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# From bone marrow mesenchymal stem cells to diseases: the crucial role of m$^6$A methylation in orthopedics

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**Abstract**

Elucidating the molecular mechanisms underlying orthopedic diseases is crucial for guiding therapeutic strategies and developing innovative interventions. N6-methyladenosine (m$^6$A)—an epitranscriptomic modification—has emerged as a key regulator of cellular fate and tissue homeostasis. Specifically, m$^6$A plays a pivotal role in several RNA biological processes such as precursor RNA splicing, 3′-end processing, nuclear export, translation, and stability. Recent advancements indicate that m$^6$A methylation regulates stem cell proliferation and osteogenic differentiation by modulating various signaling pathways. Extensive research has shown that abnormalities in m$^6$A methylation contribute significantly to the onset and progression of various orthopedic diseases such as osteoporosis (OP), osteoarthritis (OA), rheumatoid arthritis (RA), and bone tumors. This review aims to summarize the key proteases involved in m$^6$A methylation and their functions. The detailed mechanisms by which m$^6$A methylation regulates osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) through direct and indirect ways are also discussed, with a focus on specific molecular pathways. Finally, this review analyzes the roles and mechanisms of m$^6$A modification in the development and progression of multiple orthopedic diseases, offering a comprehensive understanding of the pathophysiology of these conditions and proposing new directions and molecular targets for innovative treatment strategies.

**Keywords** BMSCs, m$^6$A methylation, Epitranscriptomic regulation, Signal transduction pathways, Orthopedic diseases, Regenerative medicine

## Introduction

Bone repair is a complex biological process that involves interactions among multiple cell types, signaling molecules, and matrices [1]. Central to this process are bone marrow-derived mesenchymal stem cells (BMSCs) that play a pivotal role owing to their multidirectional differentiation potential and their ease of amplification and collection, making them a focal point in orthopedic disease research [2, 3]. Enhancement of bone formation and bone mass is achieved by promoting the osteogenic and chondrogenic differentiation of BMSCs, while concurrently inhibiting their adipogenic differentiation [4–6]. Studies indicate that in typical microenvironments,

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BMSCs undergo osteogenic differentiation and complete the bone regeneration process, effectively repairing bone injuries. However, in abnormal bone metabolism microenvironments such as OP, bone tumors, and nonunion, the osteogenic differentiation of BMSCs may be significantly inhibited, complicating the repair of bone injuries [7, 8]. At the molecular level, multiple signaling pathways crucial to the lineage differentiation of BMSCs may be disrupted in the context of disease [9, 10]. Consequently, elucidating the critical molecular mechanisms influencing BMSCs differentiation and their function in pathological microenvironments holds significant potential for advancing the treatment of orthopedic diseases.

N6-methyladenosine ($m^6A$) methylation—the most prevalent RNA modification in eukaryotes—regulates gene expression via multiple pathways including splicing, nuclear export, stability, transcription, and translation, thereby facilitating vital biological functions [11–14]. The dynamics of $m^6A$ modification are controlled by a reversible enzymatic network comprising methyltransferases (writers), demethylases (erasers), and $m^6A$-binding proteins (readers) [15]. In recent years, the advancement of high-throughput sequencing technologies has facilitated deeper investigations into the $m^6A$ methylation intricacies [16]. Numerous studies have indicated that $m^6A$ methylation is crucial for the regulation of osteogenic differentiation of BMSCs. It directly influences mRNA regulation related to BMSC differentiation, such as *Runx2* and *BMP2*, or activates associated signaling pathways, thereby influencing osteogenesis [17–19]. Indirectly, it affects non-coding RNAs (ncRNAs), which include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), crucial in biological functions [20, 21]. These ncRNAs modulate BMSCs lineage differentiation by targeting transcription factors, signaling molecules, or other ncRNAs [22]. Aberrant $m^6A$ methylation is implicated in various orthopedic-related disorders such as OP, nonunion, and bone tumors [23, 24].

In this review, the principal enzymes that regulate $m^6A$ methylation modification and their significant biological functions identified to date are summarized. The specific molecular mechanisms through which $m^6A$ methylation influences BMSCs differentiation and bone repair by targeting mRNAs and ncRNAs are subsequently discussed. Additionally, the molecular biological roles of $m^6A$ modification in various orthopedic diseases are outlined (Fig. 1). This review offers a theoretical foundation for the intricate mechanisms of BMSCs in promoting osteogenesis or bone repair and provides a molecular-level focal point for treating various orthopedic diseases.

## Enzymes and functions associated with $m^6A$ methylation

$m^6A$ methylation modification predominantly occurs within the 3' untranslated regions (3' UTRs), long introns, and near-specific coding region sequences, particularly in the DRACH sequences (D denotes A, G or U; R denotes A or G; and H denotes A, U, or C) [25, 26]. Following the discovery of the first $m^6A$ methylation transferase—methyltransferase-like 3 (METTL3)—subsequent research has progressively revealed several associated enzymes including "writers," "erasers," and "readers." These enzymes work in both temporal and spatial coordination to dynamically and reversibly regulate $m^6A$ methylation of RNAs, thereby playing a crucial role in a variety of biological processes [27] (Fig. 2).

### $m^6A$ methyltransferases: writers

Proteins that introduce $m^6A$ methylation modification at specific RNA sites are known as writers. The $m^6A$ modification in the human transcriptome is predominantly orchestrated by the $m^6A$ methyltransferase complex (MTC), which is principally situated in the cell nucleus. The MTC is composed of diverse proteins and functionally splits into two subunits: the catalytic $m^6A$-METTL complex (MAC) and the regulatory $m^6A$-METTL-associated complex (MACOM) [28]. The core of MAC consists of METTL3 and METTL14, which together form the catalytic center [29]. METTL3 provides methyltransferase activity, while METTL14 assists in RNA binding [30, 31]. MACOM includes proteins such as Wilms tumor 1-associated protein (WTAP), Vir-like $m^6A$ methyltransferase associated (VIRMA/KIAA1429), RNA Binding Motif Protein 15/15B (RBM15/RBM15B), zinc finger CCCH domain-containing protein 13 (ZC3H13), and HAKAI (also known as CBLL1), none of which possess catalytic domains and thus do not exhibit any catalytic activity. However, they interact with the core complex to ensure the precision and efficiency of methylation [32]. WTAP directs the complex to nuclear speckles enriched with precursor mRNA processing factors [33]. VIRMA targets methylation to specific regions of mRNA, especially near the 3' UTR and stop codon [34]. Similarly, RBM15 and RBM15B recognize specific RNA sequences and recruit the methyltransferase complex to these sites [35]. ZC3H13 and HAKAI affect the localization and stability of the complex components [36–38].

In addition to the primary methyltransferase complex, other enzymes—including METTL16, METTL5, TRMT112, ZCCHC4, and METTL7A—have also been identified as $m^6A$ writers [39, 40]. METTL16 influences the splicing of various nuclear RNAs [41, 42]. METTL5 and TRMT112 collaborate in the nucleus to catalyze $m^6A$ modifications on 18 S rRNA, thereby affecting protein synthesis [43]. Similarly, ZCCHC4 methylates 28 S

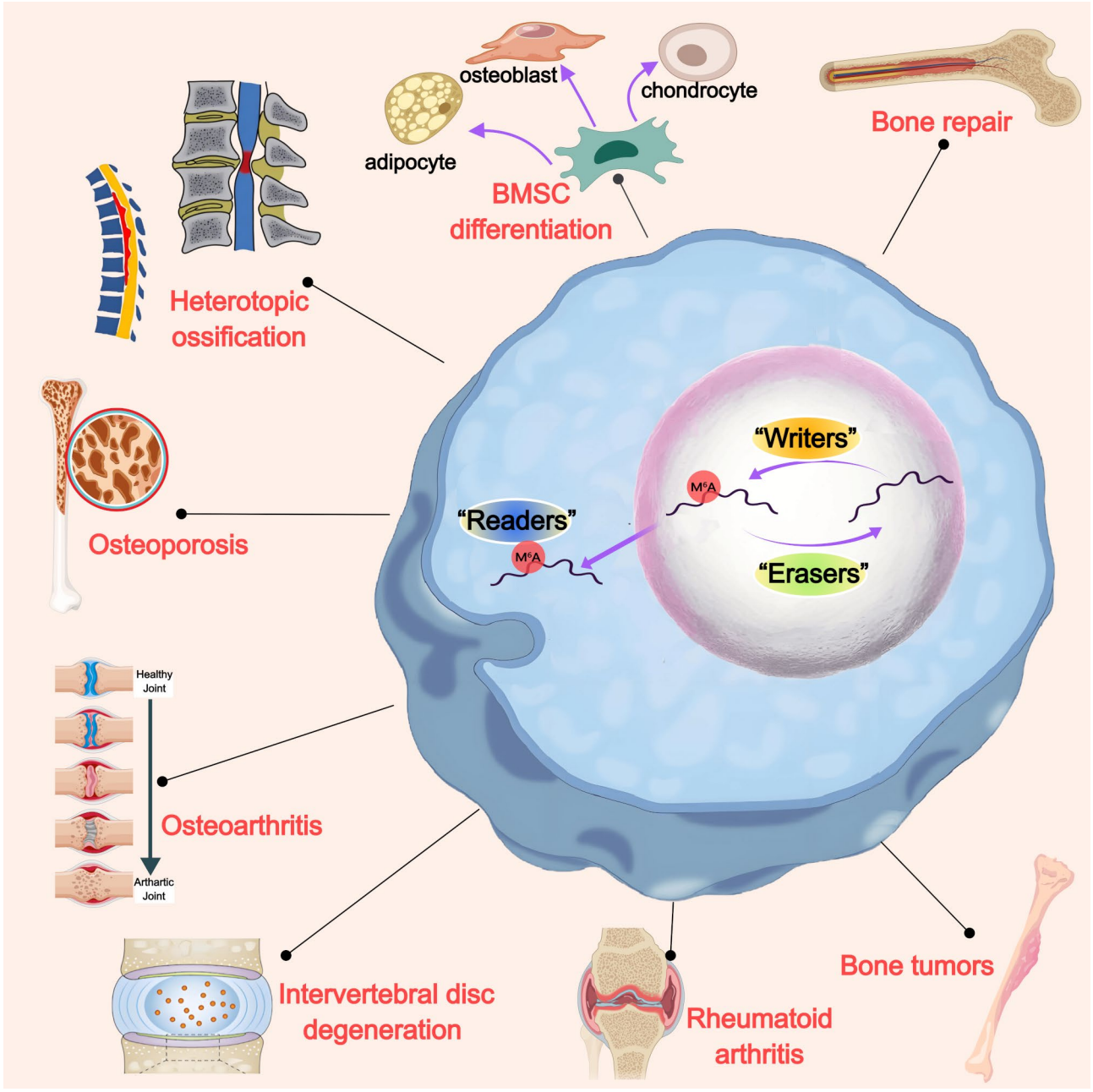


**Fig. 1** m$^6$A methylation modification in the regulation of BMSC lineage differentiation, bone repair, heterotopic ossification, degenerative bone disease, RA, and bone tumors

rRNA, thereby enhancing ribosomal function [44, 45]. Additionally, METTL7A has been found to methylate specific long non-coding RNAs (lncRNAs) [46] (Fig. 2).

#### m$^6$A demethylases: erasers

Proteins responsible for the removal of m$^6$A modification are designated as erasers. The primary erasers identified to date are Fat mass and obesity-associated protein (FTO) and alkylation repair homolog 5 (ALKBH5). FTO primarily functions in the cell nucleus, where it binds to the intronic regions of precursor mRNA (pre-mRNA). It influences pre-mRNA processing through demethylase activity, affecting selective splicing and 3′ UTR processing [47, 48]. Additionally, FTO regulates mRNA stability and translation efficiency by demethylating m$^6$Am on the mRNA 5' cap, facilitated by its movement between the nucleoplasm and cytoplasm [49, 50]. ALKBH5 aligns with particular mRNA processing factors in the nucleolus, with its demethylation activity being vital for the proper assembly or modification of these factors, thereby

