Advances in Experimental Medicine and Biology 1442

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# Hematopoietic Stem Cells

## Keystone of Tissue Development and Regenerative Medicine

Springer

# $N^6$-Methyladenosine RNA Modification in Normal and Malignant Hematopoiesis

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**Abstract**

Over 170 nucleotide variants have been discovered in messenger RNAs (mRNAs) and non-coding RNAs so far. However, only a few of them, including $N^6$-methyladenosine ($m^6A$), 5-methylcytidine ($m^5C$), and $N^1$-methyladenosine ($m^1A$), could be mapped in the transcriptome. These RNA modifications appear to be dynamically regulated, with writer, eraser, and reader proteins being identified for each modification. As a result, there is a growing interest in studying their biological impacts on normal bioprocesses and tumorigenesis over the past few years. As the most abundant internal modification in eukaryotic mRNAs, $m^6A$ plays a vital role in the post-transcriptional regulation of mRNA fate via regulating almost all aspects of mRNA metabolism, including RNA splicing, nuclear export, RNA stability, and translation. Studies on mRNA $m^6A$ modification serve as a great example for exploring other modifications on mRNA. In this chapter, we will review recent advances in the study of biological functions and regulation of mRNA modifications, specifically $m^6A$, in both normal hematopoiesis and malignant hematopoiesis. We will also discuss the potential of targeting mRNA modifications as a treatment for hematopoietic disorders.

**Keywords**

$m^6A$ RNA modifications · RNA epigenetics · Normal hematopoiesis · Malignant hematopoiesis · Targeted therapy

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## Abbreviations

| | |
|---|---|
| 2OG | 2-Oxoglutarate |
| 5-Aza | 5-Azacytidine |
| ALKBH5 | AlkB homolog 5 |
| ALL | Acute lymphoblastic leukemia |



M. Zhao, P. Qian (eds.), *Hematopoietic Stem Cells*, Advances in Experimental Medicine and Biology 1442, https://doi.org/10.1007/978-981-99-7471-9_7

| | |
|---|---|
| AML | Acute myeloid leukemia |
| Ara-C | Cytosine arabinoside |
| ATRA | All-trans retinoic acid |
| CLP | Common lymphoid progenitor |
| CML | Chronic myeloid leukemia |
| CMP | Common myeloid progenitor |
| CTCL | Cutaneous T-cell lymphoma |
| DAC | Decitabine |
| DLBCL | Diffuse large B-cell lymphoma |
| DNR | Daunorubicin |
| EHT | Endothelial-to-hematopoietic transition |
| ELAVL1 | ELAV-like RNA-binding protein 1 |
| FTO | Fat mass and obesity-associated |
| GMP | Granulocyte/macrophage progenitor |
| HNRNP | Heterogeneous nuclear ribonucleoprotein |
| HSCs | Hematopoietic stem cells |
| HSPCs | Hematopoietic stem and progenitor cells |
| IDH | Isocitrate dehydrogenase |
| IGF2BP | Insulin-like growth factor 2 mRNA-binding proteins |
| KH | K homology |
| lncRNA | Long non-coding RNA |
| LSCs/LICs | Leukemic stem cells/leukemia-initiating cells |
| $m^1A$ | $N^1$-Methyladenosine |
| $m^5C$ | 5-Methylcytidine |
| $m^6A$ | $N^6$-Methyladenosine |
| METTL14 | Methyltransferase-like 14 |
| METTL3 | Methyltransferase-like 3 |
| METTL5 | Methyltransferase-like 5 |
| MTase | Methyltransferase |
| MTC | Methyltransferase complex |
| NKTCL | Natural killer/T-cell lymphoma |
| R-2HG | R-2-Hydroxyglutarate |
| RBM15 | RNA-binding motif protein 15 |
| rRNA | Ribosomal RNA |
| SAM | S-Adenosyl-L-methionine |
| SNPs | Single-nucleotide polymorphisms |
| snRNA | Small nuclear RNA |
| TF | Transcription factor |
| TKI | Tyrosine kinase inhibitor |
| tRNA | Transfer RNA |
| VIRMA | Vir-like $m^6A$ methyltransferase associated |
| WTAP | Wilms' tumor 1-associating protein |
| YTH | YT521-B homology |
| ZC3H13 | Zinc finger CCCH domain-containing protein 13 |
| ZCCHC4 | Zinc finger CCHC-type-containing 4 |
| α-KG | α-Ketoglutarate |
| ψ | Pseudouridine |

## 7.1 Introduction

The first modified RNA nucleotide variant, pseudouridine (ψ), was discovered as the "fifth RNA nucleotide" in the 1950s (Davis and Allen 1957). Since then, over 170 types of RNA chemical modifications have been identified in both protein-coding and noncoding RNAs (Adams and Cory 1975; Alarcon et al. 2015b; Amort et al. 2013; Carlile et al. 2014; Charette and Gray 2000; Cohn and Volkin 1951; Deng et al. 2018c; Dunn 1961; El Yacoubi et al. 2012; Fu et al. 2014a; Hall 1963; Huang et al. 2020c; Huber et al. 2015; Krug et al. 1976; Roundtree et al. 2017a; Squires et al. 2012; Wei and Moss 1977). However, most of the previous research on RNA modification has focused on non-coding RNAs, such as transfer RNA (tRNA), ribosomal RNA (rRNA), and small nuclear RNA (snRNA). In 2011, Dr. He and colleagues reported that the $m^6A$ modification on mRNA can be reversibly removed by FTO (Jia et al. 2011). This groundbreaking discovery has revived the field of RNA modification research. Newly developed methods for isolating RNAs containing specific types of modified nucleosides, coupled with high-throughput sequencing, now enable the mapping of landscapes of RNA modifications, such as $m^6A$, $m^5C$, $m^1A$, inosine, and pseudouridine (ψ), in mRNA across various cellular contexts (Carlile et al. 2014; Dominissini et al. 2012, 2016; Huang et al. 2020a; Li et al. 2015; Lovejoy et al. 2014; Meyer et al. 2012; Safra et al. 2017; Schwartz et al. 2014a; Squires et al. 2012; Suzuki et al. 2015). Meanwhile, significant progress has been made, and efforts are ongoing to identify

regulators of RNA modifications (Huang et al. 2020c). Similar to modifications in histones and DNA methylation, reversible modifications in RNA involve three categories of regulatory proteins: "writer" proteins that deposit the modification, "eraser" proteins that remove the modification, and "reader" proteins that recognize the modification and mediate RNA fate decisions (Huang et al. 2020b).

