The Role of m6A Methylation in Stem Cell Regulation

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Abstract. 6-Methyladenosine (m6A) methylation is one of the most prevalent post-transcriptional RNA modifications playing a critical role in regulating stem cells. And this process is dynamically controlled by a group of proteins --- methyltransferases (writers), demethylases (erasers), and m6A binding proteins (readers) --- to drive various cellular processes, including RNA stability, splicing, and translation and so on. This review article mainly summarizes the specific roles that m6A modification has in different stem cells, such as the key functions of self-renewal, differentiation, and tissue reparation. It is also emphasized that in embryonic stem cells (ESCs), m6A is essential for balancing pluripotency and differentiation, and its dysregulation leads to impaired development. Similarly, in hematopoietic stem cells (HSCs), m6A regulates differentiation towards myeloid or lymphoid lineages. Abnormal m6A signaling has also been linked to diseases such as acute myeloid leukemia (AML), which affects leukemogenesis. And by the extensive impact of m6A modification on the regulation of stem cells, m6A is instead proposed as a prospective target for therapeutic intervention in regenerative techniques and cancer. The aims of further work is to elucidate the molecular mechanisms behind the role of m6A in stem cells, searching for potential clinical applications such as enhancing stem cell therapy and improving the efficiency of techniques such as somatic cell nuclear transplantation.

Keywords: m6A; RNA methylation; stem cells; cancer; hematopoietic stem cells; embryonic stem cells.

1. Introduction

RNA methylation is one of the most abundant post-transcriptional modifications, serving as an epigenetic alteration that does not change the underlying gene sequence. It occurs across all types of RNA and in various kingdoms of life, including archaea, bacteria, and eukaryotes. Multiple forms of RNA methylation exist, including N6-methyladenosine (m6A), 5-methylcytosine (m5C), N3-methylcytosine (m3C), N1-methyladenosine (m1A), and N7-methylguanosine (m7G), all of which represent potential therapeutic targets for various diseases. These modifications play diverse roles in regulating RNA stability, immune responses, and antibiotic susceptibility, among others. This article primarily discusses the most prevalent form of RNA methylation, m6A [1].

m6A has emerged as a critical focus of study due to its significant role in gene expression regulation. It is a chemical derivative of adenosine found in RNA, predominantly occurring in eukaryotes within the RRACH motif (where R denotes A or G, and H encompasses A, U, and C). m6A is abundantly located around termination codons, untranslated regions, and within internal exons, marking it as the most widespread internal mRNA modification in mammals, present at tens of thousands of sites throughout the transcriptome, constituting 0.15-0.6% of all adenosines [2]. Next-generation sequencing (NGS) has elucidated the distribution of m6A in the transcriptome, revealing an average of 3-5 m6A modifications per mRNA across one-third of mammalian mRNAs. m6A modifications are primarily concentrated in the 3’ untranslated region (3’ UTR) and near the stop codon of mature polyadenylated mRNAs, playing a pivotal regulatory role in eukaryotic transcription [3].

Further investigations into the biological functions and molecular mechanisms underlying m6A RNA methylation, alongside its regulators and downstream target genes, have indicated its critical yet varied roles in embryonic development, hematopoiesis, the central nervous system, reproductive systems, and cardiomyogenic differentiation. In embryonic stem cells, m6A regulation is closely associated with self-renewal, while in adult stem cells, it appears to influence differentiation and

tissue repair processes. Additionally, the expression and activity of m6A-related enzymes (e.g., METTL3, FTO, and ALKBH5) can vary with developmental stages and environmental factors, thus impacting stem cell fate. Consequently, m6A is also considered a potential target for cancer treatment. This paper will delve into the role of RNA methylation in various types of stem cells and explore its therapeutic potential in related diseases.

2. Molecular Mechanisms of m6A Modification

Several proteins serve as key players in m6A RNA biology, categorized as writers, erasers, and readers. m6A is installed in mRNA by methyltransferases (writers), removed by demethylases (erasers), and recognized by binding proteins (readers), collectively influencing the fate of RNA by regulating aspects such as decay, turnover, splicing, transport, and translation shown in Fig. 1. This interplay makes m6A methylation a reversible and dynamically controlled process.

