The Biology of m^6^A RNA Methylation in Normal and Malignant Hematopoiesis

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**ABSTRACT**

Hematopoietic development and differentiation are highly regulated processes, and recent studies focusing on m^6^A mRNA methylation have uncovered how this mark controls cell fate in both normal and malignant hematopoietic states. In this review, we focus on how writers, readers, and erasers of RNA methylation can mediate distinct phenotypes on mRNAs and on cells. Targeting the RNA methylation program has emerged as a potential novel therapeutic strategy, and we explore the role for these regulators in both normal and dysregulated cell contexts.

**Significance:** RNA methylation is required for cancer cell survival in solid tumors and in acute myeloid leukemia, and targeting this pathway has been proposed as a new therapeutic strategy in cancer. However, understanding the role for RNA methylation in both normal and malignant states is essential for understanding the potential consequences for therapeutic intervention.

**INTRODUCTION**

It is well established that messenger RNA (mRNA) is not simply an intermediate molecule that carries the genetic information from DNA to protein. In fact, posttranscriptional regulation at the level of RNA via both *cis-* and *trans*-mechanisms is critical for control of gene-expression programs that dictate cellular function and cell-fate decisions. Although posttranscriptional modifications of RNAs have long been known to exist (1–3), they have only recently emerged as an additional regulatory layer contributing to the complexity of gene-expression regulation. To date, more than 150 chemical modifications have been described for RNA (4). Although a majority of these modifications are mapped to noncoding RNAs, such as transfer RNA (tRNA) and ribosomal RNA (rRNA), mRNA is characterized by several modifications including N^6^-methyladenosine (m^6^A), N^1^-methyladenosine (m^1^A), 2'-O-methylation (m^6^Am), and 5-methylcytidine (m^5^C; refs. 5, 6). Among them, m^6^A is the most prevalent posttranscriptional modification of eukaryotic mRNAs and long noncoding RNAs (7, 8). RNA methylation on a transcript was first described back in 1974 following the discovery of poly(A) sequence at the 3'-end of an mRNA that allowed scientists to use radioactive labeled methyl nucleosides to track methylated mRNAs (1–3). However, the m^6^A RNA methylation field remained at this early stage of characterization until the recent discovery of fat mass and obesity-associated protein (FTO; ref. 9), and subsequently ALKBH5 as m^6^A demethylases (10). These exciting studies suggested that this modification can directly control gene expression. At the same time, the development of several methods incorporating next-generation sequencing techniques, e.g., m^6^A sequencing (meRIP-seq; refs. 11, 12) and single-nucleotide mapping of m^6^A (miCLIP-seq; ref. 13), enabled profiling of m^6^A modifications on specific mRNA throughout the transcriptome. Since then, the field has received enormous interest, and the term “epitranscriptomes” has been coined to attribute the importance of such regulations in comparison with the field of epigenetics (14). Similar to DNA methylation and histone modifications, the m^6^A marks on mRNAs are reversibly and dynamically regulated by “writers” and “erasers.” m^6^A is also “read” by a group of “reader” proteins that mediate recruitment of downstream functional complexes, which in turn control the ultimate fate of an mRNA. The multilayer and intricate control of m^6^A allows a wealth of regulatory modes to modulate and mediate its function.

A number of proteins have been identified in the m^6^A “writer” complex, including METTL3, METTL14, WTAP, RBM15, KIAA1429, and ZC3H13. Among them, METTL3 is the S-adenosylmethionine (SAM) binding subunit and was the first identified component of the complex (15–17). Genetic depletion of METTL3 in plants, yeast, and mammalian cells results in complete or near-complete loss of m^6^A in polyadenylated RNA, suggesting that METTL3 is the key m^6^A methyltransferase (18–20). Other members in the complex...
do not possess catalytic potential but play important roles in stabilizing the assembly as well as recruiting and localizing it to target mRNAs and specific subcellular locations. METTL14 functions as an adaptor forming a stable dimer with METTL3 in vivo (21–23). This heterodimer is essential for the catalytic activity of the functional complex. WTAP is a binding partner of METTL3–METTL14, which helps in bringing METTL3–METTL14 to nuclear speckles (20, 21, 24, 25). RBM15 and RBM15B act as mediators by binding to WTAP and assisting the complex to recognize particular binding sites on mRNA (26). KIAA1429, also named Virilizer (VIRMA), has been shown to interact with WTAP and is required for m^6^A formation (25, 27, 28). ZC3H13, the ortholog of Xio protein in *Drosophila*, was recently identified as an additional component, interacting with WTAP, KIAA1429, and RBM15 to anchor the complex in the nucleus (29–31).