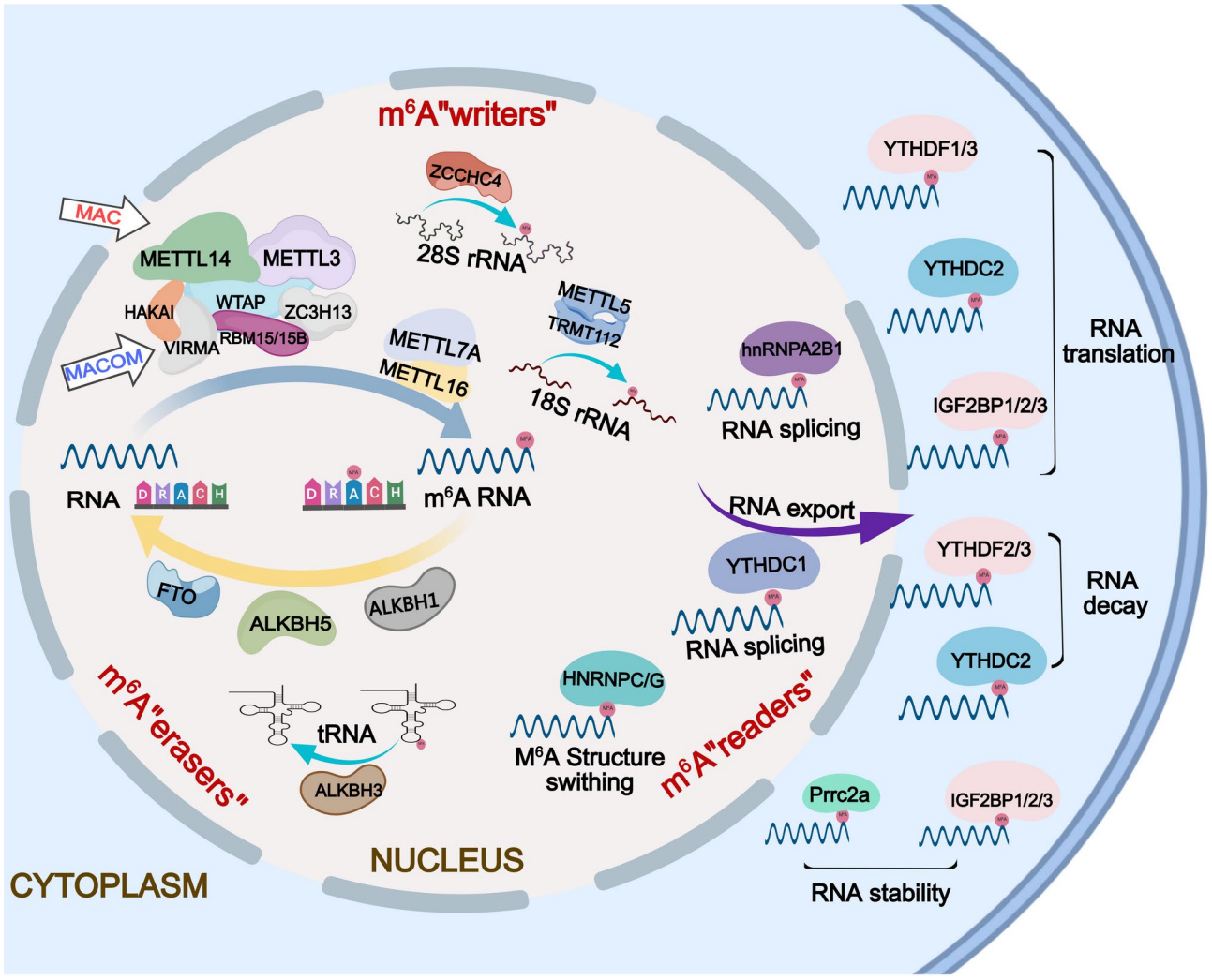


**Fig. 2** Dynamic and reversible m$^6$A methylation modification on RNAs and its functions. "Writers" are responsible for installing m$^6$A and can be removed by "erasers." "Readers" recognize m$^6$A sites and execute a series of complex biological processes

affecting mRNA nuclear export and RNA metabolism [51]. Recent studies have also shown that ALKBH1 and ALKBH3 possess m$^6$A demethylase activity, with ALKBH3 specifically targeting m$^6$A sites in tRNAs [52, 53] (Fig. 2).

### m$^6$A methylated reading proteins: readers

The m$^6$A methylation modification recruits specific proteins termed readers, which recognize and interact with m$^6$A modification sites on RNAs. This interaction substantially influences various post-transcriptional processes such as splicing, nuclear export, translation, stability, and degradation [54, 55].

The YT521-B homology (YTH) domain family proteins—including YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2—are well-known m$^6$A readers [56]. Specifically, YTHDF1 enhances translation by increasing ribosome occupancy and can initiate and elongate translation in both cap-dependent and cap-independent manners [57, 58]. It also modulates the stability of target RNAs [59]. Conversely, YTHDF2 facilitates the degradation of m$^6$A-modified mRNAs, thus reducing mRNA stability and gene expression [13, 60]. YTHDF3 synergizes with YTHDF1 to promote protein synthesis and influences the decay of methylated mRNAs mediated by YTHDF2 [61]. Notably, YTHDC1, a nuclear m$^6$A reader, affects RNA splicing and nuclear export [62, 63]. In contrast, YTHDC2, which possesses RNA helicase activity, enhances translation and regulates mRNA stability [64, 65].

Other significant m$^6$A readers include the IGF2BP family proteins—IGF2BP1, IGF2BP2, and IGF2BP3—which stabilize target mRNAs and enhance their translation [66, 67]. Moreover, Heterogeneous nuclear ribonucleoproteins (hnRNPs), such as HNRNPA2B1, HNRNPC, and HNRNPG, recognize m$^6$A modifications, influencing

RNA splicing, stability, and transport [68–71]. Recently, proteins such as Prrc2a have been identified as m$^6$A readers capable of stabilizing specific mRNAs involved in crucial cellular functions [72] (Fig. 2).

## m$^6$A methylation modulates osteogenesis by targeting mRNAs and NcRNAs

Osteogenesis is fundamentally dependent on the differentiation potential of BMSCs, characterized primarily by the formation of chondrocytes and osteoblasts, and the suppression of adipogenic differentiation [73, 74]. Additionally, the generation of osteoclasts is essential for balancing bone resorption and new bone formation [75]. These differentiation processes are governed by complex signaling pathways and gene expression patterns [76, 77]. m$^6$A methylation, a crucial regulator within these pathways, affects bone formation by modulating the stability or translation of mRNAs and ncRNAs. In the following sections, we discuss the pivotal role of m$^6$A methylation in bone formation.

### Regulating osteogenic differentiation of BMSCs

#### *Effects on osteogenic differentiation by targeting mRNAs*

Research has identified a comprehensive transcriptome-wide m$^6$A methylome in osteogenic differentiation. Differentially methylated genes are significantly enriched in signaling pathways related to BMSC osteogenesis [16]. Indeed, m$^6$A methylation modification plays a pivotal role in the complex network governing osteogenic differentiation. The Wnt/β-catenin signaling pathway is critical for osteogenic differentiation in stem cells. This pathway is activated when Wnt proteins bind to Frizzled receptors and their co-receptors, LRP5/6, stabilizing β-catenin. This stabilization facilitates the accumulation of β-catenin and nuclear translocation, where it binds to TCF/LEF transcription factors, activating essential genes for cell proliferation, differentiation, and survival, including the key osteogenic transcription factors *Runx2* and *Osterix* [78]. Multiple writers are involved in the regulation of this pathway. For instance, overexpression of METTL3 in BMSCs enhances methylation modification levels, which upregulates osteogenic factors and activates the Wnt signaling pathway, including P-Gsk-3β, β-catenin, and Lef1, subsequently enhancing osteogenesis [79]. Additionally, METTL14 enhances the stability of *PTPN6* mRNA in an m$^6$A-dependent manner, increasing PTPN6 expression. PTPN6 interacts with glycogen synthase kinase 3β (GSK3β), activating the Wnt signaling pathway and positively influencing osteogenic differentiation [80]. Furthermore, certain erasers and readers can also affect the activation of the Wnt/β-catenin pathway. Advanced glycation end-products (AGEs) elevate FTO levels, reducing the m$^6$A methylation of the sclerostin (*SOST*) transcript. Concurrently, YTHDF2 recognizes the m$^6$A modification on the *SOST* transcript and reduces *SOST* mRNA stability. SOST acts as a negative regulator of BMSC osteogenic differentiation by inhibiting the Wnt/β-catenin pathway [81]. Conversely, YTHDF1 positively regulates the osteogenic differentiation of BMSCs through autophagy and the β-catenin pathway [82].

The BMP/Smad pathway is a pivotal signaling cascade that positively influences osteogenic differentiation. BMPs initiate the pathway by attaching to receptors on the cell membrane, subsequently leading to the phosphorylation of Smad proteins. These proteins then aggregate and translocate to the nucleus, where, in conjunction with other transcription factors, they promote the expression of genes essential for the transition of MSCs to osteoblasts [83]. METTL3, through the mRNA degradation activity of YTHDF2, decreases the expression of the negative regulators Smad7 and Smurf1 within the pathway. This reduction leads to pathway activation and promotes osteogenic differentiation [84]. The complex formed by PIWI-interacting RNA-36741 (piR-36741) and PIWIL4 reduces the methylation activity of METTL3, obstructing the m$^6$A modification of BMP2 mRNA, preventing the degradation of *BMP2* mRNA mediated by YTHDF2 and enhancing BMP2 expression to accelerate BMSC osteogenic differentiation [85]. Additionally, *NOG* interrupts BMP signaling by selectively inhibiting the activity of BMP-related Smad pathways (Smad1/5), thus inhibiting osteogenic differentiation. Conversely, METTL3 accelerates the degradation of m$^6$A-marked *NOG* mRNA, thereby augmenting the process of osteogenic differentiation [86]. Similarly, METTL14 enhances the m$^6$A methylation of *Smad1* mRNA, promoting its degradation in an IGF2BP1-dependent manner, which inhibits osteogenic differentiation in BSMCs [87].

The PI3K/AKT signaling pathway is widely recognized for its role in promoting osteogenic differentiation. The pathway is initiated by the activation of cell membrane receptors, leading to PI3K activation that, in turn, activates AKT. The activated AKT facilitates cell survival, proliferation, and differentiation through various mechanisms [88]. Notably, low METTL3 expression in BMSCs inhibits Akt phosphorylation, thereby disrupting PI3K-Akt signaling and hindering osteogenic differentiation [89]. Additionally, ALKBH5 decreases m$^6$A methylation on *PRMT6* mRNA, hastening its degradation and consequently suppressing the PI3K/AKT pathway. This inhibition further reduces osteogenic differentiation [90].

Apart from these pathways, m$^6$A modification also impacts BMSC osteogenic differentiation via the NF-κB, MAPK, and AMPK pathways. For instance, METTL3 enhances m$^6$A methylation of the *MYD88* mRNA, increases MYD88 expression, and activates the NF-κB signaling pathway, which ultimately limits osteogenesis. This effect can be reversed by the ALKBH5 eraser [91].

Furthermore, diminished METTL3 expression in MSCs reduces protein synthesis of parathyroid hormone receptor-1 (Pth1r), thus decreasing translation efficiency and suppressing the activation of protein kinase A (PKA) and extracellular signal-regulated kinase (ERK) pathways, leading to reduced osteogenic differentiation [92]. FTO interacts with the p-AMPK feedback loop, inducing mild endoplasmic reticulum (ER) stress that promotes osteogenic differentiation via AMPK activation and Dlx5-dependent Runx2 expression [93] (Table 1).

#### *Effects on osteogenic differentiation by targeting NcRNAs*

Non-coding RNAs such as lncRNAs and miRNAs interact with each other, as delineated by the competing endogenous RNA (ceRNA) hypothesis, which proposes a novel interaction mechanism among these RNAs. Specifically, lncRNAs and circRNAs serve as miRNA sponges, competing with target gene mRNAs for the same miRNAs at the miRNA response elements within the 3'UTR regions. This competition diminishes the suppressive effects of miRNAs on their target genes, thereby increasing the expression levels of these targets [94]. The methylation activity of METTL3 affects the stability of various lncRNAs, which subsequently influences osteogenic differentiation in stem cells via the ceRNA mechanism. For instance, METTL3 enhances the expression of *LINC00657*, which acts as a molecular sponge for *miR-144-3p*, thereby upregulating *BMPR1B* expression and promoting the osteogenic differentiation of BMSCs [95]. Additionally, METTL3 boosts the stability and expression of *lncRNA CUTALP* in an m$^6$A-dependent manner, disrupting *miR-30b-3p*'s inhibition of *Runx2*, thus enhancing osteogenic differentiation [96]. Similarly, METTL3-mediated methylation decreases the expression of lncRNA *MIR99AHG*, which targets *miR-4660* to boost the osteogenic potential of BMSCs [97]. m$^6$A-modified lncRNAs also directly influence osteogenic differentiation through the regulation of osteogenesis-related signaling pathways. METTL3 enhances osteogenesis by increasing m$^6$A modification and expression of lncSNHG7, thereby activating the Wnt/β-catenin signaling pathway [98]. Correspondingly, METTL3 can enhance the m$^6$A modification and expression levels of lncRNA *RP11-44 N12.5*, which positively regulates the expression of serine/threonine-protein kinase 3. This elevation activates the MAPK signaling pathways (ERK, JNK, and p38), subsequently promoting osteogenic differentiation [99]. Furthermore, METTL3 promotes osteogenesis by enhancing the stability of lncRNA 4114 [100] (Fig. 3A).