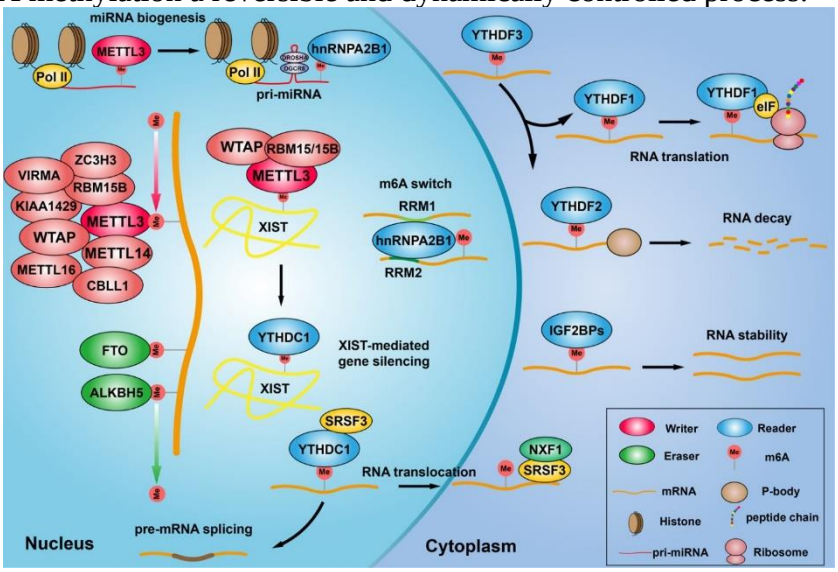

Fig. 1 The processes of m6A methylation with various proteins and functions [12].

2.1. Writers

Methyltransferases, or writers, are responsible for depositing m6A RNA modifications. They consist of three core components: METTL3, METTL14, and cofactors like Wilm's tumor-1-associated protein (WTAP) and RNA binding motif protein 15 (RBM15). METTL3 and METTL14 form a heterodimeric complex, with METTL3 acting as the prominent catalytic subunit, adding a methyl group (-CH3) to the sixth position of adenine in RNA. While METTL4 lacks enzymatic activity, it serves as an adapter protein that stabilizes the complex and enhances RNA binding by recognizing the substrate. WTAP functions as a scaffolding protein, facilitating the efficient targeting of mRNA substrates for methylation [5]. METTL16, an independent methyltransferase, can methylate specific RNA motifs and regulate the activity of other methyltransferases [6].

2.2. Erasers

Erasers are demethylases that remove m6A modifications, working alongside writers to maintain a dynamic balance within cells [7]. The two known m6A demethylases are fat mass and obesity-associated protein (FTO) and alkylation repair homolog protein 5 (ALKBH5). FTO, first identified for its association with obesity, demethylates m6A marks on transcripts such as RUNX1T1, thereby influencing differentiation. It affects mRNA stability, splicing, and translation, and plays roles in energy homeostasis, adipogenesis, and brain development. FTO has also been shown to bind various RNA species, including mRNAs and tRNAs, impacting internal and cap modifications. Dysregulation of FTO is linked to obesity, cancer, and neurodevelopmental disorders. ALKBH5 shares similar functions, particularly in spermatogenesis and stem cell differentiation, and its knockdown increases

nuclear m6A levels while decreasing cytoplasmic mRNA amounts, suggesting its role in mRNA transport [8].

2.3. Readers

m6A binding proteins, or readers, are crucial for recognizing m6A-modified RNA. These include direct binding proteins such as the YTH domain family, IGF2BPs, and eIF3, as well as indirect binding proteins like the hnRNP family. Readers regulate gene expression through various mechanisms, including mRNA stability, splicing, structure, export, translation efficiency, and miRNA biogenesis. Moreover, different proteins are responsible for different functions shown in Table 1. For instance, YTHDC1 recruits splicing factor SRSF3 and inhibits SRSF10 binding [9]. In the cytoplasm, YTHDF1, YTHDF2, and YTHDF3 exhibit distinct roles: YTHDF1 enhances translation, while YTHDF2 promotes mRNA decay. YTHDC2 is critical during spermatogenesis, facilitating the transition from mitosis to meiosis, wherease IGF2BP stabilizes mRNA by binding to m6A-modified motifs. The effects of these reader proteins can vary based on the cellular environment, sometimes producing opposing outcomes [10, 11].