To date, FTO and ALKBH5, members of the AlkB-alpha-ketoglutarate-dependent dioxygenase, are the only two identified “erasers” that can reverse m^6^A to A (9, 10). Knockdown of either FTO or ALKBH5 increases the global m^6^A level in human cells. It is noted that FTO has been shown to also have strong catalytic activities toward m^6^A_m^6^A at the 5’ cap (32) and m^A_A in tRNAs (33). As FTO targets multiple RNA substrates, it is critical to dissect the contribution of each modification to the overall functional requirement of FTO in different tissues and biological systems. To date, ALKBH5 remains the only specific m^6^A demethylase *in vivo*. Future studies might identify new m^6^A demethylase enzymes and uncover alternative mechanisms that can mediate the removal of m^6^A modifications and clearance of m^6^A marked transcripts.

m^6^A can be recognized by a number of m^6^A-binding proteins, which are responsible for exerting diverse effects of m^6^A on gene expression such as mRNA stability (34–36), mRNA splicing (37, 38), mRNA structure (39, 40), mRNA export (41), translation efficiency (42–47), and mRNA biogenesis (48). These effectors are called m^6^A “readers.” A major group of m^6^A readers is the YTH domain containing protein family, including YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 (11, 35, 49–51). Other RNA binding proteins have also been suggested to recognize m^6^A, including eIF3, HNRNP2B1, and ELAVL (43, 48, 60). Additionally, a distinct group of proteins such as HNRNPC (58) and HNRNPG (59) are considered “indirect” readers of m^6^A due to their ability to preferentially bind to an RNA structural “switch” induced by m^6^A modifications. Thus, RNA methylation is dynamically controlled by writers, readers, and erasers and potentially other proteins that can influence these regulators (Fig. 1).

Although it is recognized that RNA methylation plays a critical role in gene-expression regulation (61) and numerous cellular processes (44, 62, 63), how RNA methylation controls cell-fate decisions in diverse physiologic settings is not well understood. A recent wave of studies uncovering the role of RNA methylation in the hematopoietic system has provided critical insights into the requirement and regulation of RNA methylation during normal development and malignant transformation. The hematopoietic system has long been recognized as an ideal biological system to study stem cell biology and lineage specification. Numerous discoveries previously made in the hematopoietic field have been recapitulated in other tissue systems. In this review, we summarize these findings and discuss current perceptions as well as therapeutic implications for the RNA methylation regulatory pathways in hematopoietic malignancies.

THE ROLE OF m^6^A IN NORMAL HEMATOPOIESIS

Hematopoietic development is a delicately orchestrated process that results in maturation of hematopoietic stem and progenitor cells into a variety of terminal differentiated functional cells of the blood system (64). During development, there are multiple waves of hematopoiesis occurring in distinct spatial and temporal orders. The first wave of hematopoiesis—the primitive hematopoiesis—happens in the mammalian yolk sac to produce red blood cells and primitive megakaryocytes. The next wave of hematopoiesis—the definitive hematopoiesis—starts in the aorta-gonad mesonephros (AGM) region and placenta and subsequently occurs in fetal liver, thymus, spleen, and ultimately the bone marrow in adult mammals, where all cell lineages are generated (65).

m^6^A modifications of mRNAs have been implicated in several alternative mechanisms that can mediate the removal of m^6^A modifications and clearance of m^6^A marked transcripts.
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Figure 1. N^6-Methyladenosine (m^6A) is regulated by writers, erasers, and readers and plays a broad role in RNA processing. To date, six components of the writer complex have been identified, i.e., METTL3, METTL14, WTAP, RBM15, KIAA1429, and ZC3H13. The write complex catalyzes conversion of adenine (A) to m^6A. FTO and ALKBH5 are two erasers that function as demethylases, removing the methyl group from m^6A. Readers include a diverse group of proteins, including the YTH domain family (YTHDFs and YTHDCs), IGF2BP1/2/3 family, and other factors, i.e., eIF3, HNRNP2AB1, HNRNPC, and HNRNPG. These readers can directly bind to m^6A or are indirectly recruited to the sites to mediate downstream processes including mRNA export, mRNA stability, mRNA splicing, mRNA translation, and mRNA decay.