The RNA $m^6A$ modification has attracted the most attention in this field over the past decade. The discovery of $m^6A$ modification can be traced back to the 1970s (Adams and Cory 1975; Krug et al. 1976; Wei and Moss 1977). It is now well acknowledged that $m^6A$ is the most prevalent and abundant internal modification in eukaryotic mRNA. With the development of an antibody-based pull-down coupled with high-throughput sequencing method, it has become clear that $m^6A$ modification in the transcriptome exhibits a unique pattern, showing an enrichment around the stop codons of mRNAs and a consensus sequence of RRACH (R = G or A; H = A, C, or U) (Dominissini et al. 2012; Meyer et al. 2012). Further investigation suggests that $m^6A$ marks are installed co-transcriptionally into nascent RNAs and that histone H3K36me3 modification, along with other regulatory factors, plays a critical role in determining the site selection for $m^6A$ deposition (Huang et al. 2019a). On the other hand, functional studies have demonstrated the involvement of $m^6A$ modification in controlling normal biological and pathological processes, including stem cell biology, tissue development, circadian rhythm, sex determination, tumorigenesis, and drug response (Alarcon et al. 2015b; Barbieri et al. 2017; Chen et al. 2015; Deng et al. 2018a, b, c; Dong et al. 2021; Geula et al. 2015; Huang et al. 2018, 2020b; Li et al. 2017b; Su et al. 2018; Vu et al. 2017; Wang et al. 2014b; Weng et al. 2018; Xiang et al. 2017; Zhang et al. 2017a, b; Zhao et al. 2014, 2017a, b; Zheng et al. 2013; Zhou et al. 2015). Here, we summarize recent advances in understanding the biological functions and regulation of $m^6A$ in both normal and malignant hematopoiesis. Additionally, we discuss the potential of targeting $m^6A$ modifications as a treatment for hematopoietic disorders.

## 7.2 Regulators of $m^6A$ Modification

The main "writer" of mRNA $m^6A$ modification is a large multicomponent methyltransferase complex (MTC), in which methyltransferase-like 3 (METTL3) and methyltransferase-like 14 (METTL14) proteins form a core heterodimer, while other components, including Wilms' tumor 1-associating protein (WTAP), vir-like $m^6A$ methyltransferase associated (VIRMA, also known as KIAA1429), RNA-binding motif protein 15 (RBM15), and zinc finger CCCH domain-containing protein 13 (ZC3H13), act as regulatory subunits to facilitate $m^6A$ installation in cells (Bokar et al. 1997; Guo et al. 2018; Knuckles et al. 2018; Liu et al. 2014; Patil et al. 2016; Ping et al. 2014; Schwartz et al. 2014b; Wang et al. 2014b; Wen et al. 2018). Although both METTL3 and METTL14 belong to the MT-A70 family of S-adenosyl-L-methionine (SAM)-dependent methyltransferases (MTases), structural studies suggest that METTL3 is the only catalytic subunit in the MTC, while METTL14 provides an RNA-binding scaffold that allosterically activates and enhances the catalytic activity of METTL3 (Sledz and Jinek 2016; Wang et al. 2016a, b). METTL16 was initially identified as a methyltransferase for several structured RNAs (e.g., U6 snRNA) and pre-mRNA (Brown et al. 2016; Mendel et al. 2018; Pendleton et al. 2017). However, it has been recently shown that METTL16 could also methylate hundreds of mRNA transcripts in the nucleus, in addition to its methyltransferase-independent role in the cytosol as a facilitator of translation-initiation (Su et al. 2022). Two other $m^6A$ writers, zinc finger CCHC-type-containing 4 (ZCCHC4) and methyltransferase-like 5 (METTL5), can independently catalyze $m^6A$ modifications on 28S and 18S ribosomal RNAs (rRNAs), respectively (Ma et al. 2019; Pinto et al. 2020; van Tran et al. 2019).

Fat mass and obesity-associated (FTO) and AlkB homolog 5 (ALKBH5) are two "eraser"

proteins that have been discovered so far to catalyze the removal of $m^6A$ modification. They both belong to the AlkB subfamily of the Fe(II)/2-oxoglutarate (2OG) dioxygenase superfamily. This subfamily requires α-ketoglutarate (α-KG) and molecular oxygen as co-substrates, as well as ferrous iron Fe(II) as a cofactor, to catalyze the oxidation and demethylation of a substrate (Gerken et al. 2007; Kurowski et al. 2003). FTO was identified as the first $m^6A$ demethylase that could demethylate $m^6A$ in both DNA and RNA in vivo (Jia et al. 2011). Later on, it was reported that FTO also demethylates $m^6A_m$ (Mauer et al. 2017), a modification exclusively found at the first encoded nucleotide after the 5′ methylguanosine cap of mammalian mRNAs but with considerably lower overall abundance compared to $m^6A$ (Su et al. 2018; Wei et al. 2018). Different from FTO, ALKBH5 catalyzes the direct removal of $m^6A$ (Fu et al. 2014b).

