m^{6}A modification in skeletal system diseases (Review)

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Abstract. N6-methyladenosine (m^{6}A) modifications are key epigenetic regulatory mechanisms in mammals and serve key roles in both normal skeletal development and the pathogenesis of skeletal disorder. The dynamic and reversible regulation of m^{6}A relies on three core factors: Methyltransferases (writers), demethylases (erasers) and m6A-binding proteins (readers), which collectively ensure proper physiological functions. Despite this, the functions and regulatory mechanisms of numerous m^{6}A-associated factors in skeletal diseases remain insufficiently understood. m^{6}A modification maintains bone homeostasis during skeletal development primarily by regulating the balance between osteoblasts and osteoclasts. Under pathological conditions, dysregulated m^{6}A modification contributes to aberrant osteoclast proliferation and chondrocyte apoptosis, leading to bone loss and cartilage degeneration. These pathological changes are key contributors to common types of skeletal disorder, including osteoporosis, osteoarthritis, rheumatoid arthritis and intervertebral disc degeneration, imposing a burden on human health. Non-coding RNAs are major targets of m^{6}A modification and their interactions exert post-transcriptional regulation in skeletal biology. The present review summarizes the roles and mechanisms of m^{6}A modification in skeletal diseases and highlights its therapeutic potential, offering novel perspectives for disease prevention and treatment.

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Abbreviations: m^{6}A, N6-methyladenosine; METTL, methyltransferase-like; WTAP, Wilms tumor 1-associated protein; FTO, fat mass and obesity-associated protein; ALKBH5, AlkB homolog 5; YTHDF, YT521-B homolog domain family protein; YTHDC, YT521-B homolog domain-containing protein; IGF2BP, insulin-like growth factor 2 mRNA-binding protein; MeRIP, methylated RNA immunoprecipitation; UTR, untranslated region; CDS, coding sequence; lnc, long non-coding; OP, osteoporosis; OA, osteoarthritis; RA, rheumatoid arthritis; IVDD, intervertebral disc degeneration; BMSC, bone marrow mesenchymal stem cell; FLS, fibroblast-like synoviocyte; EMT, epithelial-mesenchymal transition; NLRP3, NLR family pyrin domain containing 3; OVX, ovariectomy; RUNX2, runt-related transcription factor 2; ATG7, autophagy-related 7; SIRT1, sirtuin 1

Key words: N6-methyladenosine methylation, epigenetics, skeletal system disease, m^{6}A regulatory factor

1. Introduction

Epigenetics refers to heritable phenotypical changes that occur without alterations in the underlying DNA sequence (1). Cell differentiation is driven by the establishment of distinct epigenetic landscapes and transcriptional programs, which determine lineage commitment and cell fate (2). Epigenetic regulation encompasses diverse mechanisms, including post-translational histone modification and non-coding (nc) RNA expression, as well as DNA and RNA methylation (3). Since Waddington (4) proposed the concept of the epigenetic landscape (4), the contribution of epigenetic regulation to the development and progression of systemic diseases has been increasingly recognized (5,6). In numerous disorders such as hematological and cardiovascular disease, Alzheimer's disease, cancer and musculoskeletal conditions, epigenetic mechanisms serve key roles in pathogenesis (7-12).

RNA modification, a key regulatory mechanism within the epigenetic landscape, acts as an intermediary between DNA, proteins and effector molecules, serving an important role in post-transcriptional gene regulation (13). In addition to the canonical nucleotides A, C, G and U, >100 distinct chemical modifications have been identified in RNA (14). For example, a classical mRNA contains a 5'cap structure that

enables ribosome recognition and provides protection from degradation, as well as a 3'poly-A tail that regulates transcription termination, facilitates nuclear export and enhances translation efficiency (15). Following the discovery of cap and tail modifications, numerous internal RNA modifications have been identified, such as N1-methyladenosine (16), N6-methyladenosine (m^{6}A) (17,18) and 5-methylcytosine (19). Among these, m^{6}A is the most abundant and dynamically regulated internal RNA modification in eukaryotic cells (20).

Skeletal system diseases, including osteoporosis (OP), osteoarthritis (OA), rheumatoid arthritis (RA), ankylosing spondylitis, lumbar disc herniation and cervical spondylosis, represent a notable category of disorders worldwide (21). OA affected 595 million people globally in 2020, rheumatoid arthritis 17.6 million, and lower back pain 619 million, with all three projected to increase further by 2050; OP is common, with a pooled global prevalence of 19.7% (22). Advances in epigenetics have provided novel perspectives for understanding the pathogenesis, diagnosis and treatment of skeletal disease (23). The present review aimed to summarize m^{6}A-mediated regulation of skeletal development and its involvement in the diagnosis and treatment of skeletal disorder, with the aim of providing novel insight and therapeutic strategies for clinical management.

2. m^{6}A modification

m^{6}A denotes adenosine modified at the nitrogen-6 position (13,17). The deposition, removal and interpretation of m6A marks on RNA are dynamically regulated by three classes of proteins: Methyltransferases, demethylases and m6A-binding readers (24-26). The methyltransferase complex, commonly referred to as the writer complex, catalyzes the addition of m6A. Its core components include methyltransferase-like (METTL)-3, METTL14 and Wilms tumor 1-associated protein (WTAP) (27,28). Reader proteins recognize m6A-modified transcripts and alter their fate by influencing RNA stability, splicing, translation efficiency and localization (29-31). Well-characterized readers include YT521-B homolog (YTH) domain family (YTHDF)-1, YTHDF2, YTHDF3, YTH domain-containing (YTHDC)-1 and YTHDC2. Demethylases, known as erasers, remove m6A modifications, rendering the process reversible (31-34). Fat mass and obesity-associated protein (FTO) (32) and AlkB homolog 5, RNA demethylase (ALKBH5) (34) are the best-characterized m6A erasers (33,35,36), underscoring m6A as a dynamic post-transcriptional regulatory mechanism.