signaling represses HSPC formation during EHT, whereas inhibition of Notch1 activity reverses the block in EHT, allowing endothelial cells to transit into HSPCs (65). YTHDF2 was identified as the reader that mediates m^6A-dependent regulation of notch1a mRNA expression. Loss of YTHDF2 phenocopies the defects observed in METTL3-deficient embryos. Overexpression of wild-type but not ythdf2 that cannot bind m^6A rescues depletion of endogenous ythdf2, indicating that YTHDF2 function depends on its ability to recognize m^6A marked transcripts. Global profiling of m^6A and transcriptomic analysis upon ythdf2 knockout confirmed more than 70% of YTHDF2 target transcripts with m^6A sites and a large number of overlapping target genes with METTL3, including notch1a. The requirement of the regulatory axis of METTL3-m^6A notch1a-YTHDF2 for specification of early HSPCs is also observed in mouse. siRNA-mediated knockdown of METTL3 expression in isolated AGM reduced colony formation (66). Reduction of METTL3 in vascular endothelial cells using the vascular specific Veg (Cdh5)-Cre Mettl3^fl/lox conditional KO mouse impairs generation and function of HSPCs. Interestingly, depletion of METTL3 at late steps of definitive hematopoiesis in Vav-Cre Mettl3^fl/lox conditional KO mice does not affect HSPCs, indicating that the requirement for METTL3 function is restricted for EHT (67). These studies established the central role for m^6A and METTL3 during specification of the hematopoietic lineage during development.

In humans, reduction of m^6A in cord blood-derived CD34^+ HSPCs (CB-HSPC) upon METTL3 depletion results in decreased proliferation and increased myeloid differentiation in an in vitro myeloid-promoting culture condition (69). Conversely, overexpression of METTL3 but not the catalytic dead mutant METTL3-CD in CB-HSPCs promotes cell proliferation and blocks myeloid differentiation. The phenotypes are attributed to modulation of m^6A levels in CB-HSPCs. An acceleration in myeloid differentiation was also observed upon METTL14 silencing in human CB-HSPCs (70). Thus, m^6A and its regulators are implicated in the maintenance of myeloid differentiation in human HSPCs.

In contrast to studies of m^6A during early development, its role in adult hematopoiesis is not well defined. Functionally, requirement of m^6A in adult hematopoietic cells was systematically evaluated only in T cells. Genetic deletion of METTL3...
in CD4-Cre conditional Mettl3ΔΔ mouse indicated that m6A controls T-cell homeostasis (69). Upon ablation of METTL3 (and METTL14) expression, m6A is markedly reduced in T cells. Interestingly, although there is no significant effect observed regarding differentiation and cellularity in the thymus, there is a substantial increase in naïve T cells in the spleen and lymph nodes of Mettl3-KO mice. However, Mettl3-depleted naïve T cells lose their ability to proliferate, expand, and differentiate to effector cells when transferred to Rag2−/− host lymphoid tissues. The effects are due to repression of IL7 signaling upon upregulation of the IL7-negative regulators Socs1, Socs3, and Cish in Mettl3-KO T cells. It is noted that in naïve T cells, loss of Mettl3 increases basal ERK and AKT signaling, allowing these cells to maintain their survival without activation of the IL7 pathway. Additionally, exposure of T cells to IL7 stimulation induces degradation of Socs1, Socs3, and Cish mRNA in a METTL3-dependent manner. These data suggest that there is a negative regulatory loop consisting of IL7, m6A –METTL3, and a group of stimulus-response genes, critical for maintaining T-cell function.