The "reader" proteins act as effectors that mediate the biological consequences of $m^6A$ modification by selectively binding to $m^6A$-modified RNAs (Deng et al. 2018c; Yang et al. 2018; Zhao et al. 2017a). Many $m^6A$ readers have been identified so far, each with diverse mechanisms for recognizing of $m^6A$ and resulting in various consequences on RNA metabolism. The YT521-B homology (YTH) domain family of proteins, including YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2, utilizes their conserved $m^6A$-binding pocket within the YTH domain to directly bind the $m^6A$ base (Dominissini et al. 2012; Hsu et al. 2017; Luo and Tong 2014; Wang et al. 2014a, 2015; Xiao et al. 2016; Xu et al. 2014; Zhu et al. 2014). Among them, YTHDF2 was the first identified and the most studied $m^6A$ reader protein that promotes the degradation of $m^6A$-modified target mRNAs (Du et al. 2016; Wang et al. 2014a). On the other hand, studies have shown that YTHDF1 promotes the translation of $m^6A$-modified mRNAs (Wang et al. 2015). In contrast, YTHDF3 and YTHDC2 can mediate mRNA decay while also enhancing translation (Bailey et al. 2017; Hsu et al. 2017; Jain et al. 2018; Li et al. 2017a; Shi et al. 2017; Wojtas et al. 2017). Unlike the reader proteins mentioned above that are located in the cytoplasm, YTHDC1 is primarily located in the nucleus. It plays a crucial role in regulating splicing, XIST-mediated X chromosome silencing, and nuclear export of $m^6A$-modified mRNAs (Patil et al. 2016; Roundtree et al. 2017b; Xiao et al. 2016). Additionally, it controls the integrity of heterochromatin by recognizing $m^6A$ modifications on transposon-derived RNAs (Chen et al. 2021a; Liu et al. 2021). In contrast to the YTH family of proteins, insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs), which include IGF2BP1/2/3, have been identified as a new family of $m^6A$ readers. These proteins promote stability and translation of their target mRNAs (Huang et al. 2018). IGF2BP proteins use their K homology (KH) domains (especially the KH3-4 di-domain) and possibly the flanking sequence to recognize $m^6A$. They play a crucial role in determining the fate of mRNA by recruiting mRNA stabilizers like ELAV-like RNA-binding protein 1 (ELAVL1), also known as HuR (Huang et al. 2018). Several heterogeneous nuclear ribonucleoproteins (HNRNPs) have also been reported as $m^6A$ readers. HNRNPC and HNRNPG have been shown to recognize $m^6A$-induced changes in mRNA secondary structures and facilitate alternative splicing of target mRNAs (Liu et al. 2015; Zhou et al. 2019). HNRNPA2B1 was previously shown to regulate alternative splicing and primary microRNA processing as an $m^6A$ reader (Alarcon et al. 2015a). However, a later study suggested a mechanism called "$m^6A$ switch" instead of direct binding to $m^6A$ (Wu et al. 2018). The list of $m^6A$ reader proteins is expanding, and other proteins are being proposed as $m^6A$ interactors, including FMR1 and LRPPRC (Arguello et al. 2017; Edupuganti et al. 2017). However, additional mechanistic studies are necessary to more accurately categorize these proteins.

## 7.3 $m^6A$ Modification in Normal Hematopoiesis

Hematopoiesis is defined as a tightly regulated process that produces mature blood cells from a

small pool of multipotent hematopoietic stem cells (HSCs) (Doulatov et al. 2012; Rosenbauer and Tenen 2007). Decades of research have provided basic knowledge on the regulation of normal hematopoiesis, highlighting a critical role of hematopoietic transcription factors (TFs) in regulating the multistep normal hematopoiesis and in determining cell fate in the hematopoietic system (Goode et al. 2016; Koschmieder et al. 2005; Rosmarin et al. 2005). For example, PU.1 (also known as SPI1) and C/EBPα are essential in generating early myeloid progenitors (i.e., common myeloid progenitors, CMPs) and granulocyte/macrophage progenitors (GMPs), respectively (Dakic et al. 2005; Rosenbauer and Tenen 2007), while PAX5 is required for the early development of the B-cell lineage (Mikkola et al. 2002). In addition, epigenetic regulatory mechanisms, including DNA methylation, histone modifications, and non-coding RNAs, have been shown to contribute to HSC homeostasis and normal hematopoiesis (Butler and Dent 2013; Challen et al. 2014; Chen et al. 2010; Guillamot et al. 2016; Moran-Crusio et al. 2011; O'Connell et al. 2010; Ooi et al. 2010; Weng et al. 2019). Emerging as a new type of epigenetic regulation, $m^6A$ RNA modification was demonstrated to be critical in governing HSC biology and hematopoiesis in recent years (Fig. 7.1).

### 7.3.1 METTL3

As the sole catalytic subunit in the $m^6A$ MTC, METTL3 has been extensively studied in the hematopoietic system across a range of species, from zebrafish to mammals. Decreased levels of $m^6A$ resulting from *mettl3* deficiency in zebrafish embryos cause blockage of HSPC emergence (Zhang et al. 2017a). Mechanistic studies have revealed that the reduction of $m^6A$ on *notch1a* mRNA suppresses YTHDF2-mediated mRNA decay, leading to the continuous activation of the Notch signaling in arterial endothelial cells. This results in the blockage of endothelial-to-hematopoietic transition (EHT) and the repression of the earliest HSPC generation in *mettl3*-deficient zebrafish embryos (Zhang et al. 2017a). This mechanism appears to be conserved in mice, as knockdown of *Mettl3* in the aorta-gonad-mesonephros impairs colony formation, likely also through activation of the Notch1 signaling pathway (Zhang et al. 2017a). Yao et al. discovered that conditional ablation of Mettl3 in the mouse hematopoietic system significantly increased the frequency of HSCs in the bone marrow and suppressed self-renewal capability of HSCs in recipient mice undergoing bone marrow transplantation (BMT) (Yao et al. 2018). Vu and colleagues reported that the knockdown of *METTL3* expression in human HSPCs inhibited cell growth and increased myeloid differentiation. On the other hand, overexpression of wild-type METTL3, but not its catalytically dead mutant, promoted proliferation and colony formation and inhibited myeloid differentiation (Vu et al. 2017).