Writers. Writers catalyze the addition of m^{6}A marks at specific sites within RNA molecules. Primary components include METTL3, METTL14 and WTAP, which assemble into the core methyltransferase complex responsible for m^{6}A deposition (27). Within this complex, the METTL3-METTL14 heterodimer exerts the primary catalytic function, while WTAP, although lacking enzymatic activity, regulates the localization and efficiency of m^{6}A modification of target RNA (27). METTL3, also known as MT-A70, encodes a 580-amino acid protein with a molecular mass of ~65 kDa (37). METTL14 shares ~43% sequence similarity with METTL3 and encodes a 456-amino acid protein (27). Both METTL3 and METTL14 contain methyltransferase domains (MTDs) located at residues 369-570 and 117-402, respectively (38,39). The MTD of METTL3 possesses a highly conserved catalytic pocket that binds the methyl donor S-adenosylmethionine (SAM), making METTL3 the catalytic core of the complex (40). In addition, METTL3 contains Cys-Cys-Cys-His zinc-binding motifs that enable RNA recognition. By contrast, METTL14 lacks a SAM-binding pocket and has no intrinsic catalytic activity. Instead, METTL14 stabilizes the conformation of METTL3 and enhances substrate recognition (41). Loss of METTL14 markedly impairs the catalytic efficiency of METTL3, demonstrating their cooperative role in m^{6}A deposition (38,41).

WTAP is a splicing-related protein encoded on chromosome 6q25-27. It consists of 396 amino acids with a molecular weight of ~46 kDa. WTAP regulates the cell cycle by stabilizing cyclin A2 and CDK2 mRNA and thereby promoting G2/M and G1/S progression (42,43). It contributes to tumorigenesis primarily through m^{6}A-dependent control of target transcripts involved in proliferation and survival, including pathways associated with glycolysis, PI3K/AKT signaling, NF-κB activation and drug resistance. In addition, WTAP is essential for sustaining the Sertoli cell-dependent spermatogonial stem cell niche, and its loss impairs spermatogonial stem cell maintenance and spermatogenesis, supporting its role in germ cell proliferation and development (42,44). WTAP itself lacks methyltransferase activity but serves as a regulatory scaffold of the m^{6}A writer complex by interacting with METTL3 and METTL14, facilitating their recruitment to target RNAs, and promoting their accumulation in nuclear speckles, thereby enabling efficient m^{6}A deposition *in vivo*. In the absence of WTAP, the binding of METTL3 to mRNA is notably decreased.

WTAP is the third subunit of the methyltransferase complex (28). Notably, METTL3 is key in maintaining WTAP stability. METTL3 exerts a bidirectional homeostatic regulation on WTAP. METTL3 overexpression increases WTAP protein levels without substantially altering WTAP mRNA abundance, primarily through enhancement of WTAP mRNA translation and protein stability. METTL3 downregulation also leads to WTAP upregulation, but this effect is mainly attributable to increased WTAP mRNA stability, resulting in coordinated elevation of both WTAP mRNA and protein levels (28,45). However, in the absence of METTL3, elevated WTAP expression alone is insufficient to promote cell proliferation (45). WTAP contains an extended N-terminal coiled-coil region and METTL3 binds the first 150 amino acids of the N-terminal of WTAP. Both METTL3 and WTAP are localized to nuclear speckles through their N-terminal localization signals (46). Nuclear speckles, also known as interchromatin granule clusters, are membraneless subnuclear structures located within the interchromatin regions of mammalian cell nuclei and typically appear as 20-50 irregular puncta under immunofluorescence microscopy. They are enriched in pre-mRNA splicing factors, small nuclear ribonucleoproteins and other proteins involved in RNA processing (47). Traditionally, nuclear speckles have been regarded as sites for the storage, assembly, and recycling of splicing factors (47,48). Other studies, however, have shown that they are spatially associated with actively transcribed genes and promote cotranscriptional splicing and gene expression by increasing

the local concentration of spliceosomal components (47,49,50). Therefore, the localization of the METTL3/WTAP complex to nuclear speckles suggests m6A deposition is coupled to pre-mRNA processing within the spatial organization of the nucleus (28). m6A methylation process comprises four primary steps. Complex assembly and nuclear localization occurs. Following synthesis in the cytoplasm, METTL3 and METTL14 translocate to the nucleus and form a stable 1:1 heterodimer. METTL3 serves as the catalytic subunit, mediating the methyl transfer reaction, while METTL14 functions as a structural scaffold, stabilizing METTL3 and facilitating RNA substrate recognition. WTAP binds the METTL3-METTL14 heterodimer to form a methyltransferase complex (MTC), with WTAP directing the catalytic core to nuclear speckles. Secondly, substrate recognition and binding occurs, whereby the MTC scans precursor (pre)-mRNA for the RRACH sequence, the canonical consensus motif for m6A modification. METTL14 directly interacts with adenine within the RRACH motif, anchoring it at the catalytic site. Thirdly, methylation catalysis occurs. METTL3 catalyzes the transfer of a methyl group from SAM to the N6 position of adenine. Following completion of the reaction, m6A-modified RNA is generated.

With regard to the sites of modification, studies have indicated that m^6A modifications are primarily enriched in coding sequences (CDSs) and 3'-untranslated regions (3'-UTRs) (51-53). m^6A marks in the 3'-UTR exhibit a high degree of conservation and display notable tissue specificity; ~36.7% of m^6A sites are tissue-specific, whereas only 5.5% are shared across tissue. Shared sites are frequently located near the stop codon, while tissue-specific sites tend to be positioned farther away. Notably, tissue-specific m^6A sites are enriched in the 5'-UTR. In this region, m^6A can promote translation initiation in a context-dependent manner, particularly by facilitating eIF3-mediated recruitment of the 43S preinitiation complex and cap-independent or eIF4F-independent translation (54,55). m^6A methylation is enriched at non-canonical cleavage sites within the 3'-UTR, indicating its key role in mRNA degradation (54). In addition to 3'-UTR-associated decay, recent studies have shown that m^6A sites within the coding sequence (CDS) trigger a distinct, mechanistically separable, and translation-dependent degradation pathway termed CDS-m^6A decay (CMD), which acts faster and more efficiently than 3'-UTR m^6A-mediated decay (56,57).

