Published in final edited form as:

Leukemia. 2021 February ; 35(2): 360–376. doi:10.1038/s41375-020-01066-4.

Targeting RNA-binding proteins in acute and chronic leukemia

Irina A. Elcheva[1,*], **Vladimir S. Spiegelman**[1]

[1]Division of Pediatric Hematology and Oncology, Department of Pediatrics, Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA;

Abstract

RNA-binding proteins (RBPs) play a crucial role in cellular physiology by regulating RNA processing, translation, and turnover. In neoplasms, RBP support of cancer-relevant expression of alternatively spliced, modified, and stabilized mRNA transcripts is essential to self-renewal, proliferation, and adaptation to stress. In this review, we assess the impact of key families of RBPs in leukemogenesis, review progress in targeting those proteins with small molecules, and discuss how multilevel composition of posttranscriptional regulation of gene expression could be used for potential therapies in acute and chronic leukemia.

Introduction

Leukemia is a blood cancer characterized by abnormal proliferation of myeloid or lymphoid progenitors in the bone marrow and their compromised ability to produce fully functional blood cells. Despite the relatively high effectiveness of current conventional and targeted therapeutics, anti-leukemia drugs are facing a number of challenges related to rapidly acquired resistance and intolerable toxicity - critical treatment factors for elderly and physically fragile patients. Mortalities associated with refractory and relapse leukemia indicate a need to optimize risk group stratification and the development of new remedies capable of overcoming resistance to therapeutics.

While alterations in protein-coding genes are considered a driving force of cancer, multiple posttranscriptional events occurring between RNA synthesis and protein production are in control of gene expression and influence cell fate. RNA processing, transport, and translation are orchestrated by various *cis*- and *trans*-acting regulatory elements. *Cis*-acting RNA regulatory elements are the internal RNA motifs recognized by the external *trans*-acting factors, such as non-coding RNAs and RNA-binding proteins (RBPs). Ribonucleoprotein (RNP) complexes are formed when RNA binds at conventional RBP RNA-binding domains, or through unconventional RNA-protein interactions[1]. Of the 1,914

[*]**Address Correspondence To:** Irina Elcheva, PhD, Department of Pediatrics, Division of Pediatric Hematology and Oncology, Pennsylvania State University, College of Medicine, PO Box 850, MC H085, 500 University Drive Hershey, Pennsylvania 17033-0850, Ph: 717-531-0003 Ext 284920; FAX: 717-531-4789; ielcheva@pennstatehealth.psu.edu;.

Conflict of interest

The authors declare no conflicts of interest.

RBPs comprising *Homo sapiens*' RNA interactome, relatively few have been associated with aberrant development and cancer.

This review provides a snapshot of key families of RBPs involved in leukemogenesis, focusing on their role in messenger RNA (mRNA) fate. We begin with RNA editing and modifying enzymes conferring changes in RNA *cis*-acting elements. We then discuss the roles of other essential *trans*-acting factors, such as RNA splicing, export, and translation regulators, as well as several oncofetal RBPs. Last, we look at the current progress and challenges in targeting these proteins with small molecules and discuss their possible applications in leukemia treatment.

RNA editing enzymes

ADAR1

RNA edits are discrete changes in RNA nucleotide sequences introduced after transcription. Hydrolytic deamination of adenine to inosine residues (so-called A-to-I editing) is one of the most prevalent edits on doublestranded mammalian RNA (dsRNA) that is carried out by the adenosine deaminases acting on RNA (ADAR) family of enzymes. ADAR1 is ubiquitously expressed and is the most studied protein of the ADAR family. The *ADAR1* gene encodes for two protein isoforms: the constitutively expressed N-terminally truncated p110 isoform, and the full length interferon (IFN)-inducible p150 isoform, both of which shuttle between the nucleus and the cytoplasm[2].

One of the adaptive rationales for RNA editing is the ability of eukaryotic cells to discriminate between "self" and "non-self" RNAs. Endogenous RNA editing occurs in transcripts from primate-specific Alu repeats, at the highly conserved regions encoding functional protein domains as well as untranslated coding and non-coding RNAs. Because editing makes the base pairing in RNA duplexes imperfect, the endogenous dsRNAs that are long and entirely aligned are not typically found in the cytoplasm of eukaryotic cells. The perfectly aligned dsRNAs are usually produced during viral replication and trigger pro-apoptotic and pro-inflammatory responses through the activation of melanoma differentiation-associated gene 5 (MDA5), protein kinase R (PKR), and other pathogen-associated molecular patterns receptors. The ADAR1 enzymes balance the immune activation and self-tolerance by attenuating MDA5 and PKR activity[3].

ADAR1 role in innate inflammation and apoptosis appears to be critical for embryonic development, especially the hematopoietic lineage, as $Adar^{-/-}$ mice die at E11.4–14 from widespread death of hematopoietic cells in the liver[4]. Their lethality can be rescued by deleting of genes encoding dsRNA-sensing, pro-inflammatory proteins e.g. *Mda5*[5]. In addition to embryonic hematopoiesis, ADAR1 is required for the repopulating capacity of hematopoietic stem cells (HSC) in adult mice[6].

Elevated mRNA and protein levels of ADAR1 were found in pediatric B-cell acute lymphoblastic leukemia (B-ALL)[7], adult acute myeloid leukemia (AML)[8], and progressed to blast crisis chronic myeloid leukemia (BC CML)[9], Table 1. Several studies indicate that ADAR1 maintains proliferation and self-renewal of myeloid leukemia stem/progenitor cells

in cooperation with WNT/β-catenin signaling. Xiao et al. reported that AML samples have significantly higher expression levels of *ADAR1* compared to complete AML remission and non-malignant myeloid blood disorders[8]. *ADAR1* knockdown led to decreased expression of WNT signaling effectors (*β-catenin, c-MYC, TCF-4, Cyclin D2*) and suppressed AML proliferation[8].

ADAR1's p150 isoform was upregulated in BC CML compared to chronic phase (CP) CML and normal cord blood progenitors[9]. Forced expression of the p150 ADAR1 isoform in CP CML cells increased production of a misspliced form of *GSK3β* implicated in leukemia stem cell (LSC) self-renewal, while ADAR1 knockdown impaired self-renewal capacity in BC CML as examined by serial *in vivo* transplantation[9]. A comprehensive mechanistic study of *ADAR1* functions in LSCs demonstrated *JAK2*- and *BCR-ABL1*-dependent activation of ADAR1-mediated RNA editing, which in turn inhibits *let-7*-mediated differentiation of CML blasts[10]. Because deregulation of RNA editing is associated with progression and therapeutic resistance of CML, Catriona Jamieson's group proposed ADAR1 as an important biomarker of CML progression and developed a clinically relevant assay for RNA editing quantification[11].

ADAR1-mediated editing influences gene expression by changing both mRNA stability and miRNA expression. Jiang Q et al. showed that A-to-I editing stabilizes *MDM2* transcript through modification of *miR-155* binding sites within its 3′ UTR region and downregulation of *pri-miR-155*[12], Figure 1 (A, C, I), Table 1. The biological consequences of non-coding RNA editing are likely to be cell type- or context-dependent, contingent on the signaling pathways they target. For example, A-to-I edits inhibiting biogenesis of the tumor suppressor *miR-26a* enhance proliferation of normal blood progenitors, but slow down the cell cycle transition in BC CML[12].

Since a loss of ADAR1 activity induces cell-intrinsic lethality and the induction of cytokines, ADAR1 presents a potentially effective therapeutic target. Gannon et al. suggested possible approaches to disrupt ADAR1 function in cancer cells through inhibition of its adenosine deaminase activity or inactivation of non-enzymatic functions specific for the p150 isoform, such as direct PKR binding[13]. In accordance with findings describing the immunomodulatory functions of ADAR1, Ishizuka et al. proposed a new strategy for overcoming the resistance to immune checkpoint blockade through ADAR1 inhibition[14]. Zipeto et al. demonstrated that the previously described inhibitory tool compound 8-azaadenosine (8-aza) reduced ADAR1's A-to-I editing activity in K562 CML cells[10]. Multiple studies defining combinatory approaches for ADAR1 inhibition, targeting ADAR-edited transcripts, and immunotherapies suggest a promising future of RNA-editing therapeutics.

RNA modification enzymes

More than 150 types of RNA modifications, ranging from simple methylation or isomerization to more complex multistep chemical transformations, occur co- and post-transcriptionally. Whereas transfer RNA (tRNAs) and ribosomal RNA (rRNA) are the most abundantly modified RNAs in a cell, mRNA is characterized by several modifications

including adenosine methylation (N^6-methyladenosine (m^6A)), which is the most prevalent modification of eukaryotic messenger and long non-coding RNAs[15].

m^6A's installation, recognition, and removal are facilitated by protein factors called writers, readers, and erasers, respectively. The main writer is a multicomponent complex that consists of a catalytic methyltransferase-like 3 (METTL3) subunit, a substrate-recognizing subunit METTL14, and other cofactors (WTAP, RBM15/15B, VIRMA, HAKAI, and ZC3H13) that enable adenosine methylation. Another writer installing m^6A in RNA sequences in a structure dependent manner is METTL16[16]. m^6A readers (e.g. YTHDCs, YTHDFs, hnRNPs, IGF2BPs) recognize m^6A modifications while conveying transcripts' processing, stability, and translation. The removal of m^6A is catalyzed by two erasers: fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5).

RNA modifications influence gene expression by changing RNA secondary structure and folding, consequently affecting functional RNA-RNA and RNA-protein interactions. For example, m^6A eraser FTO and nuclear reader YTHDC1 modulate splicing factor activity and exon inclusion[17]. The levels of m^6A RNA modifications have a remarkable effect on cell fate, but this effect is dependent on cellular context[16]. In fact, METTL3-METTL14 were reported as a tumor suppressor or oncogene in glioblastoma, a tumor suppressor in endometrial cancer, and an oncogene in lung cancer and acute myeloid leukemia[18].

METTL3-METTL14 core subunits

Two distinct genetic screens conducted by Barbieri et al. identified *METTL3* as an essential gene for AML cell growth. Downregulation of METTL3 resulted in cell cycle arrest, differentiation of leukemic cells, and failure to establish leukemia in immunodeficient mice[19]. In agreement with these data, Vu et al. demonstrated that shRNA-mediated depletion of *METTL3* in human hematopoietic stem/progenitor cells (HSPCs) and AML cell lines promotes cell differentiation, coupled with reduced cell proliferation and induction of apoptosis[20]. Weng et al. reported that a key component of m^6A methyltransferase complex, METTL14, is highly expressed in both normal HSPCs and AML cells carrying t(11q23), t(15;17), or t(8;21)[21]. *METTL14* depletion promoted terminal myeloid differentiation of normal HSPCs and AML cells and inhibited AML tumorigenicity. Therefore, both METTL3 and METTL14 are required for AML sustainability.

Single-nucleotide-resolution mapping of m^6A combined with ribosome profiling showed that m^6A promotes the translation of *c-MYC*, *BCL2*, and *PTEN* mRNAs in the human AML MOLM-13 cell line, Table 2. Loss of METTL3 led to increased levels of phosphorylated AKT that supported differentiation upon METTL3 depletion[20]. Similarly, METTL14 exerts its oncogenic role by regulating m^6A mRNA modifications and mRNA stability of master regulators of self-renewal and differentiation (e.g., *MYB* and *MYC*), whereas its expression levels are negatively regulated by myeloid transcription factor SPI1[21].