In addition, m$^6$A methylation modification impacts osteogenic differentiation in stem cells by altering miRNA maturation. METTL3 promotes the maturation of *miR-7212-5p* by binding microprocessor protein DGCR8 to pri-miRNA and inhibits osteogenic differentiation of BMSCs by targeting *FGFR3* [101]. In contrast, METTL3-dependent m$^6$A methylation suppresses the maturation of *miR-196b-5p* via DGCR8, thereby enhancing osteogenic differentiation of BMSCs [102]. METTL3 also upregulates Runx2 by impeding the maturation of *pre-miR-320* through methylation [103]. Similarly, METTL14 modulates the processing of *pri-miR-103-3p* and *pri-miR-873* by DGCR8, increasing levels of mature *miR-103-3p* and *miR-873* and consequently inhibiting osteogenic differentiation in BMSCs [104]. Furthermore, WTAP interacts with DGCR8, enhancing m$^6$A-dependent maturation of *pri-miR-29b-3p*, which reduces histone deacetylase 4 expression and promotes osteogenesis [105]. WTAP also facilitates methylation of *pri-miR-181a* and *pri-miR-181c*, which, via maturation mediated by YTHDC1, decreases *SFRP1* mRNA expression and

**Table 1** m$^6$A methylation modification regulates MSC osteogenic differentiation by targeting mRNAs

| pathways | Regulators | Effects on osteogenesis | Mechanism | Species and cells | Ref. |
|---|---|---|---|---|---|
| Wnt/β-catenin | METTL3↑ | promotion | P-Gsk-3β、β-catenin、 Lef1↑/Runx2↑ | Rat BMSCs | [79] |
| | METTL14↑ | promotion | PTPN6↑/ phosphorylation level of GSK-3β↑/β-catenin↑ | Human BMSCs | [80] |
| | FTO↑、YTHDF2 | promotion | AGEs↑/FTO↑/SOST↓/ Wnt/β-catenin↑ | Mouse BMSCs | [81] |
| | YTHDF1↑ | promotion | β-catenin↑/COL1 、 RUNX2↑ | Human BMSCs | [82] |
| BMP/Smad | METTL3↓、YTHDF2 | inhibition | LPS↑/ METTL3↓/ Smad7 、Smurf1↑/ Runx2↓ | Mouse BMSCs | [84] |
| | METTL3、YTHDF2 | promotion | piR-36741-PIWIL4 complex↑/ m$^6$A activity of METTL3/ BMP2↑/ Smad1/5/8↑ | Human BMSCs | [85] |
| | METTL3↑ | promotion | NOG↓/ Smad1/5↑ | Human MSCs | [86] |
| | METTL14↑、IGF2BP1↑ | promotion | SMAD1↑ | Human BMSCs | [87] |
| PI3K/AKT | METTL3↓ | inhibition | Akt phosphorylation↓ | Rat BMSCs | [89] |
| | ALKBH5↑ | inhibition | PRMT6↓/ p-AKT↓ | Human BMSCs | [90] |
| NF-κB | METTL3↑ | Inhibition | MYD88↑/ NF-κB↑ | Human MSCs | [91] |
| | ALKBH5 | promotion | MYD88↓/ NF-κB↓ | Human MSCs | [91] |
| MAPK | METTL3↑ | promotion | Pth1r↑/ PKA、ERK↑ | Rat BMSCs | [92] |
| AMPK | FTO↑ | promotion | positive feedback loop existed between FTO and p-AMPK/ mild ER stress | Mouse BMSCs | [93] |

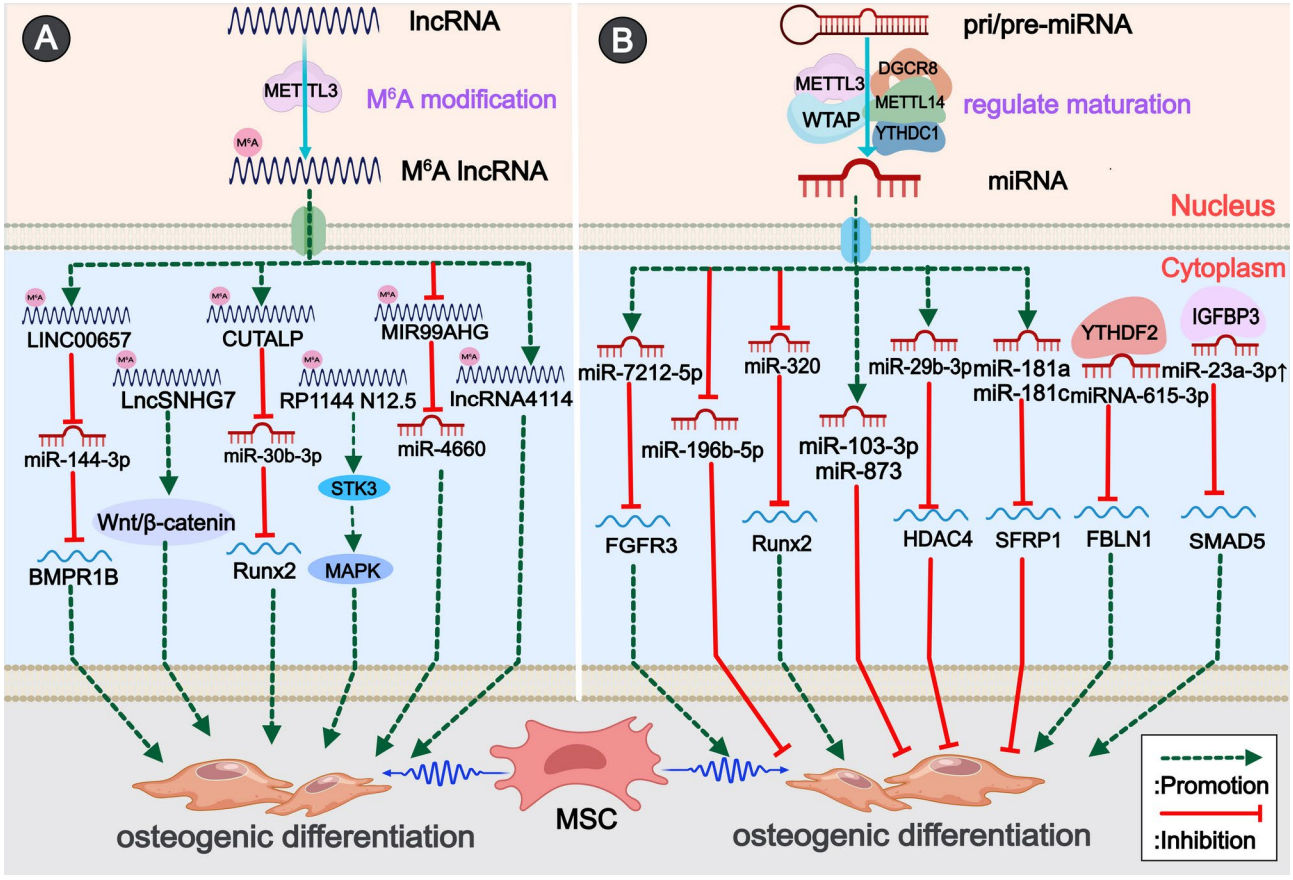


**Fig. 3** m$^6$A methylation modification regulates MSC osteogenic differentiation by targeting ncRNAs. (**A**) METTL3 affects the stability of various lncRNAs to regulate osteogenic differentiation. STK3, serine/threonine-protein kinase 3. (**B**) m$^6$A methylation modification influences osteogenic differentiation by altering miRNA maturation. HDAC4, histone deacetylase 4

contributes to osteogenesis [106]. Regarding other reader proteins, YTHDF2 targets m$^6$A sites on *FBLN1* mRNA and decreases its stability and degrades the osteogenic and regenerative capacities of MSCs. Additionally, YTHDF2 implements a novel RNA degradation pathway by forming a complex with *miRNA-615-3p*, which interacts with m$^6$A sites on *FBLN1* mRNA, thus reducing its stability and expression [107]. IGFBP3 stabilizes *miR-23a-3p* through m$^6$A modification, resulting in the downregulation of *SMAD5* mRNA, which suppresses osteogenic differentiation and delays bone fracture healing [108] (Fig. 3B).

### Regulating adipocyte differentiation of BMSCs

Serving as a shared progenitor for both osteoblasts and adipocytes, MSCs balance osteogenic and adipogenic differentiation through a dynamic interplay of coordination and competition that is influenced by a variety of regulatory factors. Factors that promote adipogenic differentiation often inhibit bone formation [109]. Additionally, abnormal adipogenic differentiation is associated with various orthopedic diseases, including femoral head necrosis [110]. m$^6$A modification plays a crucial role in the regulation of adipogenic differentiation in BMSCs, thus maintaining bone homeostasis. Research has shown that METTL3 and FTO had opposite effects on this process: FTO enhanced adipocyte differentiation, while METTL3 negatively impacted adipogenesis [111, 112]. Both proteins influence this differentiation by affecting the stability and translation of target mRNAs in critical signaling pathways such as JAK-STAT and Wnt/β-catenin. Specifically, METTL3 increases the m$^6$A modification of Janus kinase 1 (*JAK1*) mRNA, reducing its stability in a YTHDF2-dependent manner, leading to the inhibition of BMSC adipogenesis through JAK1-mediated phosphorylation. This phosphorylation activates the signaling and transcription activator factor (STAT) 5 and binds to the promoter of CCAAT/enhancer-binding protein (C/EBP)β [113]. Furthermore, METTL3 reduces AKT protein through m$^6$A modification, subsequently decreasing MSC adipogenesis [114]. By contrast, FTO decreases the m$^6$A level of *JAK2* mRNA, extends the half-life of *JAK2* transcripts via YTHDF2, and enhances JAK2 expression and phosphorylation. This increment leads to STAT3 phosphorylation and nuclear translocation, which accelerates the gene

expression and transcription of *C/EBPβ*, thereby promoting adipogenesis [115]. Conversely, ALKBH5 increases *TRAF4* mRNA and protein levels through its demethylase activity and activates the kinase activity of PKM2, which then stimulates the Wnt/β-catenin pathway and inhibits adipogenic differentiation [116].

$m^6A$ methylation modification influences adipogenic differentiation by regulating the cell cycle. FTO decreases the methylation of cyclin A2 (*CCNA2*) and cyclin-dependent kinase 2 (*CDK2*) mRNA, enhancing their stability through YTHDF2 and increasing their expression. This accelerates mitotic clonal expansion (MCE), promoting adipogenesis [117, 118]. Similarly, METTL3 inhibits cyclin D1 by facilitating YTHDF2-mediated mRNA degradation, thereby delaying the cycle and reducing adipogenesis of BMSCs [119]. Research has shown that ZFP217 directly binds to the FTO promoter, boosting FTO expression. Moreover, ZFP217 interacts with YTHDF2, disrupting its binding to $m^6A$ mRNA and enhancing the interaction between FTO and $m^6A$ mRNA, which increases adipogenic differentiation [120]. ZFP217 also suppresses METTL3 expression, further enhancing adipogenesis [119]. Beyond the role of FTO, WTAP collaborates with METTL3 and METTL14 to actively control adipogenic differentiation by upregulating CCNA2 and accelerating the cell cycle transition during MCE [121].

In terms of other regulatory mechanisms, $m^6A$ modification enhances the translation of PNPLA2 and mitochondrial carrier homolog 2 via YTHDF1, while reducing the expression of uncoupling protein-2, collectively promoting adipogenic differentiation [122, 123]. Besides, FTO influences adipogenic differentiation by modulating $m^6A$ levels near splice sites, altering the splicing of the adipogenic regulator *RUNX1T1* [124]. Subsequent studies indicated that FTO promoted adipogenesis by increasing the expression of the adipogenic isoform of *RUNX1T1* [125]. Furthermore, growth differentiation factor 11 augments FTO expression through a C/EBPα-dependent mechanism, wherein FTO demethylates *PPARG* mRNA, enhancing its expression and thus promoting adipogenesis [23] (Table 2).

### Regulating chondrocyte differentiation of BMSCs

The differentiation of BMSCs into chondrocytes is essential for the repair of cartilaginous tissues in joint and fracture healing, particularly within native bone healing mechanisms. During fracture repair, BMSCs initially migrate to the injury site and differentiate into chondrocytes. These chondrocytes produce a cartilaginous matrix that acts as a temporary repair tissue, stabilizing the fracture site and promoting new bone formation [126]. METTL3-mediated $m^6A$ modification enhances

**Table 2** $m^6A$ methylation modification regulates MSC adipogenic and chondrogenic differentiation

| Differentiation Fate | Regulators | Effects | Mechanism | Species and cells | Ref. |
|---|---|---|---|---|---|
| adipogenic differentiation | METTL3↑、YTHDF2 | inhibition | JAK1↓/ STAT5、C/EBPβ↓ | Pig BMSCs | [113] |
| | METTL3↓ | promotion | AKT↑ | AML-MSCs | [114] |
| | FTO↑、YTHDF2 | promotion | JAK2↑/ p-STAT3↑/ C/EBPβ↑ | Porcine primary preadipocytes | [115] |
| | ALKBH5↑ | inhibition | TRAF4↑/ kinase activity of PKM2↑/ β-catenin↑ | Human MSCS | [116] |
| | FTO↑、YTHDF2 | promotion | CCNA2、CDK2↑/MCE↑ | Mouse 3T3-L1 pre-adipocytes | [117, 118] |
| | METTL3↑、YTHDF2 | inhibition | ZFP217 ↓/ METTL3↑/cyclin D1↓ | Mouse 3T3-L1 pre-adipocytes | [119] |
| | WTAP-METTL3-METTL14↑ | promotion | CCNA2↑/ MCE↑ | Mouse 3T3-L1 pre-adipocytes | [121] |
| | YTHDF1 | promotion | PNPLA2↑、MTCH2↑、 | Pig intramuscular preadipocytes | [122, 123] |
| | FTO | promotion | pro-adipogenic short isoform of RUNX1T1↑ | Mouse primary adipocytes | [125] |
| | FTO | promotion | GDF11- C/EBPα↑/FTO↑/ PPARG↑ | Human BMSCS | [23] |
| chondrogenic differentiation | METTL3↑ | promotion | MMP3、MMP13、GATA3↑ | Rat SMSCs | [127] |
| | METTL3↑、YTHDF2、eEF1α-1 | promotion | Sox9↑ | Rat BMSCs | [128] |
| | YTHDF1↑ | promotion | Dmp1↑ | Mouse chondrocyte line ATDC5 | [129] |
| | YTHDF1↑ | promotion | Wnt/β-catenin↑ | Human BMSCS | [131] |
| | METTL3↑、YTHDC1 | promotion | CircRNA3634↑/ miR-124486-5↓/MAPK1↑ | C nippon antlers cells | [130] |

AML: acute myeloid leukaemia; UCP2: uncoupling protein-2; MTCH2: mitochondrial carrier homolog 2

SMSCs: Synovium-derived mesenchymal stem cells

the expression of MMP3, MMP13, and GATA3, supporting MSC differentiation into chondrocytes through post-transcriptional regulation [127]. Furthermore, METTL3 works in conjunction with Nsun4 (with m5C catalytic activity) to target the 3'-UTR of *Sox9* mRNA and recruits proteins such as YTHDF2 and eEF1α-1 to augment *Sox9* translation, thereby significantly advancing chondrogenic differentiation [128]. Dentin matrix protein 1 (*Dmp1*) mRNA is another direct target of METTL3-mediated m$^6$A modification. Under METTL3 catalysis, YTHDF1 stabilizes *Dmp1* mRNA, facilitating hypertrophic differentiation of chondrocytes [129]. METTL3 also mediates m$^6$A modification near the splicing sites of *CircRNA3634*, while the m$^6$A reader YTHDC1 promotes the nuclear export of *CircRNA3634* in an m$^6$A-dependent manner. *CircRNA3634* acts as a molecular sponge for *miR-124486-5*, competitively binding to *miR-124486-5* and elevating MAPK1 expression, thus supporting chondrocyte differentiation, proliferation, and migration [130]. Moreover, YTHDF1 promotes chondrogenesis by activating the Wnt/β-catenin signaling pathway [131] (Table 2).

