physiological processes [2–4]. Named microRNAs (miRNAs or miRs) due to their final small length (17–24 nucleotides), these endogenously-initiated molecules have now been reported across several systems, ranging from the nematode *Caenorhabditis elegans* to flies and mammals, including humans [5]. *lin-4*, the first microRNA to be discovered, was shown to play a major regulatory role in the *C. elegans* development pathway. Soon after, the miRNA *let-7* too emerged as an important regulatory component [6]. This was followed by discovery in *Arabidopsis thaliana* [7] and thereafter the floodgates were opened, with miRNAs being discovered in human systems. Though the last release of miRBase (v.22), the repository for miRNAs lists the number of entries across all species at 38,589, the number of miRNAs discovered every year has been increasing significantly [8].

1.1. *Synthesis and biogenesis*

The biogenesis of a miRNA generally begins from the transcription of the miRNA-coding gene into a large primary transcript known as the pri-miRNA [9, 10]. Deviations from this canonical pathway occurs in a few instances, as exemplified by the "mirtrons" which are produced from intronic regions of mRNAs during splicing [11]. In the canonical pathway, the pri-miRNA is cleaved into an approximately 80 nucleotide long structure known as the pre-miRNA (Fig. 1). The process of cleavage is mediated by an enzyme known as the Dicer-like 1 (which is actually an RNAse III) in a single step within the nucleus [12] in plant systems. In animals though, this processing step involves more than one enzyme and takes place both within the nucleus and also in the cytoplasm (Fig. 1). An enzyme known as Drosha (also an RNAse III) is known to cleave the

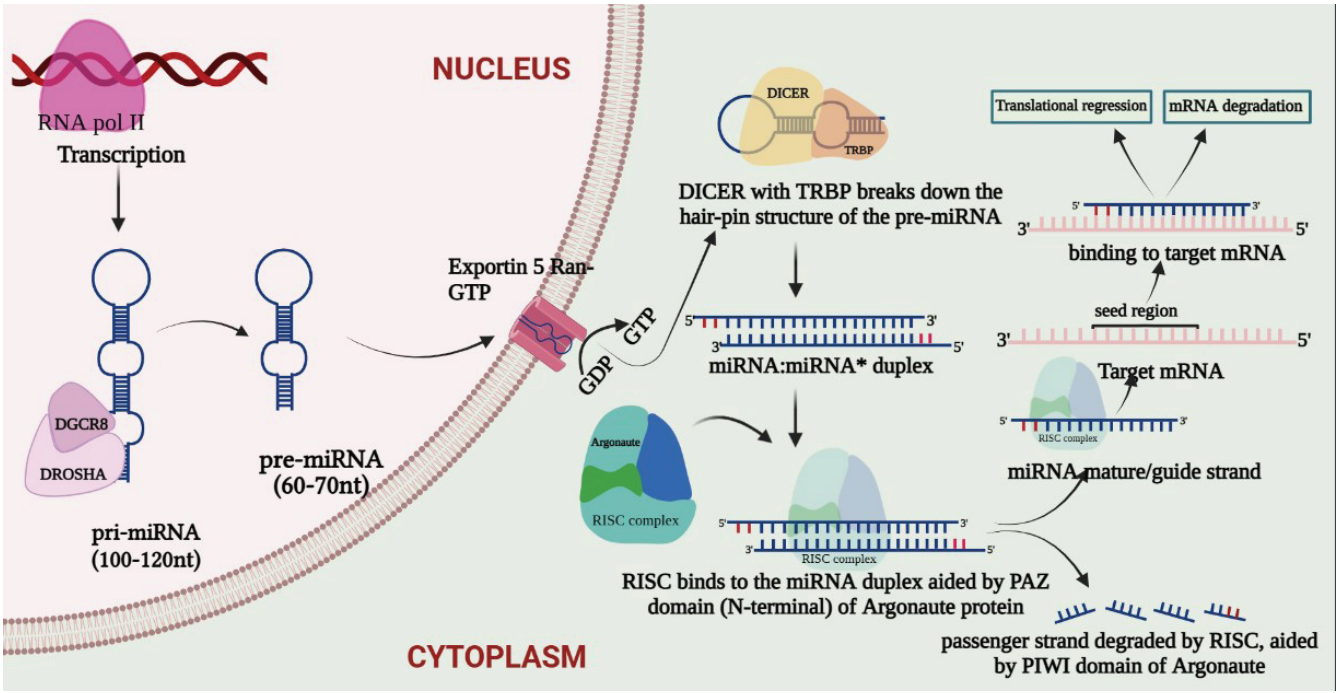

Figure 1. The biogenesis of microRNAs.

pri-miRNA in animals with the aid of a RNA-binding protein known as DGCR8 (also known as Pasha), yielding the shorter pre-miRNA [13–16].

The pre-miRNA with a stem-and-loop structure is transported from the nucleus to the cytoplasm by the Ran/GTP/Exportin5 complex, wherein it is further cleaved by another RNAseIII known as Dicer (along with its binding partner, the Transactivator RNA Binding Protein) [17]. This results in the formation of an 18–24 nucleotide long miRNA/miRNA* duplex. The duplex is the juxtaposition of the guide strand (or mature strand) to its complementary strand (*). Unwinding of the duplex is associated with the formation of an eventual microRNA-induced silencing complex (miRISC) [18]. One of the strands is incorporated into the miRISC depending on complementarity with a protein known as Argonaute, whereas the other strand is degraded by the RISC [19–21]. The RISC is guided to the target mRNA where the initial interaction between the miRNA and the mRNA occurs with the formation of an initial region of complementarity, often dubbed the "seed" region [22]. The "seed" is a 6–8 nucleotide long stretch of the miRNA that forms Watson-Crick base pairs with the complementary sequences in the target mRNA. The recent understanding is through a little divergent, where other non-specific binding sites in the duplex apart from the hypothetical seed region are also seen to form [23, 24]. Binding of the miRNA to the target often produces interesting results depending on the extent of the complementarity. Very often, when the complementarity is nearly absolute, the target mRNA can be degraded, whereas imperfect complementarity leads to an inhibition of translation of the target [4, 22].

1.2. *Implications in the cancer pathway*

In the early years of this century, Calin *et al*. noted that frequent deletions and downregulation of the genes *miR15* and *miR16* were associated with the development of chronic lymphocytic leukemia (CLL) [25]. Located in the long arm of chromosome 13 in humans, these genes were found to be downregulated or deleted in 68% of all CLL cases. This was the first indication of a possible involvement of miRNAs in cancer. A year later, the discovery of a large number of miRNA sequences associated with colonic

adenocarcinoma further cemented the association of miRNAs in cancer [26, 27]. More information about the miRNA-15/16 from other cancer types also kept on emerging, and several other reports citing similar associations were soon available to the community [28]. At the same time, microarray-based detection methods for human and murine miRNAs were also developed, and characterization of expression levels in cancerous cells became relatively simpler [29, 30]. The introduction of other technological advances like small RNA-sequencing added to the momentum, provided more and more evidences supporting the importance of miRNAs in cancer. Within a few years, the writing was on the wall — "50% of microRNA genes are located in cancer-associated genomic regions or in fragile sites, on chromosomes which are frequently deleted, amplified or rearranged in case of cancer development" [31–36]. It is more than accepted now that aberrant miRNA expression has a significant role to play in almost all malignancies.

However, it would be naïve to conclude that miRNAs are only downregulated or deleted in cancer cells; very soon, it was seen that the *miR-17–92* cluster, made up of seven miRNAs and located again in the long arm of chromosome 13, is significantly overexpressed in lung cancers [37, 38]. Similar expression levels were also seen in B-cell lymphomas for the same family of miRNAs. Even more interesting was the discovery of translocation of the *miR-17–92* cluster in Notch-induced T cell acute lymphoblastic leukemia, highlighting the possibilities of the gene rearrangement process [39].

The process of miRNA biogenesis and their involvement in cancer regulation is the result of a synchronized, tightly regulated symphony which features the interaction of multiple transcription factors. For example, the transcription of miRNA genes *mir-15a*, *miR-26*, *miR-29*, and *mir-30* (all of which are involved in tumor suppression) is repressed by c-Myc [40, 41]. Similarly, p53 regulates the expression of the mir-34a/b/c family. Binding of p53 to the promoter of *mir-34a* results in the expression of miR-34a [42, 43]. miR-34a, in turn, represses a negative regulator of p53 known as SIRT, which drives the cell towards apoptosis [44]. There are several such examples of multiple elements being involved in the regulation of expression of miRNAs. Hence, any obvious deviation or aberration in any part of the elaborate network will lead to an aberrant expression of the miRNAs [45].

Another significant breakthrough which came about in the first decade of this century was the availability of circulating miRNAs in human circulating body fluid, safeguarded from the effects of endogenous RNase activity. Mitchell *et al*. reported that the levels of the miR-141 (expressed in prostate cancer) were sufficiently high in serum to actually be used to distinguish prostate cancer patients from healthy individuals [46]. How this became a very important landmark for miRNA diagnostics will be discussed later, but more and more circulating miRNAs came to be detected in blood, serum, plasma, urine, tears, saliva, seminal fluid, cerebrospinal fluid, and other body fluids.

1.3. *Oncomirs and tumor suppressor miRNAs*

From the previous discussion, it follows that miRNAs can have two roles in cancer — either as oncogenic miRNAs or as tumor suppressors. The miRNAs which are overexpressed in cancer cells or tumors can be thought of as oncogenes, whereas the ones which are downregulated in cancerous cells maybe thought of as the tumor suppressors [47, 48]. On one hand, the oncogenic miRNAs (oncomiRs) promote cancer development by inhibiting the expression of tumor suppressor genes or other genes involved in cell cycle regulation. The mir-21, which is an oncomiR, targets Phosphatase and Tensin homolog (PTEN) [49] and Programmed Cell Death 4 (PDCD4) [50], two well-known tumor suppressors present in a host of cell types. Overexpression of mir-21 leads to the downregulation of the tumor suppressors [51] and has often been associated with proliferation and metastasis in glioblastoma [52], pancreatic [53], breast [54], and hepatocellular cancers [55].

