REVIEW ARTICLE

Blood Science

OPEN

Epigenetic modifications in hematopoietic ecosystem: a key tuner from homeostasis to acute myeloid leukemia

Shuxin Yao[a,b,c], Rongxia Guo[d], Wen Tian[a,b,c], Yanbing Zheng[c], Jin Hu[c], Guoqiang Han[b,c], Rong Yin[b,c], Fuling Zhou[b,*], Haojian Zhang[a,b,c,e,*]

[a]State Key Laboratory of Oral & Maxillofacial Reconstruction and Regeneration, Key Laboratory of Oral Biomedicine Ministry of Education, Hubei Key Laboratory of Stomatology, School & Hospital of Stomatology, Wuhan University, Wuhan, China; [b]Department of Hematology, Zhongnan Hospital, Medical Research Institute, Wuhan University, Wuhan, China; [c]Frontier Science Center for Immunology and Metabolism, Medical Research Institute, Wuhan University, Wuhan, China; [d]Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan, China; [e]Taikang Center for Life and Medical Sciences, Wuhan University, Wuhan, China

Abstract

Hematopoietic stem cells (HSCs) maintain homeostasis in the hematopoietic ecosystem, which is tightly regulated at multiple layers. Acute myeloid leukemia (AML) is a severe hematologic malignancy driven by genetic and epigenetic changes that lead to the transformation of leukemia stem cells (LSCs). Since somatic mutations in DNA methylation-related genes frequently occur in AML, DNA methylation is widely altered and functions as a starting engine for initiating AML. Additionally, RNA modifications, especially N^6-methyladenosine (m^6A), also play an important role in the generation and maintenance of the hematopoietic ecosystem, and AML development requires reprogramming of m^6A modifications to facilitate cells with hallmarks of cancer. Given the complex pathogenesis and poor prognosis of AML, it is important to fully understand its pathogenesis. Here, we mainly focus on DNA methylation and RNA m^6A modification in hematopoiesis and AML and summarize recent advances in this field.

Key Words: Acute myeloid leukemia; DNA methylation; Hematopoiesis; Hematopoietic stem cells; Leukemia stem cells; RNA modification

1. INTRODUCTION

During the entire lifespan, hematopoietic stem cells (HSCs) maintain homeostasis of the hematopoietic ecosystem through self-renewal and differentiation into all lineages of blood and immune cells.[1] These processes are tightly controlled by a series of regulatory mechanisms in multiple layers, including metabolism, protein synthesis, and epigenetic regulation.[2–4] These mechanisms function as HSC quality-control systems to maintain the hematopoietic ecosystem; dysregulation of these quality-control systems interrupts homeostasis of the hematopoietic ecosystem, which subsequently leads to abnormal hematopoiesis and blood diseases, such as leukemia and myelodysplastic syndrome.

Genetic and epigenetic changes drive the development of many hematological malignancies. Acute myeloid leukemia (AML) is a fatal disorder characterized by abnormal expansion and arrested differentiation ability of myeloid progenitor cells[5]; AML development origins from leukemia stem cells (LSCs).[6] Currently, chemotherapy remains the standard treatment for most newly diagnosed patients with AML, and the 5-year overall survival rate remains lower (~30%), as many AML patients eventually relapse after reaching remission or directly fail to achieve remission after primary therapy and unfortunately develop into refractory AML.[7] Thus, a comprehensive understanding of AML pathogenesis remains an important task in this field.

Chemical modification is a highly specific and efficient way to regulate the functions of biological macromolecules (eg, DNA, RNA, and proteins).[8] DNA methylation is one of the major types of epigenetic modifications that refers to changes in regulating gene expression without affecting the genetic sequence.[9]

*Address correspondence: Haojian Zhang, Frontier Science Center for Immunology and Metabolism, Medical Research Institute, Wuhan University, No.185, East Lake Road, Wuchang District, Wuhan, Hubei 430071, China. E-mail address: haojian_zhang@whu.edu.cn (H. Zhang); Fuling Zhou, Department of Hematology, Zhongnan Hospital, Wuhan University, No.185, East Lake Road, Wuchang District, Wuhan, Hubei 430071, China. E-mail address: zhoufuling@whu.edu.cn (F. Zhou).

Conflict of interest: The authors declare that they have no conflict of interest.

S.Y., R.G., W.T. contributed equally to this work.

This work is supported by the grants to H.Z. from the National Key R&D Program of China (2022YFA0103200), and the National Natural Science Foundation of China (82325003, 82230007). This work is supported by the grants to R.G. from the Fundamental Research Funds for the Central Universities (2042023kf0057) and the National Natural Science Foundation (NSFC) (82301997). This work is also supported by the grants to R.Y. from the National Natural Science Foundation of China (82200188) and the Special Fund of China Postdoctoral Science Foundation (2022TQ0238). This work is also supported by the grants to H.Z. from the Fundamental Research Funds for the Central Universities (2042022dx0003 and 2042024kf1022).

Blood Science (2024) 6, 1–10:e00206.

Received May 12, 2024; Accepted August 20, 2024.

http://dx.doi.org/10.1097/BS9.0000000000000206

Copyright © 2024 The Authors. Published by Wolters Kluwer Health Inc., on behalf of the Chinese Medical Association (CMA) and Institute of Hematology, Chinese Academy of Medical Sciences & Peking Union Medical College (IHCAMS). This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

RNA can also be modified using more than 170 different chemical modifications.[10] Many studies have clearly demonstrated the implication of these nucleic acid modifications in the hematopoietic ecosystem,[2] and alterations in these modifications could disrupt gene expression and cause the development of hematologic malignancies, especially AML.[11] In this review, we mainly focus on DNA methylation and RNA N^6-methyladenosine (m^6A) modification in hematopoiesis and AML and summarize recent advances in this field.

2. DNA METHYLATION

DNA methylation was first discovered in bacteria in the 1920s and has been investigated in a vast range of organisms.[12] DNA methylation occurs at the cytosine 5 carbon position of the genomic cytosines preceding guanines (CpG) dinucleotide by the incorporation of a methyl group via a covalent bond.[13] The reaction is catalyzed by DNA methyltransferases (DNMTs).[14] Among the DNMT family members, DNMT1 is mainly responsible for maintaining methylation patterns, whereas DNMT3A and DNMT3B are responsible for adding new methylation patterns (**Fig. 1**). However, the other two family members, DNMT2 and DNMT3L, do not exhibit catalytic activity. DNMT3L interacts with and stabilizes DNMT3A to facilitate DNA methylation.[15,16] DNMT2 is an RNA methyltransferase that functions as a catalyst for the methylation of aspartate cytosine sites on transfer RNAs (tRNAs).[17] DNA demethylation is catalyzed by DNA demethylases, in which methylated bases are removed in the presence of DNA glycosidases. This is equivalent to repairing damaged DNA catalyzed by glycosidases and base-free nuclease cleavage couplings. So far, active DNA demethylases include ten-eleven translocation (TET) methylcytosine dioxygenase, activation-induced cytidine deaminase/apolipoprotein B microRNA (mRNA) editing enzyme complex (AID/APOBEC), and thymine DNA glycosylase (TDG)[18–20] (**Fig. 1**). In addition, methylated CpG-binding proteins, such as MBD2, have demethylase activity.[21–24]

2.1. DNA methylation maintains HSC function via the distinct roles of DNMTs in establishing and maintaining methylation patterns

The spatiotemporal dynamics of DNA methylation and its synergistic action with transcription factors are essential for maintaining the HSC state and the hematopoietic ecosystem. First, the functional programs of HSCs are governed by gradual differences in methylation levels, and constitutive methylation is essential for HSC self-renewal, but dispensable for cell cycle control, homing, and suppression of apoptosis. For instance, conditional deletion of DNMT1 in the hematopoietic system severely reduces the genomic methylation levels of HSCs and impairs HSC self-renewal, niche retention, and differentiation potential.[25] Secondly, DNMT1 regulates distinct patterns of methylation and expression of discrete gene families in HSCs and progenitors. HSC with reduced DNMT1 activity can differentiate into myeloerythroid but not lymphoid progeny,[26] while DNMT1 deficiency in myeloid progenitor cells enhances cell cycling and causes inappropriate expression of mature lineage genes.[25]

DNMT3A and DNMT3B are de novo DNMTs responsible for establishing DNA methylation patterns. Loss of Dnmt3a results in HSC expansion and impaired differentiation.[27] Although the predominant Dnmt3b isoform in adult HSCs is catalytically inactive, its residual activity in Dnmt3a-null HSCs can drive their differentiation and generates the paradoxical hypermethylation of CpG islands. Thus, the combined loss of Dnmt3a and Dnmt3b is synergistic, resulting in enhanced HSC self-renewal and a more severe block in differentiation than in Dnmt3a-null cells,[28] indicating that DNMT3A and DNMT3B have both overlapping and distinct functions in HSCs.