#### Indirectly regulating osteogenesis in BMSCs via effect on osteoclast function

Osteoclasts originate from the monocyte/macrophage lineage of hematopoietic stem cells. Regulating osteoclast activity can alter the equilibrium between bone resorption and formation. For instance, inhibiting excessive osteoclast activity prevents OP by fostering an environment conducive to osteoblast activity and new bone formation. Moreover, osteoclasts indirectly influence the behavior of BMSCs by secreting pro-inflammatory and growth factors such as PDGF-B, that promote their differentiation into osteoblasts [132]. METTL3, aided by YTHDF2, diminishes both the stability and expression of the cell fusion-specific gene *Atp6v0d2* mRNA. Concurrently, the methylation activity of METTL3 decreases nuclear retention of *Traf6T* mRNA and its transcription products, thereby speeding up the activation of the MAPK, NF-kB, and PI3K-AKT signaling pathways. These mechanisms collectively intensify osteoclast differentiation and function, potentially leading to bone homeostasis disorders and impeding osteogenesis [133]. Additionally, low expression of METTL3 enhances the stability of *Nos2* mRNA through a YTHDF1-dependent mechanism, exacerbating iNOS/NO-mediated mitochondrial dysfunction that inhibits osteoclast differentiation [134]. METTL3 enhances osteoclast formation by boosting m$^6$A methylation and the post-transcriptional upregulation of *CHI3L1* [135]. Both METTL3 and ALKBH5 modulate RNA-protein interactions through m$^6$A-dependent RNA structural remodeling [71]. Research has shown that *Circ_0008542* disrupts *miR-185-5p*'s inhibition of *Tnfrsf11a* (*RANK*) mRNA through its sponging action, thereby amplifying osteoclast differentiation. METTL3 can modify the spatial structure of *circ_0008542* through the "m$^6$A switch" mechanism to increase its sponging effect, while ALKBH5 counteracts this modification [136]. METTL14, with the support of Hu antigen R (HuR), stabilizes GPX4 mRNA post-transcriptionally through m$^6$A modification, inhibiting RANKL-induced osteoclast differentiation [137].

In addition to writers, FTO substantially impeded osteoclast differentiation during bone regeneration, thus improving the efficiency of this process [138]. The demethylase activity of FTO enhances the phosphorylation and nuclear translocation of NF-κB p65 protein, increasing the expression of downstream targets such as c-FOS and NFATc1, which are pivotal in promoting osteoclast differentiation [139]. Furthermore, FTO augments the stability and expression of *CCNA2* and *CDK2* mRNA, key S-phase proteins in osteoclast precursors, through a YTHDF2-dependent mechanism. This action not only facilitates their proliferation and differentiation but also diminishes apoptosis [140]. Regarding reader proteins, YTHDF1 heightens the activation of ER stress-related pathways such as PERK, IRE1α, and ATF6 and elevates the stability of *Tnfrsf11a* mRNA. Consequently, this stability fosters enhanced phosphorylation of crucial proteins in the NF-κB, MAPK, and PI3K-AKT pathways, thereby promoting osteoclast differentiation [141]. In contrast, YTHDF2 obstructs osteoclast formation through the NF-κB and MAPK pathways [142]. Additionally, YTHDC1 collaborates with HuR to increase the stability and transcription of *PTPN6* mRNA by identifying m$^6$A sites, which in turn inhibits osteoclast differentiation [143] (Table 3).

## The role of m$^6$A methylation in orthopedic diseases

m$^6$A is being increasingly recognized as a critical epigenetic regulator with multifaceted roles in bone metabolism and disease progression with respect to the pathogenesis of orthopedic diseases. For example, abnormal m$^6$A regulation can impair osteoblast function and increase the activity of osteoclasts and adipocytes, thereby accelerating bone loss. Additionally, m$^6$A methylation plays a pivotal role in other orthopedic conditions, including OA and bone tumors. It influences disease progression and treatment responses by regulating inflammatory and apoptotic pathways and other key biological processes in tumor cells. The current challenge involves mapping the complete m$^6$A regulatory network and deciphering its dynamic changes in specific orthopedic disease contexts, thus enabling more precise interventions in this critical epigenetic process. In this section, we summarized the latest findings on the role of m$^6$A methylation in

**Table 3** $m^6A$ methylation modification regulates osteoclastic differentiation

| Regulators | Effects on osteoclastogenesis | Mechanism | Species and cells | Ref. |
|---|---|---|---|---|
| METTL3↑、YTHDF2 | promotion | Atp6v0d2↓, retention of Traf6 in the nucleus↓ / MAPK↑、NF-kB↑、PI3K-AKT↑ | Mouse RAW264.7 cell lines | [133] |
| METTL3↓、YTHDF1 | inhibition | Nos2↑/ iNOS/NO↑/mitochondrial dysfunction↑ | Mouse BMMs | [134] |
| METTL3↑ | promotion | EGR1↑/ METTL3↑/ CHI3L1↑ | Mouse BMMs | [135] |
| METTL3↑、ALKBH5↓ | promotion | circ_0008542↑/ miRNA-185-5p↓/ RANK↑ | Mouse RAW264.7 cell lines | [136] |
| METTL14↑ | inhibition | GPX4↑ | Mouse BMMs | [137] |
| FTO↑ | promotion | phosphorylation and nuclear translocation of NF-κB p65↑/ c-FOS↑、NFATc1↑ | Mouse BMMs and RAW264.7 cell lines | [139] |
| FTO↑、YTHDF2 | promotion | CCNA2↑、CDK2↑ | Mouse BMMs | [140] |
| YTHDF1↑ | promotion | LPS↑/ YTHDF1↑/ Tnfrsf11a↑/NF-κB↑、MAPK ↑、PI3K-AKT↑ | Mouse RAW264.7 cell lines | [141] |
| YTHDF2↑ | inhibition | NF-κB ↓、MAPK↓ | Mouse RAW264.7 cell lines | [142] |
| YTHDC1↑ | inhibition | PTPN6↑ | Mouse BMMs | [143] |

BMMs: bone marrow-derived macrophages

the pathogenesis of OA, degenerative diseases, and bone tumors. These insights will enhance our understanding of the molecular mechanisms underlying orthopedic diseases and highlight potential molecular targets for developing new therapeutic strategies.

### Heterotopic ossification

In pathological conditions, ectopic ossification significantly diverges from normal bone formation, with bone tissue abnormally developing in non-skeletal tissues such as muscles and tendons [144]. $m^6A$-mediated epigenetic modification plays a role in regulating both the incidence and progression of ectopic ossification, which predominantly occurs in ligaments. For example, studies have demonstrated that BMP4 upregulated by high expression of METTL3 and OCS3 and downregulated by low expression of FTO contributed to ectopic ossification of the posterior longitudinal ligament and ligamentum flavum [145, 146]. ALKBH5 promotes the osteogenic differentiation of ligamentum flavum cells via two mechanisms: it removes methylation from *BMP2* mRNA, thereby enhancing BMP2 expression, and it activates p-AKT [147]. Additional research has revealed two distinct molecular mechanisms involved in osteogenic differentiation in primary ligament fibroblasts: the $m^6A$ methylation modification and the ceRNA mechanism. METTL3 specifically increases the level of lncRNA *XIST1* through $m^6A$ methylation. Acting as a molecular sponge, *XIST1* prevents the inhibition of *miR-302a-3p* to ubiquitin-specific protease 8, thus facilitating osteogenic differentiation via the ceRNA mechanism [148] (Fig. 4A). $m^6A$-mediated ectopic ossification is also observed in blood vessels, where ALKBH1-mediated DNA demethylation increases Oct4 binding to the *BMP2* mRNA promoter, enhancing BMP2 transcription and leading to osteogenic reprogramming of vascular smooth muscle cells and progression of vascular calcification [149] (Fig. 4B). Additionally, overexpression of METTL3 facilitates *TWIST1* mRNA methylation, promoting its degradation in a YTHDF2-dependent manner and decreasing TWIST1 expression. This reduction augments osteogenic differentiation in human aortic valve interstitial cells and advances aortic valve calcification [150] (Fig. 4C).

### Degeneration of bones and joints

Degenerative changes in bones and joints entail pathological alterations in which skeletal and joint tissues progressively deteriorate and lose function because of aging or disease. This category encompasses OP, OA, and disc degeneration [151]. In this process, $m^6A$ modification significantly impacts RNA stability and translation; regulates critical gene expression; and influences cell differentiation, inflammatory responses, and the synthesis and degradation of the extracellular matrix (ECM).

#### *Osteoarthritis*

Osteoarthritis is characterized by degenerative damage to joint cartilage and inflammation. Abnormal $m^6A$ modification of certain ncRNAs and mRNAs in chondrocytes disrupts their function by promoting degeneration and apoptosis, thereby accelerating degenerative changes in the cartilage [152]. Specifically, increased production of interleukin-1β (IL-1β) is a principal activator in the progression of OA. In this process, METTL3 elevated by IL-1β induction interacts with DGCR8 to facilitate the maturation of *miR-126-5p*, which in turn targets and reduces *PIK3R2* mRNA expression, culminating in chondrocyte degeneration, with PIK3R2 acting as an inhibitory regulator within the PI3K/Akt pathway [153, 154]. METTL3-mediated $m^6A$ modification also increases the expression of lncRNA *IGFBP7-OT*, reducing the presence

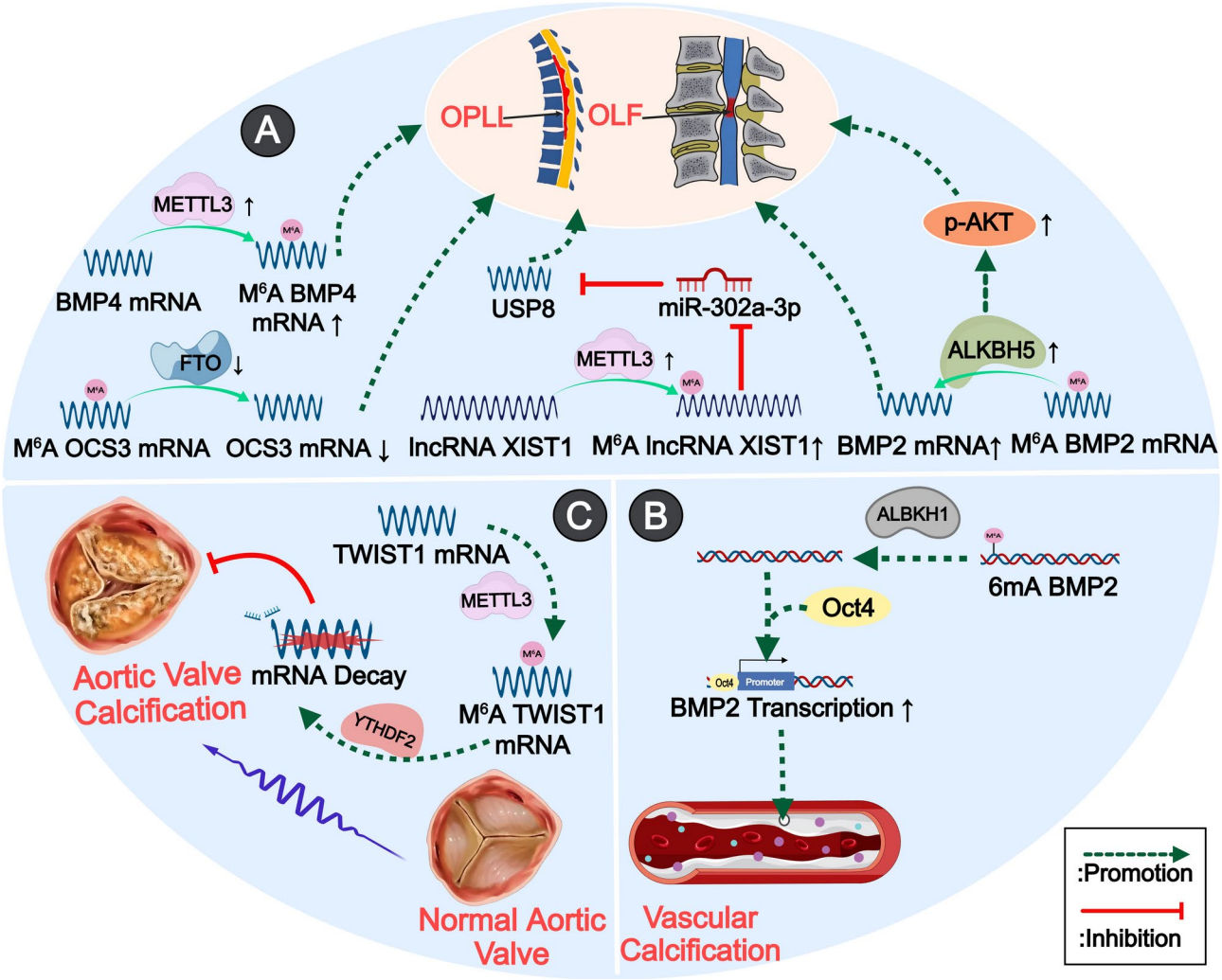


**Fig. 4** m$^6$A methylation modification in heterotopic ossification. (**A**) m$^6$A methylation modification regulates the occurrence of ligament ossification. OPLL, ossification of posterior longitudinal ligament; OLF, ossification of ligamentum flavum. USP8, ubiquitin-specific protease 8. (**B**) m$^6$A methylation modification regulates vascular calcification. (**C**) m$^6$A methylation modification regulates aortic valve calcification