In addition to previously described methyltransferase (MTase) dimer, Barbieri et al. proposed a METTL14-independent mode of METTL3 function through interaction with chromatin[19]. The study showed that CAATT-box binding protein CEBPZ recruits METTL3 to the promoters of actively transcribed genes, Figure 1 (B). The promoter bound METTL3

induces m^{6}A modification within the coding region of the associated mRNA transcripts which enhances their translation by relieving ribosome stalling. These observations are relevant to Huang et al. discovery that Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs), known to bind and stabilize coding regions of oncogenic transcripts, are m^{6}A readers[22].

METTL3 catalytic activity in the nucleus has a predominant effect on the fate of downstream targets. However, METTL3 can also locate in the cytoplasm and promote the translation of specific mRNAs as a reader. High cytoplasmic levels of METTL3 result in an increase of WTAP protein expression, which might work as a self-regulatory feedback loop necessary for sustaining MTase levels in myeloid leukemia[23].

WTAP and RBM15 regulatory subunits—Initially considered as a splicing factor, RNA-binding protein Wilms tumor 1-associated protein (WTAP) has no methyltransferase activity. As a MTase co-factor, WTAP interacts with METTL3 and METTL14, and is required for their recruitment into nuclear speckles. In the absence of WTAP, the RNA-binding capability of METTL3 and m^{6}A levels are strongly reduced, suggesting that WTAP regulates its recruitment to mRNA targets[24]. Around 30% of AML samples, especially those with *FLT3-ITD* and *NPM1* mutations, show WTAP upregulation, which possesses oncogenic properties in cooperation with functional METTL3[23, 25].

RNA-binding motif 15 (RBM15) is a multifunctional RBP with an essential role in development and normal and malignant hematopoiesis. As a MTase regulatory subunit, RBM15 binds and recruits the METTL3-METTL14 complex to specific sites of coding and non-coding RNAs[26], Table 2. As a splicing factor, RBM15 regulates pre-mRNA splicing of key erythro-megakaryocytic regulators (*GATA1*, *RUNX1*, *TAL1* etc.) by recruiting SF3B1 splicing complex to intronic regions, Table 3. Perturbations in RBM15 expression are common for infant acute megakaryoblastic leukemia (AMKL), and can potentially be rescued by inhibiting PRMT1 which determines RBM15 protein methylation and stability[27].

FTO and ALKBH5 m^{6}A erasers

The m^{6}A eraser FTO is upregulated in AML with the mixed lineage leukemia (*MLL*) gene rearrangements, *PML-RARA*, *FLT3*-ITD, and/or *NPM1* mutations[28]. The molecular analysis of FTO gain-of-function in *MLL*-rearranged MONOMAC-6 cells identified significantly up- and downregulated hypomethylated mRNAs. The upregulated hypomethylated RNA messengers were enriched in stem cell genes (*NANOG*, *SOX2*) and WNT-signaling, while most of the downregulated hypomethylated transcripts belonged to the interferon signaling and genes of the immune system. Ultimately Li et al. showed that FTO enhances leukemogenesis and inhibits all-trans-retinoic acid (ATRA)-induced AML cell differentiation by regulating expression of *ASB2* and *RARA* through reducing m^{6}A levels in these mRNAs[28], Figure 1 (B), Table 2.

Subsequently, this research group conducted a massive search for FTO inhibitory compounds, followed by *in vitro* mRNA target validation and *in vivo* studies of two highly effective FTO inhibitors, CS1 and CS2[29]. Other inhibitors, namely FB23 and FB23–2, which selectively block FTO m^{6}A demethylase activity, were recently described by Huang

et al.[30] Similar to genetic depletion, FTO pharmacologic targeting dramatically suppressed proliferation and promoted differentiation of AML cell lines and primary blast cells *in vitro* and in mouse models. Su et al. demonstrated that in addition to self-renewal and cell cycle control FTO regulates expression of immune checkpoint genes of the *LILRB4* family overexpressed in AML. Therefore, FTO inhibition suppressed *LILRB4* and sensitized leukemia cells to T cell cytotoxicity[29]. Given recent findings by Mauer et al. that FTO mediates modifications of small nuclear RNAs (U1, U2, U6 snRNAs) involved in mRNA splicing[31], FTO inhibitors may have a broad effect on gene expression.

Two independent studies showed that another RNA demethylase, alkB homologue 5 protein (ALKBH5), is highly expressed in AML[32, 33]. Wang et al. demonstrated that ALKBH5 transcription is activated by H3K9me3 demethylase KDM4C, and proto-oncogene MYB[33]. Shen et al. focused on the role of ALKBH5 in mRNA stability and identification of the direct mRNA targets by integrative omics studies of RNA-seq, m^{6}A-seq, and ALKBH5-RNA immunoprecipitant's sequencing[32]. Ultimately, both groups illustrated that ALKBH5 selectively supports leukemia stem cell proliferation, metabolism, and self-renewal by regulating essential factors of cell division and kinase signaling such as *TACC3* and *AXL*, Table 2.

YTHDF2 m^{6}A reader—m^{6}A writers and erasers determine the specifics of *cis*-acting RNA regulatory elements that are recognized and functionally interpreted by m^{6}A readers. Among three cytoplasmic YT521-B homology (YTH) domain family of proteins (YTHDF1, 2, and 3), YTHDF2 targets m^{6}A labeled mRNAs for degradation. Conversely, YTHDF1 and 3 promote translation. Other YTHD readers include nuclear YTHDC1, which regulates splicing and targets some mRNAs for nonsense mediated decay, and cytoplasmic YTHDC2 promoting translation.

Paris et al. reported that YTHDF2 levels are significantly increased in cytogenetically diverse human AML. Importantly from a potential therapeutic standpoint, inactivation of YTHDF2 in AML selectively kills LSCs (most likely by modulating essential regulators of apoptosis) but stimulates expansion of normal HSCs[34].

Chemical modulation of m^{6}A RNA methylation—Targeting abnormally overexpressed regulators of RNA methylation has emerged as a promising therapeutic strategy. Within the writer complex, RNA-binding subunit METTL3 is a key m^{6}A methyltransferase containing a targetable S-adenosyl-L-methionine (SAM)-binding pocket. Several biotechnology companies have begun development of METTL3 inhibitors with prospective clinical trials starting in 2021–2022[35]. m^{6}A erasers FTO and ALKBH5 belong to the 2-oxoglutarate and iron-dependent oxygenases respectively, and are sensitive to certain conventional inhibitors, e.g. 2OG competitor succinate and the metal chelator flavonoid[15]. Because FTO negatively regulates ATRA pathway through *ASB2* and *RARA*, FTO inhibitors can potentially supplement ATRA treatments in myeloid leukemia. Solving crystal structures of these proteins will further aid in the design of selective inhibitors that have high therapeutic potential. However, the physiological consequences of m^{6}A mRNA methylation are context-dependent and may have the opposite effect in different tissues. Another question is why writers and erasers, enzymes with the opposite effects on RNA

methylation, both have oncogenic properties. It will be important to understand how cancer cells gain advantage from hundreds of oncogenes and tumor suppressors being simultaneously methylated or demethylated. Elucidating these mechanisms and biological consequences of altering RNA modifications will be critical for the successful clinical implementation of RNA methylation-based therapies.

mRNA splicing

The precursors of eukaryotic mRNA, pre-mRNAs, contain introns that should be excluded from matured RNA messengers. Intron removal happens through splicing, which is carried out by the spliceosomes acting at the regulatory splicing sites in nascent pre-mRNA. Multiple mRNA's isoforms are usually produced from a single gene by differential exon usage during alternative splicing (AS). Cancer cells often express differentially spliced or aberrant cancer-specific isoforms favoring clonal expansion and survival.

The preferential assembly of the anti-apoptotic long isoform of B-cell lymphoma (*BCL-2*) gene, and anti-apoptotic short Caspase 9 protein are canonical examples of how acute and chronic myeloid leukemia cells utilize alternative splicing to acquire chemoresistance[36]. Along with the selective expression of physiologically normal variants, around 30% of differentially expressed transcripts in cancer cells contain products of abnormal splicing. Those events include atypical usage of exons (cassette exon), intron retention, and a disruption of functional open reading frames[37]. A genome-wide study showed that equal proportions of oncogenes and tumor suppressors are recurrently mis-spliced in AML[38]. However, distinct sets of splicing-related mutations affect expression of tumor suppressors and oncogenes[39]. For instance, intron retention, a widespread splicing alteration across various cancers, is a common mechanism for tumor suppressor inactivation[40]. Although most aberrantly spliced transcripts undergo degradation via nonsense mediated decay, and not all protein products of mis-splicing are equality important for cancer development and progression, clonal enrichments with cancer-specific variants driving chronic myeloid[41] and lymphoid[42] leukemia as well as the acquired resistance to CAR19 therapies in childhood B-ALL[43] were previously described.

The fidelity of canonical splicing hinges on the structural and functional integrity of spliceosomal subunits U1, U2, U4, U5, and U6 snRNPs (five snRNA and around 50 proteins), regulatory RNA sequences in splicing sites flanking introns at 5'-(GT/U) and (AG)-3', the intronic branch nucleotide adenine (A), exonic or intronic splicing silencers, and enhancers.

In 2011, Kenichi Yoshida et al. were among the first who described the importance of splicing factors (SF) for the pathogenesis of myelodysplasia[44]. Recurrent mutations in six components of the splicing machinery (*SF3B1, U2AF1*, *SRSF2*, *ZRSR2*, *SF3A1*, and *PRPF40B*) were found in about 55% of cases, Figure 1(D), Table 3. Importantly, the heterozygous mutations occurred in a mutually exclusive manner, indicating that the functional splicing factors are required for cell survival.

Among more than 150 proteins involved in splicing, 4 factors (SF3B1, U2AF1, SRSF2, and ZRSR2) are altered most commonly (comprehensively reviewed by Taylor and Lee[45]).

Splicing Factor 3b Subunit 1 (*SF3B1*, 155 KDa subunit) gene is the most commonly mutated splicing factor in human cancer. It encodes the largest of seven subunits of the SF3B complex, which plays a key role in U2 snRNP positioning to the branchpoint site[46]. Mutations in the *SF3B1* gene are present in about 10% to 20% of acute myeloid and lymphoid leukemia, but are significantly enriched in chronic myeloid malignancies, especially in refractory anemia with ring sideroblasts (RARS)[45]. Displaying up to 80% frequency for K700E substitution, *SF3B1* mutations are likely to be early genetic events in RARS and are associated with favorable prognosis. Conversely, *SF3B1* mutations are the subclonal events in chronic lymphoblastic or lymphocytic leukemia (CLL) tumors and linked to poor clinical outcomes. U2 small nuclear RNA auxiliary factor 1 (U2AF1, 35 kDa subunit) is also a core component of the spliceosome that, together with its partner U2AF2, recruits U2 snRNP to the branch site of pre-mRNA. U2AF1 mutations can be found in 10–15% of patients with non-RARS MDS, chronic myelomonocytic leukemia (CMML), and secondary AML (s-AML). Serine and Arginine-(R) Rich Splicing Factor 2 (SRSF2), binds to splicing enhancers and promotes splicing by recruiting a core spliceosome. SRSF2 mutations were found in 50% of CMML cases and in 15–20% of MDS and s-AML cases. The haploid, presumably loss-of-function mutations in the *ZRSR2* gene located at Xp22.1, are found in 5–10% patients with MDS, and are more common in males[45].