Interestingly, the TET family enzymes remove DNA methylation by oxidizing 5mC to 5hmC, which is also essential for hematopoietic homeostasis.[29] TET2 is abundantly expressed in HSCs. Tet2 deficiency leads to decreased genomic levels of

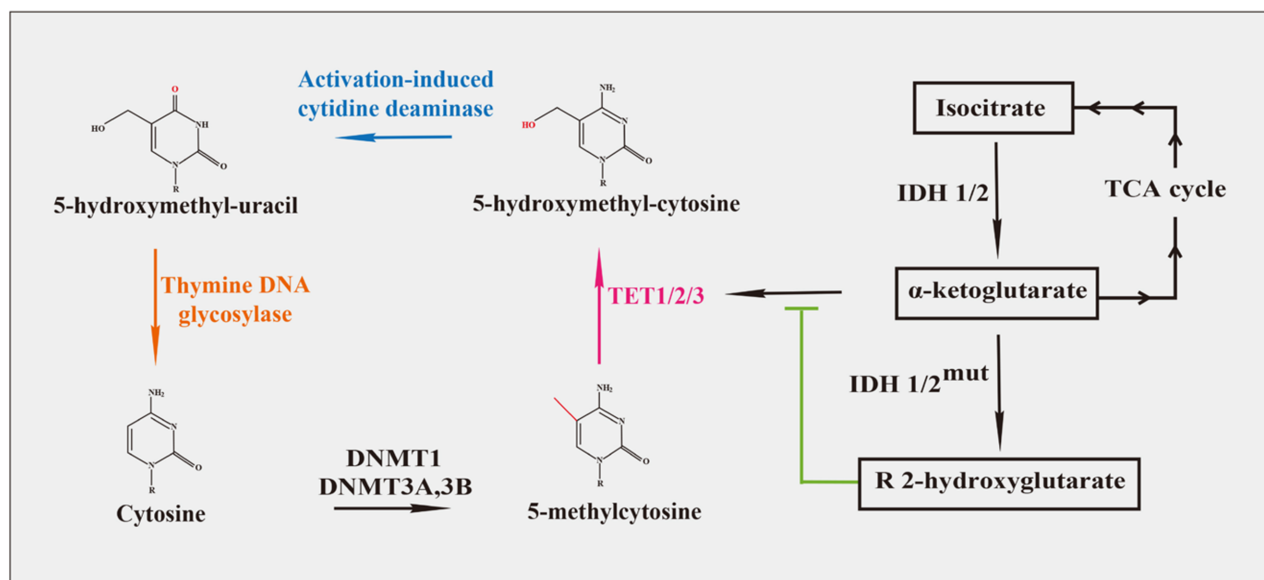

Figure 1. DNA methylation pathways. The methyl group of 5mC can be modified by the addition of a hydroxyl group mediated by Tet enzymes to generate 5hmC. 5hmC can also be chemically modified by AID/APOBEC. AID/APOBEC can deaminate 5hmC to produce 5hmU. Eventually, the 5hmU can be recognized and cleaved off to replace with a naked cytosine mediated by TDG. DNMT1 maintains methylation patterns, and DNMT3A/3B add new methylation. Isocitrate dehydrogenases (IDH1/2) are responsible for converting isocitrate to αKG. Mutant IDH proteins acquire a neomorphic enzymatic activity converting αKG to 2HG and inducing global DNA hypermethylation. Accumulation of 2HG inhibits TET proteins. 2HG = 2-hydroxyglutarate, 5hmC = 5-hydroxymethyl-cytosine, 5hmU = 5-hydroxymethyl-uracil, 5mC = 5-methylcytosine, αKG = α-ketoglutarate, AID/APOBEC = activation-induced cytidine deaminase/apolipoprotein B mRNA editing enzyme complex, DNMT = DNA methyltransferase, IDH = isocitrate dehydrogenase, TCA = Tricarboxylic acid, TDG = thymine DNA glycosylase, TET = ten-eleven translocation.

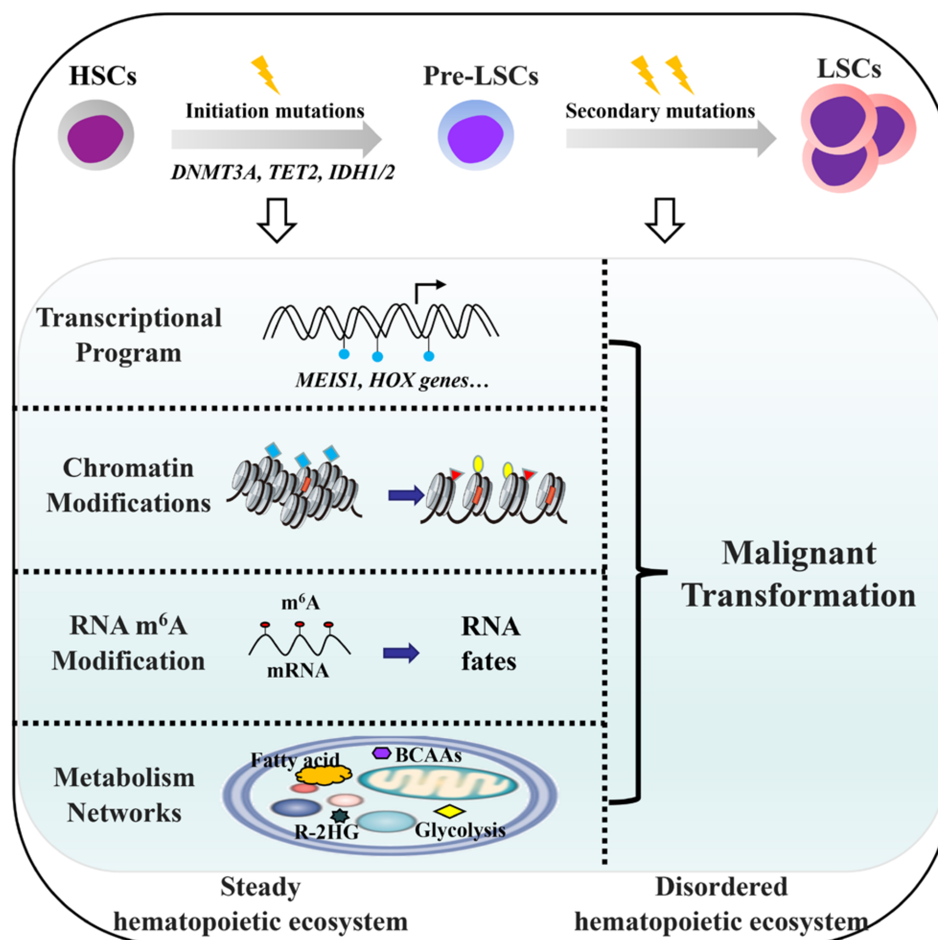

Figure 2. Aberrant DNA methylation drives AML development. Aberrant DNA methylation caused by recurrent mutations in epigenetic modifiers function as a driving force to control the fate of HSPCs and initiate AML development by tuning up a series of cellular and molecular programs including the transcriptional programs, chromatin modifications, RNA m^{6}A modification, and metabolism networks. 2HG = 2-hydroxyglutarate, AML = acute myeloid leukemia, BCAA = branched-chain amino acid, DNMT = DNA methyltransferase, HSPC = hematopoietic stem and progenitor cell, HSC = hematopoietic stem cell, IDH = isocitrate dehydrogenase, LSC = leukemia stem cell, TET = ten-eleven translocation.

5hmC and increases the size of the hematopoietic stem and progenitor cell (HSPC) pool in a cell-autonomous manner. Tet2-deficient HSCs are capable of multilineage reconstitution and possess a competitive advantage over wild-type HSCs, resulting in enhanced hematopoiesis into both the lymphoid and myeloid lineages.[30] In vitro, Tet2 deficiency delays HSC differentiation and skews development toward the monocyte/macrophage lineage.[30] Notably, the actions of DNMTs and TETs cause opposite results: the addition or removal of methylation; however, they are also epistatic, as hydroxymethylation depends on the presence of the 5mC marks introduced by DNMTs. DNMT3A and TET2 can compete and cooperate to repress lineage-specific transcription factors (eg, Klf1, Epor) in HSCs, as DNMT3A and TET2 both repress these factors, suggesting a model of cooperative inhibition by epigenetic modifiers.[31] Together, these studies indicate that DNA methylation levels tuned up by DNMTs and TETs play a key role in controlling the functional programs of HSCs.

DNA methylation also coordinates with other mechanisms (eg, histone modifications, higher-order chromatin structure, and RNA splicing) to regulate HSCs. For instance, a recent study showed that DNMT3A and the spliceosome govern HSCs exit from the stem state toward differentiation.[32] DNMT3A coordinates splicing by recruiting the core spliceosome protein SF3B1 to RNA polymerase and mRNA, and the loss of DNMT3A leads to impaired splicing during stem cell turnover. Interestingly, in this process, the DNA methylation function of DNMT3A is not required.[32] Additionally, TET2 modulates the spatial redistribution of H3K9me3-marked heterochromatin to mediate the upregulation of endogenous retroviruses and interferon-stimulated genes, thus contributing to the functional decline in aged HSCs.[33] Large DNA methylation nadirs can form long anchor chromatin loops between repressive elements and form genomic subcompartments to maintain HSC identity.[34] Overall, these studies suggest that DNA methylation is necessary for maintaining the hematopoietic ecosystem by regulating the functional programs of HSCs.

2.2. DNA methylation in AML pathogenesis

Alterations in DNA methylation are common in patients with AML. Mutations in DNA methylation-related genes, such as *DNMT3A*, *TET2*, and isocitrate dehydrogenase (*IDH*)*1/2*, lead to aberrant methylation patterns, contributing to leukemogenesis. These mutations often coexist with other genetic alterations, thereby compounding their effects.

Given the important role of DNA methylation in regulating the HSC program, aberrant DNA methylation contributes substantially to AML pathogenesis. The genomic and epigenomic landscapes of adult de novo AML were constructed using 200 clinically annotated cases (50 cases for whole-genome sequencing

and 150 cases for whole-exome sequencing).[35] Unsupervised analysis of changes in DNA methylation revealed significant differences across human AML samples, with 67% of the 160,519 CpG loci resulting in a gain of methylation, and 33% resulting in a loss. In addition, mutations in DNA methylation-related genes were detected in approximately 44% of cases[35] (**Fig. 2**). Thus, these data indicate that alterations in DNA methylation may act as a key driver of AML pathogenesis.