The requirement for m6A in stem-cell function and maintenance has been implied by several studies. High expression of m6A “writers” Mettl3 and Mettl14 mRNAs has been observed in the stem/progenitor compartments in comparison with differentiated cells in the mouse bone marrow (70, 71), suggesting a potential role of m6A in controlling HSPCs. It has been shown that loss of METTL14 reduces the ability of donor bone marrow cells to competitively engraft in recipient mice (70). However, it is not clear whether the defect is in any specific lineage or if the defect is relevant across all immature cell types. Additionally, whether the reduction in chimerism is due to impairment in cell proliferation, survival, or self-renewal of HSPCs is not clear. On the other hand, CRISPR/Cas9-mediated targeting of Mettl3 at the catalytic domain does not show any effect in the ability of mouse Lin-negative bone marrow cells to form colonies and replate in vitro (72). Although validation of Cas9-guide RNA-mediated repression of METTL3 catalytic activity and expression is required for interpretation of the observation, the result suggests an m6A-independent function for the “writers” in mouse hematopoiesis. In the Mettl3 and Mettl14 Mx1-Cre–mediated conditional KO mouse models, deletion of Mettl3 (Mettl3ΔΔ) and Mettl14 (Mettl14ΔΔ) both decreased global m6A levels in adult bone marrow cells, albeit loss of Mettl3 conferred a more severe reduction (73). Notably, only METTL3, not METTL14, depletion resulted in increased hematopoietic stem cell (HSC) frequency. Similar to the previous report, Mettl14ΔΔ bone marrow cells exhibited a mild decrease in engraftment ability in recipient animals at 4 months after transplantation. On the other hand, there was very little engraftment from Mettl3ΔΔ donor cells, indicating a central requirement of METTL3 for hematopoietic stem cell function. Staining with BrdUrd captured an increase in cycling HSCs in Mettl3ΔΔ mice, suggesting that METTL3 could be important to control quiescence of HSCs. It is unclear whether loss of METTL3 has any other effects on downstream hematopoietic progenitor cells in various lineages.

One major question that remains is how METTL3 exerts such critical regulation in HSCs and whether it is m6A dependent. It is also important to further dissect the differential requirements for METTL3 and METTL14 in HSCs and HSPCs. This could help resolve the m6A-dependent and -independent function of these regulatory factors. Interestingly, Rbm15 conditional KO mice were also characterized by an increase in long-term HSCs as the result of a block in the transition from long-term to short-term HSCs (74). However, engraftment of Rbm15 KO HSCs is severely reduced due to their inability to interact with bone marrow niches. Furthermore, normal megakaryocytic development is disrupted in the absence of RBM15. Although studies have provided detailed analysis of RBM15 loss of function in adult hematopoiesis, these phenotypes have not been directly linked with alterations in RNA methylation. Similarly, deletion of YTHDF2 in adult HSCs resulted in the expansion of HSCs that were functionally normal in their engraftment potential (75). Additionally, YTHDF2 negatively regulates m6A marked hematopoietic transcription factors, including TAL1, GATA2, RUNX1, and STAT5, by regulating their mRNA decay (75). Furthermore, depletion of YTHDF2 in human cord blood results in HSC expansion (75). Despite the emerging evidence for regulatory roles of m6A in cell-fate determination in the hematopoietic hierarchy, further studies are needed to clarify the requirement for m6A modification and the molecular mechanisms for specification of diverse cell types.

THE ROLE OF m6A IN MALIGNANT HEMATOPOIESIS

Malignant hematopoiesis is characterized by the abnormal development of blood cells accompanying a block in differentiation, abnormal self-renewal, and/or aberrant proliferation of hematopoietic cells. The deregulation of hematopoiesis can affect both lymphoid and myeloid lineages. Myeloid malignancies are clonal diseases comprised of chronic myeloid diseases including myelodysplastic syndromes, myeloproliferative neoplasms (MPN), chronic myeloid leukemia, chronic myelomonocytic leukemia (CMML), and acute myeloid leukemia (AML). In myeloid malignancies, the activities of genes involved in key processes of self-renewal, differentiation, proliferation, and survival/apoptosis are perturbed (76). Given the critical roles of m6A in regulating HSC maintenance and myeloid differentiation, it is not surprising that m6A modifications play an important role in leukemia. Currently, m6A “writer” and “eraser” complexes have been characterized only in AML.