On the other hand, tumor suppressor miRNAs bind the mRNAs of oncogenes as their targets and inhibit the oncogene, thereby preventing tumor development. Hence, cells progressing towards cancer need to make sure that these miRNAs are either downregulated or completely absent. For an example, we would again look into the expression patterns of miR-15a and miR-16-1 in chronic lymphocytic leukemia. Bcl-2 (which is an anti-apoptotic regulator) has an inverse relationship with the expression of miR-15a and miR-16-1 [25]. When the cell moves towards apoptosis, miR-15a and miR-16-1 are overexpressed, but the expression of Bcl-2 is severely repressed [56]. However, with the progression of CLL,

miR-15a and miR-16-1 levels are severely depleted while Bcl-2 levels keep on increasing. This highlights the role played by miR-15a and miR-16-1 as tumor suppressors [57]. Other examples of such tumor suppressors are miR-204 [58], miR-148a [59], and miR-365 [60], which also act on Bcl-2. A cancer cell therefore would normally upregulate an oncomiR or downregulate a tumor suppressor to progress into tumorigenesis.

2. MicroRNAs as theranostic molecules

In the previous section, we briefly mentioned about the availability of miRNAs in the circulating body fluid. While the yield of miRNAs in body fluids is fairly stable, it is also remarkable that they are found in histopathological sections (even in formalin-fixed and paraffin-embedded (FFPE) specimens) preserved for 20 years. Added to the above attributes is the tissue specificity of some of the miRNAs, which allow for easy detection using comparative profiling protocols. All these features led to miRNAs being used as novel diagnostic and prognostic blood-based biomarkers. With the continuous surge in the number of these molecules being reported in cancer and other immune responses, miRNAs were soon looked upon as excellent theranostic molecules. It would not be an exaggeration if we state that miRNAs are a very effective source of biomarkers, which can be used to detect cancer without any painful, invasive procedure. Not only that, but they can also be used for the estimation of malignancy transformation, efficacy of treatment, or even in the evaluation of recurrence.

Given their multi-dimensional attributes, microRNAs can therefore be used both as diagnostic biomarkers and prognostic biomarkers [61]. By the broad term “diagnostic,” we imply their role as biomarkers which help in the identification of cancer, whereas miRNA biomarkers that can estimate the efficacy of the treatment decisions can be categorized as “prognostic” biomarkers [62, 63]. As prognostic biomarkers, miRNAs aid in the identification of individuals who have already received treatment, but stand a chance of recurrence or further deterioration, and thereby, provide an opportunity to suitably modify the treatment regimen or go for an altogether new strategy. Representative examples of miRNA biomarkers with respect to different organ types have been listed in Table 1. Some of the

Table 1. miRNA biomarkers in different cancer cells: role as diagnostic or prognostic molecules [66–93].

	miRNA markers	
Target organ	**Diagnostic**	**Prognostic**
Breast	miR-145*#	miR-21*#
	miR-146a	miR-423-3p
	miR-210*#	miR-335
	miR-191	miR-122*#
	miR-215	miR-141**
	miR-92a*	miR-375**
	miR-192*	miR-30a*#
	miR-382	miR-768-3p
	miR-133a	miR-34a*#
	miR-30a*#	miR-210*#
Prostate	miR-222-3p*#	miR-125b-5p
	miR-24-3p	miR-200a**
	miR-30c-5p	miR-200c**
	miR-16	miR-141**
	miR-21*#	miR-375**
	miR-378*#	miR-151-5p
	miR-409-3p*#	miR-146b-3p**
	miR-1290*	miR-210*#
	miR-26a	miR-21*#
	miR-15b	miR-409-3p*#
Colorectal	miR-29a	miR-34b/c
	miR-92a*	miR-148a
	miR-221*#	miR-29c
	miR-34a*#	miR-200c**
	miR-142-5p	miR-20a
	miR-409-3p*#	miR-130
	miR-601	miR-145*#
	miR-760	miR-216
	miR-18a	miR-378*#
	miR-223*	mi-221*#
CSF	miR-19b	miR-21*#
	miR-451	
	miR-92a*	

Table 1. (*Continued*)

	miRNA markers	
Target organ	**Diagnostic**	**Prognostic**
NSCLC	miR-25	miR-34a*#
	miR-223*	miR-21*#
	miR-379	miR-486
	miR-20a-5p	miR-320b
	miR-141-3p	miR-23b-3p
	miR-145-5p	miR-10b-3p
	miR-155-5p	miR-222-3p*#
	miR-223-3p	miR-195-5p
	miR-126-3p	miR-4257-3p
	miR-210-3p	miR-21-5p
Hepatocellular	miR-101	miR-106b
	miR-126	miR-122*#
	miR-122*#	miR-125b
	miR-801	miR-1
	miR-885-5p	miR-146b-3p**
	miR-192*	miR-210*#
	miR-130b	miR-200a**
	miR-21*#	miR-200c**
	miR-378*#	miR-141**
	miR-1290*	miR-375**

Abbreviations: CSF: Cerebrospinal Fluid; NSCLC: Non small cell lung carcinoma.

'*' common diagnostic marker in more than one cancer types.

'**' common prognostic marker in more than one cancer types.

'*#' both diagnostic and prognostic marker in different cancer types.

earliest therapeutic uses of miRNAs as biomarkers were in the diagnosis of breast cancer, while their usage as prognostic molecules was most notably observed with respect to non-small cell lung cancer (NSCLC) [64, 65].

3. miRNA-based strategies to combat cancer

Over the years, a number of strategies have been derived to tackle carcinogenesis based on our improved understanding of the molecular mechanism of cancer. In the light of new targets and newer molecules being

discovered, existing strategies keep changing and novel therapies emerge. However, treatment with one drug against a selective target often leads to drug resistance in due course. A significant number of patients succumbing to cancer despite the administration of chemotherapy can be traced to the development of resistance to the drug being used. Thus, there is a dynamic demand for new strategies and new molecules to replace the traditional ones. RNA-based strategies seem to be the flavor of this decade's therapeutic approach to cancer. A look at the US patents granted for diverse types of cancers reveal that "miRNA and breast cancer" yielded the maximum number of search results. As miRNAs have the ability to simultaneously regulate multiple targets and control important cellular processes, there is immense potential in utilizing them as the strategy of this new era. While being oncogenes, they also act as tumor suppressors, allowing the flexibility to utilize any one direction while devising a suitable mechanism of treatment [94–96].

The approaches towards the design of miRNA-based molecules can be broadly categorized into four different strategies (Fig. 2):

1. RNAi inhibition strategies
2. Mimetic agents
3. miRNA inhibitors
4. Small molecule inhibitors.

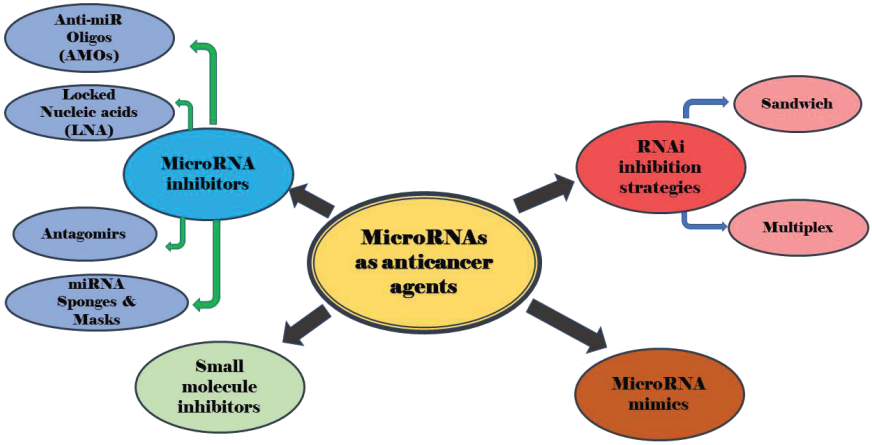

Figure 2. miRNA-based anticancer strategies.

3.1. *RNAi inhibition strategies*

RNAi inhibition refers to the targeting of a single molecular defect or several molecular defects in the complex processes of the cancer pathway using RNA-based approach. Currently, there are two approaches, *viz,* the "sandwich RNAi inhibition strategy" and the "multiplex RNAi inhibition strategy." Both these approaches have been equally successful and have been deployed across a range of cancers.

3.1.1. *Sandwich RNAi inhibition strategy*

The sandwich RNAi inhibition method, pioneered in the works of Calin and Croce (2009) [97], focused on "a major molecular interaction" in the B-cell CLL pathogenesis, using multiple agents. As noted before, CLL is usually characterized by the overexpression of the Bcl-2 protein, associated with the loss of the *miR-15a/miR-16-1* cluster [59, 60]. Calin and Croce proposed to design a strategy which would contain a cocktail of anti-Bcl-2 antisense oligonucleotides (ASO) [98] and miRNAs which are known to target Bcl-2, *viz*, the miR-15/miR-16 family members. Both the ASO and the miRNAs would target the overexpression of Bcl-2, hence the name sandwich RNAi strategy. A similar strategy was also adopted for targeting *ephA2*, an important oncogene for the development of ovarian cancer [99]. In a cocktail with other EphA2-targeting siRNAs, the miR-520d-3p (which is known to target EphA2) was supplied in an attempt to suppress the overexpression of EphA2 [100, 101]. The result was a synergistic action of the two, underlining the efficacy of the sandwich strategy rather than using a "one-drug-one-target" method.

3.1.2. *Multiplex RNA inhibition strategy*

On the other hand, the multiplex RNA inhibition strategy, again from the same group, targeted more than one molecular defect in the same pathway (such as apoptosis). At the risk of sounding cliché, we again take the example of overexpression of the anti-apoptotic Bcl-2. During the process of tumorigenesis, it was observed that the initial phase (when deletion of the *miR-15a/miR-16-1* cluster occurs) is followed by a later more

malignant phase, when cells lack another miRNA, the miR-29 resulting in an aggressive onset of CLL. miR-29 is known to regulate the expression of the *tcl1* oncogene [102]. In the multiplex inhibition approach, both the *miR-15/miR-16* family members (to inhibit Bcl-2 expression) and the *miR-29* members (to offset the expression of Tcl1) are delivered together to counteract the progression of CLL. The conjugative regimen is much more effective than the administration of any these two in isolation. Similar results were observed for the treatment of K-Ras-mutant colorectal cancer using siRNA and miRNA cocktails [103].