Among DNA methylation-related genes, somatic mutations in *DNMT3A* account for approximately 22% of AML.[36,37] Multiple missense mutations in the coding region of *DNMT3A* were identified, the most common of which was predicted to affect amino acid R882 (R882H or R882C) in the structural domain of the methyltransferase. Other types of mutations resulting in frameshift, nonsense, or alternative splicing in *DNMT3A* were also identified.[36] *DNMT3A* mutations frequently co-occur with the other four most commonly mutated genes (*FLT3*, *NPM1*, *IDH1*, and *IDH2*) in AML.[38] *DNMT3A* mutations are associated with poor event-free survival and shorter overall survival; this association is independent of the presence of *FLT3* or *NPM1* mutations, and regardless of the mutation type or genetic location.[36] Analysis of global DNA methylation using LC-MS did not reveal a significant difference in 5-methylcytosine (5mC) levels in *DNMT3A*-mutant AMLs compared with those in AML genomes without *DNMT3A* mutations.[36] Notably, DNMT3A haploinsufficiency is sufficient to contribute to myeloid transformation because *DNMT3A* mutations occur in one allele in the vast majority of AML patients.[36]

TET2 is another gene frequently mutated in myeloid malignancies (**Fig. 2**). Somatic deletions and inactivating mutations in *TET2* have been identified in 10% to 20% of MPN and MDS cases and in 7% to 23% of AML cases.[39,40] In another study, 131 somatic TET2 mutations were identified in 27.4% of AML patients (87/318).[41] *TET2* mutations concomitantly occurred with mutations in other genes (eg, *NPM1*, *FLT3*-ITD, *FLT3*-TKD, *JAK2*, *RUNX1*, *CEBPA*, and *CBL)*. Similarly, *TET2* mutations also act as important prognostic biomarkers in AML and are associated with a poorer prognosis in de novo AML patients with normal cytogenetics (CN-AML).[41–43] TET2 mutations are heterozygous and the wild-type allele is retained in most AML patients, suggesting that TET2 is a haploinsufficient tumor suppressor in leukemia. Another family member TET1 was identified as a fusion partner of the mixed lineage leukemia (*MLL*) gene in AML with t(10;11)(q22;q23).[44] Interestingly, TET1 is a direct target of MLL-fusion proteins and is significantly upregulated in MLL-rearranged leukemia, leading to a global increase in 5-hydroxymethylcytosine level.[45]

Recurring mutations in isocitrate dehydrogenases *IDH1* and *IDH2* have been frequently identified in AML.[46,47] While wild-type IDH1/2 are responsible for converting isocitrate to α-ketoglutarate (αKG), mutant IDH proteins acquire a neomorphic enzymatic activity converting αKG to 2-hydroxyglutarate (2HG) and inducing global DNA hypermethylation. This is most likely due to the inhibitory effect of 2HG on TET protein activity. Interestingly, *IDH1/2* mutations are mutually exclusive of *TET2* mutations. Thus, *IDH1/2* mutants cause epigenetic defects similar to *TET2* loss-of-function mutations,[48] implying that IDH1/2 and TET2 may, to certain extent, share some relevant mechanisms in driving AML pathogenesis.

2.3. Aberrant DNA methylation functions as a starting engine for initiating AML by changing multiple cellular and molecular programs

Over the past 15 years, extensive attention has been paid to how alterations in DNA methylation drive the progression of normal hematopoietic ecosystems to hematological diseases. Mutations in DNA methylation-related genes are considered founder mutations and early events in AML development (**Fig. 2**). A recent study provided key insights into the cellular context and functional consequences of the earliest genetic lesions of human AML and established the sequential order of mutation acquisition; *DNMT3A^mut^* occurs before *NPM1c* and *FLT3*-ITD.[49] In addition, ancestral pre-leukemic HSCs have been identified. These cells enable regeneration of the entire hematopoietic hierarchy while possessing a competitive repopulation advantage over non-leukemic HSCs; they survive induction chemotherapy and persist in the bone marrow at remission.[49] Thus, the cell of origin for $DNMT3A^{mut}$ AML is the HSC, and the DNMT3A mutation confers a pre-leukemic state with clonal expansion ability. This leukemogenic process was confirmed by retroviral transduction and bone marrow transplantation to establish a murine model of $DNMT3A^{R882H}$ hotspot mutation.[50] $DNMT3A^{R882H}$ enhances the sensitivity of HSPCs and transforms them into leukemia-initiating cells (LIC) in cooperation with *NRAS* mutations. $DNMT3A^{R882H}$ induces focal epigenetic alterations and transactivates stemness genes (*MEIS1*, *MN1*, and *HOXA* genes) (**Fig. 2**). Inhibition of DOT1L represses $DNMT3A^{R882H}$-induced gene expression programs, providing an attractive therapeutic strategy for *DNMT3A*-mutated leukemias.[50] Similarly, a mouse conditional knock-in model of Cre-mediated expression of the mutant $Dnmt3a^{R878H}$ was established.[51] Dnmt3a mutation results in enlarged $Lin^-Sca1^+cKit^+$ cells and causes hypomethylation that contributes to mammalian target of rapamycin (mTOR) upregulation, in turn leading to aberrantly increased CDK1 expression.[51] Mechanistically, hotspot mutations in *DNMT3A* act in a dominant-negative manner, and *DNMT3A* mutations disrupt the formation of a DNMT3A-associated tetramer complex required for efficient DNA methylation. For instance, hotspot $DNMT3A^{R882}$ mutations promote polymerization and a dominant-negative effect, leading to aberrant DNA methylation at specific sites.[52] The polymerization capacity of $DNMT3A^{R882mut}$ relies on the heterodimerization motif but not on its various chromatin-binding domains. Mutation of the heterodimerization motif interferes with $DNMT3A^{R882mut}$ binding to endogenous wild-type DNMT proteins and partially reverses the CpG hypomethylation phenotype caused by $DNMT3A^{R882mut}$.[53] Thus, DNMT3A acts in a dominant-negative manner during leukemia transformation.

In addition, altered DNA methylation of Hi-C and whole-genome sequencing have identified recurrent and subtype-specific 3D genome alterations in human AML. Altered DNA methylation contributes to these pathogenic chromatin interactions by displacing CCCTC-binding factor (CTCF) binding. For instance, multiple chromatin loops were detected in AML samples across the lost CTCF-binding sites, including a loop linking *WDR66* promoter to distal regions[54] (**Fig. 2**). These data revealed a delicate relationship between DNA methylation, CTCF binding, and 3D genome structure. Interestingly, a recent study identified 58 tumor-specific antigens (TSAs) by analyzing the major histocompatibility complex class I (MHC-I)-associated immunopeptidome in human AML samples and found that mutations in epigenetic modifiers correlated with TSA expression, which causes expansion of cognate T cell receptor clonotypes and accumulation of activated cytotoxic T cells,[55] indicating that epigenetic alterations in leukemia cells also change the hematopoietic ecosystem.

Mutations in both *TET2* and *IDH1/2* result in DNA hypermethylation and are considered founders for AML transformation. Genetic inactivation of *Tet2* in the mouse hematopoietic system confers HSPCs with a competitive advantage and increased susceptibility to cellular transformation; however, cooperative mutations (eg, *FLT3, KIT*) are necessary to achieve full malignant transformation. In a mouse model of human AML1-ETO-induced AML, the loss of Tet2 leads to a genome-wide increase in the DNA methylation of active enhancers, especially for several tumor suppressor genes (eg, *Mtss1*, *Las2*, *Lxn*, *Ctdspl*, *Grap2*), and this effect is progressive.[56] Similarly, mutations in *Tet2* and *Flt3* that result in fully penetrant and lethal AML,

such as $Flt3^{ITD}$ mutations and *Tet2* loss, cooperatively remodel site-specific DNA methylation and gene expression that are not observed with either mutant allele alone.[57] TET2 activity also shapes the local chromatin environment at enhancers to facilitate TF binding. Genome-wide mapping of TET2 binding sites reveals that TET2 localizes to regions of open chromatin and cell type-specific enhancers, and the deletion of *Tet2* attenuates the binding of the basic helix-loop-helix (bHLH) TF family within these regions.[58] Additionally, Tet2 deficiency may regulate RNA methylation. A recent study found that TET2 deficiency results in the accumulation of methyl-5-cytosine modification in TSPAN13 mRNA, which stabilizes the *TSPAN13* mRNA recognized by YBX1. Increased expression of TSPAN13 activates the CXCR4/CXCL12 signaling, promoting LSCs homing/migration into the BM niche.[59] Because the three TET proteins exhibit functional redundancy, a mouse model of inducible deletion of all three Tet genes (iTKO) was generated recently.[60] Interestingly, these iTKO mice developed rapid and fatal AML within 4 to 5 weeks, and a striking increase in the expression of all members of the *stefin/cystatin* gene cluster was observed. Independent of DNA methylation, the increased expression of *stefin/cystatin* cluster genes was associated with a heterochromatin-to-euchromatin compartment switch, indicating that TET deficiency can also change the 3D genomic structure (**Fig. 2**).