### 7.3.2 METTL14

In mouse bone marrow, *Mettl14* was found to be highly expressed in HSCs and $Lin^-Sca\text{-}1^+c\text{-}kit^+$ (LSK) cells and be responsible for the high $m^6A$ level in these naïve cells (Weng et al. 2018). Notably, the expression of *Mettl14* was gradually downregulated during myelopoiesis, with the lowest expression observed in mature myeloid cells (Weng et al. 2018). Consistent with this expression pattern, the knockdown of *METTL14* in human HSPCs promoted myeloid differentiation in vitro. Moreover, conditional knockout of *Mettl14* in donor cells impaired the self-renewal ability of HSCs in the BMT recipient mice (Weng et al. 2018; Yao et al. 2018). Interestingly, SPI1, which is a transcriptional master regulator of myelopoiesis, was identified as a negative regulator that controls the transcription of *METTL14* in the hematopoietic system (Weng et al. 2018). Considering the role of SPI1 (Iwasaki et al. 2005), MYB (Mucenski et al. 1991; Sandberg et al. 2005), and MYC (Satoh et al. 2004; Wilson et al. 2004) transcription factors in regulating HSC self-renewal and differentiation, the SPI1-METTL14-$m^6A$-MYB/MYC regulation axis

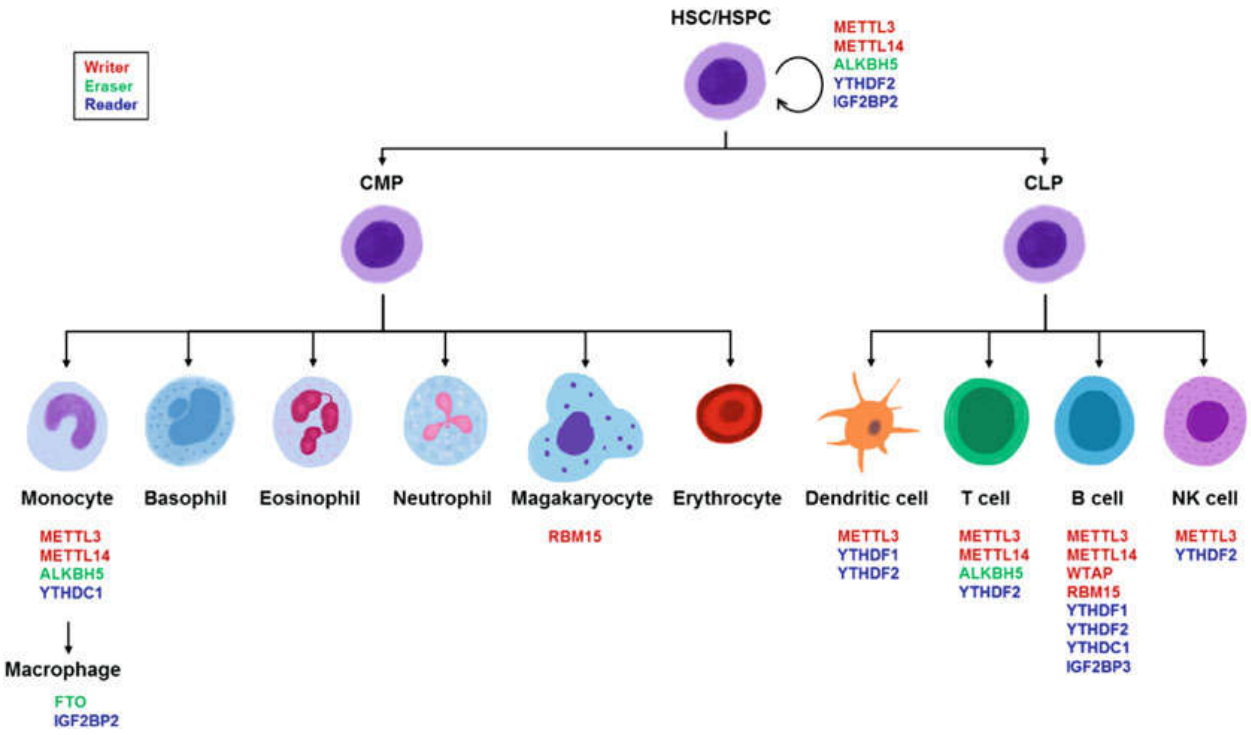


**Fig. 7.1** The involvement of m$^6$A regulators in normal hematopoiesis. The m$^6$A modification and its associated m$^6$A regulators have been demonstrated to be crucial for the self-renewal of HSCs/HSPCs and for the proper development of blood cells. Writers are represented in red, erasers in green, and readers in blue

adds a new layer of complexity to the regulatory networks involved in normal hematopoiesis.

### 7.3.3 RBM15

*RBM15*, also known as *OTT1*, was initially identified as a fusion partner of the *MKL1* gene detected in infant acute megakaryocytic leukemia with t(1;22)(p13;q13). RBM15 was recently identified as a component of the m$^6$A MTC. Early studies have shown that conditional knockout of Rbm15 resulted in an increase in HSPCs and an expansion of myeloid and megakaryocytic cells in the spleen and bone marrow while blocking B-cell differentiation (Raffel et al. 2007). Ma et al. found that Rbm15 was expressed at the highest levels in HSCs and inhibited myeloid differentiation and megakaryocytic expansion by stimulating the Notch signaling (Ma et al. 2007). Consistently, Rbm15 is required for normal interactions between HSCs and their niche, as well as for normal megakaryocyte development, at least in part through the regulation of *MYC* expression (Niu et al. 2009).