The first study directly addressing m6A roles in myeloid leukemia reported the demethylase FTO to play an oncogenic role in AML (77). FTO is highly expressed in several subtypes of AML. FTO mRNA is specifically upregulated in AML with MLL rearrangements in comparison with normal controls and non–MLL-rearranged AML. Overexpression of FTO but not the enzymatically dead mutant of FTO decreases global m6A levels, promotes cell growth and colony replating in vitro, and accelerates leukaemogenesis in vivo. Conversely, depletion of FTO by shRNAs or heterozygous Fto KO mice increased m6A levels, induced apoptosis, and delayed leukemia development in recipient animals. Mechanistically, ASB2 and RARA were identified as two main functional downstream targets. Given the role of ASB2/RARA in the ATRA pathway, the results implicate an essential function for FTO in mediating leukemia cells’ response to ATRA. It is noted that ASB2 and RARA mRNAs show decreased m6A levels due to the high expression of FTO, and their mRNA stability and expression...
is negatively regulated by FTO. Surprisingly, despite the global reduction in m^6^A levels in FTO-overexpressing cells (vs. control), m^6^AIP-seq profiling of m^6^A transcripts demonstrated that both increased and reduced number of m^6^A peaks were equivalent across the genome. Although previous studies found that m^6^A depletion increases mRNA stability and abundance, FTO overexpression reduced m^6^A and mRNA abundance compared with the control. Similarly, FTO depletion resulted in 85% of target mRNAs gaining m^6^A and increasing their mRNA abundance. It remains possible that these sites are not directly regulated by FTO and thus could explain this apparent discrepancy for increased m^6^A and increased mRNA stability. Another possible explanation comes from an independent study that uncovered FTO as the major demethylation enzyme that preferentially controls another RNA methylation modification—the m^6^Am (32). Although m^6^A labels an mRNA for degradation, m^6^Am at the increased m^6^A in diverse leukemia subtypes. Further studies are needed to resolve the role of FTO on both m^6^A and m^6^Am in myeloid leukemia.

As an α-ketoglutarate (α-KG)-dependent dioxygenase, FTO enzymatic activity can be competitively inhibited by the structurally related metabolite D-2-hydroxyglutarate (D-2HG), which aberrantly accumulates in isocitrate dehydrogenase 1 or 2 (IDH1/2)-mutant AMLs. It has been shown that in a number of IDH1/2-mutant AML patient samples, the global m^6^A is increased due to inhibition of FTO activity without any change in FTO expression (78). Thereby, in certain cases of AML, in particular AML with mutant IDH1/2, inhibition of FTO function appears to be favorable for leukemia cells. Another study found that addition of R-2-hydroxyglutarate (R-2HG), a metabolite highly produced by mutant IDH1/2, recapitulates activities of IDH mutations to promote proliferation and block differentiation of leukemia cells (79). The results indicate that R-2HG is the key effector of IDH mutations in oncogenesis. On the other hand, in certain AMLs that lack these IDH1/2 mutations, the R-2HG exhibits antileukemic activity (80). A high level of R-2HG reduces cell proliferation and viability in vitro and delays leukemogenesis in vivo (80). As expected, it was found that R-2HG binds and directly inhibits FTO enzyme activity and then leads to increases in global m^6^A levels in leukemia cells. Genome-wide profiling of transcriptome m^6^A and gene expression by RNA sequencing (RNA-seq) identified MYC as one of the top target genes of R-2HG and FTO. It was then found to maintain MYC mRNA stability and efficient translation. R-2HG treatment also reduces CEBPA expression, which in turn attenuates FTO transcription. Thus, R-2HG forms a regulatory loop with FTO/m^6^A/MYC/CEBPA to control leukemia cell survival in non-IDH-mutant lines. Overall, these studies suggest a differential requirement for FTO function and the effect of increased m^6^A in diverse leukemia subtypes. Further studies are needed to elucidate these context-dependent roles for the “m^6^A eraser” FTO in leukemia.

Although FTO’s requirement in leukemia is seemingly context-dependent, the “m^6^A writers” in myeloid malignancies have been found to be essential. Members of the m^6^A writer complex are expressed highly in AML compared with other cancer types, particularly METTL3 (69) and RBM15, exhibiting the highest expression in AML based on The Cancer Genome Atlas database (81, 82). More importantly, a genome-wide CRISPR-based screen to identify genes essential for cell survival using 14 distinct AML cell lines scored all members of the “writer” complex, including METTL3, METTL14, WTAP, and KIAA1429, among the top 10% in the essential gene list (83). m^6^A eraser ALKBH5 was not required for the survival of leukemia cell lines, and FTO was not depleted except in Mono Mac1 and EOL-1 cells. Similar observations were made in a genome-wide screen using primary mouse leukemia cells (72). These data indicate the role of the entire m^6^A writer but not the eraser complexes in most leukemia cell lines.