Readers. Readers recognize m^6A, enabling RNA to exert its biological functions. Readers primarily include the YTHDF and YTHDC. YTHDF comprises three paralogs YTHDF1, YTHDF2 and YTHDF3, which recognize cytoplasmic m^6A, whereas the YTHDC family consists of two paralogs, YTHDC1 and YTHDC2, responsible for nuclear m^6A recognition (58). All members contain a YTH domain consisting of a six-stranded β-sheet surrounded by three α-helices, forming a barrel-like structure. The domain surface is positively charged and the aromatic cage formed by three highly conserved aromatic residues serves as the recognition site for m^6A (26,59). Functionally, YTHDF1 promotes the translation of m6A-modified target transcripts in a context-dependent manner, including SON and CREBBP, EIF3C in ovarian cancer cells, TRAF6 in intestinal epithelial cells, and ATG2A/ATG14 in hypoxic hepatocellular carcinoma cells (29,60,61). YTHDF2 promotes mRNA degradation, whereas YTHDF3 enhances translation by cooperating with YTHDF1 and also facilitates mRNA decay through the YTHDF2-dependent pathway. Mechanistically, YTHDF3 binds m6A-modified transcripts at an earlier stage, promotes the target-binding specificity of YTHDF1, and facilitates the selective loading of these transcripts onto YTHDF1-associated translation machinery, thereby promoting translation initiation factor recruitment and ribosome loading. The primary role of YTHDF3 is to improve the binding specificity of YTHDF1 and YTHDF2 to target mRNAs (29-31,62,63). Collectively, YTHDF proteins coordinate the translation of m6A-modified mRNA. Recent studies have shown that YTHDF1 and YTHDF3 undergo O-GlcNAcylation, a dynamic and reversible O-linked β-N-acetylglucosamine modification on serine/threonine residues catalyzed by OGT and removed by OGA, which acts as a nutrient-sensitive regulatory mark and impairs their interactions with translation-associated proteins, thereby attenuating their translation-promoting activity and revealing a novel regulatory mechanism for YTHDF proteins (64,65). YTHDF is implicated in the regulation of global mRNA stability. Processing (P-)bodies serve as cytoplasmic hubs for RNA storage, surveillance and turnover. Depletion of YTHDF1-3 leads to increased P-body formation without a concomitant decrease in overall mRNA abundance, whereas inhibition of P-body assembly results in decreased mRNA levels (66,67). These observations indicate YTHDF proteins may contribute to mRNA stabilization through mechanisms associated with P-body dynamics that are, at least in part, independent of canonical m^6A-mediated mRNA decay (68). Notably, this translation- or stability-dominant effect without overt changes in mRNA abundance deviates from the classical view of m6A readers as primarily promoting RNA decay (66,67). However, the molecular basis regarding how YTHDF proteins regulate P-body assembly and mRNA fate, as well as the extent to which these effects depend on m^6A recognition vs. non-canonical functions of YTHDF readers, require further investigation (66).

YTHDC1 mediates the nuclear export of methylated RNA (MeR). Knockout of YTHDC1 leads to nuclear accumulation of transcripts, whereas cytoplasmic mRNA levels gradually decrease. Conversely, overexpression of YTHDC1 has been shown to reduce nuclear mRNA levels (69). YTHDC2 is highly expressed in mouse testicular tissue (31,70). Its loss causes decreased testis size, degeneration of seminiferous tubules, depletion of germ cells, loss of mature spermatozoa, and meiotic arrest, ultimately impairing spermatogenesis and causing male infertility. YTHDC2 facilitates transcript translation and accelerates mRNA degradation, although the precise mechanisms remain to be fully elucidated (31,71).

Erasers. m^6A Erasers remove methyl modifications from RNA transcripts and thereby exert demethylase activity. Among them, FTO is a member of the AlkB family of non-heme Fe(II)/α-ketoglutarate (α-KG, also known as 2-oxoglutarate)-dependent dioxygenases. Fe(II) serves as the catalytic cofactor, whereas α-KG functions as a co-substrate in the oxidative demethylation reaction. The mammalian AlkB family comprises FTO and eight AlkB homologs

(ABH1–ABH8; ABH, AlkB homolog), a group of enzymes that catalyze oxidative dealkylation of methylated nucleic acid bases. Consistent with this classification, Gerken *et al* demonstrated that FTO contains the conserved sequence motifs of Fe(II)- and 2-oxoglutarate-dependent oxygenases and functions as a nuclear nucleic acid demethylase, thereby providing the biochemical basis for its role as an RNA demethylase (72). FTO was the first identified m^{6}A demethylase (32), establishing m^{6}A as a dynamic and reversible modification (73). The demethylation activity of FTO is primarily mediated by two central functional regions: The N-terminal AlkB-like domain (residues 32-326), primarily composed of β-strands, and the C-terminal domain (residues 327-498), predominantly formed by α-helices (20). Further studies have shown that FTO catalyzes the stepwise oxidation of m^{6}A-modified mRNA into N6-hydroxymethyladenosine as an intermediate, followed by oxidation to N6-formyladenosine, which is ultimately hydrolyzed to adenosine (74,75).

The oxidative demethylation mechanism helps prevent methyltransferases from acting on RNA in nucleolar regions and ensures that demethylation is not readily reversed (74). FTO exhibits substrate selectivity, primarily catalyzing the removal of m^{6}A modifications within mRNA rather than at the 5'cap. In addition, FTO demethylates m^{6}A in nuclear mRNA and N6,2'-O-dimethyladenosine at the 5'cap in the cytoplasm (76). ALKBH5, the second m^{6}A demethylase identified after FTO, belongs to the ALKB protein family, which comprises nine homologs. ALKBH5 deficiency leads to increased m^{6}A levels in mRNA, resulting in abnormal testicular development and apoptosis of germ cells in mice (36). RNA-binding motif protein 33 (RBM33) is a key auxiliary factor for ALKBH5. RBM33 recruits ALKBH5 to m^{6}A-modified substrates and activates its demethylation activity by removing minor ubiquitin-like modifications (77). ALKBH5 is also key for oocyte meiosis, as its deficiency causes oocyte developmental arrest, disrupts RNA stability and leads to excessive translation (78). However, the precise mechanistic differences between FTO and ALKBH5 remain to be fully elucidated. Fig. 1 summarizes the regulatory effects of m^{6}A modification on mRNA.