3.2. *Mimetic agents*

The restoration or enhancement of lost or downregulated miRNAs which otherwise act as tumor suppressors in the cell, is the basic premise of the popular miRNA-based strategy known as the "restoration, replacement or overexpression therapy." The cornerstones of this therapy are the miRNA mimetic agents, which are synthetic, partially double-stranded miRNA that are identical in sequence to the endogenous precursor miRNAs. These mimics differ from the endogenous miRNAs in that they are chemically modified (2′-O′methoxy) RNA duplexes with enhanced stability and can be more easily loaded into the RISC to eventually inhibit their target mRNAs [104]. When the tumor suppressor miRNA is "lost" from the cancer cells, the miRNA mimics can be processed to behave like the endogenous miRNA in order to reverse or halt the process of tumorigenesis [105]. The mimics are also capable of downstream inhibition of the target mRNAs, a property of the endogenous ones. miRNA mimics have been widely advocated in cancer therapy and have shown significant promise in clinical trials as a treatment regimen [106].

The efficacy of miRNA mimics in prostate cancer cell lines can be used as an example to highlight their mode of action. In advanced prostate tumor cells, miR-15a and miR-16 levels are significantly repressed, concomitant with an increase in the expression of Bcl-2 and other factors [107]. The delivery of miRNA mimics similar to miR-15a in prostate cancer cell lines, showed decrease in Bcl-2 levels and the cells progressed

towards apoptosis. Similarly, the introduction of let-7 mimics in hepatic cancer cells led to a remarkable arrest of cell invasion and proliferation. Underexpression or downregulation of let-7 is a common characteristic of multiple different tumor cell types. In many of these cases, like colon cancer or laryngeal tumor, the usage of let-7 mimics has reduced proliferation and induced apoptosis [108]. A drug lead known as labeled MRX34, which is nothing but a miR-34a mimic in a liposomal formulation, has showed effective results in the treatment of CLL and other hematological malignancies. MRX34 has now completed phase I clinical trials in 2020 and is awaiting further commissioning [109].

The discussion about miRNA mimics would remain incomplete without a discussion about their delivery mechanisms. The innovative idea behind miRNA mimics would entail that these molecules should have taken the therapy space by storm, but the challenges regarding delivery of these agents have taken some time to resolve. Fortunately, now there are multiple possible delivery mechanisms which have been explored and we will take these up in a later section.

3.3. *miRNA inhibitors*

Just like a strategy to supplement tumor suppressors has been introduced as a therapy, inhibition of miRNAs which target endogenous tumor suppressor mRNAs is also a possible strategy that can be used to treat cancer. The strategy is based mainly on the design of anti-miRs which inhibit the mature miRNA from binding to its target mRNA (which are the cellular tumor suppressors), and thereby prevent those miRNAs from acting as oncogenes. Broadly, miRNA inhibitors are complementary, single-stranded oligonucleotides that prevent the binding of the endogenous miRNA to its target tumor suppressor mRNA, by themselves annealing to the miRNA, albeit with a different base-pairing. The resulting duplex is not recognized or loaded into the RISC and is degraded consequently by RNAseH mediated cleavage. miRNA inhibitors include antisense anti-miR oligonucleotides (AMO), locked nucleic acid (LNA) anti-miRNAs, antagomirs, miRNA sponges, and miRNA masks. Some of these have been depicted and summarized in Figure 3.

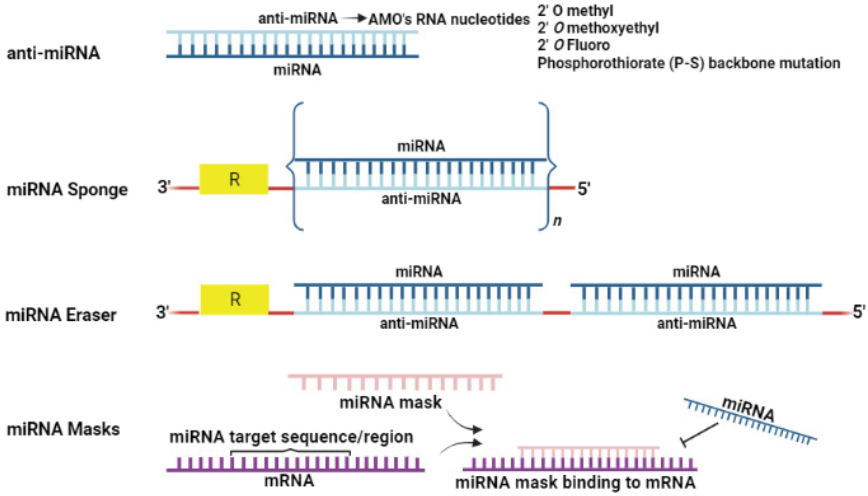

Figure 3. miRNA inhibitors.

The R group refers to a reporter element like FLuc, RLuc, GFP, or YFP, which help in the ready localization of these inhibitors.

3.3.1. *Anti-miR Oligonucleotides*

As their name suggests, AMOs are 17–22 nt single-stranded anti-sense oligonucleotides (ASOs) that are complementary to a specific miRNA [110, 111]. AMOs are chemically modified to increase their binding affinity to the target miRNA and optimized for *in vivo* delivery, thereby increasing the chances of competitive binding and ability to counter nuclease resistance in the cell [112]. The primary modifications are in the 2′ position of the ribose sugar (Fig. 4), and variations include 2′-O-methyl (2′-O-Me), 2′-O-methoxyethyl (2′-MOE), and 2′-fluoro (2′-F) groups at this position. The first report of sequence-specific miRNA inhibition by an AMO was from HeLa cells using an AMO carrying a 2′-O-Me modification. AMOs with a 2′-MOE and phosphorothioate (a sulfur atom instead of an oxygen in the phosphate) modification have been demonstrated to inhibit the synthesis of miR-122 in mouse liver [113, 114].

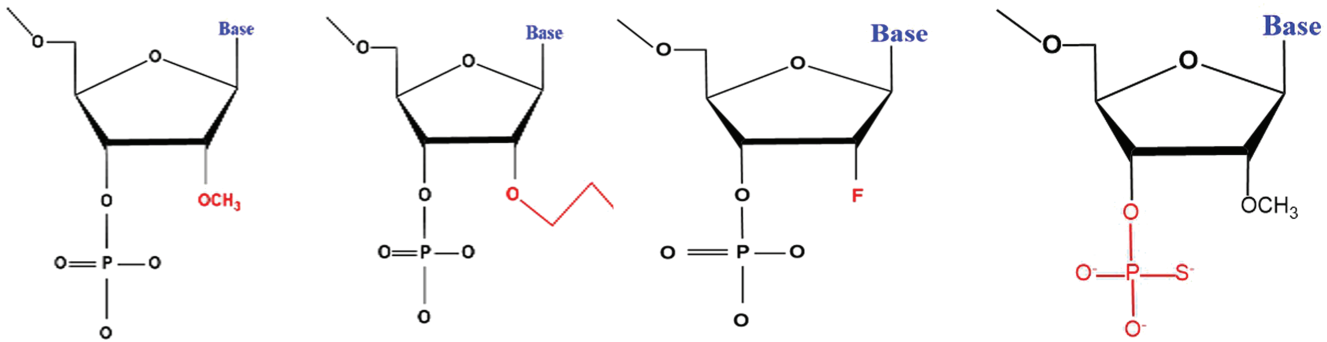

Figure 4. Modifications in the furanose ring of anti-miR oligonucleotides.
From left to right, 2′-O-Me, 2′-MOE, 2′-F, and 2′-O-Me with phosphorothioate.

3.3.2. *Locked nucleic acids*

Locked nucleic acids or LNAs are another class of AMOs which are also effective miRNA inhibitors. LNAs are synthesized with modifications in the ribose ring structure. Practically, the 2′-O and the 4′-C of the furanose ring are locked by cyclic bridging (Fig. 5) so that the resulting modified ribose resembles a C3′-*endo* puckering conformation [115–117]. Conformationally, LNAs are endowed to have better affinity towards complementary sequences of the target mRNAs and can form competitively better Watson-Crick bonds on annealing with their targets. In terms of *in vivo* delivery, LNAs are also more suitable as they have higher solubility in aqueous solutions and show comparatively higher metabolic stability. LNA-containing anti-miRs also have a short half-life and ideal tissue bioavailability, which can be understood from their minimal excretion through urine [118, 119].

LNA-modified antisense oligonucleotides have been seen to effectively silence the tumor suppressor miR-21, which is strongly overexpressed in human glioblastoma cells. Similarly, LNA-incorporated miRNAs have been effectively used as a silencing mechanism against the overexpression of the liver-expressed miR-122 in hepatitis C virus (HCV) infection [120–122]. This miRNA has been incorporated as the world's first miRNA target drug Miravirsen (SPC3649). Miravirsen which began Phase I trials early this decade has been steadily heading towards clinical approval and may soon be available as a prescribed mode of treatment

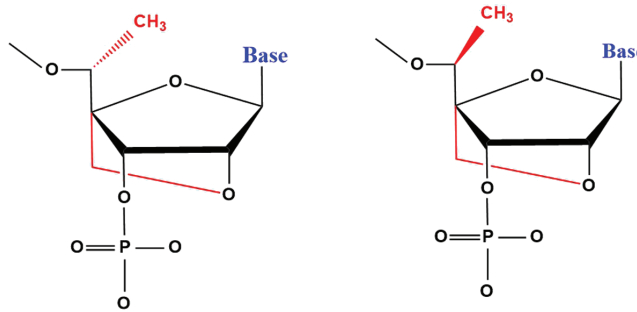

Figure 5. Locked nucleic acid conformations.