Detailed analysis of SF protein structure showed that the hotspot mutations loosen the strength of the canonical protein-protein and RNA-protein interactions therefore provoking catalytic reactions in otherwise atypical regions. For example, mutations in SF3B1 HEAT domains (HR4-HR7) have a major impact on the formation of the SF3B1 RNA-binding platform. Changes in SF3B1 tertiary structure lead to selection branchpoint sequences with a greater complementarity to U2 snRNA, a shift in the spliceosome position, and usage of cryptic 3’ splicing sites upstream of the canonical site[46].

The analysis of *SF3B1* mutations in primary human CLL revealed dysregulation of multiple cellular pathways including DNA damage response, telomere maintenance, and Notch signaling[47]. Although mis-splicing alters multiple mRNAs, dysfunction or inactivation of some factor are critical to disease development. Kim et al. identified a direct connection between SRSF2 P95 mutation, *EZH2* mis-splicing and inactivation, and myelodysplasia development. Importantly, restoring *EZH2* expression partially rescued hematopoiesis in *Srsf2* mutant cells[48].

Aberrant splicing can be a feature of leukemic cells without genomic mutations in splicing factors and is likely a result of mutations in *cis*- and *trans*-acting RNA elements or the upstream regulators of splicing. Pediatric B-cell malignances lacking genomic mutations of SF display global mRNA mis-splicing, including approximately 100 splicing regulators when compared to normal B-cells[49]. One of the mis-spliced factors, *hnRNPA1*, plays an important role in RNA metabolism. The knockdown of hnRNPA1 in B-lymphoblastoid cells initiated a broad change in hnRNPA1-regulated exon usage and production of atypical splice variants of cancer drivers including *DICER1* and *NT5C2*[49].

Although a number of *in vitro* and *in vivo* studies failed to demonstrate a uniform capacity of RBPs to initiate leukemia, three independent genome-wide studies found RBPs

indispensable for leukemia sustainability. The CRISPR/Cas9-based library designed by Wang et al. targeted RNA-binding domains of 490 classical RBPs[50]. The screen identified a network of physically interacting RBPs upregulated in AML, and the RNA splicing factor RBM39 as one of the key factors of AML dependency. RBM39 is required for efficient splicing of many mRNAs, including the *HOXA9* transcriptional targets; therefore, genetic or chemical inhibition of this splicing factor caused preferential lethality of cells with spliceosomal mutant AML. The second study by Yamauchi et al. employed a genome-wide CRISPR-Cas9 screening using AML cell lines followed by a second screen in vivo. The screening identified mRNA decapping enzyme scavenger (DCPS) as being essential for AML cell survival, interacting with components of pre-mRNA metabolic pathways including spliceosomes[51], Figure 1 (E), Table 3. Finally, a genome-wide *in vivo* CRISPR/Cas9 screen in *BCR-ABL/NUP98-HOXA9*-driven CML mouse model showed a significant enrichment with RBPs (~680 genes), suggesting a "disproportionate dependency" on RBPs in myeloid leukemias. In this study, Bajaj et al. identified dsRNA-binding protein Staufen2 (*Stau2)* as an essential regulator of chromatin modifiers[52]. The gene expression analysis identified KDM family of H3K4 demethylases being downstream targets of *Stau2*, Table 6. The biological effects of genetic and pharmacologic inhibition of KDM1A suggest its potential therapeutic value in BC CML.

Alternative cleavage and polyadenylation

Given the important functions of 3'UTR in regulating mRNA fate, mRNAs can be polyadenylated at alternative sites, which, similar to splicing, results in RNA messengers harboring 3'UTRs of different size and content. Notably, global 3'UTR shortening and high expression levels of cleavage and polyadenylation factors, often indicated as alternative polyadenylation (APA), are common for fast proliferative and cancer cells[53, 54].

A significant increase in the cleavage and polyadenylation specificity factor 1 (CPSF1) expression was found in t(8;21) AML at diagnosis, and was associated with the short 3'UTR in fusion *AML1-ETO* transcript. CPSF1 knockdown led to the extension of *AML1-ETO* 3'UTR, decreased fusion mRNA expression and suppression of leukemia cell growth[55]. Data analysis of singe cell RNA-seq of 16,843 bone marrow mononuclear cells from healthy donors and AML patients shows that *NF-κB, GATA2*, and *IAP*-family genes exhibit APA dynamics specific for altered differentiation and proliferation of leukemic cells[56].

The U1 snRNP is an essential component of a spliceosome. Independently from its role in splicing, U1 snRNP plays an important role in controlling premature cleavage and polyadenylation by inhibiting the recognition of proximal and cryptic intronal polyadenylation sites (termed telescripting)[57]. Because the base pairing between *U1 snRNA*, a component of U1 snRNP, and *pre-mRNA* is necessary both for splicing and telescripting, U1 snRNP deficiencies cause global mis-splicing[58] and 3'UTR shortening[59], Tables 3, 4. The A>C mutation of *U1* snRNA was found in eight out of 78 (10.3%) cases of CLL and other types of cancer[58], Figure 1 (D, F), Table 3.

Ubiquitously expressed human antigen R (HuR, or ELAV-like protein 1), nucleolin, and tristetraprolin protein (TTP) bind to AU-rich elements within 3'UTRs. HuR and nucleolin stabilize mRNAs and are upregulated in a variety of blood cancers, while TTP function as a

tumor suppressor by triggering mRNA decay. TTP downregulation or loss of function, reported in several human malignances including leukemia, is associated with poor prognosis[60], Figure 1 (F), Table 4.

Chemical modulation of mRNA processing

Given that splicing and RNA processing enzymes are required for cell survival, cancer cells bearing heterozygous SF mutations are dependent on wild-type alleles and are more susceptible to chemical compounds inhibiting spliceosome activity.

The first clinical trials of bacteria-derived chemicals targeting the SF3B complex (spliceostatin A, pladienolide (E7107), and GEX1) did not take into consideration the mutational status of splicing factor genes and presented severe side effects[61]. Since then, a significant scientific effort has been committed to understanding the spliceosome structure and catalytic activity for the rational design of efficient SF3B inhibitors. Recent work by Michael Seiler and colleagues describes an orally available modulator of the SF3B complex, H3B-8800, which potently and preferentially destroys spliceosome-mutant epithelial and hematologic tumor cells[62]. The safety, pharmacokinetics and pharmacodynamics of H3B-8800 might be evaluated by the end of 2020, when a phase 1 clinical trial (NCT02841540) in patients with myeloid malignancies carrying spliceosomal mutations is completed.

Whereas the majority of known spliceosome inhibitors target the SF3B complex, sulfonamide-containing compounds were shown to induce the proteasomal degradation of the accessory RNA-splicing factor RBM39. The anti-cancer properties of the molecules indisulam, E7820, and chloroquinoxaline sulfonamide have been known for decades, but the mechanism of their action through inhibiting splicing was only recently discovered[63]. Another example of possible drug repurposing is the DCPS inhibitor RG3039. A dibasic lipophilic molecule was originally developed to treat spinal muscular atrophy, and its anti-leukemic effect has been recently reported[51].

The post-translational modifiers protein arginine methyltransferases PRMT1 and PRMT5, are very promising targets for cancer treatment. These enzymes catalyze arginine methylation on many cellular proteins including histones and cooperate with oncogenic drivers and fusion proteins in promoting cancer. The selective PRMT1 inhibitors (e.g., GSK3368715 and MS023) and PRMT5 inhibitors (e.g., GSK3203591 and GSK3326595) showed a significant synergistic anti-leukemic effect in myeloid malignances[64, 65]. Mechanistically, a global deficiency of arginine methylation dramatically increased aberrant exon-skipping events[64]. This suggests that the spliceosomal mutant cancers could be the right category for treatments with PRMTs inhibitors. Indeed, distinct PRMT inhibitors preferentially killed *Srsf2*-mutant AML compared to the wild type cells[65]. In addition to spliceosome-mutant cancers, a loss of metabolic regulator *MTAP* has been shown to increase sensitivity to PRMT1 or a combinatory treatment with PRMT1,5 inhibitors[64]. The safety, tolerability, and pharmacokinetics of PRMTs inhibitors are under clinical investigation.

mRNA nuclear export and translation

Messenger RNPs are exported to the cytoplasm by a conserved export receptor NXF1-NXT1 (TAP-p15) and various adaptor proteins coupled with mRNA splicing[66]. The general protein export receptor exportin 1 (XPO1/CRM1), does not have a major role in mRNA export, although mRNAs of some proto-oncogenes and cytokines connect to the XPO1-dependent adaptors through AU-rich sequences in their 3′ UTRs[66]. Exportins (karyopherin-β proteins) play an important role in cancer including hematologic malignances by exporting ncRNAs and tumor-suppressor proteins (p53, NPM1, NFκB). High expression of XPO1 was reported for AML, ALL, CLL, CML, Non-Hodgkin lymphoma, and multiple myeloma (MM), and was linked to poor survival rates[67], Figure 1(G), Table 5. Exportin 1 inhibitor selinexor was tested in various types of cancer and is especially successful against AML and MM[68].

Among several factors of the eIF4F complex required for the initiation of canonical cap-dependent translation, cap-binding protein eIF4E stands as the most powerful oncogene capable of transforming normal cells and inducing cancer in mice[69]. It is believed that eIF4E's dual capacity of selectively transporting and initiating translation of cell cycle regulators' mRNA, e.g. *Cyclin D1*, initiates tumorigeneses[70,71], Figure 1(G, H), Table 5. Inhibition of eIF4E-dependent mRNA export with m^7G'-cap competitive inhibitor ribavirin was clinically beneficial, and did not cause significant toxicity in AML patients[72]. In a subsequent study, however, activation of factor GLI1 led to glucuronidation of ribavirin, loss of the eIF4E-ribavirin interaction, and ultimately drug resistance[73]. Several ongoing clinical studies assess the possibility of treating AML and lymphomas with ribavirin and monitoring cancer progression by Cyclin D1 levels (NCT03760666, NCT03585725).

Multifunctional oncofetal RBPs

Several multifunctional RBPs expressed in stem and progenitor cells during embryonic development are often upregulated in cancers. Although protein structures of MSI2, LIN28, and IGF2BPs do not match the criteria of well-targeted "druggable" peptides, efforts to develop small molecule inhibitors of those proteins have yielded promising results.

Musashi RNA binding protein 2

Musashi RNA-binding proteins 1 and 2 (MSI1, MSI2) belong to a family of RBPs with a pivotal role in embryonic development of multiple species. Among two homologs, MSI2 plays an essential role in normal and malignant hematopoiesis. Overexpression of MSI2 in human umbilical cord blood-derived HSCs led to a 17-fold increase in short-term repopulating cells and 23-fold *ex vivo* expansion of long-term HSCs[74]. *MSI2* knockout in mice depleted the HSCs number roughly in half, but even more severely abolished activity of LSCs that are dependent on increased levels of MSI2[75]. By mapping MSI2-mRNA binding in myeloid LSCs and normal HSCs, Nguyen et al. showed that significantly more transcripts were bound to MSI2 in cancer cells than in their normal counterparts[75]. Interestingly, MSI2 was required for maintaining protein levels of key oncogenes (e.g. *MYB, HOXA9*) rather than their mRNA abundance. These data are in line with the previous studies demonstrating that MSI2 maintains *MLL*-leukemia self-renewal programs by retaining efficient translation of *HOXA9, MYC*, and *IKZF2*, and where *IKZF2* plays a key

role in inhibiting myeloid differentiation[76, 77]. A comparative analysis of myeloid LSCs transcriptomes from *Msi*-deficient mice identified *Tspan3*, a transmembrane protein mediating signal transduction, as the important factor for leukemia development, propagation, and AML localization in the bone marrow[78].