Additionally, mutations of IDH1/2 provide an important metabolic input for driving AML pathogenesis, as IDH1/2 are the key regulator of intercellular αKG homeostasis. αKG serves as a critical cofactor for many dioxygenases, such as prolyl hydroxylases (PHDs), Egl-9 family hypoxia-inducible factor 1 (EGLN1), and TET enzymes.[61] Thus, IDH1/2 mutations reduce the level of αKG accompanied with increased production of 2HG, which inhibit the activity of TET enzymes. AML with IDH1/2 mutations displays a specific DNA hypermethylation signature, impairs hematopoietic differentiation, and increases the expression of stem/progenitor cell markers.[48] A recent study revealed another key metabolic pathway, the branched-chain amino acid (BCAA) pathway, which regulates αKG homeostasis in AML stem cells.[62] Cytosolic BCAA transaminase 1 (BCAT1) transfers the α-amino group from BCAAs to αKG, yielding glutamate and the respective branched-chain α-keto acid (BCKA). BCAT1 is overexpressed in AML stem cells and restricts αKG levels, leading to IDH^{mut}-like DNA hypermethylation. By contrast, knockdown of BCAT1 causes αKG accumulation that leads to EGLN1-mediated hypoxia-inducible factor-1α (HIF1α) protein degradation, subsequently abrogating leukemia-initiating potential.[62] IDH mutations can also affect fatty acid metabolism. The comprehensive metabolomic analysis identifies an $IDH1^{mut}$-specific reprogramming of fatty acid metabolism, which provides a targetable synthetic lethal vulnerability for $IDH1^{mut}$ AML[63] (**Fig. 2**).

Overall, aberrant DNA methylation caused by recurrent mutations function as a starting engine to control the fate of HSPCs and initiates AML development by tuning up a series of cellular and molecular programs, including the transcriptional programs, chromatin modifications, genomic structures, and metabolic networks.

3. RNA MODIFICATION

According to the central law of genetics, RNA is a key mediator in the transfer of genetic information from DNA to proteins. Over 170 types of RNA modifications have been identified over several decades; however, the revival of this field should be attributed to the discovery of RNA modification effects in regulating gene expression and to the technological developments for characterizing these modifications at the transcriptome-wide level.[64] Currently, it is known that RNA modifications substantially affect RNA fate by regulating its structure, splicing, stability, or translation. In the following paragraphs, we mainly focus on m^6A briefly discussing the modifications of pseudouridine (Ψ), 5-methylcytosine (m^5C), and N^1-methyladenosine (m^1A).

3.1. The features of pseudouridine, m^5C, and m^1A

Different RNA modifications confer distinct properties and play important roles in almost all biological processes. Pseudouridine (Ψ) is the first RNA modification identified in the 1950s,[65] and widely presents in most RNA species. A class of enzymes called pseudouridine synthases (PUSes) catalyze uridine into Ψ,[66,67] and the specific cellular localization of these enzymes confers them the potential to target their unique RNA species. For example, PUS1 predominantly localizes to the nucleus and modifies tRNA, mRNA, small nucleolar RNAs (snRNAs), and non-coding RNAs (ncRNAs).[68] Notably, the eraser and readers for Ψ remain unclear. It plays important roles in RNA biogenesis, structure, stability, and gene expression. Pseudouridine in ribosomal RNA (rRNA) is required for translational fidelity,[69] and Ψ in tRNA maintains its structure and stability.[70] However, the effect of pseudouridine on mRNA expression remains unclear.

m^5C is a rare RNA modification that was first observed in *Escherichia coli* RNA.[71] This methylation occurs at position 5 of the cytidine residue on tRNA, mRNA, rRNA, mRNA, or enhancer RNA (eRNA). The NOL1/NOP2/SUN domain (NSUN) family including NSUN1-7 has been identified as m^5C writers, and NSUN2 mainly catalyzes m^5C in mRNA.[72] Interestingly, DNMT2 can also catalyze the formation of m^5C on RNA,[73] and TET proteins not only oxidize 5-mC in DNA but also act as erasers of RNA m^5C by oxidizing m^5C to 5-hydroxymethylcytidine (hm^5C).[74,75] ALKBH1, a 2-oxoglutarate/$Fe2^+$-dependent dioxygenase, is another m^5C demethylase.[76] About the readers of m^5C, the mammalian mRNA export adaptor ALYREF was the first identified as a mRNA m^5C reader.[72] Interestingly, the RNA-binding protein (RBP) YBX1 has also been identified as another m^5C reader. YBX1 can read m^5C-modified mRNAs via its indole ring in the cold-shock domain.[77] Currently, the protein machinery of RNA m^5C remains to be investigated. Similar to other RNA modifications, m^5C plays a distinct role in these RNA subtypes. For instance, m^5C affects tRNA or rRNA structure,[78] and m^5C marks regulates mRNA export mediated by the ALYREF reader.[72]

m^1A, which is methylated at position 1 of adenosine, was first identified in mammalian and plant RNA in the 1960s.[79,80] The known writer of m^1A is a complex containing tRNA methyltransferase 6 non-catalytic subunit (TRMT6) and RNA methyltransferase catalytic 61A (TRMT61A).[81,82] RNA m^1A also shares some regulators (eg, YTHDF2) with m^6A but with a relatively low affinity.[83] ALKBH1 and ALKBH3 are m^1A erasers. ALKBH3 is the only known eraser of m^1A.[84,85] In addition to m^5C demethylation, ALKBH1 also mediates the demethylation of m^1A in tRNAs.[86] m^1A primarily affects the structures and functions of tRNA and rRNA. Since m^1A is preferentially enriched around the start codon upstream of the first splice site, it is associated with translation initiation and affects translation efficiency.[87]

The roles of these RNA modifications in the hematopoietic system and related malignancies remain elusive. A recent study revealed that RNA m^5C methyltransferases (eg, NSUN1/3) may be associated with leukemia cell drug sensitivity.[88] ALKBH3 is dispensable for HSC maintenance and differentiation; however, ALKBH3 overexpression may rectify HSC aging.[89] Therefore, it is necessary to further explore the role of these RNA modifications under various physiological and pathological conditions.

3.2. RNA m^6A modification

RNA m^6A is the most abundant and best-characterized modification in mammalian mRNA. It is known that m^6A

modification is reversible, highly dynamic, and controlled by m^{6}A modifiers, including writers, erasers, and readers. The writer is a multicomponent complex composed of two core methyltransferase-like proteins (METTL3 and METTL14) and multiple regulatory proteins (WTAP, VIRMA, CBLL1, RBM15/RBM15B, and ZC3H13).[90–94] In this complex, METTL3 acts as the sole catalytical protein for the transfer of a methyl group from S-adenosylmethionine (SAM) to the sixth N atom of RNA adenosine, and METTL14 maintains the complex structural stability.[95] Other subunits regulate the activity and specificity of this complex.[91] Both FTO and ALKBH5 are two main α-KG/Fe-dependent m^{6}A demethylases.[96,97] The m^{6}A readers include YTH domain-containing protein 1-2 (YTHDC1-2), YTH domain-containing family member 1-3 (YTHDF1-3), and insulin-like growth factor-2 mRNA-binding protein (IGF2BP) family IGF2BP1-3.[98–101] These readers recognize m^{6}A sites and perform key functions in the regulation of distinct mRNA fates. YTHDF1 and YTHDF3 mainly regulate the translation of their targets[102]; in contrast, YTHDF2 facilitates the degradation of its m^{6}A targets.[103] YTHDC1 is involved in the export of m^{6}A-tagged mRNAs from the nucleus to the cytoplasm,[98,104–106] whereas YTHDC2 regulates the translation and stability of m^{6}A targets.[107–109] Another group, IGF2BPs, mainly stabilizes their m^{6}A targets.[101] Our group revealed that the function of IGF2BPs requires YBX1 participation, as YBX1 facilitates IGF2BPs to recognize and stabilize m^{6}A-tagged transcripts.[110,111] In the future, identifying the cofactors of m^{6}A modifiers may be much more important.

3.3. RNA m^{6}A modification is essential for the generation and maintenance of hematopoietic ecosystem

HSCs are generated from embryonic precursors such as hemogenic endothelial cells and pre-HSCs during mid-gestation and progressively migrate into the fetal liver for expansion.[112,113] Before birth, HSCs migrate to the bone marrow, where they maintain a hematopoietic ecosystem via self-renewal and differentiation. Increasing evidence has shown that RNA m^{6}A plays a key role in the generation and maintenance of HSCs.

RNA m^{6}A governs HSCs generation and expansion during hematopoietic development. m^{6}A functions as a rheostat to control endothelial-to-hematopoietic transition (EHT). Activation of Notch1 signaling maintains endothelial cell identity and represses HSPC programming during EHT. m^{6}A inhibits Notch1a expression by promoting its degradation mediated by YTHDF2.[114] The m^{6}A modification is also involved in innate immune responses. Double-stranded RNA (dsRNA) from foreign pathogens such as viruses triggers the activation of the cellular innate immune response. Interestingly, the loss of m^{6}A modification results in the aberrant formation of endogenously derived dsRNA in HSCs, which induces an aberrant innate immune response and subsequently causes hematopoietic failure and perinatal lethality.[115] These studies have revealed the key role of m^{6}A in embryonic hematopoiesis.

In adult hematopoiesis, m^{6}A balances self-renewal and differentiation of HSCs. Several studies have demonstrated that m^{6}A loss blocks the normal differentiation and causes the accumulation of phenotypical HSCs with long-term hematopoietic disorders and impaired hematopoietic reconstitution potential.[116–120] This effect is mediated by fine-tuning the expression of many key factors (eg, *MYC, SON*). MYC is a major determinant of HSC differentiation, and HSCs normally maintain very low levels of MYC protein. Interestingly, MYC is a direct target of m^{6}A in HSCs, and *Mettl3*-deficient HSCs failed to upregulate MYC expression upon stimulation to differentiate.[117,118] The SON RBP is a central component of the nuclear speckles. A recent study found that SON is another essential m^{6}A target required for murine HSC self-renewal, symmetric commitment, and inflammation control.[121] Similarly, the important role of m^{6}A in adult HSCs was revealed by studying other key components of the m^{6}A pathway. For instance, YTHDF2 depletion causes HSC expansion without skewing lineage differentiation preference.[122,123] In contrast, the m^{6}A eraser, AlKBH5, is not dispensable for adult hematopoiesis and HSC function.[124,125] This might be due to the functional redundancy between ALKBH5 and FTO. Thus, these studies revealed a developmental stage-specific requirement for m^{6}A in hematopoiesis, which requires the decoding of the dynamics of m^{6}A modifications in the hematopoietic system.