of DNMT1 and DNMT3a at the IGFBP7 promoter. This reduction decreases methylation and enhances IGFBP7 expression, promoting chondrocyte degeneration [155]. Additionally, the YTHDF2-HRSP12-RNase P/MRP complex cleaves m$^6$A-modified *circRNA RERE*, catalyzed by METTL3. This cleavage can decrease the expression of IRF2BPL by targeting *miR-195-5p*, thereby inhibiting the ubiquitination and degradation of β-catenin and promoting chondrocyte apoptosis [156]. Research has also shown that METTL3 stabilizes *Bcl2* mRNA via YTHDF1, which in turn suppresses chondrocyte autophagy and apoptosis [157]. FTO and ALKBH5 exhibit a protective role in the progression of OA, and their reduced levels significantly accelerate cartilage damage. For instance, FTO reduces the m$^6$A level of *pri-miR-3591*, inhibiting its maturation. This alleviates the inhibition of *miR-3591-5p* on *PRKAA2*, thereby reducing cartilage damage in OA [158]. FTO-mediated demethylation of m$^6$A modification also downregulates *lncRNA AC008440.5* transcription, diminishing its sponging of *miR-328-3p*. This reduction inhibits AQP1 and ANKH expression and maintains chondrocyte vitality and resists apoptosis [159]. Conversely, ALKBH5 stabilizes *lncRNA HS3ST3B1-IT1* through its demethylase activity and interaction with YTHDF2, blocking ubiquitination-mediated degradation of HS3ST3B1, thus enhancing chondrocyte vitality [160] (Table 4).

m$^6$A modification can also influence the progression of OA by regulating the degradation of ECM. Specifically, abnormal upregulation of METTL3 and WTAP exacerbates the pathological changes in OA by differentially regulating this degradation process. For instance, METTL3 activates NF-κB signaling, thereby enhancing ECM degradation [161]. METTL3 also increases the

**Table 4** $m^6A$ methylation modification in OA

| Pathological Factors | Regulators | Effects | Mechanism | Species and cells | Ref. |
|---|---|---|---|---|---|
| Cartilage injury | METTL3↑ | promotion | IL-1β↑/ METTL3↑/ miR-126-5p↑/ PIK3R2↓/ PI3K/Akt↓ | Human primary chondrocytes | [153, 154] |
| | METTL3↑ | promotion | lncRNA IGFBP7-OT↑/ IGFBP7↑ | Human primary chondrocytes | [155] |
| | METTL3↑、YTHDF2 | promotion | circRNA RERE↓/ miR-195-5p↑/ IRF2BPL↓/β-catenin↑ | Human chondrocytes | [156] |
| | METTL3↑、YTHDF1 | inhibition | Bcl2↑ | Mouse chondrocyte line | [157] |
| | FTO↑ | inhibition | miR-3591-5p↓/ PRKAA2↑ | Human articular chondrocytes | [158] |
| | FTO↑ | inhibition | lncRNA AC008440.5↓/ miR-328-3p↑/ AQP1↓、ANKH↓ | Human primary chondrocytes | [159] |
| | ALKBH5↑、YTHDF2 | inhibition | lncRNA HS3ST3B1-IT1↑/ HS3ST3B1↑ | Human primary chondrocytes | [160] |
| Extracellular matrix degradation | METTL3↑ | promotion | NF-κB↑ | Mouse chondrocyte line | [161] |
| | METTL3↑ | promotion | lncRNA LINC00680↑/ SIRT1↑ | Human chondrocytes | [162] |
| | METTL3↑ | promotion | MMP1↑、MMP3↑、TIMP-1↓、TIMP-2↓ | Human chondrocytes | [163] |
| | WTAP↑ | promotion | miR-92b-5p↑/ TIMP4↓ | Human primary chondrocytes | [164] |
| Inflammatory reaction | METTL3↑ | promotion | SOCS2↓/ JAK2/STAT3↑ | Human primary chondrocytes | [167] |
| | METTL3↓ | inhibition | miR-1208↑/METTL3↓/NLRP3↓ | Human articular chondrocytes | [168] |
| | WTAP↑ | promotion | FRZB↓/ Wnt/β-catenin↑ | Human chondrocytes | [169] |
| | FTO↑ | inhibition | miR-515-5p↑/ TLR4↓/ MyD88/NF-κB↓ | Human chondrocytes | [170] |
| | ALKBH5↑ | promotion | miR-654-3p↓/ TNFRSF9↑/ NF-κB↑ | Human primary chondrocytes | [171] |
| | IGF2BP3↑ | promotion | macrophage M1 polarization↑ | Mouse BMDMs | [172] |

BMDMs: Bone marrow derived macrophages

expression of lncRNA *LINC00680*, which binds to $m^6A$ sites on the 3'-UTR of *SIRT1* mRNA, enhancing its stability and accelerating the degradation process [162]. Moreover, METTL3 influences ECM degradation by balancing TIMPs and MMPs in OA, such as increased expression of MMP1 and MMP3 and decreased expression of TIMP-1 and TIMP-2 [163]. Similarly, WTAP-mediated $m^6A$ modification promotes the maturation of *miR-92b-5p*, which subsequently strengthens its inhibition of TIMP4, leading to ECM degradation [164] (Table 4).

Although OA is often categorized as a non-inflammatory joint disease, inflammation plays a critical role in its progression. During cartilage degeneration, damaged cartilage cells and the ECM release molecular signals that trigger inflammatory cells to produce cytokines and enzymes. This exacerbates cartilage damage and accelerates the development of OA [165]. $m^6A$ methylation modification regulates the immune microenvironment in OA, particularly in terms of immune cell infiltration. YTHDF2 shows the strongest positive correlation with Treg cells, while IGFBP2 is negatively correlated with dendritic cells [166]. In addition, $m^6A$ modification is pivotal in regulating inflammatory responses. For instance, METTL3 interacts with RPL38 to inhibit SOCS2 expression through $m^6A$ modification. In OA, the abnormal elevation of METTL3 leads to a decrease in SOCS2, which intensifies the activity of the JAK2/STAT3 pro-inflammatory pathway, thereby accelerating the progression of OA [167]. Notably, extracellular vesicles derived from human umbilical cord MSCs contain *miR-1208*, which targets METTL3, reducing $m^6A$ levels and *NLRP3* mRNA expression, and subsequently diminishing inflammation [168]. Besides, augmented WTAP activity enhances *FRZB* mRNA hypermethylation, decreases FRZB expression, and activates the Wnt/β-catenin pathway, thereby aggravating inflammation [169]. $m^6A$ modification mediating the expression of miRNAs is involved in the regulation of pro-inflammatory pathways associated with OA. For instance, FTO interacts with DGCR8 to accelerate the $m^6A$-dependent maturation of *miR-515-5p*, which targets *TLR4*, deactivating the MyD88/NF-κB pathway and inhibiting synovial inflammation [170]. Conversely, ALKBH5 modulates chondrocyte inflammation by reducing *miR-654-3p* levels through $m^6A$-dependent demethylation, which elevates the expression of TNFRSF9, enhancing inflammation via the NF-κB pathway [171]. Additionally, high levels of IGF2BP3 expression supports macrophage M1 polarization, further promoting inflammation in the osteoarthritic synovium [172] (Table 4).

#### *Osteoporosis*

Osteoporosis is characterized by reduced bone density, impaired bone microarchitecture, and increased fracture risk. These pathological changes are closely associated with increased activity and differentiation of osteoclasts,

as well as decreased activity and differentiation of osteoblasts, regulated by bone metabolism [173]. In this intricate regulatory landscape, m$^6$A methylation modification plays a pivotal role by controlling the expression of critical genes involved in bone metabolism, thereby influencing cellular functionality and the progression of OP. For instance, Runx2, a vital transcription factor in bone development, boosts osteoblast differentiation but is reduced in OP. m$^6$A-related proteases can reverse this process by increasing Runx2 expression [174]. IGF2BP1 enhances the stability and expression of *Runx2* mRNA by recognizing m$^6$A sites catalyzed by METTL3 [175, 176]. YTHDF1, enhances the translation of *ZNF839* mRNA in an m$^6$A-dependent manner, wherein it interacts with Runx2 and further increases *Runx2* transcriptional activity, thereby boosting the osteogenic differentiation of BMSCs [177]. Similarly, FTO reduces m$^6$A methylation on *RBM4* mRNA, enhancing RBM4 expression, which promotes the inclusion of *Runx2* exon 5 to boost osteogenic differentiation [178]. In addition to Runx2, m$^6$A modification also regulates the expression of other osteogenesis-related mRNAs to influence OP. Especially, METTL3 stabilizes *ACLY* and *SLC25A1* mRNAs through m$^6$A-IGF2BP2/3 interactions, which enhances their expression and fosters osteogenic differentiation [179]. Conversely, disproportionate elevation of METTL3 accelerates OP progression and reduces osteoblast function in diabetes-associated OP by activating the ASK1-p38 pathway [180]. FTO reduces its stability in a YTHDF1-dependent manner by demethylating the m$^6$A sites on the 3'-UTR of *PPARG* mRNA, thereby enhancing osteogenesis [181]. FTO also enhances the stability of *Hspa1a* mRNA (encoding Hsp70) and in turn inhibits the NF-κB pathway, protecting osteoblasts from genotoxicity and cell death while maintaining bone mass [182]. In addition, the enhanced effect of osteoclasts also significantly promotes the progression of OP. In postmenopausal OP, follicle-stimulating hormone (FSH)-induced CREB phosphorylation upregulates METTL3, enhancing *CTSK* mRNA stability and translation and increasing osteoclast migration [183]. Therapeutic agents such as zoledronic acid increase METTL14 levels, which destabilizes *NFATc1* mRNA in a YTHDF2-dependent manner and inhibits osteoclast differentiation [184]. METTL14 also activates the Wnt/β-catenin pathway by upregulating TCF1 and SIRT1 and reducing osteoclast activity, thus slowing OP progression [185, 186]. Moreover, FTO may prevent diabetes-related bone loss by inhibiting TLR4-driven osteoclast differentiation [187] (Table 5).

In addition to directly regulating the activity of osteoblasts and osteoclasts, m$^6$A methylation affects the progression of OP through alternative pathways. For example, m$^6$A modification can enhance the adipogenic differentiation of BMSCs, potentially inhibiting bone formation [188, 189]. Research has also shown that elevated prednisone levels during pregnancy can increase m$^6$A modification, activate mitochondrial autophagy, and decrease FNDC5/irisin expression in skeletal muscle. This cascade of events may lead to increased bone fragility in adult offspring. S-adenosylhomocysteine (SAH), an inhibitor of m$^6$A activity, has the potential to reduce m$^6$A modification in the transcriptome, thereby mitigating these processes and potentially reversing adverse skeletal development in fetuses [190].

#### *Intervertebral disc degeneration*

Intervertebral disc degeneration is a multifaceted pathological condition characterized by the degradation of structures such as the NP, annulus fibrosus, and cartilage endplate. Recent advances in high-throughput sequencing and bioinformatics have identified alterations in m$^6$A modification patterns that occur during IVDD progression [191]. These modifications significantly affect the regulation of disc cell proliferation, apoptosis, and ECM disorders. For instance, with respect to the effect on nucleus pulposus cells (NPCs), m$^6$A-mediated methylation by abnormally elevated METTL3 enhances the stability and expression of *SIAH1* mRNA, which targets and ubiquitinates XIAP, promoting aging and apoptosis [192]. Similarly, an atypical increase in METTL14 levels enhances the stability of *NLRP3* mRNA via an IGF2BP2-mediated mechanism, elevating IL-1β and IL-18 levels and hastening the apoptosis of NPCs [193]. METTL14-mediated elevation of DIXDC1 levels also speeds up NPC degeneration and aging by activating the canonical Wnt pathway [194]. Furthermore, oxidative stress escalates apoptosis in NPCs by reducing MAT2A expression via METTL16-dependent m$^6$A modification [195]. ALKBH5 and YTHDF2 increase *FIP200* mRNA and *DNMT3B* mRNA expression through m$^6$A-dependent modification. Research has shown that although ALKBH5 expression was elevated in IVDD, it still exhibited a dual nature. FIP200 promotes autophagic flux, thus reducing apoptosis in compressed NPCs, while DNMT3B accelerates degeneration by inhibiting E4F1 expression [196, 197]. Moreover, m$^6$A modification alters the expression of ncRNAs affecting NPC activity. For instance, TNF-α enhances the expression of METTL3, which subsequently enhances *miR-143-3p* maturation through its methyltransferase activity. *MiR-143-3p* downregulates SOX5 transcription, accelerating degeneration in NPCs [198]. Similarly, METTL14 works together with DGCR8 to mature *miR-34a-5p*. This miRNA markedly reduces *SIRT1* mRNA translation, diminishing its expression, and accelerating the aging of NPCs [199]. The degradation of lncRNA *NORAD*, mediated by unusually high levels of WTAP and YTHDF2, reduces the sequestration of PUMILIO proteins, thereby intensifying PUM1/2 activity,