MSI2 pays an essential role in the development and progression of CML as a translocation partner (e.g., *MSI2-HOXA9*), or in cooperation with other fusions (e.g., *BCR-ABL1, NUP98-HOXA9*)[79, 80]. In *NUP98-HOXA9*-driven BC CML, MSI2 upregulation was accompanied by the increased expression of self-renewal regulators, *HOXA9* and *HES1*, and downregulation of differentiation factor *NUMB*[79]. In addition to control of proliferation and differentiation, MSI2 reprograms the metabolic profile of BC CML by regulating *BCAT1*[81], Table 6.

The small molecule search identified a selective MSI2 inhibitor that reduced disease burden in a murine *MLL-AF9* AML model and suppressed growth of human AML[82]. Ro 08–2750 specifically diminishes MSI2 mRNA-binding capacity and downregulates MSI2 direct translational targets (*SMAD3, c-MYC, HOXA9*)[82]. Because the transcription factor *HOXA9* regulates MSI2 expression by binding with the *MSI2* promoter[79], it is expected that the disruption of RNA-protein interaction between MSI2 and *HOXA9* mRNA and similar targets will decrease MSI2 levels and weaken the stem cell program in aggressive leukemia.

LIN28 family of proteins

The LIN28 family consists of the two proteins, LIN28A and LIN28B, which play a central role in regulating pluripotency and differentiation by controlling the fate of coding and non-coding RNA. Fetal hematopoietic progenitors express high levels of Lin28b, which, along with IGF2BP3, is at the center of the fetal-to-adult hematopoietic switch[83]. Viswanathan et al. reported *Lin28/LIN28B* upregulation in about 15% of primary human tumors and human cancer cell lines[84]. *LIN28* expression was found to be more common in peripheral blood cells from patients with BC CML or in the accelerated phase than in the chronic phase of CML. Mechanistically, LIN28 blocks maturation of the *let-7* family of microRNAs that suppress multiple proliferative factors such as *HMGA2, K-RAS*, and *c-MYC*[84]. Jiang et al. discovered a tumor suppressor *miR-150* important for *MLL*-fusion-mediated leukemogenesis, and showed that pri-miR-150/pre-miR-150 maturation is inhibited by the *MLL*-fusion/*c-MYC*/*LIN28* axis[85]. *Lin28A*, however, is required for cell differentiation and is suppressed in murine *miR-125b*-driven AML[86], Table 6.

The molecular basis of LIN28 and *let-7* interaction was thoroughly investigated[87, 88]. Several groups identified compounds disrupting the antagonistic effect of LIN28 on *miR-let-7* biogenesis[89, 90]. Wang et al. utilized the fluorescence polarization assay to identify small-molecule inhibitors for both domains of LIN28 involved in *let-7* interactions. Of 101,017 tested compounds, six inhibited LIN28/*let-7* binding and impaired LIN28-mediated *let-7* oligouridylation. The selective pharmacologic inhibition of individual domains of LIN28 provides a foundation for their therapeutic targeting in leukemia cells and other LIN28-driven diseases[91].

IGF2BPs family of proteins

Insulin-like growth factor 2 RNA-binding proteins (IGF2BPs) comprise another RBP family important for embryonic development. The family consists of three members, IGF2BP1, IGF2BP2, and IGF2BP3, where IGF2BP1 and IGF2BP3 display greater structural similarity and are often co-reactivated in cancer[92]. IGF2BPs regulate mRNA stability and translation of multiple oncogenes (e.g. *IGF2, c-MYC, LIN28B, K-RAS*) via binding with 5′UTR, 3'UTR and coding regions of messengers[92, 93]. Of note, m^{6}A RNA modifications increase the affinity of RNA-IGF2BP binding, therefore, IGF2BPs are considered as m^{6}A readers[22]. Figure 1(I).

IGF2BP1 and IGF2BP3 are upregulated in *ETV-RUNX1* B-ALL and *MLL*-rearranged leukemia, supporting leukemia proliferation through *c-MYC* and *CDK6*[94, 95], Table 6. Being a downstream target of *miR-let-7*, IGF2BP1 counteracts *let-7* and is often co-expressed with LIN28 enhancing leukemia stem cell properties[96, 97]. Therefore, upregulation of IGF2BP1 and its paralogs is associated with poor survival rates in subsets of leukemia[95, 98, 99].

Given the physiological role of IGF2BP1 in stem cell maintenance and development, we recently investigated the impact of IGF2BP1 expression on LSC properties[99]. We found that IGF2BP1 supports the LSC phenotype by maintaining levels of *HOXB4, MYB*, and metabolic factor *ALDH1A1*. The small molecule inhibitor of IGF2BP1, BTYNB, was assayed in multiple cell lines derived from solid tumors[100]. In our study, BTYNB sensitized myeloid, B-cell, and T-cell leukemia lines to chemotherapeutics, establishing a proof of principal that IGF2BPs could be successfully targeted by small molecules in leukemia cells.

Concluding Remarks

RBPs are a family of proteins playing a central role in normal cell physiology and are crucial for cancer development and progression. Whereas mutations in functional domains of splicing factors could represent early genetic events predisposing to leukemia, a large body of data depicts abnormal RBP activity as a driving force of leukemia progression and an attribute of aggressive forms of disease. Multiple studies indicate that aberrant activity of RBPs is associated with acquisition of cancer stem cell phenotypes fundamental for resistance to therapies, minimal residual disease, and relapse. Therefore, finding ways of effectively targeting major classes of RBPs, discussed in this review, could potentially improve outcomes of leukemia treatments by lowering rates of refractory and relapsed leukemia. Given the association of RBP deregulation with disease aggressiveness and poor clinical outcomes, constructing a pro-LSCs score by assessing spliceosome mutations or mis-splicing, levels of RNA editing/modifications, and oncofetal proteins expression would be a valuable addition to the existing testing platforms.

Novel molecular-genetic tools and mouse models provided compelling evidence of increased dependency of acute myeloid and blast crisis chronic myeloid leukemia on RBPs. Therefore, a search for chemical modulators of RBP activity is rapidly expanding (summarized in Table 7). The first clinical trials of splicing factor inhibitors highlighted the importance of the deep understanding of RBP functions, which are often context dependent. General toxicity and safety concerns remain a hurdle in targeting proteins that are ubiquitously expressed and are

present in normal tissues. In this regard, oncofetal RBPs, which are not widely expressed in normal adult tissues, could have a therapeutic advantage. In addition, the genetic background of leukemic cells should be taken into consideration since a mutational status of splicing factors and other genes can increase the susceptibility to RBP inhibitors. Given a supportive role of RBPs in expression of multiple oncogenes, development of relatively nontoxic compounds would be highly beneficial for combinatorial therapies that would, among other effects, allow lower dosages of conventional cytotoxic drugs in older AML patients.

Our literature review indicates that leukemia cells may experience a systemic deregulation of RNA network affecting multiple *cis*- and *trans*- acting RNA regulatory elements. It is apparent that upregulation of various classes of RBPs are required to meet the anabolic demand of fast proliferating cells. The dynamics and synergistic effect of posttranscriptional aberrations in oncogenic transformation has not been fully investigated and understood. Targeting common pathways and regulatory elements that coordinate abnormal activity of various RBPs might be essential for eradicating the most aggressive forms of leukemia and other cancers.

Acknowledgements

This study was supported in part by the NIH NCI grants CA191550 and CA243167 (V.S.S.). The authors want to thank Alexander Elchev and Rachael Mills for editorial help with the manuscript.

References:

1. Hentze MW, Castello A, Schwarzl T, Preiss T. A brave new world of RNA-binding proteins. Nat Rev Mol Cell Biol 2018 5; 19(5): 327–341. [PubMed: 29339797]
2. Nishikura K A-to-I editing of coding and non-coding RNAs by ADARs. Nat Rev Mol Cell Biol 2016 2; 17(2): 83–96. [PubMed: 26648264]
3. Lamers MM, van den Hoogen BG, Haagmans BL. ADAR1: “Editor-in-Chief” of Cytoplasmic Innate Immunity. Frontiers in Immunology 2019 7 25; 10(1763).
4. Wang Q, Khillan J, Gadue P, Nishikura K. Requirement of the RNA editing deaminase ADAR1 gene for embryonic erythropoiesis. Science 2000 12 1; 290(5497): 1765–1768. [PubMed: 11099415]
5. Liddicoat BJ, Piskol R, Chalk AM, Ramaswami G, Higuchi M, Hartner JC, et al. RNA editing by ADAR1 prevents MDA5 sensing of endogenous dsRNA as nonself. Science 2015 9 4; 349(6252): 1115–1120. [PubMed: 26275108]
6. XuFeng R, Boyer MJ, Shen H, Li Y, Yu H, Gao Y, et al. ADAR1 is required for hematopoietic progenitor cell survival via RNA editing. Proc Natl Acad Sci U S A 2009 10 20; 106(42): 17763–17768. [PubMed: 19805087]
7. Ma CH, Chong JH, Guo Y, Zeng HM, Liu SY, Xu LL, et al. Abnormal expression of ADAR1 isoforms in Chinese pediatric acute leukemias. Biochem Biophys Res Commun 2011 3 11; 406(2): 245–251. [PubMed: 21316340]
8. Xiao H, Cheng Q, Wu X, Tang Y, Liu J, Li X. ADAR1 may be involved in the proliferation of acute myeloid leukemia cells via regulation of the Wnt pathway. Cancer Manag Res 2019 9 20; 11: 8547–8555. [PubMed: 31572009]
9. Jiang Q, Crews LA, Barrett CL, Chun H-J, Court AC, Isquith JM, et al. ADAR1 promotes malignant progenitor reprogramming in chronic myeloid leukemia. Proc Natl Acad Sci U S A 2013 1 15; 110(3): 1041–1046. [PubMed: 23275297]
10. Zipeto MA, Court AC, Sadarangani A, Delos Santos NP, Balaian L, Chun HJ, et al. ADAR1 Activation Drives Leukemia Stem Cell Self-Renewal by Impairing Let-7 Biogenesis. Cell Stem Cell 2016 8 4; 19(2): 177–191. [PubMed: 27292188]