Table 1. Functions of m6A regulators in RNA metabolism [4]

Regulator	Function	Role in cancers
KIAA1429	m6A writer; Recruits and mediates the binding of methyltransferase and specific RNA site	DNA damage response, proliferation, cell survival, migration, invasion, progression, chemoresistance
METTL3	m6A writer; SOCS2 mRNA degradation, BCL2, c-MYC, MYB mRNA stabilization	
METTL14		
METTL16	m6A writer; Modify mRNA and non-coding RNA	
RBM15	m6A writer; Recruits other components during RNA methylation	
WTAP	m6A writer; Promotes the METTL3-METTL14 heterodimer	
ALKBH5	m6A eraser; Nanog mRNA expression, FOXM1 mRNA expression	DNA damage response, proliferation, cell survival, migration, invasion, progression, chemoresistance
FTO	m6A eraser; Demethylation, ASB2/RARA mRNA reduction	
HNRNPA2B1	m6A reader; Mediates primary mRNA processing	
HNRNPC	m6A reader; Relevant to RNA splicing	
IGF2BP1/2/3	m6A reader; Oncogenic mRNA stabilization	Proliferation, tumorigenesis, metastasis, chemoresistance
YTHDC1/2	m6A reader; Metastasis gene expression	
YTHDF1/2/3	m6A reader; SOCOS2 mRNA degradation, c-MYC, CEBPA mRNA stabilization	

2.4. Dynamic Regulation of m6A

The dynamic regulation of m6A is a complex and coordinated process involving writers, erasers, and readers, enabling cells to respond rapidly to internal and external signals. These methyltransferases, demethylases, and binding proteins work together to perform dynamic control as demonstrated in Fig. 1. This regulation plays a vital role in various biological functions and has significant implications for health and disease.

3. The Role of RNA Methylation in Hematopoietic Stem Cells

3.1. Impact of m6A on Hematopoietic Stem Cell Self-Renewal and Differentiation

Hematopoietic stem cells (HSCs) undergo a multi-step differentiation process, committing to either the myeloid or lymphoid lineage. Myeloid progenitors generate red blood cells, platelets, and immune cells, while lymphoid progenitors produce B cells, T cells, and natural killer cells. This differentiation is regulated by intrinsic factors, including transcription factors like PU.1, and growth factors within the bone marrow microenvironment, ensuring a continuous supply of blood cells for essential bodily functions. The m6A modification of mRNA is intricately associated with various developmental stages in hematopoiesis.

The significance of RNA methylation in hematopoietic development was first highlighted during the embryonic formation of hematopoietic stem and progenitor cells (HSPCs). METTL3 is crucial for the endothelial-to-hematopoietic transition (EHT) and, when knocked down in zebrafish models, leads to inhibited HSPC formation, whereas its overexpression can rescue this process. Additionally, the reader protein YTHDF2 regulates the m6A-target gene Notch1a, with YTHDF2 deficiency yielding effects similar to METTL3 deficiency [13,14]. In humans, depletion of METTL3 in cord blood-derived HSPCs decreases proliferation while promoting myeloid differentiation, indicating the importance of these enzymes in maintaining normal myeloid differentiation. Although the role of m6A in adult hematopoiesis remains less understood, it is implicated in T cell homeostasis. In Mettl3-deficient T cells, naive T cell populations increase, but their capacity to proliferate and differentiate into effector cells is impaired, linked to disrupted IL7 signaling. METTL3 appears essential for degrading negative regulators of IL7 signaling, thus influencing T cell survival and function [15].

In adult HSCs, both METTL3 and METTL14 are highly expressed. While METTL14 deficiency slightly affects bone marrow transplantation rates, METTL3 deficiency severely disrupts HSC function, underscoring its critical role. Interestingly, METTL3 deletion leads to increased cycling of HSCs, potentially impacting their quiescence. YTHDF2 negatively regulates key hematopoietic transcription factors by promoting mRNA decay; its deletion results in an expansion of functional HSCs [16]. Further research is needed to elucidate the distinct roles of METTL3, METTL14, and YTHDF2 in HSCs and whether their regulatory functions are dependent on m6A.