To address this fundamental question, we recently delineated a comprehensive m^{6}A landscape across the hematopoietic hierarchy by developing a super low-input m^{6}A sequencing (SLIM-seq) strategy to profile the m^{6}A landscape of HSCs and their progeny at the transcriptome-wide level. Interestingly, we observed a cell type-specific m^{6}A landscape during hematopoiesis. m^{6}A modifications arise mostly in the early stages of hematopoiesis and play distinct roles in determining mRNA fates in HSCs and committed progenitors. Furthermore, we confirmed that m^{6}A is required to balance the quiescent active states of HSCs, which is mainly mediated by the m^{6}A reader IGF2BP2. IGF2BP2 deficiency results in quiescence loss and impairs HSC function by increasing mitochondrial activity of HSCs.[126]

The interactions between hematopoietic cells and their niche are critical for hematopoietic ecosystem. m^{6}A can also affect the HSC niche, which comprises several key cell types (eg, mesenchymal stromal cells [MSCs] and osteoblasts). Previously, epitranscriptomic programs have been shown to be involved in skeletal health and diseases, such as osteoporosis mese.[127] A recent study indicated that the m^{6}A-mediated epitranscriptomic program regulates the generation, but not maintenance, of the bone marrow HSC niche.[128] Comparison of perinatal and adult bone marrow MSCs revealed that m^{6}A-related genes are enriched in MSCs at the perinatal stage, whereas Mettl3 is rapidly downregulated after birth. Deletion of Mettl3 from developing MSCs resulted in a severe defect in HSC niche formation owing to excessive osteogenic differentiation, whereas deletion of Mettl3 from MSCs postnatally did not affect the HSC niche. Overall, m^{6}A-mediated epitranscriptomic program controls the generation and maintenance of the hematopoietic ecosystem.

3.4. RNA m^{6}A modification reprogramming is required for AML development

Most myeloid leukemias are initiated by LSCs that are transformed from HSPCs along with dysregulated programs (eg, aberrant DNA methylation). The role of RNA m^{6}A in leukemia especially AML has gradually been recognized. For instance, the expression levels of m^{6}A-related genes (eg, *METTL3, METTL14, FTO, ALKBH5, YTHDF2, IGF2BP2*) are obviously increased in AML and are associated with poor prognosis.[120,125,129–131] Recently, we decoded the m^{6}A landscape during AML development and observed obvious changes in the m^{6}A methylome during leukemogenesis. Interestingly, we found that approximately 60% of the m^{6}A targets identified in LICs were newly established and LIC-specific when compared to normal HSPCs, suggesting that reprogramming of RNA m^{6}A modification occurs during cellular transformation.[131,132] Importantly, these m^{6}A-tagged targets are involved in the regulation of many hallmarks of cancer, such as sustaining proliferation, resisting cell death, metabolic adaptation, epigenetic reprogramming, and immune evasion (**Fig. 3**). In this section, we highlight three aspects.

RNA m^{6}A regulates various metabolic adaptations that are employed by AML cells to meet their metabolic requirements. For instance, we found that the m^{6}A reader IGF2BP2 restricts the uptake of docosahexaenoic acid levels via the PRMT6-MFSD2A axis to balance the fatty acid metabolism in LSCs.[131] In addition, IGF2BP2 controls glutamine metabolism by regulating

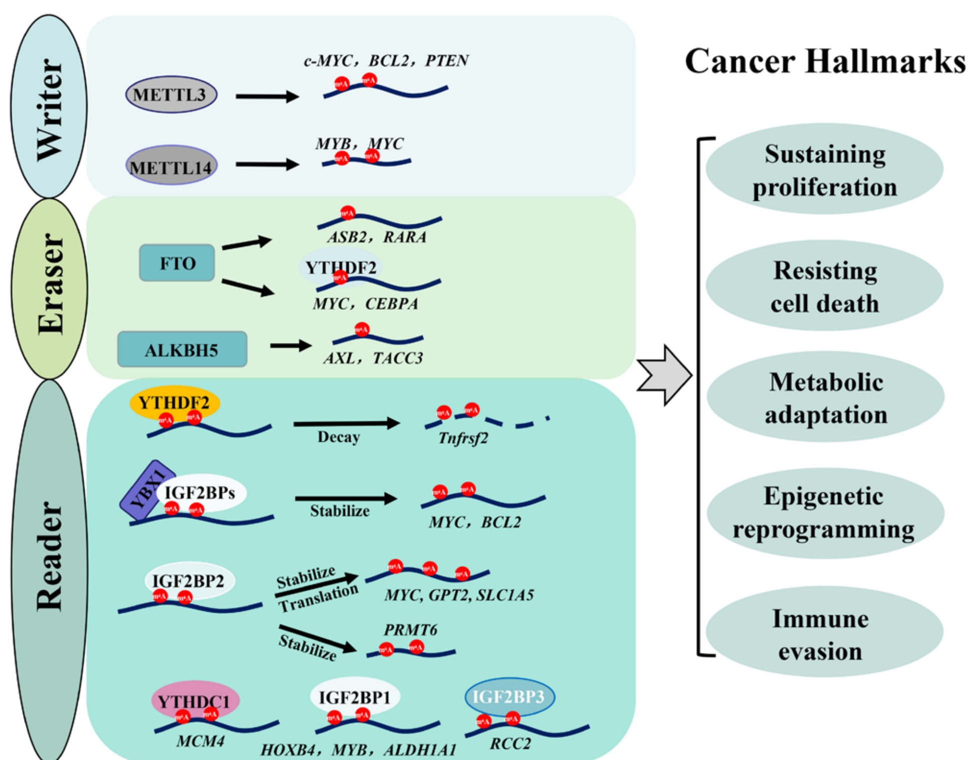

Figure 3. RNA m^{6}A modification regulates cancer hallmarks. m^{6}A modification determines mRNA fates of many key regulators, which involves regulating AML properties including sustaining proliferation, resisting cell death, metabolic adaptation, epigenetic reprogramming, and immune evasion. AML = acute myeloid leukemia.

the expression of critical targets (eg, MYC, GPT2, and SLC1A5) in an m^{6}A-dependent manner.[133] As discussed previously, IDH^{mut} catalyzes the production of the oncometabolite R-2HG. Interestingly, R-2HG abrogated the FTO-m^{6}A-YTHDF2-mediated post-transcriptional upregulation of phosphofructokinase platelet (PFKP) and lactate dehydrogenase B (LDHB), two critical glycolytic genes, thereby suppressing aerobic glycolysis in R-2HG-sensitive leukemia cells.[134] Collectively, these studies revealed a key role of m^{6}A in fine-tuning the metabolic adaptations of three key nutrients (glycolysis, amino acids, and lipids) in AML (**Fig. 3**).

RNA m^{6}A interwines and cooperates with epigenetic alterations to promote AML development. By analyzing chromatin accessibility during leukemogenesis, we found that ALKBH5 was regulated by chromatin state alteration, which were mediated by the histone demethylase KDM4C. H3K9me3 around the *ALKBH5* promoter region is removed by KDM4C to increase chromatin accessibility and recruitment of MYB and Pol II.[125] ALKBH5 is required for LSCs and AML development, but not for adult hematopoiesis and HSC function. Alkbh5 loss significantly inhibits AML development and progression by affecting *AXL* mRNA stability in an m^{6}A-dependent manner and the downstream PI3K/AKT pathway in AML. In addition, IGF2BP2 regulates the protein arginine methyltransferase PRMT6, which subsequently catalyzes the asymmetric dimethylation of histone H3R2 (H3R2me2a).[131] Therefore, RNA m^{6}A involves in the epigenetic reprogramming of AML cells (**Fig. 3**).

Immune evasion is another important hallmark of cancer, and m^{6}A is involved in cancer immunology via multiple mechanisms. m^{6}A modifiers upregulated in cancer cells may regulate the expression of the inhibitory immune checkpoint proteins (eg, PD-L1), or influence the function of immune cells in the tumor microenvironment (TME). For instance, FTO facilitates the immune surveillance escape of cancer cells through regulating glycolytic metabolism.[135] In addition, FTO upregulates the expression of immune checkpoint genes including *PD-L1* and *LILRB4* in AML cells, thereby contributing to tumor immune evasion. Pharmacological inhibition of FTO sensitizes leukemia cells to CD8$^+$ T cell cytotoxicity and overcomes hypomethylating agent-induced immune evasion.[136] RNA m^{6}A can also modulate the function of different immune cell populations (eg, T cells and dendritic cells) and shape the TME, which favors cancer growth. m^{6}A controls CD8$^+$ T cell differentiation and sustains T_{reg} suppressive functions.[137] m^{6}A also regulates durable neoantigen-specific immunity. Loss of YTHDF1 in classical dendritic cells enhances cross-presentation of tumor antigens and cross-priming of CD8$^+$ T cells.[138] Thus, these data implicate RNA m^{6}A in cancer immunology. Overall, the m^{6}A modification plays an important role in modulating the programs that confer cancer hallmarks (**Fig. 3**).