**Table 5** $m^6A$ methylation modification in OP and intervertebral disc degeneration (IVDD)

| Diseases | Regulators | Effects | Mechanism | Species and cells | Ref. |
|---|---|---|---|---|---|
| Osteoporosis | METTL3↑、IGF2BP1 | inhibition | Runx2↑ | Human BMSCs | [175, 176] |
| | YTHDF1↑ | inhibition | ZNF839↑/ Runx2↑ | Human BMSCs | [177] |
| | FTO↑ | inhibition | RBM4↑/ Runx2↑ | Human DPSCs | [178] |
| | METTL3↑、IGF2BP2/3 | inhibition | ACLY↑、SLC25A1↑ | Human DPSCs | [179] |
| | METTL3↑ | promotion | ASK1-p38↑、SLC7A11 ↑、GPX4↑ | Mouse MC3T3-E1 cells | [180] |
| | FTO↑、YTHDF1 | inhibition | PPARG↓ | Human MSCs | [181] |
| | FTO↑ | inhibition | Hsp70↑/NF-κB↓ | Mouse Osteoblasts | [182] |
| | METTL3↑ | promotion | FSH↑/ CREB phosphorylation↑/ METTL3↑/ CTSK↑ | Mouse Osteoblasts | [183] |
| | METTL14↑、YTHDF2 | inhibition | zoledronic acid↑/ METTL14↑/ NFATc1↓ | Mouse RAW264.7 monocytic cells | [184] |
| | METTL14↑ | inhibition | TCF1↓、SIRT1↓/ Wnt/β-catenin↑ | Mouse BMSCs and BMM s | [185, 186] |
| | FTO↑ | inhibition | TLR4↓ | Mouse RAW264.7 monocytic cells | [187] |
| IVDD | METTL3↑ | promotion | SIAH1↑/ XIAP↓ | Human NPCs | [192] |
| | METTL14↑、IGF2BP2 | promotion | NLRP3↑/ IL-1β↑、IL-18↑ | Human NPCs | [193] |
| | METTL14↑、IGF2BP1 | promotion | DIXDC1↑/ Wnt/β-catenin↑ | Human NPCs | [194] |
| | METTL16↑ | promotion | oxidative stress↑/ METTL16↑/MAT2A↓ | Human NPCs | [195] |
| | ALKBH5↑、YTHDF2 | inhibition | FIP200↑/ autophagic flux↑ | Human NPCs | [196] |
| | ALKBH5↑、YTHDF2 | Promotion | DNMT3B↑/E4F1↓ | Human NPCs | [197] |
| | METTL3↑ | Promotion | TNF-α↑/ METTL3↑/ miR-143-3p↑/ SOX5↓ | Human NPCs | [198] |
| | METTL14↑ | Promotion | miR-34a-5p↑/ SIRT1↓ | Human NPCs | [199] |
| | WTAP↑、YTHDF2 | Promotion | lncRNA NORAD↓/PUMILIO sequestraion↓/ PUM1/2 activity↑/ E2F3↓ | Human NPCs | [200] |
| | YTHDF2↑ | inhibition | CirGPATCH2L↓/ phosphorylation of TRIM28↑/ p53↑ | Human NPCs | [201] |
| | METTL3↑ | Promotion | SOX9↓/ COL2A1↓ | Human endplate chondrocytes | [202] |
| | METTL3↑ | Promotion | miR-126-5p↑/PIK3R2↓/PI3K/AKT↓ | Human endplate chondrocytes | [154] |

DPSCs: human dental stem pulp cells METTL3; NPCs: nucleus pulposus cells; COL2A1: type II collagen α1

which suppresses *E2F3* mRNA expression and accelerates aging in NPCs [200]. Additionally, *CircGPATCH2L* eliminates phosphorylation of TRIM28, which prevents p53 degradation, leading to DNA damage and increased apoptosis in NPCs. Concurrently, the YTHDF2-RPL10-RNase P/MRP complex targets and degrades $m^6A$-methylated *circGPATCH2L*, slowing IVDD progression [201]. Augmented levels of $m^6A$ methylation can contribute to the degeneration of cartilaginous endplates. For example, METTL3 facilitates the methylation of *SOX9* mRNA, which destabilizes *SOX9* mRNA and diminishes the expression of type II collagen α1 chain, reducing the tensile strength of endplate chondrocytes and accelerating disc degeneration [202]. Moreover, METTL3 aids in the maturation of *miR-126-5p* through $m^6A$ methylation. *MiR-126-5p* inhibits PIK3R2 expression, disrupting the PI3K/AKT signaling pathway and further promoting degeneration in endplate chondrocytes [154] (Table 5).

### Rheumatoid arthritis

In orthopedic research, $m^6A$ modification is crucial in regulating inflammation, particularly in the initiation and progression of RA [203]. Fibroblast-like synoviocytes are critical in RA, essential to both synovial hyperplasia and inflammatory responses. The $m^6A$ modification influences the pathological behavior of FLSs by regulating the stability and translation of key genes associated with cell proliferation, migration, and the release of inflammatory mediators. For instance, increased $m^6A$ methylation of *TGM2* mRNA promotes the proliferation of RA-FLSs by stimulating DNA replication, facilitating cell cycle transition, and activating the NF-κB pathway to inhibit apoptosis [204]. Despite the observed decrease in METTL14 concentrations in individuals with RA, this factor demonstrates bifunctional characteristics. METTL14 increases the expression of LASP1 and TNFAIP3 via $m^6A$ modification. LASP1 activates the SRC/AKT signaling pathway, thereby augmenting the activity and inflammatory responses of FLSs. Conversely, TNFAIP3 reduces inflammatory responses by disrupting the NF-κB signaling pathway [205, 206]. Furthermore, elevated expression of METTL3 enhances $m^6A$ modification of *AMIGO2* mRNA and *PGC-1α* mRNA. With YTHDC2 involvement, *AMIGO2* mRNA displays increased expression,

enhancing the proliferation, migration, and invasiveness of FLSs. In contrast, *PGC-1α* mRNA undergoes rapid degradation by YTHDF2, causing mitochondrial dysfunction and intensifying inflammatory responses in RA [207, 208]. Additionally, WTAP upregulates lncRNA *MAPKAPK5-AS1* expression through demethylation, disrupting the inhibition of SIRT1 by *miR-146a-3p*. As a result, SIRT1 inhibits the NF-κB pathway, leading to apoptosis in synovial cells and reduced inflammation in RA [209]. For erasers, FTO and ALKBH5 exhibit opposite expression patterns and functions in RA. FTO exhibits low expression levels and suppresses NSUN2 expression, increases SFRP1 protein levels, and inhibits the Wnt/β-catenin pathway via its demethylase activity, thereby mitigating the progression of RA [210]. Still, upregulated ALKBH5 increases the stability and protein expression of *CH25H* and *MYO1C* mRNAs by its demethylase activity, leading to cytoskeletal remodeling and enhanced migration and invasion of FLSs [211, 212]. ALKBH5 also downregulates the stability and expression of *JARID2* mRNA, facilitated by IGF2BP3, and reduces *NLRP3* mRNA degradation under YTHDC2 regulation. These mechanisms collectively advance the proliferation, migration, and invasion of FLSs, intensifying the severity of arthritis [213, 214] (Fig. 5).

## Bone tumors

### *Osteosarcoma*

During the development of osteosarcoma, m$^6$A methylation modification markedly affects cancer cell behavior, mainly mediated by methyltransferases and associated readers. METTL3, a central m$^6$A methyltransferase, significantly regulates the malignant phenotype of osteosarcoma by modifying various mRNAs and ncRNAs. On the one hand, METTL3 enhances cell proliferation by upregulating the expression of mRNAs like *ZBTB7C*, *ATAD2*,

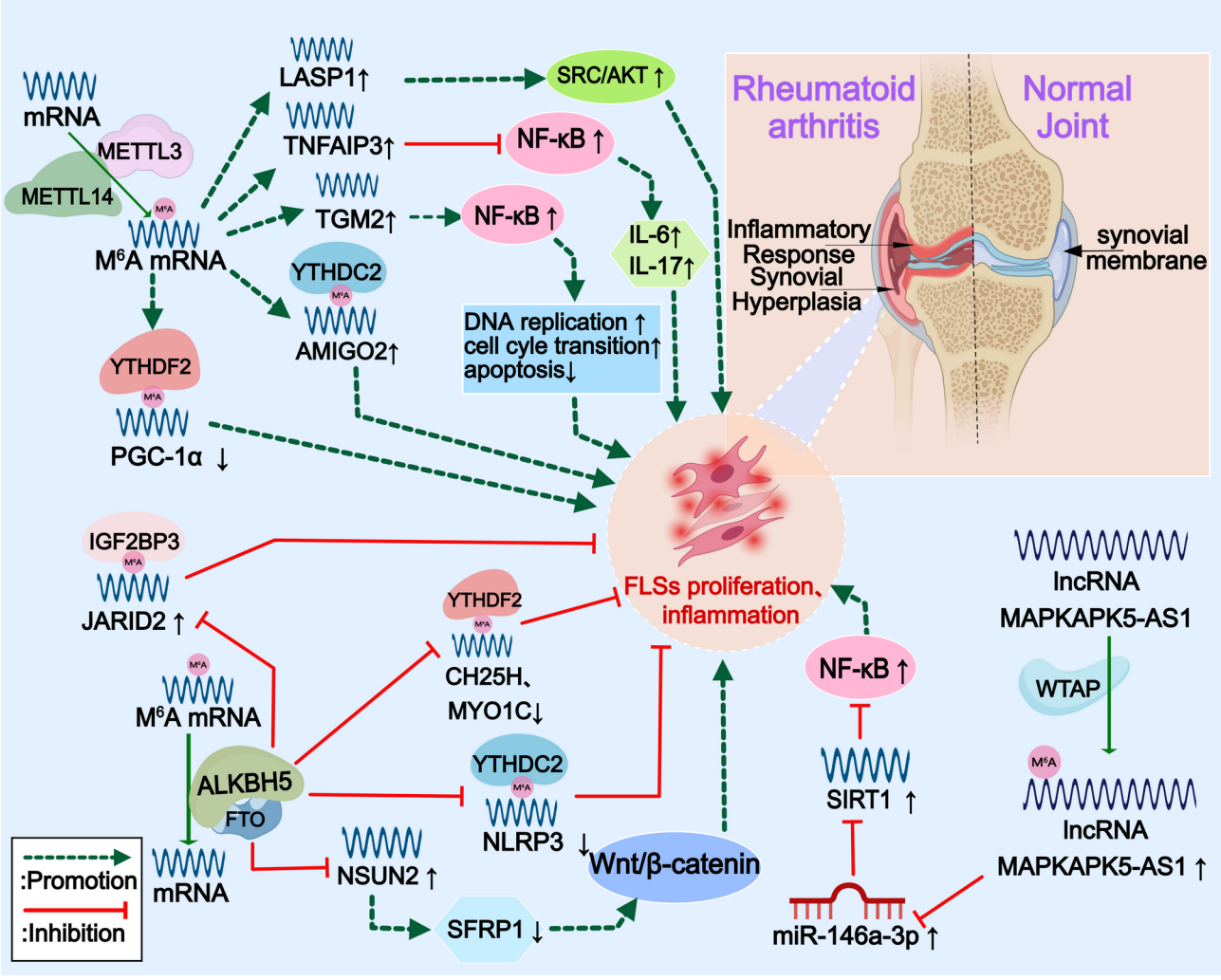


**Fig. 5** m$^6$A methylation modification in RA. m$^6$A modification influences the pathological behavior of fibroblast-like synoviocytes (FLSs) and inflammation reaction

and *HDAC5*. Specifically, HDAC5 downregulates *miR-142-5p* to increase ARMC8 expression [215–217]. On the other hand, METTL3 collaborates with YTHDF2 to degrade *TRIM7* mRNA, thereby reducing metastasis and chemoresistance in osteosarcoma. Notably, TRIM7 induces the ubiquitination and degradation of BRMS1, a metastasis inhibitor [218]. METTL3 also elevates the levels of *circRNF220*, which acts as a sponge for *miR-330-5p* and increases survivin expression, further regulating the progression of osteosarcoma [219]. In addition, METTL14 and WTAP are involved in the m$^6$A modification network that increases osteosarcoma malignancy. METTL14 prevents *MN1* mRNA degradation by enhancing its m$^6$A modification and recognition by IGF2BP2, thereby promoting the progression of osteosarcoma and resistance to all-trans retinoic acid. Meanwhile, WTAP accelerates cancer progression by enhancing the m$^6$A modification of lncRNA *FOXD2-AS1*, which interacts with *FOXM1* mRNA via m$^6$A sites to increase its stability [220, 221]. By contrast, METTL14 induces apoptosis in osteosarcoma by activating caspase-3, thus inhibiting the proliferation and migration of osteosarcoma cells [222]. The markedly upregulated demethylase ALKBH5 and associated readers also contribute to the enhanced malignancy of osteosarcoma. ALKBH5 collaborates with YTHDF2 to enhance lncRNA *PVT1* expression through demethylation, promoting osteosarcoma development [223]. Moreover, YTHDF1 and YTHDF2, which are crucial for m$^6$A modification, play distinct roles in cellular processes. YTHDF1 stabilizes *YAP* transcripts, while YTHDF2 degrades *pre-miR-181b-1* transcripts, collectively facilitating cell proliferation [224]. YTHDC1 and YTHDF3 increase mRNA stability of *PFKM*, *LDHA*, and *PGK1* by recognizing m$^6$A sites, thereby promoting glycolysis and osteosarcoma progression [225, 226].

m$^6$A modification regulates osteosarcoma cell behavior by activating multiple signaling pathways. For example, METTL3 upregulates LEF1, activating the Wnt/β-catenin pathway and advancing osteosarcoma progression [227]. By contrast, FTO promotes osteosarcoma growth and metastasis by inhibiting DACT1 and activating the Wnt/β-catenin pathway [228]. Conversely, ALKBH5 hampers osteosarcoma proliferation and malignancy by blocking the STAT3 pathway and decreasing SOCS3 expression via YTHDF2 [229]. With respect to the PI3K/AKT pathway, METTL16 and WTAP lower VPS33B and HMBOX1 expression, respectively, thus promoting osteosarcoma growth and metastasis via the PI3K/AKT pathway [230, 231]. Meanwhile, YTHDC1 stabilizes *PDPK1* mRNA via m$^6$A-dependent regulation, activating the AKT/mTOR pathway and fostering osteosarcoma progression [232] (Table 6).