METTL3 is more abundant in AML cell lines and primary AML patient samples in comparison with normal CD34^+ HSPCs. Two independent studies on METTL3 demonstrated a requirement for METTL3 and regulation of m^6^A in myeloid leukemia development (69, 72). This is consistent with the function of METTL3 in attenuating normal differentiation of myeloid cells. Increased abundance of METTL3 correlates with high global m^6^A level in primary AML cells. In myeloid leukemia cell lines, loss of METTL3 significantly reduced m^6^A levels, inhibited cell growth, and induced differentiation and apoptosis. Ablation of METTL3 expression in leukemia cells delayed onset of disease in mice. In another study, METTL3 and its catalytic activity was found to be essential in AML through two CRISPR screens: genome-wide dropout screens and a domain-targeted screen for RNA modifying enzymes using MLL-AF9 FLT3 ITD mouse leukemia cells (73). Depletion of METTL3 results in cell-cycle arrest, differentiation of leukemic cells, and delayed leukemogenesis in vivo. Profiling of methylated mRNAs in leukemia cells using single-nucleotide resolution mapping of m^6^A (mCLIP) coupled with genome-wide characterization of transcriptional and translational landscapes by RNA-seq, ribosome profiling, and reverse phase protein array (RPPA) of METTL3-depleted leukemia cells uncovered key molecular pathways regulated by m^6^A. Although METTL3 depletion increased abundance of m^6^A marked transcripts, ribosome profiling revealed that transcripts with m^6^A sites have reduced translation efficiency in leukemia cells. m^6^A targets are largely enriched in gene sets critical in stem cells. METTL3-depleted cells exhibit activated PI3K/AKT and apoptotic pathways. The phenotypes are at least in part due to METTL3 regulation of translation of MYC, BCL2, and PTEN, which are central regulators of cell survival and differentiation. Inhibition of PI3K/AKT signaling partially reverses induction of differentiation in METTL3 knockdown cells (69). Interestingly, in the other study, METTL3 was found to bind to chromatin (by chromatin immunoprecipitation sequencing) and is recruited to transcriptional start sites of a set of target genes highly enriched for CAATT-box binding protein CEBPZ binding motifs. Presence of CEBPZ is required for localizing METTL3 to those targets. However, METTL3 depletion does not reduce transcriptional activity of these transcripts. Instead, promoter-bound METTL3 marks coding regions of target genes, i.e., SPI1 and SP2, with m^6^A, which
in turn controls their translational efficiency. To determine whether binding of METTL3 to promoter regions is required for its regulation, METTL3 was fused with GAL4 to be tethered to an upstream GAL4 binding region of a promoter that drives expression of m^A target luciferase. Expression of the fusion protein with METTL3 wild-type but not catalytic dead METTL3 enhances luciferase activity, indicating that only promoter-bound METTL3 with enzymatic activity can modulate translation of the transcripts in an m^A-dependent manner (72). This provides evidence that RNA methylation is directly linked to the transcriptional program and to the translational control of these key target genes.

Other “writer” proteins have also been implicated in myeloid leukemia. METTL14 was reported to promote leukemogenesis (70). Depletion of METTL14 recapitulates the effects of loss of METTL3 function in both human and mouse leukemia cells. Transcriptome analysis using human AML cells identified MYC and MYB as METTL14 and m^A targets. Expression of MYC and MYB is dramatically decreased upon overexpression of MYC or MYB can partially rescue differentiation phenotypes in METTL14-depleted cells. WTAP was also identified as an oncogene in AML (84). Knockdown of WTAP in an AML cell line reduces proliferation and increases myeloid differentiation and apoptosis. RBM15–MKL1 (also named OTT–MAL) fusion protein frequently occurs in pediatric acute megakaryoblastic leukemia (AMKL; refs. 85–87). In the translocation t(1,22) in AMKL, RBM15–MKL1 (also named OTT–MAL) gene is fused to an almost complete MKL1 gene, producing an RBM15–MKL1 chimeric protein of 1,883 amino acids. In the RBM15–MKL1 conditional knockin mouse model, RBM15–MKL1 expression is sufficient to cause development of AMKL with a low penetrance and long latency. The diseases found in the sick animals are characterized with many features observed in human AMKL. In addition, RBM15–MKL1 can cooperate with thrombopoietin receptor myelo-proliferative leukemia virus oncogene (MPL) to induce and accelerate development of AMKL. The effects are attributed to activation of the Notch signaling pathway (88). However, it is unclear whether RBM15–MKL1 affects RNA methylation and how the effects contribute to the pathogenesis of AMKL. Overall, these studies strongly support the critical requirement for function of the m^A “writer” complex and the RNA methylation program in myeloid leukemia.