[123]. Currently the drug is completing Phase II trials in various countries and is awaiting U.S. Food and Drug Administration (FDA) approval.

3.3.3. *Antagomirs*

A third category of AMOs which carry an additional cholesterol conjugated with the usual 2′-O-Me modifications are the antagomirs. Antagomirs are short single-stranded stretches (~23 nucleotides) having sequence complementarity with their targets. The cholesterol additive accords stability to the molecule and protects it from degradation (might help in escaping an immune response). As an instance, miR-10b which is overexpressed in breast cancer can be selectively inhibited by antagomir-10b. Other antagomirs like antagomir-122 have also been successful in significantly reducing the expression levels of miR-122 in liver cells. Similar silencing effects were also observed when antagomir-16 was delivered to reduce miR-16 levels in the liver, kidney, lung, heart, skeletal muscle, colon, fat, skin, ovaries, adrenal glands, and bone marrow of murine models. The double-stranded antagomir/miR duplexes lead to the degradation of the miRNA and the antagomir is recycled for further availability [124].

3.3.4. *miRNA sponges*

The miRNA sponges are a class of miRNA inhibitors which contain complementary binding sites to a miRNA of interest and are produced from transgenes within cells. These sponges or decoys can competitively bind to the endogenous miRNA, preventing their binding to the original mRNA targets and allowing expression from the target mRNAs [125]. Since the binding sites on the sponge are specific to the miRNA seed region, the sponge can inhibit the activity of a family of miRNAs sharing a common seed. It is possible to generate transgenic sponge-expressing animals which can continuously debilitate a target miRNA throughout the lifetime of the animal. miRNA sponges in human myeloma cells have been shown to increase the induction of p53 and Suppressor of Cytokine Signaling-1 (SOCS1) by soaking up miR-19 and miR-155 [126], which are

overexpressed in myeloma cells. Inside the cell, there are multiple examples of some non-coding RNA sequences, as well as some pseudogenes that act as natural sponges. However, there is a factor of variability in the activity of the sponges in response to different conditions inside the cell.

3.3.5. *miRNA masks*

"miRNA masks" are anti-miRNAs that can selectively inhibit the interaction of the target miRNA with a specific mRNA. The mask specifically binds to the miRNA binding site in the 3′ UTR of the target mRNA, thereby masking the binding site and preventing binding of the endogenous miRNA. Like the miRNA sponge, the miRNA mask is also a 22-nt single-stranded stretch with a 2′-O-methyl-modified oligoribonucleotide (or some other modification), that has complementarity to a protein-coding mRNA. Designing a miRNA mask targeting the binding site of miR-522 led to reduced proliferation of non-small cell lung cancer [127]. However, the action of miRNA masks is sequence-specific and there are inconsistencies in their accuracy as well as potential issues with their toxicity.

3.4. *Small molecule inhibitors*

Another strategy aimed at blocking the activities of very specific miRNA targets is through the use of small molecule inhibitors. These are designed and synthesized keeping a miRNA structure in mind and can be used to block either the precursor or the mature miRNA. Small molecule inhibitors of specific miRNAs (or SMIRs) are preferred because of their ease-of-delivery and the ease of synthesis and validation when compared to the more expensive and elaborate antagomirs. Though the activity of most SMIRs is associated with inhibition of transcription or the biogenesis of endogenous miRNAs, there are a few which function by inhibiting miRNA-target binding. The first small molecule inhibitor was reported in 2008 [128], when a screening of organic compounds revealed that an azobenzene derivative could successfully inhibit the expression of miR-21. A SMIR against miR-21 was a very interesting prospect as this miR is over-expressed in multiple cancer cells — breast, colon, brain, lungs,

liver, prostate and others. Also, the mode of action of this first SMIR was found to be the transcriptional regulation of miR-21 and not interference in the target binding of miR-21. Hence, this SMIR could be effective in a number of different cancer types.

Two years after the discovery of the first SMIR, the miR-122, which is associated with the infection as well as replication of HCV, was targeted using a library of compounds. Two very potent SMIRs were found to target the transcription of the *miR-122* and were subsequently shown to inhibit the replication of the virus in hepatic cells. Similarly, enoxacin, an antibacterial fluoroquinolone which binds to TRBP, was also found to be an effective small molecule inhibitor [129]. Enoxacin has been subsequently proven to enhance the production of tumor suppressor miRNAs and inhibit tumor growth in a number of different cancer types. Polylysine (PLL) and trypaflavine (TPF) are two other compounds which have been shown to reduce tumorigenesis *in vitro*. While PLL inhibits Dicer-mediated processing, TPF acts by inhibiting the binding of miRNA with Argonaute proteins [130].

Another advantage that SMIRs provide is that they can be used even for targeting miRNAs in microvesicles or exosomes. Transport of miRNAs is known to occur via exosomes and microvesicles, and the SMIR can penetrate these vehicles to inhibit the miRNAs carried within them. Additionally, SMIRs can also be designed to prevent the production of exosomes and microvesicles [131, 132]. Likewise, GW4869 has been shown to be effective in blocking exosome secretion as well as in inhibiting miRNA production at the same time. Nowadays, high throughput assays and screens are in place to systematically scan and search for SMIRs against specific miR structures [133]. However, as a note of caution, the flexibility of the target miRNA structure and hence the absence of a proper X-ray crystallography data, make the specific targeting difficult at times, and most of these SMIRs are based on *in silico* models.

4. miRNA delivery strategies

Synthesis and activity of the molecules mentioned in the previous section would all remain futile if the delivery of the molecule to its target is not achieved. Hence, delivery mechanisms and strategies are an integral part

to the miRNA-based therapies. In the past decade and a half, a number of methodologies and exciting new vehicles have been formulated for the delivery of different AMOs or SMIRs. To create a successful delivery system, the model should be able to protect the AMO or mimic from premature degradation while in the circulating fluid, able to bring the molecule to its desired location, able to support the cellular uptake of the molecule, able to elicit a minimal immunogenic response, and easily degraded or excreted without any toxicity to the host [134]. This section outlines a few of the delivery strategies keeping in mind the significant improvements and some of their possible disadvantages.

4.1. *Viral-based delivery systems*

An efficient method for transferring RNAi substrates into target cells is the use of viral vectors. Viral vectors can efficiently transfer miRNA-based therapeutic molecules (miRNA mimics or antagonists) into the designated tumor and cause their prolonged expression. The four most popular viral vectors used for miRNA delivery are adenoviral vectors, adenovirus-like vectors, lentiviral vectors, and retroviral vectors.

4.1.1. *Adenoviral vectors*

Adenoviruses are non-enveloped viruses that contain linear double-stranded DNA genomes with two inverted terminal repeats (ITRs). Adenovirus (Ad) vectors have long been applied for RNAi-based gene silencing mechanisms. To reduce the immunogenicity of the Ad inside the cell, the viral protein-coding sequences have been attenuated to construct helper-dependent Ad vectors [135]. The tendency of Ad to localize in the hepatic region makes them a delivery mechanism of choice for targeted gene delivery in the liver. The inhibition of Hepatitis B Virus replication using a pri-miRNA mimic was achieved by transporting the mimic on an Ad helper-dependent vector. However, helper-dependent Ad vectors strongly stimulate the host innate and adaptive immune responses, which comes across as a major disadvantage of using such vectors.

4.1.2. *Adeno-associated viral vectors*

Adeno-associated viruses (AAV) are also non-enveloped viruses that have single-stranded DNA genomes. These viruses belonging to the Parvoviridae family also offer the advantage of long-term gene expression. A self-complementary AAV was used as the vehicle to deliver miR-26a to hepatoma cancer cells (where the respective miRNA was lost). The AAV was used to clone the sequence of miR-26, conjugated with a green fluorescent protein, and the formulation was administered in a hepatoma murine model with successful delivery [136]. Sustained gene expression was observed in the mice after such AAV treatment.

4.1.3. *Retroviral vectors*

Unlike adenoviruses, retroviruses are enveloped and contain two copies of a single-stranded RNA. When a retrovirus enters a host cell, the RNA is reverse transcribed to double-stranded DNA which is then integrated into the host genome. Retroviruses are therefore very good models for the sustained expression of the miRNA mimic that is inserted in it. Retroviral vectors have been used to increase the expression levels of miR-21 in cardiac nonmyocyte cells. miR-21 was cloned into a Moloney Murine Leukemia Virus (MMLV) vector [137], and adult mice were treated with the MMLV encoding miRNA-21 in the process.

4.1.4. *Lentiviral vectors*

In recent studies, a lentiviral vector was also used to deliver miR-15–16 in the xenograft prostate cancer mice model. Lentiviruses, a class of retroviruses are enveloped viruses as well, and contain two additional regulatory genes, *tat* and *rev* (other than the *gag-pol-env* basic entity in retroviruses). Lentiviruses, like retroviruses, can stably insert themselves into the genomes of recipient cells and allow for persistent gene expression. In other instances, lentiviral delivery of the miR-133b improved functional recovery in mice with an injured spinal cord [138].

Although the delivery efficiency of viral vectors remains undoubtedly unmatched, there are issues with the generation of strong immunogenic response in the host and often high toxicity. Therefore, the quest for non-viral delivery mechanisms remains pertinent, and various non-viral vectors have been used to complement the gap left by viral vectors.

4.2. *Non-viral delivery systems*

A number of delivery systems have been designed with non-viral substrates which have proven to be less toxic and more biocompatible as compared to viral vectors. These systems are less expensive, but effective and accurate in delivering the AMO/mimic to the target cells. Surface modified nanoparticles conjugated with ASOs or miRNA vectors have long been in vogue. Currently the repertoire of non-viral delivery systems is vast, and we will focus on a few in this section.

4.2.1. *Inorganic material-based delivery systems*

Inorganic materials like Fe_3O_4-conjugated nanoparticles, gold nanoparticles (AuNPs), mesoporous silica, nanodiamonds and graphene oxide have been widely used as carriers to deliver miRNA molecules. These inorganic material-based vectors can be utilized for the efficient and precise delivery of miRNAs to the target cell.