11. Crews LA, Jiang Q, Zipeto MA, Lazzari E, Court AC, Ali S, et al. An RNA editing fingerprint of cancer stem cell reprogramming. J Transl Med 2015 2 12; 13: 52. [PubMed: 25889244]
12. Jiang Q, Isquith J, Zipeto MA, Diep RH, Pham J, Delos Santos N, et al. Hyper-Editing of Cell-Cycle Regulatory and Tumor Suppressor RNA Promotes Malignant Progenitor Propagation. Cancer Cell 2019 1 14; 35(1): 81–94. [PubMed: 30612940]
13. Gannon HS, Zou T, Kiessling MK, Gao GF, Cai D, Choi PS, et al. Identification of ADAR1 adenosine deaminase dependency in a subset of cancer cells. Nature Commun 2018 12 21; 9(1): 5450. [PubMed: 30575730]
14. Ishizuka JJ, Manguso RT, Cheruiyot CK, Bi K, Panda A, Iracheta-Vellve A, et al. Loss of ADAR1 in tumours overcomes resistance to immune checkpoint blockade. Nature 2019 1; 565(7737): 43–48. [PubMed: 30559380]
15. Vu LP, Cheng Y, Kharas MG. The Biology of m(6)A RNA Methylation in Normal and Malignant Hematopoiesis. Cancer Discov 2019 1; 9(1): 25–33. [PubMed: 30578356]
16. Shi H, Wei J, He C. Where, When, and How: Context-Dependent Functions of RNA Methylation Writers, Readers, and Erasers. Mol Cell 2019 5 16; 74(4): 640–650. [PubMed: 31100245]
17. Martinez NM, Gilbert WV. Pre-mRNA modifications and their role in nuclear processing. Quant Biol 2018 9; 6(3): 210–227. [PubMed: 30533247]
18. Han SH, Choe J. Diverse molecular functions of m(6)A mRNA modification in cancer. Exp Mol Med 2020 5 13; 52(5): 738–749. [PubMed: 32404927]
19. Barbieri I, Tzelepis K, Pandolfini L, Shi J, Millan-Zambrano G, Robson SC, et al. Promoter-bound METTL3 maintains myeloid leukaemia by m(6)A-dependent translation control. Nature 2017 12 7; 552(7683): 126–131. [PubMed: 29186125]
20. Vu LP, Pickering BF, Cheng Y, Zaccara S, Nguyen D, Minuesa G, et al. The N(6)-methyladenosine (m(6)A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. Nat Med 2017 11; 23(11): 1369–1376. [PubMed: 28920958]
21. Weng H, Huang H, Wu H, Qin X, Zhao BS, Dong L, et al. METTL14 Inhibits Hematopoietic Stem/Progenitor Differentiation and Promotes Leukemogenesis via mRNA m(6)A Modification. Cell Stem Cell 2018 2 1; 22(2): 191–205. [PubMed: 29290617]
22. Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, et al. Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. Nat Cell Biol 2018 3; 20(3): 285–295. [PubMed: 29476152]
23. Sorci M, Ianniello Z, Cruciani S, Larivera S, Ginistrelli LC, Capuano E, et al. METTL3 regulates WTAP protein homeostasis. Cell Death Dis 2018 7 23; 9(8): 796. [PubMed: 30038300]
24. Ping XL, Sun BF, Wang L, Xiao W, Yang X, Wang WJ, et al. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Res 2014 2; 24(2): 177–189. [PubMed: 24407421]
25. Bansal H, Yihua Q, Iyer SP, Ganapathy S, Proia DA, Penalva LO, et al. WTAP is a novel oncogenic protein in acute myeloid leukemia. Leukemia 2014 5; 28(5): 1171–1174. [PubMed: 24413322]
26. Patil DP, Chen CK, Pickering BF, Chow A, Jackson C, Guttman M, et al. m(6)A RNA methylation promotes XIST-mediated transcriptional repression. Nature 2016 9 15; 537(7620): 369–373. [PubMed: 27602518]
27. Zhang L, Tran N-T, Su H, Wang R, Lu Y, Tang H, et al. Cross-talk between PRMT1-mediated methylation and ubiquitylation on RBM15 controls RNA splicing. Elife 2015 11 17; 4: e07938. [PubMed: 26575292]
28. Li Z, Weng H, Su R, Weng X, Zuo Z, Li C, et al. FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N(6)-Methyladenosine RNA Demethylase. Cancer Cell 2017 1 9; 31(1): 127–141. [PubMed: 28017614]
29. Su R, Dong L, Li Y, Gao M, Han L, Wunderlich M, et al. Targeting FTO Suppresses Cancer Stem Cell Maintenance and Immune Evasion. Cancer Cell 2020 7 13; 38(1): 79–96. [PubMed: 32531268]
30. Huang Y, Su R, Sheng Y, Dong L, Dong Z, Xu H, et al. Small-Molecule Targeting of Oncogenic FTO Demethylase in Acute Myeloid Leukemia. Cancer Cell 2019 4 15; 35(4): 677–691. [PubMed: 30991027]

31. Mauer J, Sindelar M, Despic V, Guez T, Hawley BR, Vasseur JJ, et al. FTO controls reversible m(6)Am RNA methylation during snRNA biogenesis. Nat Chem Biol 2019 4; 15(4): 340–347. [PubMed: 30778204]
32. Shen C, Sheng Y, Zhu AC, Robinson S, Jiang X, Dong L, et al. RNA Demethylase ALKBH5 Selectively Promotes Tumorigenesis and Cancer Stem Cell Self-Renewal in Acute Myeloid Leukemia. Cell Stem Cell 2020 7 2; 27(1): 64–80. [PubMed: 32402250]
33. Wang J, Li Y, Wang P, Han G, Zhang T, Chang J, et al. Leukemogenic Chromatin Alterations Promote AML Leukemia Stem Cells via a KDM4C-ALKBH5-AXL Signaling Axis. Cell Stem Cell 2020 7 2; 27(1): 81–97. [PubMed: 32402251]
34. Paris J, Morgan M, Campos J, Spencer GJ, Shmakova A, Ivanova I, et al. Targeting the RNA m(6)A Reader YTHDF2 Selectively Compromises Cancer Stem Cells in Acute Myeloid Leukemia. Cell Stem Cell 2019 7 3; 25(1): 137–148. [PubMed: 31031138]
35. Cully M Chemical inhibitors make their RNA epigenetic mark. Nat Rev Drug Discov 2019 11; 18(12): 892–894. [PubMed: 31780844]
36. El Marabti E, Younis I. The Cancer Spliceome: Reprograming of Alternative Splicing in Cancer. Front Mol Biosci 2018 9 07; 5: 80–80. [PubMed: 30246013]
37. Obeng EA, Stewart C, Abdel-Wahab O. Altered RNA Processing in Cancer Pathogenesis and Therapy. Cancer Discov 2019 11; 9(11): 1493–1510. [PubMed: 31611195]
38. Adamia S, Haibe-Kains B, Pilarski PM, Bar-Natan M, Pevzner S, Avet-Loiseau H, et al. A genome-wide aberrant RNA splicing in patients with acute myeloid leukemia identifies novel potential disease markers and therapeutic targets. Clin Cancer Res 2014 3 1; 20(5): 1135–1145. [PubMed: 24284058]
39. Park S, Supek F, Lehner B. Systematic discovery of germline cancer predisposition genes through the identification of somatic second hits. Nature Commun 2018 7 04; 9(1): 2601. [PubMed: 29973584]
40. Jung H, Lee D, Lee J, Park D, Kim YJ, Park WY, et al. Intron retention is a widespread mechanism of tumor-suppressor inactivation. Nat Genet 2015 11; 47(11): 1242–1248. [PubMed: 26437032]
41. Abrahamsson AE, Geron I, Gotlib J, Dao K-HT, Barroga CF, Newton IG, et al. Glycogen synthase kinase 3β missplicing contributes to leukemia stem cell generation. Proceedings of the National Academy of Sciences 2009 3 10; 106(10): 3925–3929.
42. Puente XS, Beà S, Valdés-Mas R, Villamor N, Gutiérrez-Abril J, Martín-Subero JI, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. Nature 2015 10 22; 526(7574): 519–524. [PubMed: 26200345]
43. Asnani M, Hayer KE, Naqvi AS, Zheng S, Yang SY, Oldridge D, et al. Retention of CD19 intron 2 contributes to CART-19 resistance in leukemias with subclonal frameshift mutations in CD19. Leukemia 2020 4; 34(4): 1202–1207. [PubMed: 31591467]
44. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 2011 9 11; 478(7367): 64–69. [PubMed: 21909114]
45. Taylor J, Lee SC. Mutations in spliceosome genes and therapeutic opportunities in myeloid malignancies. Genes Chromosomes Cancer 2019 12; 58(12): 889–902. [PubMed: 31334570]
46. Cretu C, Schmitzova J, Ponce-Salvatierra A, Dybkov O, De Laurentiis EI, Sharma K, et al. Molecular Architecture of SF3b and Structural Consequences of Its Cancer-Related Mutations. Mol Cell 2016 10 20; 64(2): 307–319. [PubMed: 27720643]
47. Wang L, Brooks AN, Fan J, Wan Y, Gambe R, Li S, et al. Transcriptomic Characterization of SF3B1 Mutation Reveals Its Pleiotropic Effects in Chronic Lymphocytic Leukemia. Cancer Cell 2016 11 14; 30(5): 750–763. [PubMed: 27818134]
48. Kim E, Ilagan JO, Liang Y, Daubner GM, Lee SC, Ramakrishnan A, et al. SRSF2 Mutations Contribute to Myelodysplasia by Mutant-Specific Effects on Exon Recognition. Cancer Cell 2015 5 11; 27(5): 617–630. [PubMed: 25965569]
49. Black KL, Naqvi AS, Asnani M, Hayer KE, Yang SY, Gillespie E, et al. Aberrant splicing in B-cell acute lymphoblastic leukemia. Nucleic Acids Res 2018 11 30; 46(21): 11357–11369. [PubMed: 30357359]