### 7.3.4 ALKBH5

Two independent studies reported that ALKBH5 is dispensable for normal hematopoiesis (Shen et al. 2020; Wang et al. 2020). Using the Alkbh5 constitutive knockout mouse model, researchers demonstrated that Alkbh5 deletion did not result in significant changes in the total number of bone marrow cells or the percentages of different subpopulations of HSPCs or differentiated lineages in either bone marrow or peripheral blood. Moreover, the knockout of Alkbh5 did not affect the self-renewal capacity or the long-term function of HSCs (Shen et al. 2020; Wang et al. 2020).

### 7.3.5 YTHDF2

YTHDF2 is the first well-characterized m$^6$A reader that promotes the degradation of mRNA transcripts containing m$^6$A modification (Wang et al. 2014a). Li et al. reported a remarkable increase in functional HSCs in the bone marrow of *Ythdf2* conditional knockout mice and in human umbilical cord blood upon *YTHDF2* knockdown, which was at least partially attributed to the enhanced stability of mRNA transcripts that encode TFs critical for stem cell self-renewal (Li et al. 2018). It was also reported by another group that deficiency in YTHDF2 enhanced HSC activity, and YTHDF2 did not appear to be essential for normal HSC function (Paris et al. 2019). However, the same group recently investigated the long-term effects of YTHDF2 deletion on HSC maintenance and multi-lineage hematopoiesis, and the results suggest that YTHDF2 acts as a repressor of inflammatory pathways in HSCs and is a key factor for long-term HSC maintenance (Mapperley et al. 2021).

### 7.3.6 YTHDC1

Loss of Ythdc1 resulted in rapid hematopoietic failure in mice, leading to their death within 3 weeks (Sheng et al. 2021). Further studies have shown that induced deletion of Ythdc1 compromises hematopoiesis and HSC functions. The numbers of HSPCs, mature myeloid cells, and B cells all decrease dramatically in Ythdc1 KO recipient mice compared to Ythdc1 haploinsufficient or wild-type recipient mice (Sheng et al. 2021).

## 7.4 m$^6$A Modification in Malignant Hematopoiesis

The pioneering work by Ernest McCulloch and James Till in identifying HSC and characterizing their properties strongly suggests that maintaining HSC homeostasis requires a precise balance between self-renewal and multi-lineage differentiation (McCulloch and Till 2005; Siminovitch et al. 1963). Disrupting this balance, whether through the well-studied mutations or aberrant expression of TFs or through dysregulation of epigenetic modifications, places HSC at a higher risk of developing hematopoietic diseases, such as leukemia (Chen et al. 2010; Huang et al. 2013; Qing et al. 2021; Weng et al. 2019). Dysregulation of m$^6$A regulators has been observed in hematopoietic malignancies (Fig. 7.2).

### 7.4.1 Acute Myeloid Leukemia (AML)

AML is a common subtype of leukemia that is commonly diagnosed in both adults and children. Unfortunately, it has the lowest 5-year survival rate (<30%) among all types of leukemia. This makes AML a fatal subtype of leukemia. Accumulating evidence in the past few years has closely linked RNA m$^6$A modification with the initiation and development of AML.

*FTO* is the first gene known to play a role in malignant hematopoiesis, specifically AML, through an m$^6$A-dependent mechanism (Li et al. 2017b). Previously, *FTO* was recognized as a gene related to fat mass, adipogenesis, and body weight (Church et al. 2010; Fischer et al. 2009; Merkestein et al. 2015). However, recent large-scale epidemiology studies have revealed a connection between FTO and cancers such as leukemia and lymphoma, as evidenced by single-nucleotide polymorphisms (SNPs) in *FTO* among patients (Castillo et al. 2012; Hernandez-Caballero and Sierra-Ramirez 2015; Soderberg et al. 2009). We found that *FTO* is highly expressed in AML subtypes carrying t(11q23)/*MLL* rearrangements, t(15;17)/*PML-RARA*, *FLT3*-ITD, and/or *NPM1* mutations (Li et al. 2017b). Functioning as an m$^6$A eraser, FTO removes m$^6$A marks from the transcripts of *ASB2* and *RARA*, two genes with reported roles in myeloid cell differentiation and drug response of leukemia cells. This results in decreased mRNA stability of *ASB2* and *RARA*, therefore promoting AML cell survival and leukemogenesis (Li et al.