4. CONCLUSION AND PERSPECTIVE

Epigenetic modifications act as key regulators of the hematopoietic system, and epigenetic alterations are widely considered as common genetic events in hematological malignancies. It should be noted that here we mainly discussed DNA methylation and RNA m^{6}A modification, as DNA methylation alteration is the key driver of many myeloid malignancies, and the field of RNA m^{6}A is exponentially growing. This striking evidence clearly shows the potential of targeting these epigenetic alterations in AML treatment.

Although their physiological and pathological roles have been well established, emerging interesting questions need to be elucidated. First, therapeutic strategies targeting epigenetic modifications are insufficient, and the development of more effective drugs is urgently needed for AML therapy. Second, it is necessary to further clarify the distinct roles of different m^{6}A modifiers in different contexts, particularly how these modifiers sense various environments. Our recent work implies that RBPs

or related cofactors in the regulatory machinery are key to determine their target specificity. Third, large gaps still exist between the current research and future clinical applications. The efficient translation of the findings on RNA m^{6}A modifications and DNA methylation into real treatment strategies remains challenging. For instance, several inhibitors of RNA m^{6}A modifiers (eg, STM2457, CS1, CS2, and CWI1-2) have been developed, but there is still a long way to go before the clinical application of these inhibitors.[133,136,139] Collectively, epigenetics has attracted considerable attention, and it is promising to improve therapeutic efficiency by targeting epigenetic modifications.

ACKNOWLEDGMENTS

This work is supported by the grants to H.Z. from the National Key R&D Program of China (2022YFA0103200), and the National Natural Science Foundation of China (82325003, 82230007). This work is supported by the grants to R.G. from the Fundamental Research Funds for the Central Universities (2042023kf0057) and the National Natural Science Foundation (NSFC) (82301997). This work is also supported by the grants to R.Y. from the National Natural Science Foundation of China (82200188) and the Special Fund of China Postdoctoral Science Foundation (2022TQ0238). This work is also supported by the grants to H.Z. from the Fundamental Research Funds for the Central Universities (2042022dx0003 and 2042024kf1022).

We appreciate the members of our laboratory for discussion.

AUTHOR CONTRIBUTIONS

H.Z. wrote the manuscript with the help of S.Y. and R.G. All the other authors contributed to providing information, reviewing and revising this manuscript.

REFERENCES

[1] Haas S, Trumpp A, Milsom MD. Causes and consequences of hematopoietic stem cell heterogeneity. *Cell Stem Cell* 2018;22(5):627–638.
[2] Zhao A, Zhou H, Yang J, Li M, Niu T. Epigenetic regulation in hematopoiesis and its implications in the targeted therapy of hematologic malignancies. *Signal Transduct Target Ther* 2023;8(1):71.
[3] Rattigan KM, Zarou MM, Helgason GV. Metabolism in stem cell-driven leukemia: parallels between hematopoiesis and immunity. *Blood* 2023;141(21):2553–2565.
[4] Park IK, Qian D, Kiel M, et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* 2003;423(6937):302–305.
[5] Short NJ, Ravandi F. Acute myeloid leukemia: past, present, and prospects for the future. *Clin Lymphoma Myeloma Leuk* 2016;16(Suppl):S25–S29.
[6] Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. *Science* 2019;366(6465):eaan4673.
[7] Jiffry MZM, Kloss R, Ahmed-khan M, et al. A review of treatment options employed in relapsed/refractory AML. *Hematology* 2023;28(1):2196482.
[8] Barbieri I, Kouzarides T. Role of RNA modifications in cancer. *Nat Rev Cancer* 2020;20(6):303–322.
[9] Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. *Nat Rev Genet* 2016;17(8):487–500.
[10] Ma J, Zhang L, Chen S, Liu H. A brief review of RNA modification related database resources. *Methods* 2022;203:342–353.
[11] Mahmud M, Stebbing J. Epigenetic modifications in AML and MDS. *Leuk Res* 2010;34(2):139–140.
[12] Johnson TB, Coghill RD. Researches on pyrimidines C111. The discovery of 5-methylcytosine in tuberculinic acid, the nucleic acid of the tubercle bacillus. *J Am Chem Soc* 1925;47(11):2838–2844.
[13] Wu H, Zhang Y. Reversing DNA methylation: mechanisms, genomics, and biological functions. *Cell* 2014;156(1-2):45–68.
[14] Dura M, Teissandier A, Armand M, et al. DNMT3A-dependent DNA methylation is required for spermatogonial stem cells to commit to spermatogenesis. *Nat Genet* 2022;54(4):469–480.
[15] Zhang ZM, Lu R, Wang P, et al. Structural basis for DNMT3A-mediated de novo DNA methylation. *Nature* 2018;554(7692):387–391.
[16] Veland N, Lu Y, Hardikar S, et al. DNMT3L facilitates DNA methylation partly by maintaining DNMT3A stability in mouse embryonic stem cells. *Nucleic Acids Res* 2019;47(1):152–167.
[17] Goll MG, Kirpekar F, Maggert KA, et al. Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. *Science* 2006;311(5759):395–398.
[18] Iqbal K, Jin SG, Pfeifer GP, Szabo PE. Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. *Proc Natl Acad Sci U S A* 2011;108(9):3642–3647.
[19] Inoue A, Zhang Y. Replication-dependent loss of 5-hydroxymethylcytosine in mouse preimplantation embryos. *Science* 2011;334(6053):194.
[20] Bhutani N, Brady JJ, Damian M, Sacco A, Corbel SY, Blau HM. Reprogramming towards pluripotency requires AID-dependent DNA demethylation. *Nature* 2010;463(7284):1042–1047.
[21] Zhang F, Pomerantz JH, Sen G, Palermo AT, Blau HM. Active tissue-specific DNA demethylation conferred by somatic cell nuclei in stable heterokaryons. *Proc Natl Acad Sci U S A* 2007;104(11):4395–4400.
[22] Paroush Z, Keshet I, Yisraeli J, Cedar H. Dynamics of demethylation and activation of the alpha-actin gene in myoblasts. *Cell* 1990;63(6):1229–1237.
[23] Oswald J, Engemann S, Lane N, et al. Active demethylation of the paternal genome in the mouse zygote. *Curr Biol* 2000;10(8):475–478.
[24] Mayer W, Niveleau A, Walter J, Fundele R, Haaf T. Demethylation of the zygotic paternal genome. *Nature* 2000;403(6769):501–502.
[25] Trowbridge JJ, Snow JW, Kim J, Orkin SH. DNA methyltransferase 1 is essential for and uniquely regulates hematopoietic stem and progenitor cells. *Cell Stem Cell* 2009;5(4):442–449.
[26] Broske AM, Vockentanz L, Kharazi S, et al. DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. *Nat Genet* 2009;41(11):1207–1215.
[27] Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet* 2011;44(1):23–31.
[28] Challen GA, Sun D, Mayle A, et al. Dnmt3a and Dnmt3b have overlapping and distinct functions in hematopoietic stem cells. *Cell Stem Cell* 2014;15(3):350–364.
[29] Hashimoto H, Liu Y, Upadhyay AK, et al. Recognition and potential mechanisms for replication and erasure of cytosine hydroxymethylation. *Nucleic Acids Res* 2012;40(11):4841–4849.
[30] Ko M, Bandukwala HS, An J, et al. Ten-eleven-translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. *Proc Natl Acad Sci U S A* 2011;108(35):14566–14571.
[31] Zhang X, Su J, Jeong M, et al. DNMT3A and TET2 compete and cooperate to repress lineage-specific transcription factors in hematopoietic stem cells. *Nat Genet* 2016;48(9):1014–1023.
[32] Ramabadran R, Wang JH, Reyes JM, et al. DNMT3A-coordinated splicing governs the stem state switch towards differentiation in embryonic and haematopoietic stem cells. *Nat Cell Biol* 2023;25(4):528–539.
[33] Hong T, Li J, Guo L, et al. TET2 modulates spatial relocalization of heterochromatin in aged hematopoietic stem and progenitor cells. *Nat Aging* 2023;3(11):1387–1400.
[34] Zhang X, Jeong M, Huang X, et al. Large DNA methylation nadirs anchor chromatin loops maintaining hematopoietic stem cell identity. *Mol Cell* 2020;78(3):506–521.e6.
[35] Cancer Genome Atlas Research Network; Ley TJ, Miller C, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med* 2013;368(22):2059–2074.
[36] Ley TJ, Ding L, Walter MJ, et al. DNMT3A mutations in acute myeloid leukemia. *N Engl J Med* 2010;363(25):2424–2433.
[37] Yan XJ, Xu J, Gu ZH, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. *Nat Genet* 2011;43(4):309–315.
[38] Brunetti L, Gundry MC, Goodell MA. DNMT3A in Leukemia. *Cold Spring Harb Perspect Med* 2017;7(2):a030320.
[39] Delhommeau F, Dupont S, Della Valle V, et al. Mutation in TET2 in myeloid cancers. *N Engl J Med* 2009;360(22):2289–2301.
[40] Langemeijer SM, Kuiper RP, Berends M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. *Nat Genet* 2009;41(7):838–842.
[41] Weissmann S, Alpermann T, Grossmann V, et al. Landscape of TET2 mutations in acute myeloid leukemia. *Leukemia* 2012;26(5):934–942.
[42] Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet* 2012;44(11):1179–1181.
[43] Nibourel O, Kosmider O, Cheok M, et al. Incidence and prognostic value of TET2 alterations in de novo acute myeloid leukemia achieving complete remission. *Blood* 2010;116(7):1132–1135.
[44] Lorsbach RB, Moore J, Mathew S, Raimondi SC, Mukatira ST, Downing JR. TET1, a member of a novel protein family, is fused to