3. m^{6}A modification of ncRNA

ncRNA refers to RNA molecules that do not encode proteins and are classified into two primary types: Structural and regulatory ncRNA. Regulatory ncRNA includes long ncRNA (lncRNA), microRNA (miRNA) and circular RNA (circRNA) (79). miRNA is a single-stranded RNA molecule ~22 nucleotides in length. Its biogenesis begins in the nucleus, where primary (pri)-miRNA is cleaved by Drosha in conjunction with DiGeorge critical region 8 (DGCR8) to generate pre-miRNA. DGCR8 facilitates localization by directing Drosha to specific cleavage sites. The second processing step occurs in the cytoplasm, whereby Dicer cleaves pre-miRNA to form a double-stranded miRNA duplex. Subsequently, one strand of this duplex associates with target mRNA to form the miRNA effector within the miRNA-induced silencing complex, while the other strand is released and degraded (80). At the molecular level, m^{6}A regulates ncRNAs primarily through two mechanisms: It reshapes local RNA secondary structures to alter the accessibility of RNA-binding motifs and recruits specific RNA-binding proteins, such as DGCR8 and HNRNPA2B1, thereby controlling ncRNA processing, stability, localization, and function. In mammals, METTL3 deposits m^{6}A on pri-miRNAs, which promotes their recognition and processing by the Microprocessor component DGCR8. Accordingly, METTL3 depletion reduces DGCR8 binding to pri-miRNAs, leads to the accumulation of unprocessed pri-miRNAs, and globally decreases mature miRNA abundance (20,81,82). In addition, HNRNPA2B1 acts as a nuclear m^{6}A reader that binds a subset of methylated pri-miRNAs and facilitates microprocessor-dependent miRNA processing (20,81). Studies have shown that the m6A reader protein heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1) participates in pri-miRNA processing (81,82). Mechanistically, HNRNPA2B1 binds a subset of m6A-marked pri-miRNAs and interacts with the microprocessor component DGCR8, thereby facilitating DGCR8 association with these pri-miRNA transcripts and promoting their processing (81-84). Consistently, depletion of HNRNPA2B1 impairs DGCR8 binding, causes the nuclear accumulation of unprocessed pri-miRNAs and reduces the levels of a subset of mature miRNAs (81,82). These findings indicate that HNRNPA2B1 functions as a nuclear m6A reader that mediates, at least in part, METTL3/m6A-dependent pri-miRNA maturation (82). m6AHNRNPA2B1 participates in the processing of a subset of pri-miRNAs rather than globally regulating all miRNAs. Knockdown of HNRNPA2B1 decreases the levels of these HNRNPA2B1-dependent miRNAs, at least in part because HNRNPA2B1 facilitates DGCR8 association with specific m6A-marked pri-miRNA transcripts and thereby promotes their processing. In addition, HNRNPA2B1 has been reported to bind m6A-modified RNAs and mediate m6A-dependent nuclear RNA processing events (81,82). Notably, miRNAs promote m6A modification and influence methylation-site selection by guiding METTL3 to target mRNAs through sequence pairing. After Dicer-dependent maturation, some miRNAs relocalize to the nucleus, where they pair with target transcripts near candidate m6A motifs, facilitate METTL3 recruitment, and enhance local m6A deposition (85,86). Accordingly, perturbation of miRNA abundance or sequence alters m6A formation at cognate sites, while Dicer depletion reduces, and Dicer overexpression increases, overall m6A levels (87). These findings indicate a reciprocal association between miRNA and m6A modification, whereby miRNAs regulate and are regulated by m6A. However, m6A-dependent regulation of miRNA biogenesis has been studied predominantly at the initiation stage, where METTL3-mediated m6A deposition on pri-miRNAs promotes DGCR8/microprocessor recognition and processing (81,82). By contrast, the role of m6A in later pre-miRNA cleavage remains less well characterized and appears to be context dependent, with limited reports showing that m6A readers can facilitate the processing of selected pre-miRNAs through AGO2- or DICER-associated mechanisms (81,88).

lncRNA is typically >200 nucleotides and performs diverse biological functions, including cis- and trans-regulation, formation of nuclear domains and regulation of RNA function (89). The X-inactive specific transcript (XIST) mediates gene silencing on the X chromosome. Methylation of XIST

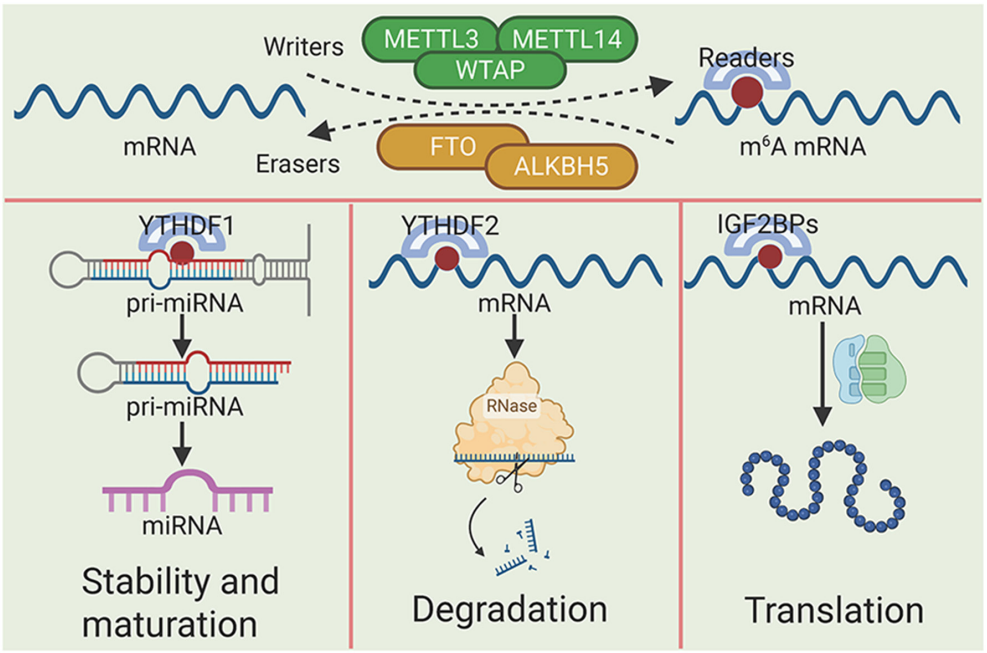

Figure 1. Dynamic regulation of m^{6}A. m^{6}A is written into the RNA sequence by the METTL3, METTL14 and WTAP methylation complexes, with FTO and ALKBH5 able to realize the demethylation function, making m^{6}A methylation a dynamic and reversible process. Reader proteins serve different roles by recognizing m^{6}A. For example, YTHDF1 promotes miRNA stabilization and maturation, YTHDF2 degrades mRNA and IGF2BPs can promote the translation of mRNAs into protein. m^{6}A, N6-methyladenosine; METTL, methyltransferase-like; WTAP, Wilms tumor 1-associated protein; FTO, fat mass and obesity-associated protein; ALKBH5, AlkB homolog 5, RNA demethylase; YTHDF, YTH domain family; miRNA, microRNA; IGF2BP, insulin-like growth factor 2 mRNA-binding protein; pri-, primary; RNase, ribonuclease.