TARGETING RNA METHYLATION AS A THERAPEUTIC VULNERABILITY IN LEUKEMIA

Given the central role of m^A pathways in leukemia, targeting the RNA modification pathway offers a promising avenue to develop innovative treatments against the disease. High expression of multiple subunits of the “writer” complex allows for a therapeutic window for the killing of leukemia cells while sparing normal blood cells. Among these subunits, METTL3 is the most attractive candidate to modulate RNA methylation, as depletion of METTL3 resulted in the greatest reduction in m^A levels. The detailed crystal structure of METTL3 in complex with METTL14 and mRNA (89) provides a basis to identify small molecules that can inhibit METTL3 enzymatic activity as well as interfere with its interaction with the functional partner METTL14. Understanding the structure of the writer complex provides a theoretical basis for developing inhibitors as for other SAM-dependent protein methyltransferases (90, 91). Ongoing efforts toward identifying highly potent and specific inhibitors for METTL3 will be critical for development of therapeutic strategies for AML and other diseases. As RNAi therapy is making progress in clinical settings (92), it could become feasible to directly suppress expression of the m^A writers to induce differentiation and apoptosis in leukemia cells.

Alternatively, interfering with the recruitment of the RNA methylation complex to mRNA targets could also abrogate the RNA methylation program. Given the possibility to intervene with protein–mRNA interactions (93, 94), targeting binding of the RNA binding subunits of the complex, i.e., RBM15, to mRNAs may represent another strategy. Targeting the m^A reader family may provide another exciting avenue for therapeutic intervention, which may control a subset of modified mRNAs. However, the effect of modulating m^A readers in either normal or malignant hematopoiesis has not been established. It is also important to prove that interference with their function would result in suppression of relevant downstream pathways.

Although the requirement for “eraser” activity might be context-dependent, increasing m^A levels could be beneficial in certain leukemia subtypes such as AML with IDH1/2 mutations (70). Because ALKBH5 and FTO belong to the 2-oxoglutarate and iron-dependent oxygenases, they are pharmacologically sensitive to pan-inhibitors of these enzymes such as ZOG competitors, e.g., 2-HG, succinate, and metal chelators, e.g., flavonoids. On the other hand, significant efforts have been devoted to development of selective RNA demethylase inhibitors. The first FTO inhibitor, a natural compound Rhein, was identified using virtual screening with the crystal structure of FTO in complex with N^3-methylthymidine (m3T) and validated in vivo (71). To increase m^A levels, Rhein also demonstrated inhibition toward other demethylases, including ALKBHs and Jumonji-domain containing 2 histone demethylases (JMJD2). The next generation of more selective FTO inhibitors was developed by exploiting differences in the nucleotide binding sites between FTO and other members of the ALKB family (96). An active compound was shown to exhibit more than 100-fold selectivity for FTO over ALKBH5 at a low micromolar range in binding assays. Although these compounds were demonstrated to increase global m^A levels and elicit antitumor effects in cells, the pharmacologic activities were not validated in vivo. Assessment of their potency, selectivity, and toxicity in vivo is required to develop efficacious inhibitors for clinical applications.

CONCLUSIONS AND PERSPECTIVES

In summary, recent studies have positioned RNA methylation as a major pathway in modulating the development and regulation of normal and malignant hematopoiesis (Fig. 2). What remains to be established is the detailed and precise
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**Figure 2.** m6A is essential in normal hematopoiesis and leukemia development. A, During embryogenesis, Notch1α is deposited with m6A and targeted by YTHDF2 for decay in zebrafish and mouse. Reduction of Notch1α allows for HSC formation. B, In adult hematopoiesis, loss of METTL3 results in expansion of defective HSCs. Depletion of YTHDF2 also results in HSC expansion, but HSC function is preserved. In lineage differentiation of T cells, decreased m6A of SOCS in METTL3-depleted T cells promotes mRNA stability and represses normal T-cell differentiation. METTL3 depletion in primary human CD34+ cells induces the myeloid differentiation. C, In human leukemia cells, high expression of METTL3 and METTL14 increases the global m6A level. RNA methylation of defective HSCs. Depletion of YTHDF2 also results in HSC expansion, but HSC function is preserved. In lineage differentiation of T cells, decreased m6A of SOCS in METTL3-depleted T cells promotes mRNA stability and represses normal T-cell differentiation. METTL3 depletion in primary human CD34+ cells induces the myeloid differentiation.

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