#### *Multiple myeloma*

m$^6$A methylation and its regulatory enzymes are crucial in the pathogenesis of MM. These enzymes, abnormally elevated in the tumor environment, alter gene expression and signaling pathways, thereby promoting MM progression and resistance to therapy. METTL3 elevates the levels of oncogenes such as *THRAP3*, *RBM25*, *USP4*, and *BZW2*, which accelerate the proliferation of MM cells and inhibit apoptosis [233, 234]. Besides, METTL3 facilitates the maturation of *pri-miR-182* and *pri-miR-27* in collaboration with DGCR8 and promotes the proliferation of MM cells. This process includes *miR-182-5p* specifically targeting and inhibiting the expression of CAMK2N1 [235, 236]. Furthermore, WTAP contributes to the complexity of MM by influencing energy metabolism and extracellular communication. WTAP reduces the expression of *NDUFS6* mRNA via YTHDF2, suppressing the activation of oxidative phosphorylation and tumor growth [237].

The roles of erasers ALKBH5 and FTO in tumor cell survival and proliferation are mediated through demethylation. ALKBH5 enhances lncRNA *SNHG15* expression and stability, thereby recruiting SETD2 and increasing chromatin accessibility, which promotes tumor growth [238]. ALKBH5 also stabilizes *TRAF1* mRNA, activating NF-κB and MAPK pathways to cause the same effect [239]. In parallel, FTO diminishes the stability of *SOD2* mRNA, thereby augmenting bortezomib resistance, and activates HSF1 to promote proliferation, migration, and invasion through an m$^6$A-YTHDF2-dependent mechanism [24, 240]. Additionally, FTO elevates WNT7B expression, thereby activating the Wnt pathway and fostering the progression of MM [241].

In addition, the HNRNPA2B1 and YTHDF2 readers, regulate tumor cell proliferation via m$^6$A-dependent post-transcriptional mechanisms. HNRNPA2B1 stabilizes *ILF3* and *TLR4* mRNA, thereby activating the PI3K-AKT signaling pathway and enhancing MM cell proliferation [242, 243]. It also disrupts the balance between osteoclasts and osteoblasts by increasing the expression of *miR-92a-2-5p* and *miR-373-3p*, thereby exacerbating osteolytic lesions. Specifically, *miR-92a-2-5p* increases NFATc1 levels by suppressing IRF8, and *miR-373-3p* reduces Runx2 expression [244]. Additionally, YTHDF2 degrades *EGR1* mRNA via m$^6$A modification, hence disrupting the transcription of p21$^{cip1/waf1}$ and increasing the expression of CDK2-cyclinE1, which promotes the proliferation of MM cells [245]. YTHDF2 also degrades *STAT5A* mRNA, while STAT5A inhibits MM cell proliferation by binding to the transcriptional sites of *MAP2K2* mRNA, consequently reducing ERK phosphorylation levels [246] (Table 6).

**Table 6** $m^6A$ methylation modification in bone tumors

| Tumor types | Regulators | Effects | Mechanism | Species and cells | Ref. |
|---|---|---|---|---|---|
| Osteosarcoma | METTL3↑ | promotion | ZBTB7C↑, ATAD2↑, HDAC5↑/ miR-142-5p↓/ ARMC8↑ | Human osteosarcoma cell | [215–217] |
| | METTL3↑、METTL14↑YTHDF2 | inhibition | TRIM7↓/ BRMS1↑ | Human osteosarcoma cell | [218] |
| | METTL3↑ | promotion | circRNF220↑/ miR-330-5p↓/ Survivin↑ | Human osteosarcoma cell | [219] |
| | METTL14↑、IGF2BP2 | promotion | MN1↑ | Human osteosarcoma cell | [220] |
| | WTAP↑ | promotion | lncRNA FOXD2-AS1↑/ FOXM1↑ | Human osteosarcoma cell | [221] |
| | METTL14↑ | inhibition | caspase-3↑ | Human osteosarcoma cell | [222] |
| | ALKBH5↑、YTHDF2 | promotion | lncRNA PVT1↑ | Human osteosarcoma cell | [223] |
| | ALKBH5↑YTHDF1 | inhibition | YAP↓ | Human osteosarcoma cell | [224] |
| | ALKBH5↑YTHDF2 | inhibition | miR-181b-1↑/ YAP↓ | Human osteosarcoma cell | [224] |
| | YTHDC1↑ | promotion | LDHA↑、PFKM↑ | Human osteosarcoma cell | [225] |
| | YTHDF3↑ | promotion | PGK1↑ | Human osteosarcoma cell | [226] |
| | METTL3↑ | promotion | LEF1↑/ Wnt/β-catenin↑ | Human osteosarcoma cell | [227] |
| | FTO↑ | promotion | DACT1↓/ Wnt/β-catenin↑ | Human osteosarcoma cell | [228] |
| | ALKBH5↑、YTHDF2 | inhibition | SOCS3↓/JAK2/ STAT3↓ | Human osteosarcoma cell | [229] |
| | METTL16↑ | promotion | VPS33B↓/PI3K/AKT↑ | Human osteosarcoma cell | [230] |
| | WTAP↑ | promotion | HMBOX1↓/PI3K/AKT↑ | Human osteosarcoma cell | [231] |
| | YTHDC1↑ | promotion | PDPK1↑/ AKT/mTOR↑ | Human osteosarcoma cell | [232] |
| Multiple myeloma | METTL3↑ | promotion | THRAP3↑、RBM25↑、USP4↑、BZW2↑ | Human MM cell lines H929 | [233, 234] |
| | METTL3↑ | promotion | miR-182-5p↑/CAMK2N1↓, miR-27a-3p↑, YY1↑ | Human MM cell lines H929 | [235, 236] |
| | WTAP↑、YTHDF2 | inhibition | NDUFS6↓/ oxidative phosphorylation↓ | Human MM cell lines | [237] |
| | ALKBH5↑ | promotion | lncRNA SNHG15↑/SETD2↑ | Human MM cell lines | [238] |
| | ALKBH5↑ | promotion | TRAF1↑/NF-κB↑、MAPK↑ | Human MM cell lines | [239] |
| | FTO↑、YTHDF2 | promotion | SOD2↓, HSF1↑ | Human MM cell lines | [24, 240] |
| | FTO↑ | promotion | WNT7B↑/ Wnt/β-catenin↑ | Human MM cell lines | [241] |
| | HNRNPA2B1↑ | promotion | ILF3↑、TLR4↑/ PI3K-AKT↑ | Human MM cell lines | [242, 243] |
| | hnRNPA2B1↑ | promotion | miR-92a-2-5p↑/ IRF8↓/ NFATc1↑, miR-373-3p↑/ Runx2↓ | Human MM cell lines | [244] |
| | YTHDF2↑ | promotion | EGR1↓/ CDK2-cyclinE1↑ | Human MM cell lines | [245] |
| | YTHDF2↑ | promotion | STAT5A↓/ MAP2K2/ p -ERK↑ | Human MM cell lines | [246] |
| Metastatic bone tumors | | | | | |
| metastatic prostate cancer | | promotion | $m^6A$-lncRNA NEAT1-1↑/ CYCLINL1/CDK19↑/ p-RNPII ser2↑/ Runx2↑ | Human prostate cancer cell | [247] |
| | METTL3↑、IGF2BP2 | promotion | lncRNA PCAT6↑/ IGF1R↑ | Human prostate cancer cell | [249] |
| | METTL3↑、RBM3 | inhibition | CTNNB1↓/Wnt/β-catenin↓ | Human prostate cancer cell | [250] |
| Metastatic hepatocellular carcinoma | METTL3↑、YTHDF1 | promotion | ANLN↑/KIF2C↑/mTORC1↑/RANKL↑ | Human hepatocellular carcinoma cell | [251] |
| Metastatic breast cancer | YTHDF2↑ | | lncRNA FGF14-AS2↓/ eIF4E/eIF4G↑、p-eIF4E↑/ Runx2↑/ RANKL↑ | Human breast cancer cell | [252] |

***Metastatic bone tumors***

Bone metastasis commonly complicates advanced-stage cancers, especially breast and prostate cancers. $m^6A$ significantly influences the bone microenvironment by disrupting the balance between bone destruction and formation, altering tumor cell secretion of inflammatory factors and proteases and promoting bone metastases. In metastatic prostate cancer (mPCa), the $m^6A$-modified lncRNA *NEAT1-1* activates the CYCLINL1/CDK19 complex when in an elevated state. This complex is then recruited to the Runx2 promoter, leading to RNPII ser2 phosphorylation. Activation of the Runx2 pathway stimulates tumor growth and metastasis [247]. KHSRP binds to $m^6A$ within enhancer RNA (eRNA) and $m^6A$ m in the 5'-UTR of *PSMD9* mRNA, thereby inhibiting *PSMD9* mRNA degradation mediated by *XRN2*. This interaction significantly enhances tumor growth and radiotherapy resistance in mPCa [248]. The $m^6A$ methyltransferase

activity of METTL3 is significant in this context; specifically, METTL3 enhances lncRNA *PCAT6* levels through m$^6$A modifications, in an *IGF2BP2*-dependent manner. *PCAT6* stabilizes *IGF1R* mRNA via the PCAT6/IGF2BP2/IGF1R complex, increasing IGF1R expression and thereby promoting bone metastasis and tumor growth in prostate cancer [249]. Additionally, METTL3 increases m$^6$A methylation on *CTNNB1* mRNA in a *RBM3*-dependent manner, reducing its stability and inhibiting the Wnt signaling pathway, which reduces the stemness and plasticity of tumor cells [250]. Similarly, aberrantly elevated METTL3 is strongly linked to bone metastasis in hepatocellular carcinoma. METTL3-mediated m$^6$A modification augments *ANLN* expression via YTHDF1, forming a transcription complex with SP1 that enhances *KIF2C* transcriptional activity and activates the mTORC1 pathway. This activation elevates RANKL levels, disrupts the RANKL-OPG balance in the bone microenvironment, and facilitates liver cancer invasion into bone [251]. In addition, YTHDF2 degrades lncRNA *FGF14-AS2* through m$^6$A, which inhibits Runx2 translation by disrupting the eIF4E/eIF4G complex and phosphorylation of eIF4E, subsequently reducing RANKL transcription and inhibiting osteolytic bone metastasis in breast cancer [252] (Table 6).

## Conclusions

In the field of epigenetics, m$^6$A modification can significantly influence gene expression and cellular fate decisions by regulating RNA splicing, stability, and translational efficiency. Moreover, this modification is pivotal in determining the onset, progression, and therapeutic response of orthopedic diseases [253]. This review comprehensively explores the profound effect of m$^6$A modification in the differentiation of BMSCs and its regulatory mechanisms in orthopedic diseases. By analyzing the complex interaction network among writers, erasers, and readers, primarily involving METTL3/14/16, WTAP, FTO, ALKBH5, YTHDF1/2/3, YTHDC1/2, and IGF2BP1/2/3, the article reveals how this epigenetic modification intricately regulates key gene expressions, thereby influencing cell destiny and disease progression. Primarily, m$^6$A modification modulates mRNA stability and translation efficiency, either directly or indirectly, affecting the differentiation of BMSCs into osteoblasts, adipocytes, and chondrocytes, as well as regulating the formation and activity of osteoclasts. Additionally, m$^6$A modification controls the biological activity of bone cells by influencing the expression and stability of molecules within crucial signaling pathways, including Wnt/β-catenin, BMP/Smad, and PI3K/AKT. This regulatory mechanism plays a significant role in the progression of diseases like OP and OA. The dynamic alterations and regulatory mechanisms of m$^6$A modification underscore its crucial role in maintaining skeletal health and addressing pathological conditions such as OP and non-healing fractures. However, current research on the role of m$^6$A modification in RNA stability, cellular activities, and orthopedic disease progression has produced conflicting results. Specifically, m$^6$A modification exhibits dual effects on RNA stability, cell proliferation and differentiation, apoptosis, inflammatory responses, and tumorigenicity. These contradictions may arise from several factors, primarily the diversity and functional overlap of m$^6$A reader proteins. Different reader proteins can exert distinct or opposing effects on the same modification. For example, YTHDF2 promotes mRNA degradation, YTHDF1 facilitates translation, and the IGF2BP family enhances mRNA stability and translation efficiency. Competitive binding among reader proteins and changes in their expression levels can lead to functional differences [254]. The same m6A modification may be recognized by different readers depending on context and cell state [67, 255]. Additionally, m$^6$A regulation is significantly influenced by cell type and tissue specificity, as m$^6$A -related enzyme and reader protein expression patterns differ among cells, leading to inconsistent functions under different physiological and pathological conditions [256, 257]. Furthermore, differences in experimental conditions and models may also contribute to these inconsistencies, as varying methodologies and environmental factors (e.g., hypoxia, inflammation) significantly affect m$^6$A function [258]. To resolve these contradictions, future research should elucidate the functional characteristics of m$^6$A reader proteins, especially their roles in different cell types and microenvironments. Single-cell sequencing (e.g., scRNA-seq, scMeRIP-seq) can reveal variations in m$^6$A modifications and reader protein expression, while CRISPR/Cas9 gene editing offers precise functional validation [259, 260]. Integrating multi-omics analysis will help construct comprehensive regulatory models of m$^6$A, further elucidating its diverse regulatory mechanisms [57].