The ferric oxide-based NP was used as a nanovehicle in human mesenchymal cells. AuNPs have been effectively used as nanovehicles for the delivery of antagomirs and some mimics. AuNPs can be very easily modified by the attachment of thiol, amino, or imino groups. For example, antagomir-miRNA-155 conjugated to thiol-modified AuNPs reduced inflammatory mediators and ultimately recovered cardiac function in a murine model. AuNPs harboring thiol modifications have also been successful in delivering the pre-miR-145 into multiple cancer cell types. Mesoporous silica also provides an effective medium for miRNA vectors as these nanoparticles are thermally more stable and provide a larger surface area with ease-of-surface-modifications. Anti-miRNA-155-loaded

mesoporous silica based nanocomplexes have shown promising results by inhibiting tumorigenesis in colorectal cancer. Graphene oxide (GO) has a unique honeycomb network which allows it to easily adsorb nucleotides. Recently, an antisense miRNA-21 was successfully loaded onto a hyaluronic acid-conjugated GO to decrease proliferation and induce apoptosis in a human epithelial breast cancer cell line.

4.2.2. *Lipid based delivery systems*

Lipid-based delivery systems are widely used to deliver drugs and nucleic acids. As such, lipid-based systems improve the bioavailability and the solubility of the molecule they carry. Lipids provide ultimate ease-of-modification and can be conjugated with miRNAs as well as fluorescent probes. Successful delivery of mimics of the tumor suppressors miR-34a and let-7 using neutral lipid emulsion to lung cancer cells has been demonstrated, and resulted in a 60% reduction in tumor size [139]. Researchers using lipid-based systems also have the luxury of choosing the vehicle from a wide range of available products in the market such as Lipofectamine®. Cationic lipid components of Lipofectamine® form complexes with the negatively-charged RNA moieties and can overcome the electrostatic repulsion of the cell membrane to be fused with the cell. Use of liposomes for the delivery of miR-29b (a tumor-suppressor which is downregulated in lung cancer) using cationic lipoplexes showed a significant reduction of tumor growth in lung cancer cells. Low delivery efficiency sometimes hampers the delivery mediated by such cationic lipids. To circumvent the problem, these have been conjugated with polyethylene glycol (PEG) at times, as in the case of the miRNA-126-loaded PEG-modified liposomes. Another novel lipid-based delivery system used for miRNA transport is the solid lipid nanoparticles (SLNs). Using SLNs, miR-34a mimics were successfully delivered into cancer stem cells which led to the induction of apoptosis in these cells. However, cationic lipids have sometimes been associated with increased toxicity and increased immunogenic response.

4.2.3. *Polymeric vectors*

Polymeric vectors utilize either synthetic biodegradable polymers, or natural polymers such as collagen, chitosan, and gelatin. Polymeric vectors have multiple advantages — high stability, controllable size, flexibility of addition of functional groups, and they mostly evoke little immunogenic response. Polyethylenimines (PEIs) are cationic polymers which are rich in amine groups and are the most frequently used polymer-based vectors for miRNA therapeutic delivery. Nanodelivery vehicles involving PEIs (Fig. 6) bound to miRNAs can improve cellular uptake and prevent degradation of the miRNA at the same time. Several examples of miRNA mimics conjugated to PEIs have been found to be effective in different cancer cell types. Some notable ones are miR-34a in hepatocellular carcinoma, miR-145 in glioblastoma, and miR-145 in colon cancer [140].

Once again, the use of PEIs has been impaired by their toxicity and low biological degradation in serum. Though addition of polymers like PEG or PLL could somewhat circumvent the problem of biocompatibility of PEIs, the extent of toxicity due to the presence of so many positively-charged moieties have restricted their use as clinical vectors. As an alternative, some researchers have used poly(lactide-co-glycolide) (PLGA), which is a copolymer of polylactic acid and polyglycolic acid. PLGA nanoparticles have much more biocompatibility than PEIs and can be used as sustained release vehicles. PLGA-based nanoparticles could effectively deliver miRNA-99a mimics to hepatic carcinoma and successfully suppress tumor growth.

Chitosan is a polymeric delivery vehicle which is derived from natural sources. Chitosan is derived from chitin, which is a structural component

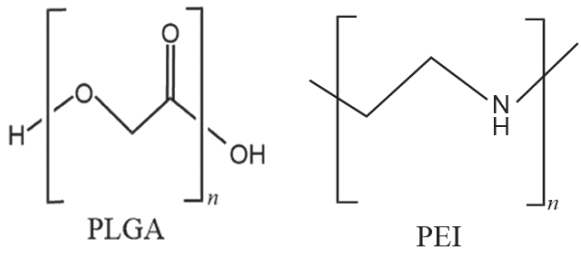

Figure 6. Monomeric units of PLGA and PEI.

of the exoskeletons of crustaceans, molluscs, insects, and fungi. Chitosan being a natural polymer is more biocompatible and has little cytotoxicity, while it can prevent nucleic acid degradation and allow for slow sustained release. Galactosylated low molecular weight chitosan (G-LMWC) has been combined with miR-16 precursors and showed promising improvement in miR-16 levels in colonic macrophages [141].

Polymeric micelles, another popular delivery vehicle, are derived by joining a hydrophilic and a hydrophobic polymer. Mixed nanosized polymeric micelles have been used as part of a codelivery system for the delivery of the doxorubicin and endogenous tumor suppressor miRNA-34a into cancer cells with good effect.

4.2.4. *Some other delivery systems*

There are some more effective delivery vectors which have been tried recently. Three notable ones are degradable dendrimers, polyamidoamine dendrimers, and atelocollagen. Dendrimers are nano-sized, radially symmetric molecules with a symmetric core surrounded by an inner shell and an outer shell. Dendrimers have a branched structure and generally have a spherical morphology, hence the name.

Degradable dendrimers allow multiple modifications and have flexibility with respect to surface functional groups as well as their chemical composition. Modular degradable dendrimers were created with the addition of ester bonds to deliver let-7g mimics. The dendrimer-launched mimic could successfully inhibit tumor growth and had very low toxicity due to its inherent design.

Polyamidoamine (PAMAM) dendrimers are yet another positively-charged delivery vehicle, which binds the negatively-charged miRNAs. Since the surface has high positive charge density, PAMAMs are conjugated with negatively-charged PEG to alleviate the associated toxicity. PAMAM dendrimers have been used for targeted delivery of miR-34a expressing plasmid to NSCLC and have shown remarkable activity without any cytotoxicity.

Lastly, atelocollagen (ATE) is a pepsin-derived type I collagen polymer that has the attributes of a positively-charged carrier. An ATE complex with miR-34a showed reduction in tumor growth when administered

to a xenograft model of colon cancer. ATE-miR-16 has also been used for the *in vivo* amelioration of prostate cancer and could efficiently inhibit tumorigenesis.

5. miRNA drugs in the pipeline

We would like to close this discussion here on an optimistic note. Having listed several methods of countering carcinogenesis and debated about the advantages and disadvantages of multiple delivery vehicles, we should finally conclude with a ray of hope. As cited before in the case of Miravirsen or MRX-34, there are multiple lead molecules which are in different stages of clinical trials. Again, not all the leads that went into trial have been successful, but it is a healthy sign that we can enlist more than fifteen therapeutic molecules at this stage (Table 2). It is our belief that this is only perhaps the tip of the iceberg, and miRNA-based therapeutics has a prolonged future in this decade and beyond.

Table 2. Chronological list of therapeutic miRNA molecules throughout the world.

Therapeutic molecule	Country of origin	Target miRNA	Year of entry into trial	Current status
Miravirsen (SPC3649)	Copenhagen, Denmark	miR-122	13 September 2010	Phase II CT
MRX34	Texas, USA	miR-34a	11 April 2013	Phase I CT
MGN-1374	Massachussets, USA	miRNA-15 and miR195	23 August 2013	Pre-clinical trails
MGN-9103	Massachussets, USA	miR-208	23 August 2013	Pre-clinical trails
MGN-4938	Massachussets, USA	miR-451	23 August 2013	Pre-clinical trails
RG-101	California, USA	miR-122	4 June 2014	Phase IB CT
RG-012	California, USA	miRNA-21	4 September 2014	Phase I completed
Cobomarsen (MRG-106)	Massachussets, USA	miR-155	20 October 2015	Phase II completed
Remlarsen (MRG-201)	Massachussets, USA	miR-29	11 November 2015	Phase II completed

Table 2. (*Continued*)

Therapeutic molecule	Country of origin	Target miRNA	Year of entry into trial	Current status
MRG-107	Massachussets, USA	miR-155	2 June 2016	Pre-clinical Trial completed
MGN-4220	Massachussets, USA	miR-29	8 September 2017	Pre-clinical trails
MGN-5804	Massachussets, USA	miR-378	8 September 2017	Pre-clinical trails
MGN-6114	Massachussets, USA	miR-92	21 March 2018	Pre-clinical trails
MGN-2677	Massachussets, USA	miR-143/145	21 March 2018	Pre-clinicBeal trails
MRG-110	Massachussets, USA	miR-92a	7 May 2019	Phase I completed
RGLS4326	California, USA	miR-17	3 September 2020	Phase IB CT

Source- https://clinicaltrials.gov/

References

1. Lee RC, Feinbaum RL and Ambros V. (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*, **75**(5), 843–854.
2. Lagos-Quintana M, Rauhut R, Lendeckel W and Tuschl T. (2001) Identification of novel genes coding for small expressed RNAs. *Science*, **294**(5543), 853–858.
3. Lau NC, Lim LP, Weinstein EG and Bartel DP. (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis Elegans*. *Science*, **294**(5543), 858–862.
4. Vasudevan S, Tong Y and Steitz JA. (2007) Switching from repression to activation: MicroRNAs can up-regulate translation. *Science*, **318**, 1931–1934.
5. Rodriguez A, Griffiths-Jones S, Ashurst JL and Bradley A. (2004) Identification of mammalian microRNA host genes and transcription units. *Genome Research*, **14**(10A), 1902–1910.
6. Lee RC and Ambros V. (2001) An extensive class of small RNAs in *Caenorhabditis Elegans*. *Science*, **294**(5543), 862–864.
7. Brenda J, Reinhart EG, Weinstein MW, Rhoades BB and David PB. (2002) MicroRNAs in plants. *Genes & Development*, **16**(13), 1616–1626.
8. Kozomara A and Griffiths-Jones S. (2014) miRBase: Annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Research*, **42**, D68–D73.
9. Wahid F, Shehzad A, Khan T and Kim YY. (2010) MicroRNAs: Synthesis, mechanism, function, and recent clinical trials. *Biochimica et Biophysica Acta*, **1803**(11), 1231–1243.