MLL in acute myeloid leukemia containing the t(10;11)(q22;q23). *Leukemia* 2003;17(3):637–641.
[45] Huang H, Jiang X, Li Z, et al. TET1 plays an essential oncogenic role in MLL-rearranged leukemia. *Proc Natl Acad Sci U S A* 2013;110(29):11994–11999.
[46] Mardis ER, Ding L, Dooling DJ, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med* 2009;361(11):1058–1066.
[47] Marcucci G, Maharry K, Wu YZ, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. *J Clin Oncol* 2010;28(14):2348–2355.
[48] Figueroa ME, Abdel-Wahab O, Lu C, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* 2010;18(6): 553–567.
[49] Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature* 2014;506(7488):328–333.
[50] Lu R, Wang P, Parton T, et al. Epigenetic perturbations by Arg882-mutated DNMT3A potentiate aberrant stem cell gene-expression program and acute leukemia development. *Cancer Cell* 2016;30(1):92–107.
[51] Dai YJ, Wang YY, Huang JY, et al. Conditional knockin of Dnmt3a R878H initiates acute myeloid leukemia with mTOR pathway involvement. *Proc Natl Acad Sci U S A* 2017;114(20):5237–5242.
[52] Lu J, Guo Y, Yin J, et al. Structure-guided functional suppression of AML-associated DNMT3A hotspot mutations. *Nat Commun* 2024;15(1):3111.
[53] Lu R, Wang J, Ren Z, et al. A model system for studying the DNMT3A hotspot mutation (DNMT3A(R882)) demonstrates a causal relationship between its dominant-negative effect and leukemogenesis. *Cancer Res* 2019;79(14):3583–3594.
[54] Xu J, Song F, Lyu H, et al. Subtype-specific 3D genome alteration in acute myeloid leukaemia. *Nature* 2022;611(7935):387–398.
[55] Ehx G, Larouche JD, Durette C, et al. Atypical acute myeloid leukemia-specific transcripts generate shared and immunogenic MHC class-I-associated epitopes. *Immunity* 2021;54(4):737–752.e10.
[56] Rasmussen KD, Jia G, Johansen JV, et al. Loss of TET2 in hematopoietic cells leads to DNA hypermethylation of active enhancers and induction of leukemogenesis. *Genes Dev* 2015;29(9):910–922.
[57] Shih AH, Jiang Y, Meydan C, et al. Mutational cooperativity linked to combinatorial epigenetic gain of function in acute myeloid leukemia. *Cancer Cell* 2015;27(4):502–515.
[58] Rasmussen KD, Berest I, Kebetaler S, et al. TET2 binding to enhancers facilitates transcription factor recruitment in hematopoietic cells. *Genome Res* 2019;29(4):564–575.
[59] Li Y, Xue M, Deng X, et al. TET2-mediated mRNA demethylation regulates leukemia stem cell homing and self-renewal. *Cell Stem Cell* 2023;30(8):1072–1090.e10.
[60] Yuita H, Lopez-Moyado IF, Jeong H, et al. Inducible disruption of Tet genes results in myeloid malignancy, readthrough transcription, and a heterochromatin-to-euchromatin switch. *Proc Natl Acad Sci U S A* 2023;120(6):e2214824120.
[61] Baksh SC, Finley LWS. Metabolic coordination of cell fate by alpha-ketoglutarate-dependent dioxygenases. *Trends Cell Biol* 2021;31(1):24–36.
[62] Raffel S, Falcone M, Kneisel N, et al. BCAT1 restricts alphaKG levels in AML stem cells leading to IDHmut-like DNA hypermethylation. *Nature* 2017;551(7680):384–388.
[63] Thomas D, Wu M, Nakauchi Y, et al. Dysregulated lipid synthesis by oncogenic IDH1 mutation is a targetable synthetic lethal vulnerability. *Cancer Discov* 2023;13(2):496–515.
[64] Zhang Y, Lu L, Li X. Detection technologies for RNA modifications. *Exp Mol Med* 2022;54(10):1601–1616.
[65] Davis FF, Allen FW. Ribonucleic acids from yeast which contain a fifth nucleotide. *J Biol Chem* 1957;227(2):907–915.
[66] Borchardt EK, Martinez NM, Gilbert WV. Regulation and function of RNA pseudouridylation in human cells. *Annu Rev Genet* 2020;54:309–336.
[67] Karijolich J, Yi C, Yu YT. Transcriptome-wide dynamics of RNA pseudouridylation. *Nat Rev Mol Cell Biol* 2015;16(10):581–585.
[68] Penzo M, Guerrieri AN, Zacchini F, Trere D, Montanaro L. RNA pseudouridylation in physiology and medicine: for better and for worse. *Genes (Basel)* 2017;8(11):301.
[69] Liang XH, Liu Q, Fournier MJ. rRNA modifications in an intersubunit bridge of the ribosome strongly affect both ribosome biogenesis and activity. *Mol Cell* 2007;28(6):965–977.
[70] Lorenz C, Lunse CE, Morl M. tRNA modifications: impact on structure and thermal adaptation. *Biomolecules* 2017;7(2):35.
[71] Amos H, Korn M. 5-Methyl cytosine in the RNA of Escherichia coli. *Biochim Biophys Acta* 1958;29(2):444–445.
[72] Yang X, Yang Y, Sun BF, et al. 5-methylcytosine promotes mRNA export—NSUN2 as the methyltransferase and ALYREF as an m(5)C reader. *Cell Res* 2017;27(5):606–625.
[73] Bohnsack KE, Hobartner C, Bohnsack MT. Eukaryotic 5-methylcytosine (m(5)C) RNA methyltransferases: mechanisms, cellular functions, and links to disease. *Genes (Basel)* 2019;10(2):102.
[74] Delatte B, Wang F, Ngoc LV, et al. RNA biochemistry. Transcriptome-wide distribution and function of RNA hydroxymethylcytosine. *Science* 2016;351(6270):282–285.
[75] Shen Q, Zhang Q, Shi Y, et al. Tet2 promotes pathogen infection-induced myelopoiesis through mRNA oxidation. *Nature* 2018;554(7690): 123–127.
[76] Kawarada L, Suzuki T, Ohira T, Hirata S, Miyauchi K, Suzuki T. ALKBH1 is an RNA dioxygenase responsible for cytoplasmic and mitochondrial tRNA modifications. *Nucleic Acids Res* 2017;45(12): 7401–7415.
[77] Chen X, Li A, Sun BF, et al. 5-methylcytosine promotes pathogenesis of bladder cancer through stabilizing mRNAs. *Nat Cell Biol* 2019;21(8):978–990.
[78] Schosserer M, Minois N, Angerer TB, et al. Methylation of ribosomal RNA by NSUN5 is a conserved mechanism modulating organismal lifespan. *Nat Commun* 2015;6:6158.
[79] Dunn DB. The occurrence of 1-methyladenine in ribonucleic acid. *Biochim Biophys Acta* 1961;46:198–200.
[80] Hall RH. Isolation of 2'-O-methylribonucleosides from the RNA of mammalian tissues and from E. coli. *Biochem Biophys Res Commun* 1963;12:429–431.
[81] Bar-Yaacov D, Frumkin I, Yashiro Y, et al. Mitochondrial 16S rRNA Is methylated by tRNA methyltransferase TRMT61B in all vertebrates. *PLoS Biol* 2016;14(9):e1002557.
[82] Chujo T, Suzuki T. Trmt61B is a methyltransferase responsible for 1-methyladenosine at position 58 of human mitochondrial tRNAs. *RNA* 2012;18(12):2269–2276.
[83] Lei Y, Xu K, Li J, et al. Feasibility of space-division-multiplexed transmission of IEEE 802.11n/ac-compliant wireless MIMO signals over OM3 multimode fiber. *J Lightwave Technol* 2018;36(11): 2076–2082.
[84] Duncan T, Trewick SC, Koivisto P, Bates PA, Lindahl T, Sedgwick B. Reversal of DNA alkylation damage by two human dioxygenases. *Proc Natl Acad Sci U S A* 2002;99(26):16660–16665.
[85] Aas PA, Otterlei M, Falnes PO, et al. Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. *Nature* 2003;421(6925):859–863.
[86] Liu F, Clark W, Luo G, et al. ALKBH1-mediated tRNA demethylation regulates translation. *Cell* 2016;167(3):816–828.e16.
[87] Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S, et al. The dynamic N(1)-methyladenosine methylome in eukaryotic messenger RNA. *Nature* 2016;530(7591):441–446.
[88] Cheng JX, Chen L, Li Y, et al. RNA cytosine methylation and methyltransferases mediate chromatin organization and 5-azacytidine response and resistance in leukaemia. *Nat Commun* 2018;9(1): 1163.
[89] He H, Wang Y, Wang J. ALKBH3 is dispensable in maintaining hematopoietic stem cells but forced ALKBH3 rectified the differentiation skewing of aged hematopoietic stem cells. *Blood Sci* 2020;2(4):137–143.
[90] Liu J, Yue Y, Han D, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. *Nat Chem Biol* 2014;10(2):93–95.
[91] Ping XL, Sun BF, Wang L, et al. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. *Cell Res* 2014;24(2):177–189.
[92] Hu Y, Ouyang Z, Sui X, et al. Oocyte competence is maintained by m(6)A methyltransferase KIAA1429-mediated RNA metabolism during mouse follicular development. *Cell Death Differ* 2020;27(8): 2468–2483.
[93] Knuckles P, Lence T, Haussmann IU, et al. Zc3h13/Flacc is required for adenosine methylation by bridging the mRNA-binding factor Rbm15/Spenito to the m(6)A machinery component Wtap/Fl(2)d. *Genes Dev* 2018;32(5-6):415–429.
[94] Wen J, Lv R, Ma H, et al. Zc3h13 regulates nuclear RNA m(6)A methylation and mouse embryonic stem cell self-renewal. *Mol Cell* 2018;69(6):1028–1038.e6.