3.2. Role in Acute Myeloid Leukemia (AML)

The role of m6A RNA methylation in malignant hematopoiesis is pivotal, particularly in myeloid malignancies such as acute myeloid leukemia (AML). Characterized by the rapid proliferation of abnormal myeloid cells, AML disrupts normal blood cell production, leading to symptoms like fatigue and shortness of breath. Recent insights into m6A modification suggest promising therapeutic avenues for AML.

FTO, an m6A demethylase, is upregulated in AML, decreasing overall m6A levels, promoting leukemic cell growth, and reducing apoptosis. Conversely, FTO depletion elevates m6A levels and curtails leukemogenesis, primarily targeting genes involved in leukemic cell survival, such as ASB2 and RARA. In IDH1/2 mutant AML, FTO activity can be inhibited by d-2-hydroxyglutarate (D-2HG), raising m6A levels and potentially benefiting leukemia cells by counteracting FTO activity [17].

METTL3 is also implicated in AML, where its overexpression correlates with increased m6A levels, leading to enhanced cell growth and decreased differentiation. METTL3 regulates key

leukemia-associated genes, including MYC, BCL2, and PTEN, thus influencing cell survival. Similarly, METTL14 stabilizes and translates transcripts like MYC and MYB, facilitating leukemia progression [18]. WTAP functions as an oncogene in AML, promoting cell proliferation while inhibiting differentiation [19]. The RBM15-MKL1 fusion protein, resulting from chromosomal translocation, is critical in acute megakaryoblastic leukemia (AMKL), particularly in pediatric cases, promoting leukemia development and enhancing oncogenic activity through Notch signaling. However, the specific role of RBM15-MKL1 in m6A methylation and AMKL pathogenesis remains to be clarified [20].

3.3. Potential Therapeutic Strategies

Targeting RNA methylation, especially m6A modification, presents a promising strategy for leukemia treatment. The RNA “writer” complexes, particularly METTL3 and METTL14, are key targets due to their roles in regulating m6A levels. FTO, as a scavenger of m6A, is another focus, particularly in AML with IDH1/2 mutations, where FTO inhibition raises m6A levels and suppresses leukemic cells [21]. Inhibitors like Rhein have been identified, but more selective compounds are necessary to enhance efficacy and safety.

While therapies targeting RNA methylation in leukemia show great potential, challenges remain, including identifying biomarkers for therapeutic efficacy and combining RNA methylation therapies with existing treatments. Continued research is essential to refine these strategies and facilitate their clinical application.

4. Role of RNA Methylation in Embryonic Stem Cells

4.1. Importance of m6A in Embryonic Stem Cells

Embryonic stem cells (ESCs) are specialized cells derived from early embryonic blastocysts, capable of developing into all embryonic tissues, a property known as pluripotency. The main factors of m6A methylation and the related regulation pathway are shown in Fig. 2. In vitro, these cells can replicate indefinitely while maintaining their pluripotent state. ESCs exist in two primary states: the "naïve" state, located in the inner cell mass of the blastocyst, and the "primed" state, which is poised for differentiation and resides in the ectoderm. Key transcription factors, including NANOG, OCT4, SOX2, ESRRB, KLF4, and TFAP2C, form an intricate network that governs pluripotency [22]. The role of m6A RNA modifications in early development has been explored through m6A RNA immunoprecipitation sequencing (RIP-seq), revealing that m6A modifications are prevalent in the transcriptomes of both mouse (mESCs) and human embryonic stem cells (hESCs). In mESCs, 9,754 m6A peaks were identified across 5,578 transcripts, including 5,461 mRNAs and 117 long noncoding RNAs (lncRNAs), indicating a substantial modification of transcripts. In hESCs, 16,943 m6A peaks were identified in 7,871 genes, with significant dynamics observed during differentiation, suggesting a regulatory role for m6A during cell fate transitions.