50. Wang E, Lu SX, Pastore A, Chen X, Imig J, Chun-Wei Lee S, et al. Targeting an RNA-Binding Protein Network in Acute Myeloid Leukemia. Cancer Cell 2019 3 18; 35(3): 369–384. [PubMed: 30799057]
51. Yamauchi T, Masuda T, Canver MC, Seiler M, Semba Y, Shboul M, et al. Genome-wide CRISPR-Cas9 Screen Identifies Leukemia-Specific Dependence on a Pre-mRNA Metabolic Pathway Regulated by DCPS. Cancer Cell 2018 3 12; 33(3): 386–400. [PubMed: 29478914]
52. Bajaj J, Hamilton M, Shima Y, Chambers K, Spinler K, Van Nostrand EL, et al. An in vivo genome-wide CRISPR screen identifies the RNA-binding protein Staufen2 as a key regulator of myeloid leukemia. Nature Cancer 2020 4 20; 1(4): 410–422.
53. Tian B, Manley JL. Alternative polyadenylation of mRNA precursors. Nat Rev Mol Cell Biol 2017 1; 18(1): 18–30. [PubMed: 27677860]
54. Danckwardt S, Hentze MW, Kulozik AE. 3' end mRNA processing: molecular mechanisms and implications for health and disease. EMBO J 2008 2 6; 27(3): 482–498. [PubMed: 18256699]
55. Shima T, Davis AG, Miyauchi S, Kochi Y, Johnson DT, Stoner SA, et al. CPSF1 Regulates AML1-ETO Fusion Gene Polyadenylation and Stability in t(8;21) Acute Myelogenous Leukemia. Blood 2017 12 7; 130(Abstract): 2498–2498.
56. Ye C, Zhou Q, Hong Y, Li QQ. Role of alternative polyadenylation dynamics in acute myeloid leukaemia at single-cell resolution. RNA Biol 2019 3; 16(6): 785–797. [PubMed: 30810468]
57. Venters CC, Oh JM, Di C, So BR, Dreyfuss G. U1 snRNP Telescripting: Suppression of Premature Transcription Termination in Introns as a New Layer of Gene Regulation. Cold Spring Harb Perspect Biol 2019 2 1; 11(2): 1–15.
58. Shuai S, Suzuki H, Diaz-Navarro A, Nadeu F, Kumar SA, Gutierrez-Fernandez A, et al. The U1 spliceosomal RNA is recurrently mutated in multiple cancers. Nature 2019 10; 574(7780): 712–716. [PubMed: 31597163]
59. Oh J-M, Venters CC, Di C, Pinto AM, Wan L, Younis I, et al. U1 snRNP regulates cancer cell migration and invasion in vitro. Nature Commun 2020 1 7; 11(1): 1–8. [PubMed: 31911652]
60. Baou M, Norton JD, Murphy JJ. AU-rich RNA binding proteins in hematopoiesis and leukemogenesis. Blood 2011 11 24; 118(22): 5732–5740. [PubMed: 21917750]
61. Salton M, Misteli T. Small Molecule Modulators of Pre-mRNA Splicing in Cancer Therapy. Trends Mol Med 2016 1; 22(1): 28–37. [PubMed: 26700537]
62. Seiler M, Yoshimi A, Darman R, Chan B, Keaney G, Thomas M, et al. H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers. Nat Med 2018 5; 24(4): 497–504. [PubMed: 29457796]
63. Han T, Goralski M, Gaskill N, Capota E, Kim J, Ting TC, et al. Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. Science 2017 4 28; 356(6336): 3755.
64. Fedoriw A, Rajapurkar SR, O'Brien S, Gerhart SV, Mitchell LH, Adams ND, et al. Anti-tumor Activity of the Type I PRMT Inhibitor, GSK3368715, Synergizes with PRMT5 Inhibition through MTAP Loss. Cancer Cell 2019 7 8; 36(1): 100–114. [PubMed: 31257072]
65. Fong JY, Pignata L, Goy PA, Kawabata KC, Lee SC, Koh CM, et al. Therapeutic Targeting of RNA Splicing Catalysis through Inhibition of Protein Arginine Methylation. Cancer Cell 2019 8 12; 36(2): 194–209. [PubMed: 31408619]
66. Kohler A, Hurt E. Exporting RNA from the nucleus to the cytoplasm. Nat Rev Mol Cell Biol 2007 10; 8(10): 761–773. [PubMed: 17786152]
67. Mahipal A, Malafa M. Importins and exportins as therapeutic targets in cancer. Pharmacol Ther 2016 8; 164: 135–143. [PubMed: 27113410]
68. Talati C, Sweet KL. Nuclear transport inhibition in acute myeloid leukemia: recent advances and future perspectives. Int J Hematol Oncol 2018 10; 7(3): IJH04–IJH04. [PubMed: 30405902]
69. Wendel H-G, Silva RLA, Malina A, Mills JR, Zhu H, Ueda T, et al. Dissecting eIF4E action in tumorigenesis. Genes Dev 2007 12 15; 21(24): 3232–3237. [PubMed: 18055695]
70. Topisirovic I, Guzman ML, McConnell MJ, Licht JD, Culjkovic B, Neering SJ, et al. Aberrant eukaryotic translation initiation factor 4E-dependent mRNA transport impedes hematopoietic differentiation and contributes to leukemogenesis. Mol Cell Biol 2003 12; 23(24): 8992–9002. [PubMed: 14645512]

71. Culjkovic-Kraljacic B, Fernando TM, Marullo R, Calvo-Vidal N, Verma A, Yang S, et al. Combinatorial targeting of nuclear export and translation of RNA inhibits aggressive B-cell lymphomas. Blood 2016 2 18; 127(7): 858–868. [PubMed: 26603836]
72. Borden KL, Culjkovic-Kraljacic B. Ribavirin as an anti-cancer therapy: acute myeloid leukemia and beyond? Leuk Lymphoma 2010 10; 51(10): 1805–1815. [PubMed: 20629523]
73. Zahreddine HA, Culjkovic-Kraljacic B, Assouline S, Gendron P, Romeo AA, Morris SJ, et al. The sonic hedgehog factor GLI1 imparts drug resistance through inducible glucuronidation. Nature 2014 7 3; 511(7507): 90–93. [PubMed: 24870236]
74. Rentas S, Holzapfel N, Belew MS, Pratt G, Voisin V, Wilhelm BT, et al. Musashi-2 attenuates AHR signalling to expand human haematopoietic stem cells. Nature 2016 4 28; 532(7600): 508–511. [PubMed: 27121842]
75. Nguyen DTT, Lu Y, Chu KL, Yang X, Park SM, Choo ZN, et al. HyperTRIBE uncovers increased MUSASHI-2 RNA binding activity and differential regulation in leukemic stem cells. Nat Commun 2020 4 24; 11(1): 2026. [PubMed: 32332729]
76. Park S-M, Gönen M, Vu L, Minuesa G, Tivnan P, Barlowe TS, et al. Musashi2 sustains the mixed-lineage leukemia-driven stem cell regulatory program. J Clin Invest 2015 3; 125(3): 1286–1298. [PubMed: 25664853]
77. Park SM, Cho H, Thornton AM, Barlowe TS, Chou T, Chhangawala S, et al. IKZF2 Drives Leukemia Stem Cell Self-Renewal and Inhibits Myeloid Differentiation. Cell Stem Cell 2019 1 3; 24(1): 153–165. [PubMed: 30472158]
78. Kwon HY, Bajaj J, Ito T, Blevins A, Konuma T, Weeks J, et al. Tetraspanin 3 Is Required for the Development and Propagation of Acute Myelogenous Leukemia. Cell Stem Cell 2015 8 6; 17(2): 152–164. [PubMed: 26212080]
79. Ito T, Kwon HY, Zimdahl B, Congdon KL, Blum J, Lento WE, et al. Regulation of myeloid leukaemia by the cell-fate determinant Musashi. Nature 2010 8 5; 466(7307): 765–768. [PubMed: 20639863]
80. Kharas MG, Lengner CJ, Al-Shahrour F, Bullinger L, Ball B, Zaidi S, et al. Musashi-2 regulates normal hematopoiesis and promotes aggressive myeloid leukemia. Nat Med 2010 8; 16(8): 903–908. [PubMed: 20616797]
81. Hattori A, Tsunoda M, Konuma T, Kobayashi M, Nagy T, Glushka J, et al. Cancer progression by reprogrammed BCAA metabolism in myeloid leukaemia. Nature 2017 5 25; 545(7655): 500–504. [PubMed: 28514443]
82. Minuesa G, Albanese SK, Xie W, Kazansky Y, Worroll D, Chow A, et al. Small-molecule targeting of MUSASHI RNA-binding activity in acute myeloid leukemia. Nature Commun 2019 6 19; 10(1): 2691. [PubMed: 31217428]
83. Wang S, Chim B, Su Y, Khil P, Wong M, Wang X, et al. Enhancement of LIN28B-induced hematopoietic reprogramming by IGF2BP3. Genes Dev 2019 8 1; 33(15–16): 1048–1068. [PubMed: 31221665]
84. Viswanathan SR, Powers JT, Einhorn W, Hoshida Y, Ng TL, Toffanin S, et al. Lin28 promotes transformation and is associated with advanced human malignancies. Nature Genet 2009 7; 41(7): 843–848. [PubMed: 19483683]
85. Jiang X, Huang H, Li Z, Li Y, Wang X, Gurbuxani S, et al. Blockade of miR-150 maturation by MLL-fusion/MYC/LIN-28 is required for MLL-associated leukemia. Cancer Cell 2012 10 16; 22(4): 524–535. [PubMed: 23079661]
86. Chaudhuri AA, So AY, Mehta A, Minisandram A, Sinha N, Jonsson VD, et al. Oncomir miR-125b regulates hematopoiesis by targeting the gene Lin28A. Proc Natl Acad Sci U S A 2012 3 13; 109(11): 4233–4238. [PubMed: 22366319]
87. Ransey E, Björkbom A, Lelyveld VS, Biecek P, Pantano L, Szostak JW, et al. Comparative analysis of LIN28-RNA binding sites identified at single nucleotide resolution. RNA Biol 2017 12 2; 14(12): 1756–1765. [PubMed: 28945502]
88. Yamashita S, Nagaike T, Tomita K. Crystal structure of the Lin28-interacting module of human terminal uridylyltransferase that regulates let-7 expression. Nat Commun 2019 4 29; 10(1): 1960. [PubMed: 31036859]

89. Roos M, Pradere U, Ngondo RP, Behera A, Allegrini S, Civenni G, et al. A Small-Molecule Inhibitor of Lin28. ACS Chem Biol 2016 10 21; 11(10): 2773–2781. [PubMed: 27548809]

90. Yu C, Wang L, Rowe RG, Han A, Ji W, McMahon C, et al. A nanobody targeting the LIN28:let-7 interaction fragment of TUT4 blocks uridylation of let-7. Proc Natl Acad Sci U S A 2020 3 3; 117(9): 4653–4663. [PubMed: 32060122]

91. Wang L, Rowe RG, Jaimes A, Yu C, Nam Y, Pearson DS, et al. Small-Molecule Inhibitors Disrupt let-7 Oligouridylation and Release the Selective Blockade of let-7 Processing by LIN28. Cell Rep 2018 6 5; 23(10): 3091–3101. [PubMed: 29874593]

92. Degrauwe N, Suva ML, Janiszewska M, Riggi N, Stamenkovic I. IMPs: an RNA-binding protein family that provides a link between stem cell maintenance in normal development and cancer. Genes Dev 2016 11 15; 30(22): 2459–2474. [PubMed: 27940961]

93. Huang X, Zhang H, Guo X, Zhu Z, Cai H, Kong X. Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) in cancer. J Hematol Oncol 2018 6 28; 11(1): 88. [PubMed: 29954406]

94. Stoskus M, Vaitkeviciene G, Eidukaite A, Griskevicius L. ETV6/RUNX1 transcript is a target of RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive acute lymphoblastic leukemia. Blood Cells Mol Dis 2016 3; 57: 30–34. [PubMed: 26852652]

95. Palanichamy JK, Tran TM, Howard JM, Contreras JR, Fernando TR, Sterne-Weiler T, et al. RNA-binding protein IGF2BP3 targeting of oncogenic transcripts promotes hematopoietic progenitor proliferation. J Clin Invest 2016 4 1; 126(4): 1495–1511. [PubMed: 26974154]

96. Busch B, Bley N, Müller S, Glaß M, Misiak D, Lederer M, et al. The oncogenic triangle of HMGA2, LIN28B and IGF2BP1 antagonizes tumor-suppressive actions of the let-7 family. Nucleic acids research 2016 5 5; 44(8): 3845–3864. [PubMed: 26917013]

97. Zhou J, Bi C, Ching YQ, Chooi J-Y, Lu X, Quah JY, et al. Inhibition of LIN28B impairs leukemia cell growth and metabolism in acute myeloid leukemia. Journal of hematology & oncology 2017 7 11; 10(1): 138–138. [PubMed: 28693523]

98. He X, Li W, Liang X, Zhu X, Zhang L, Huang Y, et al. IGF2BP2 Overexpression Indicates Poor Survival in Patients with Acute Myelocytic Leukemia. Cell Physiol Biochem 2018 12; 51(4): 1945–1956. [PubMed: 30513526]

99. Elcheva IA, Wood T, Chiarolanzio K, Chim B, Wong M, Singh V, et al. RNA-binding protein IGF2BP1 maintains leukemia stem cell properties by regulating HOXB4, MYB, and ALDH1A1. Leukemia 2020 5; 34(5): 1354–1363. [PubMed: 31768017]