by the METTL3-WTAP complex is key for its function and the m^{6}A reader YTHDC1 promotes XIST-mediated gene silencing by binding the methylated transcript, demonstrating m^{6}A involvement in lncRNA functional regulation (90). In colorectal cancer (CRC), METTL14 knockdown decreases m^{6}A modification on XIST, leading to increased XIST expression and enhanced proliferation, migration, and invasion of CRC cells (90,91). YTHDF2, rather than the other m^{6}A readers tested, recognized m^{6}A-modified XIST and promoted its degradation m^{6}A m^{6}A (62,90-92). circRNA lacks a free 5'cap and 3'poly-A tail, forming a covalently closed loop structure. circRNA can be categorized into three types: Exonic, exonic-intronic and intronic. Exonic circRNAs are the most abundant and are primarily localized in the cytoplasm, whereas exonic-intronic and intronic circRNAs are mainly found in the nucleus. circRNAs perform multiple functions, including serving as miRNA sponges, regulating transcription and translation, facilitating protein splicing and transport, modulating protein-protein interactions and serving as templates for protein translation (93). Yang *et al* (94) demonstrated that m^{6}A modification promotes the translational function of circRNAs. Specifically, the METTL3-METTL14 complex may mediate m^{6}A modification of circRNAs without affecting their stability. Translation of circRNAs requires eukaryotic translation initiation factor 4 γ-2 (eIF4G2), which is key for the initiation of eukaryotic circRNA translation; deficiency of eIF4G2 decreases circRNA translation. YTHDF3 serves as a key recognition factor, recruiting eIF4G2 to m^{6}A-modified circRNAs to initiate translation, while FTO serves as a negative regulator in this process (94). Subsequent research has shown that m^{6}A modification may not only facilitate circRNA translation but also serve a key role in circRNA degradation, innate immune responses and tumor development (95).

4. Role of m^{6}A in skeletal system development

As m^{6}A modification governs key RNA fate decisions such as mRNA stability, translation and cell state transitions, this epitranscriptomic regulation may serves a key role in orchestrating skeletal system development. Skeletal development relies on coordinated processes including mesenchymal stem cell (MSC) commitment, lineage specification and tissue maturation, all of which are highly sensitive to post-transcriptional regulation (29,62,76). Recent studies have begun to elucidate how m^{6}A-mediated mechanisms contribute to skeletal development and homeostasis (96,97). The skeletal system provides structural support, enables movement, protects internal organs, and participates in endocrine regulation. It also serves as the attachment framework for soft tissues, including muscles, tendons, and ligaments, thereby contributing to normal body function. Despite its diverse physiological roles, the skeletal system is composed of a limited number of major cell types, primarily osteoblasts, osteoclasts, osteocytes, and chondrocytes, which collectively maintain skeletal homeostasis. Bone formation occurs through two principal processes, intramembranous ossification and endochondral ossification. In intramembranous ossification, MSCs directly condense and differentiate into osteoprogenitor cells without a cartilage intermediate; these osteoprogenitors subsequently mature into osteoblasts, which synthesize bone matrix and eventually differentiate into osteocytes (98-103). In endochondral ossification, mesenchymal condensations do not form bone

directly; they first generate chondrocytes that form a transient cartilaginous template of the future bone. This cartilage template undergoes hypertrophy and matrix mineralization, followed by vascular invasion and osteoblast-mediated bone deposition, resulting in progressive replacement of cartilage by bone (104). A number of transcription factors regulate bone cell formation. Runt-related transcription factor 2 (RUNX2) is key in bone formation (105,106). In intramembranous ossification, RUNX2 type I is broadly expressed in osteoprogenitor cells and activated osteoblasts, whereas RUNX2 type II is expressed solely in activated osteoblasts (106). In endochondral ossification, RUNX2 expression increases prior to cartilage mineralization but is absent during cartilage proliferation, revealing the spatiotemporal characteristics of RUNX2 expression (107). Further studies demonstrate that RUNX2 interacts with core binding factor-β (Cbf-β), which enhances RUNX2 DNA binding and transcriptional activity (108,109). Loss of Cbf-β impairs RUNX2-dependent transcription and leads to defective skeletal development and ossification in mouse embryos (108-111).

Regulation of bone marrow MSCs (BMSCs) by m^{6}A. BMSCs are multipotent stromal cells capable of differentiating into osteoblasts, chondrocytes and adipocytes (112). Their lineage allocation is key for skeletal development and homeostasis, and a shift toward adipogenesis is closely associated with increased marrow adiposity and bone loss (113). METTL3 is a key epitranscriptomic regulator of BMSC fate through multiple downstream pathways (114,115). *In vivo, c*onditional deletion of Mettl3 in mesenchymal stem cells impairs bone formation, reduces osteogenic differentiation potential, and increases marrow adiposity, whereas Mettl3 overexpression in MSCs protects mice from estrogen deficiency-induced OP (114). Mechanistically, METTL3 promotes the translation of Pth1r and maintains the PTH/PTH1R signaling axis in MSCs; it also suppresses adipogenic differentiation through the m6A-YTHDF2-JAK1/STAT5/C/EBPβ pathway, and enhances osteogenic differentiation by regulating m6A modification of RUNX2 and precursor miR-320 (102,113-116). These findings identify METTL3 as a central regulator of the osteogenic-adipogenic balance in BMSCs (113-115). The parathyroid hormone (PTH)/PTH receptor-1 (PTH1R) signaling pathway is downstream of METTL3 action and reduced METTL3 expression impairs PTH1R translation efficiency (114). In addition, METTL3 expression is markedly downregulated in ovariectomy (OVX)-induced OP models, with *in vitro* overexpression of METTL3 restoring the osteogenic potential of BMSCs (117). Tian *et al* (118) demonstrated that METTL3 downregulation decreases both early and late stages of osteoblast differentiation in BMSCs, accompanied by decreased alkaline phosphatase (ALP) activity and mineralized nodule formation. This suggests METTL3-mediated m6A modification is pivotal in osteoblast differentiation. Downstream targets of m6A, including osteogenesis-associated genes such as RUNX2 and osterix, exhibit decreased expression upon METTL3 downregulation (114,118).