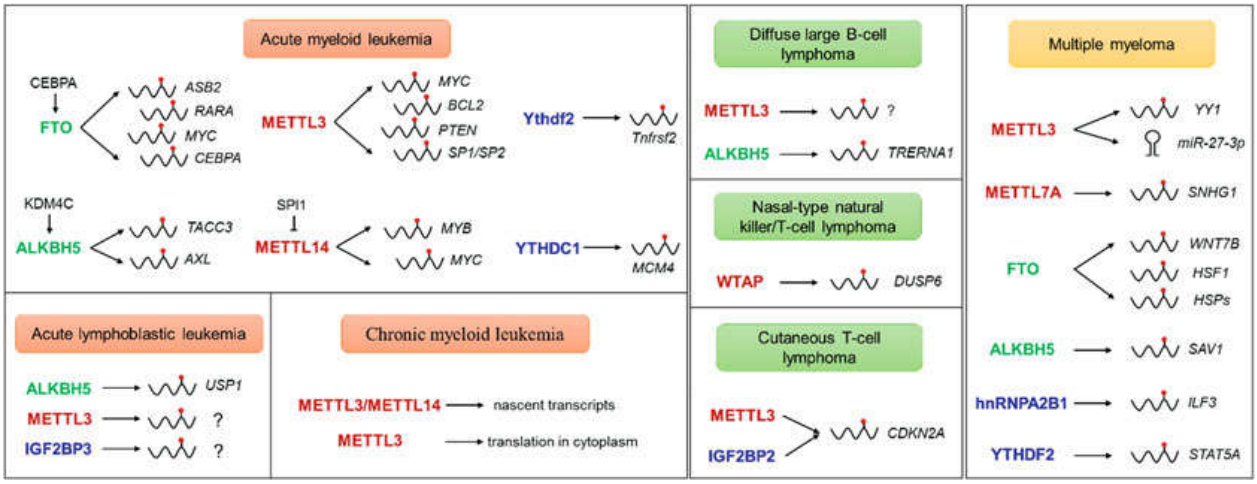


**Fig. 7.2** Roles and mechanisms of m$^6$A regulators in malignant hematopoiesis. Deregulation of m$^6$A regulators has been identified in various hematopoietic disorders, including leukemia (acute myeloid leukemia, acute lymphoblastic leukemia, and chronic myeloid leukemia), lymphoma (diffuse large B-cell lymphoma, nasal-type natural killer/T-cell lymphoma, and cutaneous T-cell lymphoma), and multiple myeloma. Although some studies have yet to characterize the m$^6$A-modified target transcripts responsible for the function of m$^6$A regulators, critical target transcripts regulated by m$^6$A modification have been identified and illustrated in such hematopoietic malignancies

2017b). Interestingly, it was later discovered that FTO mediates the anti-leukemia activity of R-2-hydroxyglutarate (R-2HG), a metabolite produced in high levels by mutant isocitrate dehydrogenase 1/2 (IDH1/2) enzymes and that was previously reported to cause leukemia (Losman et al. 2013; Sasaki et al. 2012; Wang et al. 2013). R-2HG binds directly to FTO to inhibit its demethylase activity, leading to the hypomethylation and accelerated decay of *MYC* and *CEBPA* mRNAs (Su et al. 2018). Moreover, treatment with R-2HG or inhibition of FTO could sensitize AML cells to first-line chemotherapy drugs (Su et al. 2018). These studies collectively suggest that targeting FTO signaling could be a promising therapeutic approach for AML.

As another known m$^6$A eraser, *ALKBH5* has been found to be aberrantly overexpressed in AML, and its increased expression is associated with poor prognosis in AML patients (Shen et al. 2020; Wang et al. 2020). In line with this, *ALKBH5* was preferentially overexpressed in LSCs, and its m$^6$A demethylation activity was necessary for the survival of AML cells both in vitro and in vivo. Mechanistic study revealed that KDM4C depletion of the repressive histone mark H3K9me3 around the *ALKBH5* locus increases chromatin accessibility, resulting in the overexpression of *ALKBH5* in leukemia cells (Wang et al. 2020). *TACC3* and *AXL* have been identified as functional targets of ALKBH5. The stability of mRNA for both targets decreased when ALKBH5 was deleted. Importantly, loss of function of either *TACC3* or *AXL* could mimic the effects of ALKBH5 depletion, while the forced expression of *TACC3* or *AXL* could at least partially rescue the growth inhibition or clonogenic defect caused by ALKBH5 deficiency (Shen et al. 2020; Wang et al. 2020). Considering that ALKBH5 is dispensable for normal hematopoiesis, the essential role of ALKBH5 in AML cells and LSCs may represent a therapeutic vulnerability.

In AML, the oncogenic roles of METTL3 and METTL14 as m$^6$A writers have been reported by us and other groups. Both METTL3 and METTL14 were highly expressed in AML compared to the vast majority of other cancer types (Barbieri et al. 2017; Vu et al. 2017; Weng et al. 2018). METTL3 promoted AML cell proliferation and AML development in an m$^6$A-dependent manner by methylating its target transcripts, including *MYC*, *BCL2*, and *PTEN* (Vu et al. 2017). In addition, it has been reported that

METTL3 can be recruited to chromatin by CEBPZ, a transcription factor critical for hematopoietic differentiation (Barbieri et al. 2017). Promoter-bound METTL3 introduces m$^6$A modifications within the coding region of associated mRNA transcripts, such as *SP1* and *SP2*, and enhances their translation by relieving ribosome stalling (Barbieri et al. 2017). We found that METTL14 is aberrantly overexpressed in certain AML subtypes, including those carrying t(11q23)/*MLL* rearrangements, t(15;17)/*PML-RARA*, and t(8;21)/*AML1-ETO*, and that similar to *METTL3*, *METTL14* could be upregulated by corresponding oncofusion genes (Weng et al. 2018). In vitro and in vivo gain- and loss-of-function studies have demonstrated that METTL14 plays a critical oncogenic role in the initiation and development of AML (Weng et al. 2018). Importantly, *MYB* and *MYC*, two well-known TFs involved in leukemogenesis, were found to be direct targets of METTL14 and mediate the effect of *METTL14* knockdown/knockout in AML (Weng et al. 2018). Furthermore, we have identified SPI1 as a negative regulator of *METTL14* expression in both HSCs and AML cells (Weng et al. 2018). Taken together, these findings highlight the critical roles of the m$^6$A MTC core and m$^6$A modification in malignant hematopoiesis.