100. Mahapatra L, Andruska N, Mao C, Le J, Shapiro DJ. A Novel IMP1 Inhibitor, BTYNB, Targets c-Myc and Inhibits Melanoma and Ovarian Cancer Cell Proliferation. Transl Oncol 2017 10; 10(5): 818–827. [PubMed: 28846937]

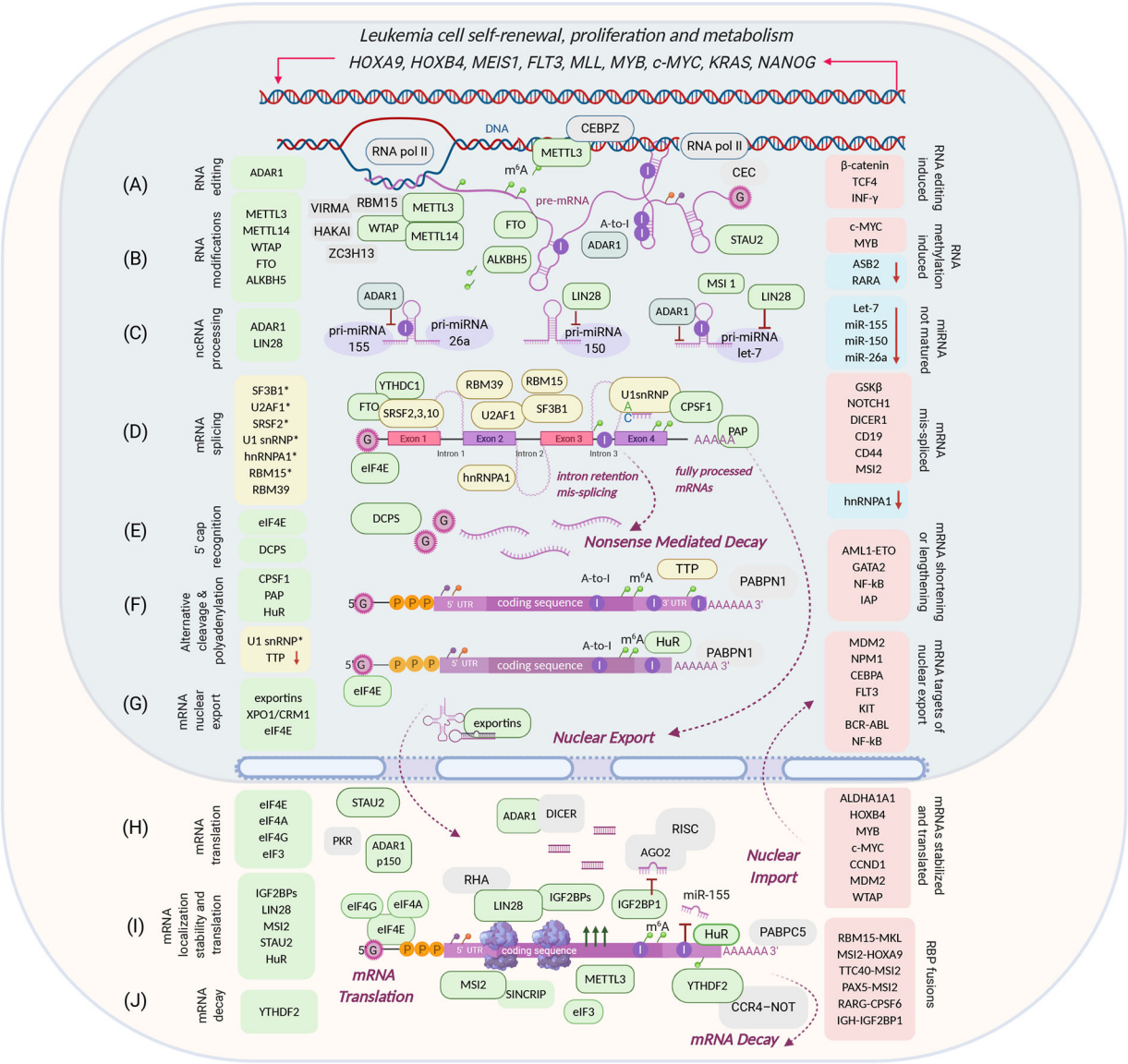

Figure 1, RNA-binding proteins involved in leukemogenesis.

RNA-binding proteins are listed on the left side of the diagram: upregulation, gain-of-function (green), loss- or change-of-function (yellow, arrow down, *mutation). Target genes are listed on the right side of the diagram: upregulated oncogenes (red boxes), tumor suppressor gene inactivation (blue boxes, arrow down). **(A)** ADAR1 regulates miRNA biogenesis in an A-to-I editing-dependent manner (C), A-to-I editing affects mRNA stability (I); **(B)** RNA modifying enzymes facilitate m^{6}A methylation (METTL3/14), demethylation (FTO, ALKBH5), substrate recognition (WTAP, RBM15); METTL3 can co-localize with DNA in the nucleus and enhance mRNA translation in the cytoplasm; **(C)** noncoding RNA processing: ADAR1 and LIN28 suppress maturation of miRNA *let-7*, *miR-155*, *miR-150*, and *miR-26a*; **(D)** RNA splicing factors are often mutated in chronic leukemia and/or mis-spliced in acute leukemia producing more mis-spliced pro-oncogenic mRNA isoforms; **(E)** 5’ cap recognizing enzymes that either promote mRNA nuclear export and translation

(eIF4E) or destabilize mRNA (DCPS); **(F)** alternative cleavage and polyadenylation, occurring during splicing (D), is characterized by mRNA 3'UTRs shortening or lengthening; shorter 3'UTRs increase stability and expression of oncogenic transcripts; downregulation or deactivation of 3'UTR-binding protein TTP increases mRNA abundance; **(G)** high expression levels of nuclear export regulators (exportins, XPO1/CRM1, eIF4E) increase transport and translation of oncogenic factors; **(H)** high levels of eIF4E promote nuclear export and translation of selective proto-oncogenic targets; **(I)** increased mRNA stability and translation of oncogenic transcripts through multiple post-transcriptional events, including reactivation of oncofetal proteins LIN28 and IGF2BPs; **(J)** m^6A reader YTHDF2 targets mRNAs for CCR4-NOT-dependent deadenylation and degradation.

Table 1.

RNA editing enzyme, ADAR1

Gene	Protein/RNP function	Target Genes	Biological consequences	Type of cancer	Expression in cancer	Ref
ADAR1	A-to-I substitutions in dsRNAs, writer	*miR-26a, miR-155, let-7*	cell cycle regulation via block of miRNA processing	CML,BC CML	upregulation	9, 10, 12
		GSK-β	mis-spliced mRNA			
		MDM2	mRNA stabilization through 3'UTR modification			
		INF-γ pathway	immune response activation			
		β-catenin, TCF-4, CCND2	WNT activation	AML		8
		not studied	high ADAR1 expressors were in standard-risk groups	pediatric ALL		7

Abbreviations used: Acute myeloid leukemia (AML); acute promyeloblastic leukemia (APL); acute megakaryoblastic leukemia (AMKL); acute lymphoblastic or lymphocytic (ALL); B-cell acute lymphoblastic leukemia (B-ALL); adult T-cell leukemia/lymphoma (ATL); diffuse large B-cell lymphomas (DLBCLs); chronic myeloid leukemia (CML), chronic phase (CP), accelerated phase (AP), blast crisis (BC); chronic lymphoblastic or lymphocytic leukemia (CLL); myelodysplastic syndromes (MDS); multiple myeloma (MM); hepatocellular carcinoma (HCC); leukemia stem cell (LSC); wild type (WT); patient-derived xenograft (PDX); bone marrow (BM); hematopoietic stem/progenitor cells (HSCs, HSPCs).

Table 2.

RBPs involved in RNA modification

Gene	Protein/RNP function	Target Genes	Biological consequences	Type of cancer	Expression in cancer	Ref
METTL3	RNA hypermethylation, m^{6}A writer	*c-MYC, BCL2, PTEN*	promotes oncogenes translation	AML MOLM-13	upregulation	20
	RNA m^{6}A writer, DNA promoter binding through binding CEBPZ	*global*	methylation of coding regions of mRNAs, m^{6}A-dependent translation, relieving ribosome stalling	AML		19
	Attenuate translation, cytoplasmic localization	*WTAP*	translation, interaction with eIF3, upregulated expression	K562, HeLa		23
METTL14	RNA recognition	*MYB, MYC*	enables m^{6}A methylation by METTL3, regulates self-renewal and differentiation	AML (LSCs)	upregulation	21
WTAP	RNA recognition	*global analysis, transcription and RNA processing genes*	promotes m^{6}A methylation by METTL3 enables METTL3 nuclear localization	HEK293 cells, HeLa	upregulation	24
		CD4, CD44, CEBPA, CSF1R, MPO, ABCG2, TCL1A, CYP1A1, CYP3A4, FGFR1, PTPRC (CD45), CD83, CD86, CD9 and CCR4.	abnormal proliferation and arrested differentiastion	AML, HL-60, K562		25
RBM15	RNA recognition	genes on the X chromosome	transcriptional silencing by lncRNA *XIST*	n/a	n/a	26
FTO	RNA hypomethylation, m^{6}A eraser	*NANOG, SOX2 WNT signaling*	stem cell genes, oncogenes upregulated	AML: *MLL*r, *PML-RARA*, *FLT3-ITD, NPM1* mut.	upregulation	28
		ASB2, RARA	differentiation factors are downregulated			
		immune checkpoint genes *e.g. LILRB4*	immune evasion	AML (LSCs)		29
		U1, U2, U6 snRNAs	demethylation, FTO inhibition leads to altered splicing	human TF-1 erythroleukemia cells	tumor suppressor?	31
ALKBH5	RNA hypomethylation, m^{6}A eraser	*TACC3*	functions as an oncogene in AML regardless of TP53 mutation status; significantly associated with shorter overall survival and poor prognosis in AML, similar to solid tumors	NOMO-1 (TP53-mutant), MV4;11 (TP53-WT) and MA9.3-ITDcells (TP53-WT), in vivo	upregulation	32
			promotes LSCs self-renewal through MYC-p21 axis	AML (LSCs)		
		receptor tyrosine kinase AXL	*AXL* mRNA stability in m^{6}A-dependent manner; MYB, Pol II activity	AML (LSCs)		33
YTHDF2	m^{6}A reader, cytoplasmic, targets mRNA for degradation	*TNFR2*	Inhibition of apoptosis, enhanced self-renewal	AML (LSCs)	upregulation	34

Abbreviations used: Acute myeloid leukemia (AML); acute promyeloblastic leukemia (APL); acute megakaryoblastic leukemia (AMKL); acute lymphoblastic or lymphocytic (ALL); B-cell acute lymphoblastic leukemia (B-ALL); adult T-cell leukemia/lymphoma (ATL); diffuse large B-cell lymphomas (DLBCLs); chronic myeloid leukemia (CML), chronic phase (CP), accelerated phase (AP), blast crisis (BC); chronic lymphoblastic or lymphocytic leukemia (CLL); myelodysplastic syndromes (MDS); multiple myeloma (MM); hepatocellular carcinoma (HCC); leukemia stem cell (LSC); wild type (WT); patient-derived xenograft (PDX); bone marrow (BM); hematopoietic stem/progenitor cells (HSCs, HSPCs).