METTL3 enhances m^{6}A methylation of RUNX2 and pre-miR-320, whereas METTL3 silencing or knockout suppresses these modifications. Notably, downregulation of mature miR-320 rescues the bone mass reduction induced by METTL3 silencing or knockout, indicating that METTL3 promotes osteogenic differentiation of BMSCs through both direct and indirect regulation of RUNX2 (115). In addition, inhibiting adipogenic differentiation of BMSCs may indirectly promote osteogenesis. METTL3 knockout decreases m^{6}A levels on JAK-1 mRNA, thereby enhancing YTHDF2-dependent JAK1 mRNA stability. JAK1 activates STAT5, which binds the promoter of CCAAT/enhancer-binding protein-β, ultimately promoting adipogenesis (113). These findings suggest that increasing METTL3-mediated m^{6}A methylation may reduce adipocyte formation and enhance osteogenesis (113). ALKBH1, a DNA demethylase, also regulates BMSC differentiation. Cai *et al* (119) demonstrated that ALKBH1 expression decreases with BMSC aging, coinciding with a shift toward adipogenic differentiation and decreased osteogenic potential. Furthermore, *in vitro* knockout of ALKBH1 recapitulates this phenotype, with optineurin identified as a downstream target of ALKBH1 (120).

Regulation of osteoblasts by m^{6}A. m^{6}A modification regulates osteoblast function (120). METTL3-mediated m^{6}A modification of HAP1A is implicated in the suppression of osteoblast senescence. YTHDF2 may participate in this process by recognizing methylated HAP1A transcripts (121). A tert-butyl hydroperoxide-induced osteoblast senescence model revealed a marked decrease in METTL3 expression. Mechanistically, METTL3-mediated m^{6}A modification enhances the stability of sirtuin 1 (SIRT1) mRNA, a direct METTL3 target, through YTHDF2 recognition of m^{6}A-modified SIRT1 transcripts, thereby suppressing osteoblast senescence; conversely, METTL3 knockdown decreases SIRT1 stability, whereas METTL3 overexpression markedly attenuates osteoblast senescence and increases bone mass in aged mice (122,123). Similarly, following lipopolysaccharide (LPS) stimulation, METTL3 knockdown in osteoblasts results in the decreased expression of osteoblast markers, ALP activity and phosphorylation of SMAD1, SMAD5 and SMAD9. By contrast, mRNA expression and stability of SMAD signaling negative regulators, SMAD7 and SMURF1, are increased (124). METTL3 deficiency also induces proinflammatory cytokine expression and enhances phosphorylation of ERK, p38, JNK and p65 in the MAPK and NF-κB signaling pathways, highlighting the positive regulatory role of METTL3 in osteoblast-mediated bone formation (124).

METTL14 exhibits similar regulatory functions. A recent study showed that METTL14 alleviates H_2O_2-induced impairment of osteoblast differentiation in MC3T3-E1 murine calvaria-derived clonal preosteoblastic/osteoblast-like cell line) (125). Mechanistically, GLUT3 was identified as an m^{6}A-modified target of METTL14, and YTHDF1 participated in promoting GLUT3 expression, thereby enhancing osteogenesis under oxidative stress conditions (125,126). The demethylase FTO serves an important role in normal bone development. Zhang *et al* (127) demonstrated that FTO expression is key in bone formation: FTO knockout mice exhibit reduced trabecular bone volume and number, resulting in bone formation defects. FTO is key in osteoblast differentiation; its deficiency increases osteoblast apoptosis and renders cells more susceptible to physical and chemical stressors such as

ultraviolet radiation and H_2O_2, partly via the NF-κB signaling pathway. These findings underscore the importance of FTO in maintaining normal bone formation (127).

Regulation of m⁶A in osteoclasts. Osteoclasts are multinucleated cells derived from hematopoietic SCs and differentiate from osteoclast precursors upon stimulation by macrophage colony-stimulating factor and receptor activator of NF-κB ligand (RANKL) (103). In the skeletal system, osteoblasts synthesize and secrete RANKL and osteoprotegerin (OPG). Osteoclast precursors express RANK, which binds RANKL, promoting differentiation into osteoclasts and enhancing bone resorption. OPG serves as a decoy receptor by competing with RANKL, thereby delaying osteoclast precursor differentiation and inhibiting bone resorption, maintaining the balance between osteoblast and osteoclast activity (128).

METTL3 knockout results in enlarged osteoclasts with decreased resorptive capacity. METTL3 deficiency suppresses the expression of osteoclast-specific genes, including nuclear factor of activated T cells 1 (NFATC1), c-Fos, cathepsin K, acid phosphatase 5 and dendrocyte-expressed seven transmembrane protein, while upregulating the cell fusion-specific gene ATP6V0D2, METTL3 knockout enhances ATP6V0D2 mRNA stability, thereby inhibiting osteoclast differentiation and bone resorption activity (129). Similarly, during LPS-induced osteoclastogenesis, both total m⁶A content and METTL3 expression decrease. METTL3 knockdown decreases osteoclast numbers, the expression of osteoclast-related genes and bone resorption area, while increasing osteoclast apoptosis and expression of pro-apoptotic proteins. Mechanistically, METTL3 deficiency stabilizes nitric oxide synthase 2 mRNA, thereby inhibiting osteoclast differentiation and promoting apoptosis (130). However, as aforementioned, METTL3 deficiency also impairs osteoblast differentiation and maturation. The mechanism by which METTL3 coordinates the maturation of osteoblasts and osteoclasts to regulate bone formation remains unclear. m⁶A modification within the 1916-1992 bp region of osteoblast-derived exosomal circ_0008542, particularly at the A1956 site, promotes osteoclast differentiation and bone resorption, and that these effects are attenuated by METTL3 inhibition or ALKBH5 overexpression, highlighting an m⁶A-dependent mechanism of osteoblast-osteoclast crosstalk m⁶A m⁶A (131).

m⁶A modification serves a dynamic role in normal skeletal development. Methylation and demethylation are necessary for bone formation and their dynamic balance determines the equilibrium between osteoblasts and osteoclasts.