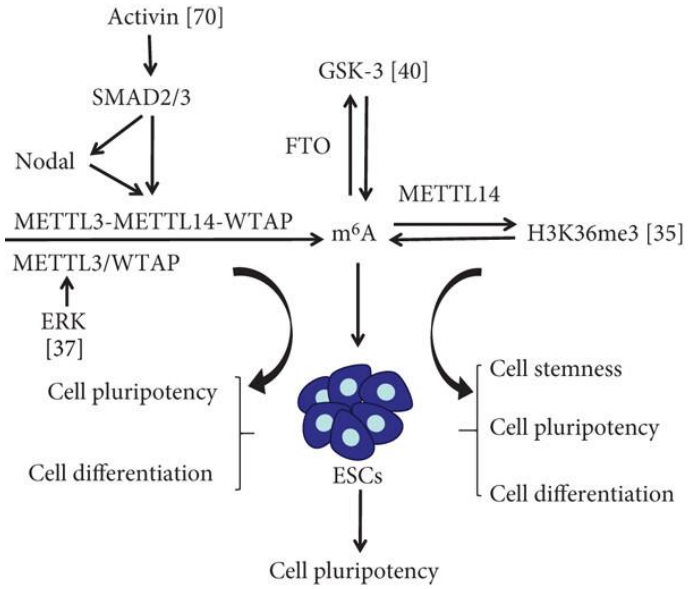

Fig. 2. m6A RNA methylation interacts with H3K36me3, ERK, GSK-3, and Activin/Nodal signaling pathway to regulate ESC self-renewal, differentiation, cell stemness, and pluripotency [4].

P. Batista's research demonstrated that m6A is enriched in pluripotency-related genes and plays a crucial role in regulating their expression during differentiation. In vitro and in vivo studies show that inactivation of the core m6A methylase METTL3 leads to decreased m6A levels, prolonged expression of pluripotency factors such as Nanog, and impaired ESC differentiation. These findings highlight the critical role of m6A in maintaining stem cell self-renewal and guiding differentiation into specific lineages [23].

4.2. Effect on Differentiation of Embryonic Stem Cells

In ESCs, m6A is vital for managing the transition from a pluripotent state to differentiation. The key enzymes METTL3 and METTL14 function as "writers," depositing m6A on the mRNAs of crucial pluripotency factors like NANOG and SOX2. Knockdown of METTL3 results in reduced m6A levels, leading to prolonged expression of pluripotent genes, which inhibits proper differentiation and delays development. In METTL3 knockout mouse models, embryos typically fail to differentiate and die around days 3.5 - 6.5 due to a lack of specific cell type development, underscoring the necessity of m6A in embryonic growth [24]. A similar effect is observed with METTL14 knockdown, confirming the synergistic role of both enzymes in regulating stem cell fate [25]. m6A regulation is dynamic, and during neural differentiation, it facilitates the decay of mRNAs that inhibit the Jak/Stat signaling pathway, crucial for functional neuron formation [26]. These findings illustrate the extensive influence of m6A in directing stem cell differentiation across various lineages.

4.3. Importance in Early Embryonic Development

During pre-implantation development, the zygotic genome activation (ZGA) occurs alongside the degradation of maternal RNA during the maternal-to-zygotic transition (MZT). METTL3 knockout (KO) mice exhibit normal pre-implantation morphology but have abnormalities in ZGA and oocyte maturation, highlighting m6A's role in maternal mRNA degradation control [27]. The m6A reader hnRNPA2B1, regulated by METTL3, influences the expression of pluripotent genes. In contrast, YTHDF2 promotes mRNA decay during ZGA while inhibiting maternal mRNA breakdown, and the absence of IGF2BPs, which stabilize mRNA, adversely affects early embryogenesis [27].

Post-implantation, the transition from the naïve to primitive pluripotent state relies on m6A modifications. Deletion of m6A leads to a "hyper" naïve state, halting lineage differentiation and causing embryo lethality [28]. WTAP is essential for mesodermal and endodermal differentiation, while METTL3 and METTL14 KO embryos show impaired germ layer differentiation. METTL16

influences embryonic development by regulating MAT2A expression, whereas YTHDC1 is crucial for mRNA processing and early embryo viability [29]. Overall, m6A modifications are essential for both fertility and embryonic development; however, the regulatory mechanisms of m6A-related enzymes remain largely unexplored due to their lethal effects in early development stages.
with existing treatments. Continued research is essential to refine these strategies and facilitate their clinical application.