Table 3.

RBPs involved in mRNA splicing

Gene	Protein/RNP function	Target Genes	Biological consequences	Type of cancer	Expression in cancer	Ref
SF3B1 U2AF1(35) SRSF2 ZRSR2 SF3A1 PRPF40B	splicing factors 3' splice site recognition in pre-mRNA	17 genes NMD (*SMG1,5,6,7,8 9, DHX34, UPFs, BTZ, Y14, PYM, hNAG, MAGOH, eIF4A3*).	apoptosis, G1/M phase arrest, compromise reconstitution capacity	MDS, HeLa	loss or change-of-function due to mutations and mis-splicing	44
U1 snRNP	splicing factor 5' splice site recognition thru U1 snRNA:pre-mRNA base pairing	*MSI2, POLD1, CD44, ABCD3*, global splicing	mis-splicing, intron retention;downregulated genes related to apoptosis, more aggressive CLL	CLL, HCC	mutations in canonical *U1 snRNA genes*, change-of-function	58
hnRNPA1	splicing factor	*DICER, NT5C2*, global splicing, RNA metabolism	global mis-splicing, BM failure	pediatric B-ALL	loss or change-of-function due to mis-splicing	49
RBM15	RNA splicing, erythro-megakaryocytic lineage factors	*GATA1, RUNX1, MPL, TAL1 RBM15-MKL1*	altered splicing, abolished megakaryocytic differentiation	AMKL	deletions, fusion (tumor suppressor?)	27
RBM39	splicing factor	*HOXA9 transcriptional targets*	RBM39 inactivation leads to mis-splicing and downregulation of *GATA2, BMI-1, MYB*	AML, MOLM-13 (MLL-AF9, FLT3ITD), K562	non-oncogenic "addiction", upregulated	50
DCPS	decapping	spliceosomes, transcription, export, nuclear pore complexes	DCPS inactivation causes pre-mRNA mis-splicing, induces a type I interferon response in AML	CALM/AF10 or MLL/AF9 leukemia, AML MOLM-13, AML PDX	AML dependency, upregulated	51

Abbreviations used: Acute myeloid leukemia (AML); acute promyeloblastic leukemia (APL); acute megakaryoblastic leukemia (AMKL); acute lymphoblastic or lymphocytic (ALL); B-cell acute lymphoblastic leukemia (B-ALL); adult T-cell leukemia/lymphoma (ATL); diffuse large B-cell lymphomas (DLBCLs); chronic myeloid leukemia (CML), chronic phase (CP), accelerated phase (AP), blast crisis (BC); chronic lymphoblastic or lymphocytic leukemia (CLL); myelodysplastic syndromes (MDS); multiple myeloma (MM); hepatocellular carcinoma (HCC); leukemia stem cell (LSC); wild type (WT); patient-derived xenograft (PDX); bone marrow (BM); hematopoietic stem/progenitor cells (HSCs, HSPCs).

Table 4.

RBPs involved in mRNA polyadenylation

Gene	Protein/RNP function	Target Genes	Biological consequences	Type of cancer	Expression in cancer	Ref
U1 snRNP	telescripting - inhibits premature cleavage and polyadenylation	*global transcription elongation*	3' UTR shortening, truncated mRNA, increased migration and invasion	HeLa	loss or change-of-function	[59] reviewed in[57]
PAP	Poly(A) Polymerase	n/a (PAP activity in cell extracts)	PAP activity is higher in acute leukemia than in chronic leukemia	AML, ALL, CML	upregulation	reviewed in[54]
APA (process)	alternative polyadenylation	*NF-kB, GATA2, IAP-family of genes*	global RNA shortening or lengthening	BM AML	upregulation	[56]
CPSF1	cleavage and polyadenylation, recruits nuclear export	*AML1-ETO*	oncogenic mRNA stability	AML	upregulation	[55]
HuR	AU-rich RNA binding protein, mRNA stability	*eIF4E, cEBPβ, p21, FOXO3, MEK1, MEK2, DUSP10, ZFP36L1, MYC*	mRNA stability	AML, BC CML	upregulation	reviewed in[60]
TTP	mRNAs degradation via the exosome or via Xrn1 exonuclease	*VEGF, cytokines, c-IAP-2*	proapoptotic function in cancers	BC CML, DLBCL, acute phase ATL	loss-of-function, tumor suppressor	reviewed in [60]

Abbreviations used: Acute myeloid leukemia (AML); acute promyeloblastic leukemia (APL); acute megakaryoblastic leukemia (AMKL); acute lymphoblastic or lymphocytic (ALL); B-cell acute lymphoblastic leukemia (B-ALL); adult T-cell leukemia/lymphoma (ATL); diffuse large B-cell lymphomas (DLBCLs); chronic myeloid leukemia (CML), chronic phase (CP), accelerated phase (AP), blast crisis (BC); chronic lymphoblastic or lymphocytic leukemia (CLL); myelodysplastic syndromes (MDS); multiple myeloma (MM); hepatocellular carcinoma (HCC); leukemia stem cell (LSC); wild type (WT); patient-derived xenograft (PDX); bone marrow (BM); hematopoietic stem/progenitor cells (HSCs, HSPCs).

Table 5.

RBPs involved in nuclear transport and translation.

Gene	Protein/RNP function	Target Genes	Biological consequences	Type of cancer	Expression in cancer	Ref
XPO1/ CRM1	Exportin 1, ubiquitous nuclear export	protein export p53, NPM1, NFκB	anti-apoptotic properties	AML, ALL, CML, CLL, lymphoma, MM	upregulation	reviewed in[67]
eIF4E	cap-dependent mRNA nuclear export and translation	*CCND1*	delays granulocytic and monocytic differentiation, promotes leukemogenesis	AML, BC CML, ALL	upregulation	[70]
		MYC, BCL2, BCL6, B-cell receptor signaling, metabolism, and epigenetic regulation	promotes proliferation, aggressiveness	aggressive double- and triple-hit (DH/TH) DLBCL with active Hsp90 stress pathway		[71]

Abbreviations used: Acute myeloid leukemia (AML); acute promyeloblastic leukemia (APL); acute megakaryoblastic leukemia (AMKL); acute lymphoblastic or lymphocytic (ALL); B-cell acute lymphoblastic leukemia (B-ALL); adult T-cell leukemia/lymphoma (ATL); diffuse large B-cell lymphomas (DLBCLs); chronic myeloid leukemia (CML), chronic phase (CP), accelerated phase (AP), blast crisis (BC); chronic lymphoblastic or lymphocytic leukemia (CLL); myelodysplastic syndromes (MDS); multiple myeloma (MM); hepatocellular carcinoma (HCC); leukemia stem cell (LSC); wild type (WT); patient-derived xenograft (PDX); bone marrow (BM); hematopoietic stem/progenitor cells (HSCs, HSPCs).

Table 6.

Multifunctional oncofetal RNA-binding proteins

Gene	Protein/RNP function	Target Genes	Biological consequences	Type of cancer	Expression in cancer	Ref
STAU2	mRNA transport, localization, translation	*KDM1A,1B,5B Kras, Wnt, PTEN, KLF6, VHL*	chromatin reorganization, global histone methylation	mouse BCR-ABL, NUP98/ HOXA9 CML, human BC CML, AML relapse, AML LSCs	upregulation	52
MSI2	HSCs self-renewal, multilineage differentiation and engraftment	*Ikzf2, Hoxa9, Myc, Meis1*	promoted self-renewal, LSCs survival	AML, K562 CML	upregulation	76
		Tetraspanin 3 TSPAN3	CXCR4-mediated chemokine responses	mouse AML, BC CML primary human AML		78
		NUMB	Suppressed differentiation in BC CML	BC CML		79, 80
		BCAT1	reprogrammed cellular metabolism	CP CML, BC CML, *de novo* AML		81
LIN28 LIN28B	HSCs self-renewal, differentiation, ncRNA processing, mRNA stability and translation	*KRAS, c-MYC, HMGA2, let-7*	promotes proliferation	CP, AP and BC CML	upregulation	84
		miR-150	suppresses *miR-150* maturation, stimulates leukemogenesis	*MLL*-associated AML, 293T		85
LIN28A		*pre-B-cell lineage*	preleukemic state of highly invasive myeloid leukemia	*miR-125b*-driven mouse AML	Lin28A is suppressed by *miR-125b*	86
IGF2BP1	stemness, proliferation, metabolism, mRNA localization, cell adhesion	*ETV6/RUNX1*	survival, proliferation	B-ALL	upregulation	94
IGF2BP1,3		*HOXB4, MYB, ALDH1A1*	leukemia stem cell properties	K562 CML, HL60 AML, 697 B-ALL		99
IGF2BP2		*n/a*	poor prognosis	AML		98
IGF2BP3		*MYC, CDK6*	proliferation, survival, B-cell/ myeloid programing	*MLL*-rearranged B-ALL		95

Abbreviations used: Acute myeloid leukemia (AML); acute promyeloblastic leukemia (APL); acute megakaryoblastic leukemia (AMKL); acute lymphoblastic or lymphocytic (ALL); B-cell acute lymphoblastic leukemia (B-ALL); adult T-cell leukemia/lymphoma (ATL); diffuse large B-cell lymphomas (DLBCLs); chronic myeloid leukemia (CML), chronic phase (CP), accelerated phase (AP), blast crisis (BC); chronic lymphoblastic or lymphocytic leukemia (CLL); myelodysplastic syndromes (MDS); multiple myeloma (MM); hepatocellular carcinoma (HCC); leukemia stem cell (LSC); wild type (WT); patient-derived xenograft (PDX); bone marrow (BM); hematopoietic stem/progenitor cells (HSCs, HSPCs).

Table 7.

Small molecule inhibitors of RNA-binding and modifying proteins

Function	Gene Name	Inhibitor	CAS#	References
RNA editing	*ADAR1* *JAK2* *BCR-ABL*	8-azaadenosine SAR302503 Dasatinib	10299-44-2 936091-26-8 302962-49-8	[10]
RNA modification	*METTL3* *METTL14*	In development		reviewed in[15, 35]
	FTO	CS1 (Bisantrene) CS2 (Brequinar)	78186-34-2 96187-53-0	[29]
		FB23/FB23–2	2243736-45-8	[30]
RNA splicing	*SF3B1*	Spliceostatins A-G Pladienolides A(E7107) Herboxidiene (GEX1A)	391611-36-2 445493-23-2 142861-00-5	reviewed in[37, 45, 61]
	RBM39	E7820 Indisulam Tasisulam	289483-69-8 165668-41-7 519055-62-0	[50, 63]
	PRMT1 *(type I PRMTs)*	GSK3368715 MS023 (pan type I PRMTs inh.)	1629013-22-4 1831110-54-3	[64, 65]
	PRMT5	GSK3203591 (GSK591) GSK3326595	1616391-87-7 1616392-22-3	[64, 65]
Decapping	*DCPS*	RG3039	1005504-62-0	[51]
Nuclear export & translation	*XPO1/CRM1*	Selinexor	1393477-72-9	reviewed in[68]
	eIF4E	Ribavirin	36791-04-5	reviewed in[72]
Oncofetal RBPs	*MSI2*	Ro 08-2750 (Ro)	37854-59-4	[82]
	LIN28	C1632 ($C_{15}H_{15}N_5O$)	108825-65-6	[89]
		TPEN, LI71		[91]
	IGF2BP1	BTYNB	304456-62-0	[100]