10. Kim VN, Han J and Siomi MC. (2009) Biogenesis of small RNAs in animals. *Nature Reviews Molecular Cell Biology Biol*, **10**(2), 126–139.
11. O'Brien J, Hayder H, Zayed Y and Peng C. (2018) Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front Endocrinol (Lausanne)*, **9**, 402.
12. Jones-Rhoades WM, Bartel DP and Bartel B. (2006) MicroRNAs and their regulatory roles in plants, *Annual Review of Plant Biology*, **57**, 19–53.
13. Borchert GM, Lanier W and Davidson BL. (2006) RNA polymerase III transcribes human microRNAs. *Nature Structural & Molecular Biology*, **13**, 1097–1101.
14. Lee Y, Kim M, Han J, Yeom KH and Lee S. (2004) MicroRNA genes are transcribed by RNA polymerase II. *EMBO Journal*, **23**, 4051–4060.
15. Han J, Lee Y, Yeom KH, Kim YK, Jin H and Kim VN. (2004) The Drosha–DGCR8 complex in primary microRNA processing. *Genes & Development*, **18**, 3016–3027.
16. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N and Shiekhattar R. (2004) The Microprocessor complex mediates the genesis of microRNAs. *Nature*, **432**, 235–240.
17. Ha M and Kim VN. (2014) Regulation of microRNA biogenesis. *Nature Reviews Molecular Cell Biology*, **15**(8), 509–524.
18. Carthew RW and Sontheimer EJ. (2009) Origins and mechanisms of miRNAs and siRNAs. *Cell*, **136**(4), 642–655.
19. Medley JC, Panzade G and Zinovyeva AY. (2021) microRNA strand selection: Unwinding the rules. *Wiley Interdisciplinary Reviews: RNA*, **12**(3), e1627.
20. Macfarlane LA and Murphy PR. (2010) MicroRNA: Biogenesis, function and role in cancer. *Current Genomics*, **11**, 537–561.
21. Lund E, Guttinger S, Calado A, Dahlberg JE and Kutay U. (2004) Nuclear export of microRNA precursors. *Science*, **303**, 95–98.
22. Bartel DP. (2009) MicroRNAs: Target recognition and regulatory functions. *Cell*, **136**, 215–233.
23. Kothandan R and Biswas S. (2015) Identifying microRNAs involved in cancer pathways using support vector machines. *Computational Biology and Chemistry*, **55**, 31–36.
24. Kothandan R and Biswas S. (2022) MicRooN — An ensemble-based classifier for identifying miRNAs associated with cancer. *IEEE Explore*, 9675112.
25. Calin, GA, Dumitru, CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K and Rassenti L. (2002) Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences of the United States*, **99**(24), 15524–15529.
26. Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP and James RJ. (2003) Reduced accumulation of specific microRNAs in colorectal neoplasia. *Molecular Cancer Research*, **1**(12), 882–891.

27. Peng Y and Croce CM. (2016) The role of microRNAs in human cancer. *Signal Transduction and Targeted Therapy*, **1**, 15004.
28. Croce CM. (2009) Causes and consequences of microRNA dysregulation in cancer. *Nature Reviews Genetics*, **10**, 704–714.
29. Munker R and Calin GA. (2011) MicroRNA profiling in cancer. *Clinical Science (London)*, **121**, 141–158.
30. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, *et al.* (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. *Proceedings of the National Academy of Sciences of the United States*, **103**, 2257–2261.
31. Calin GA. (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proceedings of the National Academy of Sciences*, **101**, 2999–3004.
32. Calin GA and Croce CM. (2007) Chromosomal rearrangements and microRNAs: A new cancer link with clinical implications. *Journal of Clinical Investigation*, **117**, 2059–2066.
33. Garzon R, Calin GA and Croce CM. (2009) MicroRNAs in cancer. *Annual Review of Medicine*, **60**, 167–179.
34. Sevignani C, Calin GA, Nnadi SC, Shimizu M, Davuluri RV, Hyslop T, Demant P, Croce CM and Siracusa LD. (2007) MicroRNA genes are frequently located near mouse cancer susceptibility loci. *Proceedings of the National Academy of Sciences*, **104**, 8017–8022.
35. Cho WC. (2007) OncomiRs: The discovery and progress of microRNAs in cancers. *Molecular Cancer*, **6**, 60–66.
36. Drakaki A and Iliopoulos D. (2009) MicroRNA gene networks in oncogenesis. *Current Genomics*, **10**, 35–41.
37. Tagawa H and Seto M. (2005) A microRNA cluster as a target of genomic amplification in malignant lymphoma. *Leukemia*, **19**, 2013–2016.
38. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, *et al.* (2005) A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Research*, **65**, 9628–9632.
39. Mavrakis KJ, Wolfe AL, Oricchio E, Palomero T, Keersmaecker K, McJunkin K, Zuber, J, James T, Khan AA, Leslie CS and Parker JS. (2010) Genome-wide RNA-mediated interference screen identifies miR-19 targets in Notch-induced T-cell acute lymphoblastic leukaemia. *Nature Cell Biology*, **12**, 372–379.
40. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV and Mendell JT. (2005) c-Myc-regulated microRNAs modulate E2F1 expression. *Nature*, **435**, 839–843.
41. Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE and West KM. (2008) Widespread microRNA repression by Myc contributes to tumorigenesis. *Nature Genetics*, **40**, 43–50.
42. Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N and Moskovits N. (2007) Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Molecular Cell*, **26**, 731–743.

43. Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M and Lee KH. (2007) Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Molecular Cell*, **26**, 745–752.
44. Yamakuchi M and Lowenstein CJ. (2009) MiR-34, SIRT1, and p53: The feedback loop. *Cell Cycle*, **8**, 712–715.
45. Hermeking H. (2010) The miR-34 family in cancer and apoptosis. *Cell Death & Differentiation*, **17**, 193–199.
46. Mitchell PS, *et al.* (2008) Circulating microRNAs as stable blood-based markers for cancer detection. *Proceedings of the National Academy of Sciences of the United States*, **105**(30), 10513–8.
47. Esquela-Kerscher A and Slack FJ. (2006) Oncomirs-microRNAs with a role in cancer. *Nature Reviews Cancer*, **6**(4), 259–269.
48. Sotillo E and Thomas-Tikhonenko A. (2011) Shielding the messenger (RNA): MicroRNA-based anticancer therapies. *Pharmacology & Therapeutics*, **131**(1), 18–32.
49. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST and Patel T. (2007) MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology*, **133**, 647–658.
50. Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A and Lund AH. (2008) Programmed cell death 4(PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. *Journal of Biological Chemistry*, **283**, 1026–1033.
51. Krichevsky AM and Gabriely G. (2009) miR-21: A small multi-faceted RNA. *Journal of Cellular and Molecular Medicine*, **13**(1), 39–53.
52. Ciafrè SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G, Negrini M, Maira G, Croce CM and Farace MG. (2005) Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochemical and Biophysical Research Communications*, **334**, 1351–1358.
53. Bloomston M, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP, Liu CG, Bhatt D, Taccioli C and Croce CM. (2007) MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA*, **297**, 1901–1908.
54. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M and Campiglio M. (2005) MicroRNA gene expression deregulation in human breast cancer. *Cancer Research*, **65**, 7065–7070.
55. Karagonlar ZF, Korhan P and Atabey N. (2015) Targeting c-met in cancer by microRNAs: Potential therapeutic applications in hepatocellular carcinoma. *Drug Development Research*, **76**, 357–367.
56. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, AqeilanRI, Zupo S and Dono M. (2005) miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proceedings of the National Academy of Sciences*, **102**, 13944–13949.

57. Kumar MS, Lu J, Mercer KL, Golub TR and Jacks T. (2007) Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nature Genetics*, **39**, 673–677.
58. Sacconi A, Biagioni F, Canu V, Mori F, Di Benedetto A and Lorenzon L. (2012) miR-204 targets Bcl-2 expression and enhances responsiveness of gastric cancer. *Cell Death & Disease*, **3**, e423.
59. Zhang H, Li Y, Huang Q, Ren X, Hu H and Sheng H. (2011) MiR-148a promotes apoptosis by targeting Bcl-2 in colorectal cancer. *Cell Death & Differentiation*, **18**, 1702–1710.
60. Nie J, Liu L, Zheng W, Chen L, Wu X and Xu Y. (2012) microRNA-365, down-regulated in colon cancer, inhibits cell cycle progression and promotes apoptosis of colon cancer cells by probably targeting Cyclin D1 and Bcl-2. *Carcinogenesis*, **33**, 220–225.
61. Anfossi S, Babayan A, Pantel K and Calin GA. (2018) Clinical utility of circulating non-coding RNAs — An update. *Nature Reviews Clinical Oncology*, **15**(9), 541–563.
62. Etheridge A, Lee I, Hood L, Galas D and Wang K. (2011) Extracellular microRNA: A new source of biomarkers. *Mutation Research*, **717**(1-2), 85–90.
63. Lin HM, Nikolic I, Yang J, Castillo L, Deng N, Chan CL, Yeung NK, Dodson E, Elsworth B, *et al.* (2018) MicroRNAs as potential therapeutics to enhance chemo sensitivity in advanced prostate cancer. *Scientific Reports*, **8**(1), 7820.
64. Iorio MV and Croce CM. (2009) MicroRNAs in cancer: Small molecules with a huge impact. *Journal of Clinical Oncology*, **27**(34), 5848–5856.
65. Cho WC. (2010) MicroRNAs: Potential biomarkers for cancer diagnosis, prognosis and targets for therapy. *International Journal of Biochemistry & Cell Biology*, **42**(8), 1273–1281.
66. Sempere LF, Christensen M, Silahtaroglu A, Bak M, Heath CV, Schwartz G, Wells W, Kauppinen S and Cole CN. (2007) Altered MicroRNA expression confined to specific epithelial cell subpopulations in breast cancer. *Cancer Research*, **67**(24), 11612–11620.
67. Volinia S, Galasso M, Sana ME, Wise TF, Palatini J, Huebner K and Croce CM. (2012) Breast cancer signatures for invasiveness and prognosis defined by deep sequencing of microRNA. *Proceedings of the National Academy of Sciences*, **109**(8), 3024–3029.
68. Medina PP, Nolde M and Slack FJ. (2010) OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. *Nature*, **467**, 86–90.
69. Farazi TA, Horlings, HM, ten Hoeve J, Mihailovic A, Halfwerk H, Morozov P, Brown M, Hafner M, Reyal F, van Kouwenhove M and Kreike B. (2011) MicroRNA sequence and expression analysis in breast tumors by deep sequencing. *Cancer Research*, **71**, 4443–4453.
70. Png KJ, Yoshida M, Zhang XHF, Shu W, Lee H, Rimner A, Chan TA, Comen E, Andrade VP, Kim SW and King TA. (2011) MicroRNA-335 inhibits tumor