In addition to erasers and writers, the roles of m$^6$A readers have also been investigated in AML. *YTHDF2* has recently been proposed as another potential therapeutic target for AML. *YTHDF2* was found to be overexpressed in a wide range of human AML cases, and its expression was found to be correlated with LSC activity (Paris et al. 2019). Depletion of Ythdf2 selectively compromises AML initiation and propagation by extending the half-life of diverse m$^6$A-containing transcripts that contribute to the overall integrity of LSC function, including the tumor necrosis factor receptor Tnfrsf2 (Paris et al. 2019). In AML with t(8;21), expression of YTHDF2 was upregulated by the AML1/ETO-HIF1 alpha loop, and high expression of YTHDF2 was associated with a higher risk of relapse and inferior relapse-free survival (Chen et al. 2021b). YTHDC1 was found to be significantly upregulated in AML samples compared to healthy controls and is required for the growth of AML cells in vitro and AML development in vivo (Sheng et al. 2021). Mechanistic studies have revealed that YTHDC1 can stabilize mRNAs (e.g., MCM4) of cell cycle-associated genes to support the proliferation of malignant cells (Sheng et al. 2021). IGF2BP2 has recently been shown to be aberrantly overexpressed in AML cells, especially in LSCs, likely due to transcriptional activation by the AML-associated oncoproteins, especially MLL fusions. By recognizing m$^6$A modifications on key transcripts in the glutamate metabolism pathway, such as *MYC*, *SLC1A5*, and *GPT2*, IGF2BP2 stabilizes these mRNAs and enhances their translation. As a result, IGF2BP2 supports the high demand for glutamate in AML cells, thereby promoting the initiation and development of AML (Weng et al. 2022).

### 7.4.2 Acute Lymphoblastic (or Lymphocytic) Leukemia (ALL)

ALL is a type of cancer that originates in the bone marrow. It is characterized by the abnormal proliferation of early lymphoid precursors, which replace the normal hematopoietic cells in the marrow. ALL accounts for approximately 80% of childhood leukemia. Although the 5-year survival rate has increased to over 70% due to significant advances in treatment protocols in recent years, recurrence still occurs in 15–20% of cases.

High expression of USP1 is correlated with poor prognosis in T-ALL patients. It mediates T-ALL chemoresistance by interacting with and deubiquitinating Aurora B. It has recently been discovered that ALKBH5 can enhance the expression of USP1 by decreasing the m$^6$A level and mRNA stability of *USP1* mRNA. Therefore, inhibiting ALKBH5 reduced USP1 levels and improved glucocorticoid resistance in T-ALL cells by suppressing Aurora B expression (Gong et al. 2021). This suggests a new therapeutic approach for ALL.

Other regulators of m$^6$A modification have been shown to be dysregulated in ALL. For example, a study by Sun et al. found that ETV6/RUNX1-positive B-ALL had low expression of METTL3, which was associated with a high recurrence rate (Sun et al. 2019). However, it has yet to be investigated whether these regulators play a role in ALL.

### 7.4.3 Chronic Myeloid Leukemia (CML)

CML is a type of myeloproliferative neoplasm that is characterized by the presence of the Philadelphia chromosome and the resulting BCR-ABL1 oncofusion protein. Although tyrosine kinase inhibitors (TKIs) have been developed to successfully block the enzymatic activity of BCR-ABL1, these medications must be taken for life to keep the disease under control (Ben-Neriah et al. 1986; Druker et al. 2006, 1996; Goldman and Melo 2001; Rowley 1973). In addition, resistance to TKIs remains a challenge in curing CML patients. Therefore, further mechanistic studies are necessary to develop improved therapeutic options for CML.

Recently, Ianniello and colleagues found that the expression of METTL3/METTL14 was upregulated in CML patients. This upregulation was required for the proliferation of primary CML cells or CML cell lines, regardless of their sensitivity to the first-generation TKI imatinib (Ianniello et al. 2021). They further proposed a model in which METTL3/METTL14 modifies nascent transcripts in the nucleus, while METTL3 promotes translation of certain transcripts in the cytoplasm independent of its catalytic activity, highlighting a role of METTL3/METTL14 in CML and suggesting that inhibiting the METTL3/METTL14 complex could hold therapeutic potential for CML cells that have escaped from TKI treatment. Another group has reported that the downregulation of the lncRNA nuclear-enriched abundant transcript 1 (NEAT1) in CML is partly attributed to the METTL3-mediated m$^6$A modification, which in turn promotes the progression of CML (Yao et al. 2021).

### 7.4.4 Lymphoma

Expression of METTL3, as well as m$^6$A level, was found to be upregulated in diffuse large B-cell lymphoma (DLBCL), the most common subtype of lymphoma (Cheng et al. 2020). By regulating the m$^6$A level of pigment epithelium-derived factor (PEGF), METTL3 promotes the proliferation of DLBCL cells in vitro and the progression of DLBCL cells in nude mice (Cheng et al. 2020). ALKBH5 mediates the demethylation of lncRNA *TRERNA1*, promoting its expression in DLBCL. *TRERNA1* acts as a scaffold, recruiting EZH2 to epigenetically silence p21, thereby contributing to DLBCL cell proliferation both in vitro and in vivo (Song et al. 2022). In contrast to that in DLBCL, the expression of METTL3 was significantly downregulated in cutaneous T-cell lymphoma (CTCL) cells. The decline of METTL3 resulted in reduced methylation on *CDKN2A* mRNA, which in turn blocked the interaction between IGF2BP2 and *CDKN2A*, leading to the degradation of *CDKN2A* mRNA transcripts and the proliferation and migration of CTCL cells (Wang et al. 2022a). In nasal-type natural killer/T-cell lymphoma (NKTCL), WTAP increases the m$^6$A level of the dual-specificity phosphatase 6 (*DUSP6*) mRNA transcript, leading to enhanced mRNA stability of *DUSP6*. This contributes to the progression of NKTCL and chemotherapy resistance to cisplatin (Ma et al. 2021).