5. m⁶A in skeletal system diseases

Disruption of regulatory mechanisms that govern skeletal development typically predisposes tissue to degenerative and inflammatory disease later in life. Given the key role of m⁶A modification in skeletal development and cell homeostasis, dysregulation of m⁶A regulators is increasingly implicated in the pathogenesis of skeletal disorder. Aberrant m⁶A regulation is associated with notable skeletal diseases, including OP, OA, RA and intervertebral disc degeneration (IVDD) (97,132-134).

m⁶A in OP. OP is a skeletal disorder characterized primarily by decreased bone mass, deterioration of bone microarchitecture, decreased bone strength and an increased risk of fractures (135). A decrease in bone mineral density ≥2.5 SD compared with age- and sex-matched adults is diagnostic for OP (136). With advancing age, OP becomes more common, and its prevalence is notably higher in women than in men. Based on US NHANES 2017-2018 data cited in the 2025 USPSTF Recommendation Statement, the age-adjusted prevalence of OP among adults aged 50 years or older was 12.6%, including 19.6% in females and 4.4% in men; among those aged 65 years or older, the prevalence increased to 27.1% in women and 5.7% in men (137). OP markedly increases fracture risk, imposing a health burden on patients (138). A key cause of OP is an imbalance between osteoblast and osteoclast activity. Therefore, strategies that promote osteoblast differentiation while inhibiting osteoclast activity are key in the prevention and treatment of OP (139). Daily supplementation with calcium and vitamin D improves bone health, while representative pharmacological treatments include bisphosphonates (140), calcitonin (141) and strontium (142).

Changes in bone density serve as the primary diagnostic criterion for OP. Advances in epigenetic research have revealed additional molecular mechanisms underlying OP, among which m⁶A-mediated regulation serves a key role, offering novel insights for diagnosis and therapeutic intervention (132,143). Mesenchymal stem cells (MSCs) are multipotent progenitors whose lineage allocation is key for skeletal homeostasis. A shift in MSC fate from osteogenesis toward adipogenesis disrupts bone homeostasis and contributes to osteoporosis. In this context, METTL3 is a key regulator of the osteogenic-adipogenic balance in bone marrow MSCs. Conditional loss of Mettl3 in MSCs impairs bone formation, decreases bone mass, and increases marrow adiposity, whereas Mettl3 overexpression protects mice from ovariectomy-induced osteoporosis. Mechanistically, METTL3 promotes translation of Pth1r mRNA and maintains PTH/PTH1R signaling, thereby favoring osteogenic commitment over adipogenic differentiation (114). RUNX2, a member of the RUNT-related transcription factor family, serves a key role in osteoblast differentiation and is regulated by numerous miRNAs (144). Studies have reported that METTL3 decreases the abundance of miRNA-320 by enhancing m⁶A modification of pre-miRNA-320, thereby increasing RUNX2 expression and promoting osteogenesis, exerting an anti-osteoporotic effect (145,146). In addition, METTL3 mediates m⁶A modification of long intergenic non-protein coding (LINC)-00657, promoting bone formation by serving as a competing endogenous RNA to upregulate bone morphogenetic protein receptor type 1B by sponging miRNA-144-3p (147).

Beyond MSC lineage commitment, m⁶A-mediated regulation influences osteoclast activity and bone resorption. Global m⁶A levels and METTL14 expression were significantly lower in patients with OP (148). Similarly to METTL3, knockdown of METTL14 inhibits the osteogenic potential of MSCs. METTL14 improves bone mass in OVX mice and increases m⁶A modification of SMAD1, a process regulated by insulin-like growth factor 2 mRNA-binding protein (IGF2BP)-1 (148). Extracellular vesicles (EVs) are key mediators of intercellular communication, regulating cell functions and maintaining homeostasis by transporting biologically active components, including DNA, RNA, protein

and lipids (149,150). A recent study reported that overexpression of METTL14 in MC3T3-E1 cells promotes release of exosomes, which increases the m^{6}A modification of NFATC1, thereby inhibiting osteoclast activity and mitigating OP (151).

Autophagy and signaling pathways involved in bone remodeling are subject to m^{6}A-dependent control (152). YTHDF2 facilitates this process by recognizing m^{6}A and promoting NFATC1 mRNA degradation (153). Autophagy, the lysosomal degradation of cytoplasmic components, is key in maintaining cell homeostasis (154). Within the skeletal system, autophagy regulates the balance between osteoblasts and osteoclasts, with inhibition of autophagy-associated genes impairing bone formation. METTL14 promotes autophagy and directs bone marrow cell differentiation toward osteoblasts, with beclin 1 serving as a key target (155). The stability of beclin 1 m^{6}A modification is maintained by IGF2BP1, IGF2BP2 and IGF2BP3 (152). T cell factor 1 (TCF1), a member of the TCF family containing a high-mobility group domain, serves as an effector of the Wnt signaling pathway. Activation of this pathway promotes osteoblast differentiation while inhibiting osteoclast formation (156). METTL14 exerts anti-osteoporotic effects by promoting m^{6}A-dependent TCF1 upregulation, which increases RUNX2 expression and osteogenic activity (157). SIRTs, members of the class III histone/lysine deacetylase family, regulate biological processes, including the cell cycle, immune responses and inflammation. SIRT1 promotes osteogenesis and mitigates osteoblast aging (158). Overexpression of METTL14 increases the m^{6}A modification of SIRT1 mRNA in BMSCs, enhancing osteogenesis while inhibiting osteoclast differentiation of bone marrow mononuclear macrophages, thereby highlighting the role of SIRT1 in maintaining bone metabolism balance (159). WTAP, similarly to METTL3 and METTL14, is downregulated in osteoporotic bone tissues from patients and in ovariectomized (OVX) mice. WTAP promotes osteogenic differentiation while suppressing adipogenic differentiation of BMSCs by enhancing m^{6}A modification of pri-miR-181a and pri-miR-181c; YTHDC1 then recognizes these methylated pri-miRNAs and facilitates their maturation, leading to increased miR-181a/miR-181c levels, suppression of SFRP1, and enhanced osteogenesis (160). microRNA-29b-3p has been identified as a potential WTAP target mediating anti-osteoporotic effects (161). Machine learning analyses and clinical studies support the diagnostic value of WTAP in postmenopausal OP (160-162).