reinitiation and is silenced through genetic and epigenetic mechanisms in human breast cancer. *Genes & Development*, **25**, 226–231.

71. Fredsøe J, Rasmussen AKI, Thomsen AR, Mouritzen P, Høyer S, Borre M, Ørntoft TF and Sørensen KD. (2017) Diagnostic and prognostic microRNA biomarkers for prostate cancer in cell-free urine. *European Urology Focus*, **4569**(17), 30066–300674.
72. Dong Q, Meng P, Wang T, Qin W, Wang F, Yuan J, Chen Z, Yang A and Wang H. (2010) MicroRNA let-7a inhibits proliferation of human prostate cancer cells in vitro and in vivo by targeting E2F2 and CCND2. *PLoS One*, **5**(4), e10147.
73. Baraniskin A, Kuhnhenn J, Schlegel U, Chan A, Deckert M, Gold R, Maghnouj A, Zöllner H, Reinacher-Schick A, Schmiegel W and Hahn SA. (2011) Identification of microRNAs in the cerebrospinal fluid as marker for primary diffuse large B-cell lymphoma of the central nervous system. *Blood*, **117**, 3140–3146.
74. Drusco A, Bottoni A, Laganà A, Acunzo M, Fassan M, Cascione L, Antenucci A, Kumchala P, Vicentini C, Gardiman MP and Alder H. (2015) A differentially expressed set of microRNAs in cerebro-spinal fluid (CSF) can diagnose CNS malignancies. *Oncotarget*, **6**, 20829–20839.
75. Kopkova A, Sana J, Fadrus P and Slaby O. (2018) Cerebrospinal fluid microRNAs as diagnostic biomarkers in brain tumors. *Clinical Chemistry and Laboratory Medicine*, **56**(6), 869–879.
76. Teplyuk NM, Mollenhauer B, Gabriely G, Giese A, Kim E, Smolsky M, Kim RY, Saria, MG, Pastorino S, Kesari S and Krichevsky AM. (2012) MicroRNAs in cerebrospinal fluid identify glioblastoma and metastatic brain cancers and reflect disease activity. *Neuro-Oncology*, **14**, 689–700.
77. Huang Z, Huang D, Ni S, Peng Z, Sheng W and Du X. (2010) Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *International Journal of Cancer*, **127**(1), 118–126.
78. Ayaz L, Görür A, Yaroğlu HY, Özcan C and Tamer L. (2009) Differential expression of microRNAs in plasma of patients with colorectal cancer: A potential marker for colorectal cancer screening. *Journal of Cancer Research and Clinical Oncology*, **58**(10), 1375–1381.
79. Pu XX, Huang GL, Guo HQ, Guo CC, Li H, Ye S, Ling S, Jiang L, Tian Y and Lin TY. (2010) Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. *Journal of Gastroenterology and Hepatology*, **25**(10), 1674–1680.
80. Cho WC. (2011) Epigenetic alteration of microRNAs in feces of colorectal cancer and its clinical significance. *Expert Review of Molecular Diagnostics*, **11**(7), 691–694.
81. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X and Li Q. (2008) Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Research*, **18**(10), 997–1006.

82. Cazzoli R, Buttitta F, Di Nicola M, Malatesta S, Marchetti A and Pass HI. (2013) MicroRNAs derived from circulating exosomes as non-invasive biomarkers for screening and diagnose lung cancer. *Journal of Thoracic Oncology*, **8**(9), 1156–1162.
83. Yu SL, Chen HY, Chang GC, Chen CY, Chen HW, Singh, S, Cheng CL, Yu CJ, Lee YC, Chen HS and Su TJ. (2008) MicroRNA signature predicts survival and relapse in lung cancer. *Cancer Cell,* **13**(1), 48–57.
84. Markou A, Tsaroucha EG, Kaklamanis L, Fotinou M, Georgoulias V and Lianidou ES. (2008) Prognostic value ofmature microRNA-21 andmicroRNA-205 overexpression innon-small cell lung cancer byquantitative real-time RT-PCR. *Clinical Chemistry*, **54**(10), 1696–704.
85. Gallardo E, Navarro A, Viñolas N, Marrades RM, Diaz T, Gel B, Quera A, Bandres E, Garcia-Foncillas J, Ramirez J and Monzo M. (2009) miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. *Carcinogenesis*, **30**(11), 1903–1909.
86. Florczuk M, Szpechcinski A and Chorostowska-Wynimko J. (2017) miRNAs as biomarkers and therapeutic targets in non-small cell lung cancer: Current perspectives. *Target Oncology*, **12**(2), 179–200.
87. Xie Y, Yao Q, Butt AM, Guo J, Tian Z, Bao X, Li H, Meng Q and Lu J. (2014) Expression profiling of serum microRNA-101 in HBV-associated chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. *Cancer Biology & Therapy*, **15**(9), 1248–1255.
88. Peveling-Oberhag J, Seiz A, Döring C, Hartmann S, Köberle V, Liese J, Zeuzem S, Hansmann ML and Piiper A. (2014) MicroRNA profiling of laser-microdissected hepatocellular carcinoma reveals an oncogenic phenotype of the tumor capsule. *Translational Oncology*, **7**(6), 672–680.
89. Zhou J, Lu S, Yang S, Chen H, Shi H, Miao M and Jiao B. (2014) MicroRNA-127 post-transcriptionally downregulates Sept7 and suppresses cell growth in hepatocellular carcinoma cells. *Cellular Physiology and Biochemistry*, **33**(5), 1537–1546.
90. Li BK, Huang PZ, Qiu JL, Liao YD, Hong J and Yuan YF. (2014) Upregulation of microRNA-106b is associated with poor prognosis in hepatocellular carcinoma. *Diagnostic Pathology*, **9**(1), 226.
91. Xu Q, Zhang M, Tu J, Pang L, Cai W and Liu X. (2015) MicroRNA-122 affects cell aggressiveness and apoptosis by targeting PKM2 in human hepatocellular carcinoma. *Oncology Reports*, **34**(4), 2054–2064.
92. Tsang FH, Au V, Lu WJ, Shek FH, Liu AM, Luk JM, Fan ST, Poon RT and Lee NP. (2014) Prognostic marker microRNA-125b inhibits tumorigenic properties of hepatocellular carcinoma cells via suppressing tumorigenic molecule eIF5A2. *Digestive Diseases and Sciences*, **59**(10), 2477–2487.
93. Shen S, Lin Y, Yuan X, Shen L, Chen J, Chen L, Qin L and Shen B. (2016) Biomarker MicroRNAs for diagnosis, prognosis and treatment of hepatocellular carcinoma: A functional survey and comparison. *Science Reports*, **6**, 38311.

94. Burnett JC and Rossi JJ. (2012) RNA-based therapeutics: Current progress and future prospects. *Chemistry & Biology*, **19**(1), 60–71.
95. Labatut AE and Mattheolabakis G. (2018) Non-viral based miR delivery and recent developments. *European Journal of Pharmaceutics and Biopharmaceutics*, **128**, 82–90.
96. Ho PY, Duan Z, Batra N, Jilek JL, Tu MJ, Qiu JX, Hu Z, Wun T, Lara PN, DeVere-White RW, Chen HW and Yu AM. (2018) Bioengineered noncoding RNAs selectively change cellular miRNome profiles for cancer therapy. *Journal of Pharmacology and Experimental Therapeutics*, **365**(3), 494–506.
97. Calin GA and Croce CM. (2009) Chronic lymphocytic leukemia: Interplay between noncoding RNAs and protein-coding genes. *Blood*, **114**, 4761–4770.
98. Esau CC. (2008) Inhibition of microRNA with antisense oligonucleotides. *Methods*, **44**(1), 55–60.
99. Nishimura M. (2013) Therapeutic synergy between microRNA and siRNA in ovarian cancer treatment. *Cancer Discovery*, **3**, 1302–1315.
100. Li X, Wang L, Gu JW, Li B, Liu WP, Wang YG, Zhang X, Zhen HN and Fei Z. (2010) Up-regulation of EphA2 and down-regulation of EphrinA1 are associated with the aggressive phenotype and poor prognosis of malignant glioma. *Tumour Biology*, **31**(5), 477–488.
101. Lu C, Shahzad MM, Wang H, Landen CN, Kim SW, Allen J, Nick AM, Jennings N, Kinch MS, Bar-Eli M and Sood AK. (2008) EphA2 overexpression promotes ovarian cancer growth. *Cancer Biology & Therapy*, **7**(7), 1098–1103.
102. Pekarsky Y, Santanam U, Cimmino A, Palamarchuk A, Efanov A, Maximov V, Volinia S, Alder H, Liu CG, Rassenti L, Calin GA, Hagan JP, Kipps T and Croce CM. (2006) Tcl1 expression in chronic lymphocytic leukemia is regulated by miR-29 and miR-181. *Cancer Research*, **66**(24), 11590–11593.
103. Shah MY, Ferrajoli A, Sood AK, Lopez-Berestein G and Calin GA. (2016) microRNA Therapeutics in Cancer - An Emerging Concept. *EBioMedicine*, **12**, 34–42.
104. Wang Z. (2011) The guideline of the design and validation of MiRNA mimics. *Methods in Molecular Biology*, **676**, 211–223.
105. Thomson DW, Bracken CP, Szubert JM and Goodall GJ. (2013) On measuring miRNAs after transient transfection of mimics or antisense inhibitors. *PLoS One*, **8**(1), e55214.
106. Jin HY, Gonzalez-Martin A, Miletic AV, Lai M, Knight S, Sabouri-Ghomi M, Head SR, Macauley MS, Rickert RC and Xiao C. (2015) Transfection of microRNA mimics should be used with caution. *Frontiers in Genetics*, **6**, 340.
107. Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, D'urso L, Pagliuca A, Biffoni M, Labbaye C, Bartucci M, Muto G, Peschle C and de Maria R. (2008) The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. *Nature Medicine*, **14**, 1271–1277.
108. Goldgraben MA, Russell R, Rueda OM, Caldas C and Git A. (2016) Double-stranded microRNA mimics can induce length- and passenger strand–dependent effects in a cell type–specific manner. *RNA*, **22**(2), 193–203.