[95] Wang P, Doxtader KA, Nam Y. Structural basis for cooperative function of Mettl3 and Mettl14 methyltransferases. *Mol Cell* 2016;63(2):306–317.

[96] Jia G, Fu Y, Zhao X, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat Chem Biol* 2011;7(12):885–887.

[97] Zheng G, Dahl JA, Niu Y, et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. *Mol Cell* 2013;49(1):18–29.

[98] Xiao W, Adhikari S, Dahal U, et al. Nuclear m(6)A reader YTHDC1 regulates mRNA splicing. *Mol Cell* 2016;61(4):507–519.

[99] Hsu PJ, Zhu Y, Ma H, et al. Ythdc2 is an N(6)-methyladenosine binding protein that regulates mammalian spermatogenesis. *Cell Res* 2017;27(9):1115–1127.

[100] Theler D, Dominguez C, Blatter M, Boudet J, Allain FH. Solution structure of the YTH domain in complex with N6-methyladenosine RNA: a reader of methylated RNA. *Nucleic Acids Res* 2014;42(22): 13911–13919.

[101] Huang H, Weng H, Sun W, et al. Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. *Nat Cell Biol* 2018;20(3):285–295.

[102] Shi H, Wang X, Lu Z, et al. YTHDF3 facilitates translation and decay of N-methyladenosine-modified RNA. *Cell Res* 2017;27(3): 315–328.

[103] Du H, Zhao Y, He J, et al. YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. *Nat Commun* 2016;7:12626.

[104] Xu C, Wang X, Liu K, et al. Structural basis for selective binding of m6A RNA by the YTHDC1 YTH domain. *Nat Chem Biol* 2014;10(11):927–929.

[105] Zhang Z, Theler D, Kaminska KH, et al. The YTH domain is a novel RNA binding domain. *J Biol Chem* 2010;285(19):14701–14710.

[106] Roundtree IA, Luo G-Z, Zhang Z, et al. YTHDC1 mediates nuclear export of N-methyladenosine methylated mRNAs. *Elife* 2017;6:e31311.

[107] Kretschmer J, Rao H, Hackert P, Sloan KE, Höbartner C, Bohnsack MT. The mA reader protein YTHDC2 interacts with the small ribosomal subunit and the 5'-3' exoribonuclease XRN1. *RNA* 2018;24(10):1339–1350.

[108] Xu C, Liu K, Ahmed H, Loppnau P, Schapira M, Min J. Structural basis for the discriminative recognition of N6-methyladenosine RNA by the human YT521-B homology domain family of proteins. *J Biol Chem* 2015;290(41):24902–24913.

[109] Wojtas MN, Pandey RR, Mendel M, Homolka D, Sachidanandam R, Pillai RS. Regulation of mA transcripts by the 3'→5' RNA helicase YTHDC2 is essential for a successful meiotic program in the mammalian germline. *Mol Cell* 2017;68(2):374–387.e12.

[110] Feng M, Xie X, Han G, et al. YBX1 is required for maintaining myeloid leukemia cell survival by regulating BCL2 stability in an m6A-dependent manner. *Blood* 2021;138(1):71–85.

[111] Chai J, Wang Q, Qiu Q, et al. YBX1 regulates the survival of chronic myeloid leukemia stem cells by modulating m(6)A-mediated YWHAZ stability. *Cell Oncol (Dordr)* 2023;46(2):451–464.

[112] Zhou F, Li X, Wang W, et al. Tracing haematopoietic stem cell formation at single-cell resolution. *Nature* 2016;533(7604):487–492.

[113] Yokomizo T, Ideue T, Morino-Koga S, et al. Independent origins of fetal liver haematopoietic stem and progenitor cells. *Nature* 2022;609(7928):779–784.

[114] Zhang C, Chen Y, Sun B, et al. m(6)A modulates haematopoietic stem and progenitor cell specification. *Nature* 2017;549(7671): 273–276.

[115] Gao Y, Vasic R, Song Y, et al. m(6)A modification prevents formation of endogenous double-stranded RNAs and deleterious innate immune responses during hematopoietic development. *Immunity* 2020;52(6):1007–1021.e8.

[116] Yao QJ, Sang L, Lin M, et al. Mettl3-Mettl14 methyltransferase complex regulates the quiescence of adult hematopoietic stem cells. *Cell Res* 2018;28(9):952–954.

[117] Lee H, Bao S, Qian Y, et al. Stage-specific requirement for Mettl3-dependent m(6)A mRNA methylation during haematopoietic stem cell differentiation. *Nat Cell Biol* 2019;21(6):700–709.

[118] Cheng Y, Luo H, Izzo F, et al. m(6)A RNA methylation maintains hematopoietic stem cell identity and symmetric commitment. *Cell Rep* 2019;28(7):1703–1716.e6.

[119] Martin GH, Park CY. Meddling with METTLs in normal and leukemia stem cells. *Cell Stem Cell* 2018;22(2):139–141.

[120] Vu LP, Pickering BF, Cheng Y, et al. The N(6)-methyladenosine (m(6)A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. *Nat Med* 2017;23(11):1369–1376.

[121] Luo H, Cortes-Lopez M, Tam CL, et al. SON is an essential m(6)A target for hematopoietic stem cell fate. *Cell Stem Cell* 2023;30(12): 1658–1673.e10.

[122] Li Z, Qian P, Shao W, et al. Suppression of m(6)A reader Ythdf2 promotes hematopoietic stem cell expansion. *Cell Res* 2018;28(9): 904–917.

[123] Paris J, Morgan M, Campos J, et al. Targeting the RNA m(6)A reader YTHDF2 selectively compromises cancer stem cells in acute myeloid leukemia. *Cell Stem Cell* 2019;25(1):137–148.e6.

[124] Shen C, Sheng Y, Zhu AC, et al. RNA demethylase ALKBH5 selectively promotes tumorigenesis and cancer stem cell self-renewal in acute myeloid leukemia. *Cell Stem Cell* 2020;27(1):64–80.e9.

[125] Wang J, Li Y, Wang P, et al. Leukemogenic chromatin alterations promote AML leukemia stem cells via a KDM4C-ALKBH5-AXL signaling axis. *Cell Stem Cell* 2020;27(1):81–97.e8.

[126] Yin R, Chang J, Li Y, et al. Differential m(6)A RNA landscapes across hematopoiesis reveal a role for IGF2BP2 in preserving hematopoietic stem cell function. *Cell Stem Cell* 2022;29(1):149–159.e7.

[127] Wu Y, Xie L, Wang M, et al. Mettl3-mediated m(6)A RNA methylation regulates the fate of bone marrow mesenchymal stem cells and osteoporosis. *Nat Commun* 2018;9(1):4772.

[128] Gao L, Lee H, Goodman JH, Ding L. Hematopoietic stem cell niche generation and maintenance are distinguishable by an epitranscriptomic program. *Cell* 2024;187(11):2801–2816.e17.

[129] Barbieri I, Tzelepis K, Pandolfini L, et al. Promoter-bound METTL3 maintains myeloid leukaemia by m(6)A-dependent translation control. *Nature* 2017;552(7683):126–131.

[130] Li Z, Weng H, Su R, et al. FTO plays an oncogenic role in acute myeloid leukemia as a N(6)-Methyladenosine RNA demethylase. *Cancer Cell* 2017;31(1):127–141.

[131] Cheng Y, Gao Z, Zhang T, et al. Decoding m(6)A RNA methylome identifies PRMT6-regulated lipid transport promoting AML stem cell maintenance. *Cell Stem Cell* 2023;30(1):69–85.e7.

[132] Liu W, Wang Y, Yao S, et al. Reprogramming of RNA m6A modification is required for acute myeloid leukemia development. *Genomics Proteomics Bioinformatics* 2024;22:qzae049. Online ahead of print.

[133] Weng H, Huang F, Yu Z, et al. The m(6)A reader IGF2BP2 regulates glutamine metabolism and represents a therapeutic target in acute myeloid leukemia. *Cancer Cell* 2022;40(12):1566–1582.e10.

[134] Qing Y, Dong L, Gao L, et al. R-2-hydroxyglutarate attenuates aerobic glycolysis in leukemia by targeting the FTO/m(6)A/PFKP/LDHB axis. *Mol Cell* 2021;81(5):922–939.e9.

[135] Liu Y, Liang G, Xu H, et al. Tumors exploit FTO-mediated regulation of glycolytic metabolism to evade immune surveillance. *Cell Metab* 2021;33(6):1221–1233.e11.

[136] Su R, Dong L, Li Y, et al. Targeting FTO suppresses cancer stem cell maintenance and immune evasion. *Cancer Cell* 2020;38(1):79–96.e11.

[137] Guo W, Wang Z, Zhang Y, et al. Mettl3-dependent m(6)A modification is essential for effector differentiation and memory formation of CD8(+) T cells. *Sci Bull (Beijing)* 2024;69(1):82–96.

[138] Han D, Liu J, Chen C, et al. Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells. *Nature* 2019;566(7743):270–274.

[139] Yankova E, Blackaby W, Albertella M, et al. Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. *Nature* 2021;593(7860):597–601.