### 7.4.5 Multiple Myeloma (MM)

Studies on RNA m$^6$A modification in MM have emerged in the past 2 years. METTL3 has been reported to be upregulated in MM and to promote tumorigenesis by enhancing the stability of *Yin Yang 1* (*YY1*) mRNA and facilitating the maturation of primary miR-27a-3p in an m$^6$A-dependent manner (Che et al. 2022). METTL7A was

identified as an RNA methyltransferase in MM cells. It mediates $m^6A$ methylation of lncRNAs LOC606724 and SNHG1, promoting their packaging into adipocyte exosomes (Wang et al. 2022b). The increased levels of lncRNA in MM cells have been found to be positively correlated with poor prognosis in MM patients (Wang et al. 2022b). Isocitrate dehydrogenase 2 (IDH2) activates FTO in MM, which leads to a decrease in $m^6A$ levels on *WNT7B* mRNA transcripts, resulting in the increase in WNT7B expression and the activation of the Wnt signaling pathway to promote tumorigenesis and progression of MM (Song et al. 2021). Upregulation of FTO has been reported by another group to be responsible for the decreased $m^6A$ level in plasma cells from MM patients (Xu et al. 2022). FTO promotes MM cell proliferation, migration, and invasion by targeting HSF1/HSPs in a YTHDF2-dependent manner. Additionally, inhibiting FTO showed a synergistic effect with bortezomib treatment in inhibiting extramedullary myeloma formation. These findings highlight the FTO-HSF1/HSP axis as a potential therapeutic target in MM (Xu et al. 2022). The other $m^6A$ eraser, ALKBH5, was also found to play a role in MM. Yu and colleagues found that ALKBH5 was highly expressed in $CD138^+$ plasma cells from MM patients. They also found that reducing ALKBH5 expression impeded MM cell survival and invasion, most likely through restoring the $m^6A$ level of the *SAV1* transcript (Yu et al. 2022). HNRNPA2B1 has been found to be elevated in MM patients and is associated with poor prognosis. By recognizing $m^6A$ modifications on *ILF3* mRNA, HNRNPA2B1 stabilizes *ILF3* mRNA and promotes *AKT3* expression in an ILF3-dependent manner (Jiang et al. 2021). The same group also reported an oncogenic role of YTHDF2 in MM, in which YTHDF2 destabilizes its target transcript *STAT5A* in an $m^6A$-dependent manner to activate the ERK signaling pathway (Hua et al. 2022).

## 7.5 Targeting of $m^6A$ Modification in Malignant Hematopoiesis

Given the significant roles of $m^6A$ regulators in hematopoietic malignancies, there has been a recent development of inhibitors that target these $m^6A$ regulators. As the first $m^6A$ regulator linked to malignant hematopoiesis, FTO has become the most attractive target for developing inhibitors to treat hematopoietic malignancies (Deng et al. 2018b). R-2HG, a major metabolic product of mutant IDH1/2, has been found to inhibit the $m^6A$ demethylase activity of FTO and thereby exhibit anti-leukemia efficacy both in vitro and in vivo (Su et al. 2018). In addition, R-2HG could sensitize AML cells to a range of first-line therapeutic agents, including all-trans retinoic acid (ATRA), 5-azacytidine (5-Aza), decitabine (DAC), daunorubicin (DNR), and cytosine arabinoside (Ara-C) (Su et al. 2018). More potent FTO inhibitors, such as FB23-2, CS1 (also known as bisantrene), and CS2 (also known as brequinar), have been developed later. These inhibitors have shown remarkable suppression of AML progression in xeno-transplanted mice, including PDX AML models (Huang et al. 2019b; Su et al. 2020). Recently, inhibitors of METTL3 have also been reported. Among them, STM2457 has been shown to be a highly potent and selective first-in-class catalytic inhibitor of METTL3 and could specifically target stem cell subpopulations of AML (Yankova et al. 2021). In addition to targeting $m^6A$ erasers and writers, inhibitors for $m^6A$ readers have recently been developed. An inhibitor named CWI1-2, which targets IGF2BP2, has been developed and shown to suppress glutamate metabolism and inhibit the growth of AML cells both in vitro and in vivo (Weng et al. 2022). Such findings demonstrate that $m^6A$ regulators hold promise as therapeutic targets for the treatment of hematopoietic malignancies.

## 7.6 Conclusions and Perspectives

In the past few years, it has been shown that RNA $m^6A$ modification plays critical roles in almost every type of cancers, particularly in hematopoietic malignancies. However, whether the level of total cellular $m^6A$ correlates with cancer states remains controversial in the field of RNA cancer epigenetics. In leukemia studies, evidence is accumulating that the abundance of $m^6A$ on specific target transcripts, as well as on special target sites of certain target transcripts with important functions, is important for AML pathogenesis, rather than the total level of cellular $m^6A$ (Deng et al. 2018c). This could explain the oncogenic roles of METTL3/METTL14 and FTO/ALKBH5, which represent $m^6A$ writers and erasers, respectively, in AML. It suggests that tipping the balance of methylation and demethylation toward either direction could lead to deleterious effects for cancers. This information could be utilized for the development of novel targeted therapies for hematopoietic malignancies and other cancer types. Future investigations should focus on identifying critical $m^6A$ sites on specific target transcripts. This will enable the manipulation of a single $m^6A$ site for cancer therapy, rather than influencing the total cellular $m^6A$ level. In addition, it is still unclear how various $m^6A$ reader proteins recognize $m^6A$ sites within the same cellular context and how this impacts the development of cancer.

**Acknowledgment** This work was supported in part by the National Key R&D Program of China 2020YFA0112403 (H.W.) from the Ministry of Science and Technology of the People's Republic of China, 82173058 (H.H.) and 82270168 (H.W.) from the National Natural Science Foundation of China, 2021A1515010425 (H.H.) from the Guangdong Basic and Applied Basic Research Foundation, the Pearl River Talent Recruitment Program 2021ZT09Y233 (H.W.), and the National Institutes of Health (NIH) grants R01 CA271497 (J.C.), R01 CA243386 (J.C.), R01 CA236399 (J.C.), and DK124166 (J.C.), as well as the Simms/Mann Family Foundation (J.C.). J.C. is a Leukemia & Lymphoma Society (LLS) scholar.

**Competing Financial Interests** All the authors have no competing interests.

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