109. Filip AA, Grenda A, Popek S, Koczkodaj D, Michalak-Wojnowska M, Budzyński M, Wąsik-Szczepanek E, Zmorzyński S, Karczmarczyk A and Giannopoulos K. (2017) Expression of circulating miRNAs associated with lymphocyte differentiation and activation in CLL—another piece in the puzzle. *Annals of Hematology*, **96**(1), 33–50.
110. Garzon R, Marcucci G and Croce CM. (2010) Targeting microRNAs in cancer: Rationale, strategies and challenges. *Nature Reviews Drug Discovery*, **9**, 775–789.
111. Lennox KA and Behlke MA. (2011) Chemical modification and design of anti-miRNA oligonucleotides. *Gene Therapy*, **18**(12), 1111–1120.
112. Stenvang J, Petri A, Lindow M, Obad S and Kauppinen S. (2012) Inhibition of microRNA function by antimiR oligonucleotides. *Silence*, **3**, 1.
113. Hutvagner G, Simard MJ, Mello CC and Zamore PD. (2004) Sequence-specific inhibition of small RNA function. *PLoS Biology*, **2**, E98.
114. Meister G, Landthaler M, Dorsett Y and Tuschl T. (2004) Sequence-specific inhibition of microRNA- and siRNA-induced RNA silencing. *RNA*, **10**, 544–550.
115. Petersen M and Wengel J. (2003) LNA: A versatile tool for therapeutics and genomics. *Trends in Biotechnology*, **21**, 74–81.
116. Davis S, Lollo B, Freier S and Esau C. (2006) Improved targeting of miRNA with antisense oligonucleotides. *Nucleic Acids Research*, **34**, 2294–2304.
117. Davis S, Propp S, Freier SM, Jones LE, Serra MJ, Kinberger G, Bhat B, Swayze EE, Bennett CF and Esau C. (2009) Potent inhibition of microRNA in vivo without degradation. *Nucleic Acids Research*, **37**, 70–77.
118. Obika S, Nanbu D, Hari Y, Andoh Ji, Morio Ki, Doi T and Imanishi T. (1998) Stability and structural features of the duplexes containing nucleoside analogues with a fixed N-type conformation, 2′-O,4′-C-methyleneribonucleosides. *Tetrahedron Letters*, **39**, 5401–5404.
119. Nielsen KE, Rasmussen J, Kumar R, Wengel J, Jacobsen JP and Petersen M. (2004) NMR studies of fully modified locked nucleic acid (LNA) hybrids: Solution structure of an LNA:RNA hybrid and characterization of an LNA:DNA hybrid. *Bioconjugate Chemistry*, **15**, 449–457.
120. Lindow M and Kauppinen S. (2012) Discovering the first microRNA-targeted drug. *Journal of Cell Biology*, **199**, 407–412.
121. Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, van der Meer AJ, Patick AK, Chen A, Zhou Y, Persson R, King BD, Kauppinen S, Levin AA and Hodges MR. (2013) Treatment of HCV infection by targeting microRNA. *New England Journal of Medicine*, **368**, 1685–1694.
122. Lieberman J and Sarnow P. (2013) Micromanaging hepatitis C virus. *New England Journal of Medicine*, **368**, 1741–1743.
123. Chakraborty C, Sharma AR, Sharma G, Doss CGP and Lee S. (2017) Therapeutic miRNA and siRNA: Moving from bench to clinic as next generation medicine. *Molecular Therapy — Nucleic Acids*, **8**, 132–143.
124. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M and Stoffel M. (2005) Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature*, 438, 685–689.

125. Ebert MS, Neilson JR and Sharp PA. (2007) MicroRNA sponges: Competitive inhibitors of small RNAs in mammalian cells. *Nature Methods*, **4**, 721–726.
126. Mignacca L, Saint-Germain E, Benoit A, Bourdeau V, Moro A and Ferbeyre G. (2016) Sponges against miR-19 and miR-155 reactivate the p53-Socs1 axis in hematopoietic cancers. *Cytokine*, **82**, 80–86.
127. Zhang T, Hu Y, Ju J, *et al.* (2016) Downregulation of miR-522 suppresses proliferation and metastasis of non-small cell lung cancer cells by directly targeting DENN/MADD domain containing 2D. *Scientific Reports*, **6**, 19346.
128. Gumireddy K, Young DD, Xiong X, Hogenesch JB, Huang Q and Deiters A. (2008) Small-molecule inhibitors of microrna miR-21 function. *Angewandte Chemie*, **47**, 7482–7484.
129. Melo S, Villanueva A, Moutinho C, Davalos V, Spizzo R, Ivan C, Rossi S, Setien F, Casanovas O, Simo-Riudalbas L, Carmona J, Carrere J, Vidal A, Aytes A, Puertas S, Ropero S, Kalluri R, Croce CM, Calin GA and Esteller M. (2011) Small molecule enoxacin is a cancer-specific growth inhibitor that acts by enhancing TAR RNA-binding protein 2-mediated microRNA processing. *Proceedings of the National Academy of Sciences*, **108**, 4394–4399.
130. Watashi K, Yeung ML, Starost MF, Hosmane RS and Jeang KT. (2010) Identification of small molecules that suppress microRNA function and reverse tumorigenesis. *Journal of Biological Chemistry*, **285**, 24707–24716.
131. Junker K, Heinzelmann J, Beckham C, Ochiya T and Jenster G. (2016) Extracellular vesicles and their role in urologic malignancies. *European Urology*, **70**(2), 323–331.
132. Verma M, Lam TK, Hebert E and Divi RL. (2015) Extracellular vesicles: Potential applications in cancer diagnosis, prognosis, and epidemiology. *BMC Clinical Pathology*, **15**, 6.
133. Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, Lovat F, Fadda P, Mao C, Nuovo GJ, Zanesi N, Crawford M, Ozer GH, Wernicke D, Alder H, Caligiuri MA, Nana-Sinkam P, Perrotti D and Croce CM. (2012) MicroRNAs bind to toll-like receptors to induce prometastatic inflammatory response. *Proceedings of the National Academy of Sciences*, **109**, E2110–E2116.
134. Roberts TC, Langer R and Wood MJA. (2020) Advances in oligonucleotide drug delivery. *Nature Reviews Drug Discovery,* **19**(10), 673–694.
135. Ibrišimović M, Kneidinger D, Lion T and Klein R. (2013) An adenoviral vector-based expression and delivery system for the inhibition of wild-type adenovirus replication by artificial microRNAs. *Antiviral Research*, **97**(1), 10–23.
136. Kota J, *et al.* (2009) Therapeutic delivery of miR-26a inhibits cancer cell proliferation and induces tumor-specific apoptosis. *Cell*, **137**(6), 1005–1017.
137. Ramanujam D, Sassi Y, Laggerbauer B and Engelhardt S. (2016) Viral vector-based targeting of miR-21 in cardiac nonmyocyte cells reduces pathologic remodeling of the heart. *Molecular Therapy*, **24**(11), 1939–1948.

138. Theis T, Yoo M, Park CS, Chen J, Kügler S, Gibbs KM and Schachner M. (2017) Lentiviral delivery of miR-133b improves functional recovery after spinal cord injury in mice. *Molecular Neurobiol*ogy, **54**(6), 4659–4671.
139. Wei JS, Song YK, Durinck S, Chen Q, Cheuk ATC, Tsang P, Zhang Q, Thiele CJ, Slack A, Shohet J and Khan J. (2008) The MYCN oncogene is a direct target of miR-34a. *Oncogene*, **27**(39), 5204–5213.
140. Gaur S, Wen Y, Song JH, Parikh NU, Mangala LS, Blessing AM, Ivan C, Wu SY, Varkaris A, Shi Y, Lopez-Berestein G, Frigo DE, Sood AK and Gallick GE. (2015) Chitosan nanoparticle-mediated delivery of miRNA-34a decreases prostate tumor growth in the bone and its expression induces non-canonical autophagy. *Oncotarget*, **6**, 29161–29177.
141. Han HD, Mangala LS, Lee JW, Shahzad MM, Kim HS, Shen D, Nam EJ, Mora EM, Stone RL, Lu C, Lee SJ, Roh JW, Nick AM, Lopez-Berestein G and Sood AK. (2010) Targeted gene silencing using RGD-labeled chitosan nanoparticles. *Clinical Cancer Research*, **16**, 3910–3922.

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