Current studies on m$^6$A methylation in diseases have expanded to include its regulatory roles in various orthopedic conditions (including bone tumors) and other pathological states, such as cancers. In orthopedic conditions, m$^6$A methylation regulates osteogenesis, cartilage degeneration, inflammatory responses, and bone tumors. In cancer, m$^6$A methylation influences cell proliferation, invasion, and immune evasion [261]. Future research should investigate the role of m$^6$A regulatory factors in bone-related cells, including bone marrow mesenchymal stem cells, as well as the regulatory mechanisms in osteoblasts, osteoclasts, and chondrocytes, to enable precise targeted interventions. This will support the development of novel therapeutic approaches for bone formation and cartilage repair. CRISPR/Cas gene-editing

technology can precisely modulate m$^6$A methylation, enhancing the treatment of orthopedic diseases [262]. For instance, combining gene-editing technologies with delivery systems can target bone tissue cells precisely, improving editing efficiency and therapeutic outcomes [184]. Emerging technologies, such as single-cell sequencing and spatial transcriptomics, can reveal cell-type-specific m$^6$A regulatory patterns in bone tissues, especially dynamic changes in osteoblasts, osteoclasts, and chondrocytes [263]. These techniques are crucial for understanding the contributions of different cells in bone formation and repair, providing a basis for personalized therapies. Another key direction is integrating material science to explore the synergistic effects of m$^6$A methylation and biomaterials in bone regeneration. For example, functionalizing bone repair scaffolds with m$^6$A -regulated bioactive factors may promote bone regeneration and healing. Combining delivery systems with functionalized biomaterials allows precise delivery of bioactive factors within scaffolds, enhancing bone regeneration [264, 265]. These research directions advance orthopedic disease treatment and open new avenues for applications at the intersection of material science and epigenetics. Advancing these directions will provide a comprehensive understanding of m$^6$A methylation in orthopedic diseases, laying a foundation for future therapeutic research.

In future research studies, the molecular mechanisms of m$^6$A and other RNA modifications in orthopedic diseases need to be explored via both in vivo and in vitro experiments, with a focus on their effects on cellular behaviors and signaling pathways. This review emphasizes the need for ongoing research into epigenetic mechanisms in orthopedic diseases through in-depth discussions and analyses. We believe this review will enhance our comprehension of the disease's nature and foster the development of new therapeutic methods, ultimately improving clinical outcomes for patients.

**Abbreviations**

| | |
|---|---|
| m$^6$A | N6-methyladenosine |
| BMSC | Bone marrow mesenchymal stem cell |
| 3' UTRs | 3′ Untranslated Regions |
| METTL3 | Methyltransferase-like 3 |
| MTC | m$^6$A methyltransferase complex |
| MAC | m$^6$A -METTL complex |
| MACOM | m$^6$A -METTL-associated complex |
| SAM | S-adenosyl methionine |
| WTAP | Wilms tumor 1-associated protein |
| VIRMA/KIAA1429 | Vir-like m$^6$A methyltransferase associated |
| RBM15/RBM15B | RNA Binding Motif Protein 15/15B |
| ZC3H13 | Zinc finger CCCH domain-containing protein 13 |
| HAKAI | E3 ubiquitin ligase HAKAI |
| SMAD | Mothers against decapentaplegic homolog |
| Nanog | Nanog homeobox |
| CEBPZ | CCAAT/enhancer binding protein zeta |
| SP1 | Specificity protein 1 |
| ZFP217 | Zinc-finger protein 217 |
| MTD | N-terminal methyltransferase domain |
| VCRs | C-terminal vertebrate-conserved regions |
| TRMT112 | tRNA methyltransferase 11 – 2 homolog |
| ZCCHC4 | Zinc finger CCHC-type containing 4 |
| FTO | Fat mass and obesity-associated protein |
| ALKBH5 | Alkylation repair homolog 5 |
| NTD | N-terminal domain |
| pre-mRNA | Precursor mRNA |
| YTH domain | YT521-B homology domain |
| SRSF3 | Serine/arginine-rich splicing factor 3 |
| NXF1 | Nuclear export factor 1 |
| XRN1 | 5′–3′ ribonuclease 1 |
| IGF2BPs | Insulin-like Growth Factor 2 mRNA-binding proteins |
| RRM | RNA Recognition Motif |
| KH domains | K-Homology domains |
| hnRNPs | Heterogeneous nuclear ribonucleoproteins |
| Prrc2a | Proline rich coiled-coil 2 A |
| LRP5/6 | Low-density lipoprotein receptor-related protein 5/6 |
| TCF/LEF | T-cell factor/lymphoid enhancer factor |
| Runx2 | Runt-related transcription factor 2 |
| Osterix | Sp7 transcription factor |
| P-Gsk-3β | Phosphorylated glycogen synthase kinase 3 beta |
| PTPN6 | Protein tyrosine phosphatase, non-receptor type 6 |
| AGEs | Advanced glycation end-products |
| SOST | Sclerostin |
| PIWIL4 | Piwi-like protein 4 |
| BMP2 | Bone morphogenetic protein 2 |
| NOG | Noggin |
| PI3K | Phosphoinositide 3-kinase |
| AKT | AKT serine/threonine kinase |
| PRMT6 | Protein arginine methyltransferase 6 |
| NF-κB | Nuclear factor kappa-light-chain-enhancer of activated B cells |
| MAPK | Mitogen-activated protein kinase |
| AMPK | AMP-activated protein kinase |
| MYD88 | Myeloid differentiation primary response 88 |
| Pth1r | Parathyroid hormone receptor-1 |
| PKA | Protein kinase A |
| ERK | Extracellular signal-regulated kinase |
| ER | Endoplasmic reticulum |
| Dlx5 | Distal-less homeobox 5 |
| ceRNA | Competing endogenous RNA |
| BMPR1B | Bone morphogenetic protein receptor type 1B |
| DGCR8 | DiGeorge syndrome critical region gene 8 |
| FGFR3 | Fibroblast growth factor receptor 3 |
| SFRP1 | Secreted frizzled-related protein 1 |
| FBLN1 | Fibulin 1 |
| JAK | Janus kinase |
| STAT | Signaling and transcription activator factor |
| C/EBP | CCAAT/enhancer-binding protein |
| TRAF | TNF receptor-associated factor |
| CCNA2 | Cyclin A2 |
| CDK2 | Cyclin-dependent kinase 2 |
| MCE | Mitotic clonal expansion |
| PNPLA2 | Patatin-like phospholipase domain-containing protein 2 |
| RUNX1T1 | Runt-related transcription factor 1, translocated to 1 |
| PPARG | Peroxisome proliferator-activated receptor gamma |
| MMP | Matrix metalloproteinase |
| GATA3 | GATA binding protein 3 |
| Nsun | NOP2/Sun RNA methyltransferase family member |
| eEF1α-1 | Eukaryotic elongation factor 1 alpha 1 |
| Sox9 | SRY-box transcription factor 9 |
| Dmp1 | Dentin matrix protein 1 |
| Atp6v0d2 | ATPase $H^+$ transporting V0 subunit d2 |
| Nos2 | Nitric oxide synthase 2 |
| iNOS | Inducible nitric oxide synthase |
| CHI3L1 | Chitinase 3-like 1 |
| RANK | Receptor activator of nuclear factor κB |
| RANKL | Receptor activator of nuclear factor κB ligand |
| HuR | Hu antigen R |
| GPX4 | Glutathione peroxidase 4 |
| c-FOS | Cellular FOS proto-oncogene |
| NFATc1 | Nuclear factor of activated T-cells, cytoplasmic 1 |
| PERK | Protein kinase RNA-like endoplasmic reticulum kinase |

| | |
|---|---|
| IRE1α | Inositol-requiring enzyme 1 alpha |
| ATF6 | Activating transcription factor 6 |
| OCS3 | O-GlcNAc-specific hydrolase 3 |
| Oct4 | Octamer-binding transcription factor 4 |
| TWIST1 | Twist family bHLH transcription factor 1 |
| ECM | Extracellular matrix |
| OA | Osteoarthritis |
| IL-1β | Interleukin-1β |
| DNMT | DNA methyltransferase |
| HRSP | Heat-responsive protein |
| MRP | Multidrug resistance-associated protein |
| Bcl2 | B-cell lymphoma 2 |
| PRKAA2 | Protein kinase AMP-activated catalytic subunit alpha 2 |
| AQP1 | Aquaporin 1 |
| ANKH | ANKH inorganic pyrophosphate transport regulator |
| HS3ST3B1 | Heparan sulfate-glucosamine 3-sulfotransferase 3B1 |
| SIRT1 | Sirtuin 1 |
| TIMPs | Tissue inhibitors of metalloproteinases |
| SOCS2 | Suppressor of cytokine signaling 2 |
| NLRP3 | NOD-like receptor family pyrin domain containing 3 |
| FRZB | Frizzled-related protein |
| TLR4 | Toll-like receptor 4 |
| TNFRSF9 | Tumor necrosis factor receptor superfamily member 9 |
| OP | Osteoporosis |
| RBM4 | RNA binding motif protein 4 |
| ACLY | ATP-citrate lyase |
| SLC25A1 | Solute carrier family 25 member 1 |
| Hspa1a | Heat shock protein family A member 1 A |
| FSH | Follicle-stimulating hormone |
| CTSK | Cathepsin K |
| TCF1 | T-cell factor 1 |
| FNDC5 | Fibronectin type III domain containing 5 |
| SAH | S-adenosylhomocysteine |
| IVDD | Intervertebral disc degeneration |
| NPCs | Nucleus pulposus cells |
| SIAH1 | Seven in absentia homolog 1 |
| XIAP | X-linked inhibitor of apoptosis protein |
| DIXDC1 | Dishevelled-Axin domain containing 1 |
| FIP200 | FAK family-interacting protein of 200 kDa |
| DNMT3B | DNA methyltransferase 3 beta |
| SOX5 | SRY-box transcription factor 5 |
| PUMILIO | Pumilio RNA-binding family member |
| PUM1/2 | Pumilio RNA-binding family member 1/2 |
| E2F3 | E2F transcription factor 3 |
| TRIM28 | Tripartite motif-containing 28 |
| RPL | Ribosomal protein, large subunit |
| RA | Rheumatoid arthritis |
| FLSs | Fibroblast-like synoviocytes |
| TGM2 | Transglutaminase 2 |
| TNFAIP3 | Tumor necrosis factor alpha-induced protein 3 |
| LASP1 | LIM and SH3 domain protein 1 |
| SRC | Proto-oncogene tyrosine-protein kinase Src |
| AMIGO2 | Adhesion molecule with Ig like domain 2 |
| PGC-1α | Peroxisome proliferator-activated receptor gamma coactivator 1-alpha |
| CH25H | Cholesterol 25-hydroxylase |
| MYO1C | Myosin IC |
| JARID2 | Jumonji and AT-rich interaction domain containing 2 |
| ZBTB7C | Zinc finger and BTB domain containing 7 C |
| ATAD2 | ATPase family AAA domain-containing 2 |
| HDAC5 | Histone deacetylase 5 |
| ARMC8 | Armadillo repeat containing 8 |
| TRIM7 | Tripartite motif containing 7 |
| BRMS1 | Breast cancer metastasis suppressor 1 |
| MN1 | Meningioma 1 |
| FOXM1 | Forkhead box M1 |
| PVT1 | Plasmacytoma Variant Translocation 1 |
| YAP | Yes-associated protein |
| PFKM | Phosphofructokinase, muscle |
| LDHA | Lactate dehydrogenase A |
| PGK1 | Phosphoglycerate kinase 1 |
| DACT1 | Dishevelled associated antagonist of beta-catenin 1 |
| VPS33B | Vacuolar protein sorting 33B |
| HMBOX1 | Homeobox containing 1 |
| PDPK1 | 3-phosphoinositide dependent protein kinase 1 |
| mTOR | Mammalian target of rapamycin |
| MM | Multiple myeloma |
| THRAP3 | Thyroid hormone receptor associated protein 3 |
| RBM25 | RNA binding motif protein 25 |
| USP4 | Ubiquitin-specific protease 4 |
| BZW2 | Basic leucine zipper and W2 domain containing 2 |
| CAMK2N1 | Calcium/calmodulin-dependent protein kinase 2 inhibitor 1 |
| SETD2 | SET domain containing 2 |
| SOD2 | Superoxide dismutase 2 |
| HSF1 | Heat shock factor 1 |
| WNT7B | Wingless-type MMTV integration site family member 7B |
| ILF3 | Interleukin enhancer-binding factor 3 |
| IRF8 | Interferon regulatory factor 8 |
| EGR1 | Early growth response 1 |
| mPCa | Metastatic prostate cancer |
| RNPII | RNA polymerase II |
| KHSRP | KH-type splicing regulatory protein |
| eRNA | Enhancer RNA |
| PSMD9 | Proteasome 26 S subunit, non-ATPase 9 |
| XRN2 | 5′-3′ exoribonuclease 2 |
| PCAT6 | Prostate cancer-associated transcript 6 |
| IGF1R | Insulin-like growth factor 1 receptor |
| CTNNB1 | Catenin beta 1 |
| ANLN | Anillin,, actin-binding protein |
| KIF2C | Kinesin family member 2 C |
| OPG | Osteoprotegerin |

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**Author contributions**
YC conceived the project; all authors wrote the manuscript; DW, and FG revised the manuscript.

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**Data availability**
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## Declarations

**Ethics approval and consent to participate**
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**Consent for publication**
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**Competing interests**
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