5. Role of RNA Methylation in Other Types of Stem Cells

5.1. Bone Marrow Stem Cells

Bone marrow stem cells (BMSCs) play essential roles in hematopoiesis, tissue regeneration, and maintaining immune privilege, making them valuable for stem cell therapies. The m6A methyltransferase METTL3 is significantly upregulated in porcine BMSCs (pBMSCs) during adipogenic differentiation. The absence of METTL3 leads to increased STAT5 activity, which promotes the production of adipogenic genes by stabilizing JAK1 mRNA in a manner dependent on m6A and the YTHDF2 reader protein. This illustrates how m6A guides BMSCs towards adipocyte differentiation. Conversely, METTL3 is also vital for osteogenic differentiation; its deficiency results in reduced osteogenic potential and increased adipogenic differentiation. METTL3 enhances osteogenesis by facilitating the m6A methylation of runt-related transcription factor 2 (RUNX2). Silencing METTL3 diminishes osteogenic differentiation and bone mass, while overexpression can counteract osteoporosis linked to low estrogen levels. In BMSCs, the PTH/PTH1R signaling pathway plays a critical role in these m6A-related mechanisms [30].

The demethylase FTO is also essential for mesenchymal stem cell (MSC) development. Exposure to TNF-α inhibits FTO expression, leading to increased methylation of Nanog mRNA and reduced expression, which subsequently lowers the differentiation capacity of MSCs [31]. Thus, these enzymes are critical for regulating BMSC development and functionality.

5.2. Neural Stem Cells

m6A modifications are crucial for adult neurogenesis and neural development. In adult neural stem cells (aNSCs), the lack of METTL3 results in decreased m6A levels, inhibiting NSC proliferation and shifting differentiation towards glial cells, ultimately affecting new neuron development. METTL3 also regulates the expression of histone methyltransferase Ezh2, and overexpression of Ezh2 can compensate for the neurogenic deficits caused by METTL3 loss. Elevated METTL3 expression has been observed in mouse models of spinal cord injury, indicating its involvement in spinal cord regeneration [32].

Similarly, the m6A methyltransferase METTL14 is vital for maintaining NSC proliferation and an undifferentiated state; its deletion promotes early differentiation and decreases proliferation. FTO deficiency significantly impacts neurodevelopment, resulting in reduced brain growth and impaired aNSC proliferation and differentiation, which affect learning and memory processes. Through the Pdgfra/Socs5-Stat3 pathway, FTO deficiency decreases long-term neurogenesis while temporarily enhancing aNSC proliferation [33].

Furthermore, the ability of neural stem and progenitor cells (NSPCs) to self-renew relies on the m6A reader protein YTHDF2. The deletion of YTHDF2 results in delayed mRNA degradation, leading to neurogenesis issues by disrupting the growth and differentiation of NSPCs [26]. Collectively, these findings underscore the pivotal role of m6A modifications in NSC development.

6. Conclusion

m6A methylation is a crucial mechanism for the precise temporal and spatial regulation of gene expression, significantly impacting cell fate and early development. While transcription is modulated by epigenetic factors such as DNA and histone methylation, m6A primarily influences gene

expression at the post-transcriptional level. This dynamic and reversible modification is vital for maintaining the balance between stem cell pluripotency and differentiation, as well as for processing various physiological and environmental signals. However, the roles of cytoplasmic m6A readers, particularly YTHDF proteins, remain contentious, with evidence suggesting they have opposing effects on translational regulation and mRNA degradation. Further research is essential to elucidate how m6A readers identify target transcripts during stem cell fate determination and early embryonic development.

The significance of m6A methylation in stem cell research opens avenues for regenerative medicine by influencing cell fate and early development. Understanding the molecular mechanisms governing m6A's control over stem cell behavior could enhance the application of pluripotent stem cells in cancer therapy and organ transplantation. Notably, m6A has been associated with key processes such as reprogramming and embryonic development in somatic cell nuclear transfer, indicating that modulating m6A levels may improve the efficiency of this technique.

Recent advances in m6A sequencing technologies, including low-input and antibody-independent methods, provide exciting opportunities to explore the m6A epitranscriptome, particularly in challenging contexts such as early embryonic and clinical samples with limited RNA availability. Additionally, innovations in m6A editing technologies, such as CRISPR-based systems that facilitate the targeted addition or removal of methylation, offer promising prospects for regenerative and precision medicine. The potential applications of m6A in reproductive development, organ transplantation, and cancer therapy underscore its importance in clinical interventions.

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