Collectively, the aforementioned findings demonstrate that m^{6}A modification regulates OP through coordinated control of MSC fate determination, osteoclast activity, autophagy and key osteogenic signaling pathways. Rather than acting through isolated regulators, m^{6}A-dependent networks integrate multiple post-transcriptional mechanisms to maintain bone remodeling homeostasis. Table I and Fig. 2 summarize the mechanisms by which m^{6}A modification regulates OP.

m^{6}A in OA. OA is a degenerative joint disease characterized by joint pain, swelling, stiffness and restricted mobility. OA affects ~15% of individuals aged 30 years or older worldwide, and the number of people living with OA is projected to approach 1 billion by 2050, driven primarily by population ageing, population growth, and increasing obesity (163). OA pathogenesis primarily involves joint inflammation, cartilage degradation and deformation and osteophyte formation (164). Therapeutic strategies for alleviating OA pain primarily rely on cyclooxygenase-2 selective inhibitors, such as celecoxib (165) and meloxicam (166), as well as traditional non-steroidal anti-inflammatory drugs including diclofenac (167). However, these treatments are symptomatic, providing pain relief without slowing disease progression or promoting cartilage repair and fail to improve the long-term quality of life for patients. Consequently, identifying molecular targets that inhibit chondrocyte apoptosis, improve the inflammatory microenvironment and promote cartilage regeneration has become a key research focus (168,169). m^{6}A RNA modification represents a promising avenue in this regard. Studies investigating the role of m^{6}A in OA pathogenesis have primarily focused on autophagy, fibrosis, oxidative stress and associated processes (170,171).

Aberrant activation of fibroblast-like synoviocytes (FLSs) is a key inflammatory driver in OA. Under physiological conditions, FLSs reside in the synovial intimal lining and contribute to joint homeostasis. Following activation by inflammatory stimuli, FLSs acquire an aggressive phenotype characterized by enhanced proliferation, migration and invasion (172). In this state, FLSs participate in joint destruction by promoting synovial hyperplasia and pannus formation, and by directly invading adjacent cartilage and bone (173). Mechanistically, the DDR2/annexin A2/MMP-13 loop promotes FLS migration and invasion, whereas RasGRP4 contributes to pathological FLS proliferation, driving persistent synovitis and structural joint damage (174,175). Impaired FLS homeostasis and defective autophagy are associated with OA development. Reduced autophagy has been documented in OA tissues and patient-derived cells, and in surgically induced OA, particularly in articular cartilage (171). In OA-FLS, METTL3-mediated m^{6}A modification of autophagy-related gene 7 (ATG7) promotes cellular senescence (176). Mechanistically, YTHDF2 recognizes m^{6}A-modified ATG7 mRNA and decreases its RNA stability, thereby reducing ATG7 protein expression, impairing autophagic flux, and accelerating OA progression (171,176,177). In osteoarthritis, m^{6}A modification directly regulates chondrocyte survival and extracellular matrix (ECM) homeostasis by modulating the stability, maturation, or translation of key RNAs that control apoptotic, inflammatory-catabolic, and anabolic pathways. For example, increased METTL3-mediated m^{6}A promotes NF-κB activation in chondrocytes, enhances apoptosis and inflammatory responses, and shifts ECM metabolism toward degradation, as reflected by increased MMP13 and collagen X and reduced aggrecan and collagen II. By contrast, FTO-mediated demethylation stabilizes SMAD2 mRNA, thereby preserving anabolic signaling and restraining cartilage catabolism. In addition, WTAP-dependent m^{6}A regulation aggravates chondrocyte injury either by enhancing CA12 mRNA stability or by promoting pri-miR-92b maturation and YTHDF2-dependent TIMP4 downregulation, leading to reduced chondrocyte viability, increased apoptosis, and ECM degradation (178-180).

Unlike miRNAs, lncRNAs do not encode proteins but regulate gene expression at multiple levels. In OA, lncRNA IGFBP7-OT is upregulated in osteoarthritic cartilage and is positively correlated with its sense gene, IGFBP7.

Table I. Roles of m^{6}A methylation related regulators in osteoporosis.

Writer	Reader	Expression of m^{6}A regulators	Target genes or pathways	Research objects	Biological function	(Refs.)
METTL3	Not reported	Upregulated	PTH/PTH1R	Mice/BMSCs	Promotes osteogenesis and inhibits lipogenesis	(114)
METTL3	Not reported	Upregulated	Wnt/β-catenin	Rats/BMSCs	Promotes osteogenesis	(117)
METTL3	Not reported	Upregulated	Pre-miR-320/ miR-320/RUNX2	Human BMSCs/mice	Promotes osteogenesis	(145)
METTL3	Not reported	Upregulated	LINC00657/ miR-144-3p/ BMPR1B	Human BMSCs	Promotes osteogenesis	(147)
METTL14	IGF2BP1	Upregulated	SMAD1	Human BMSCs/mice	Promotes osteogenesis	(148)
METTL14	YTHDF2	Upregulated	NFATC1	Raw264.7 monocytic/ Mc3t3-E1 cell lines/ alveolar bone/mice	Inhibits osteoclasts	(151)
METTL14	IGF2BP1, IGF2BP2 and IGF2BP3	Upregulated	Beclin 1	Mice/BMSCs	Promotes osteogenesis and inhibits osteoclasts	(152)
METTL14	Not reported	Upregulated	TCF1/RUNX2	Mice/Mc3t3-E1 cells	Promotes osteogenesis and inhibits osteoclasts	(157)
METTL14	Not reported	Upregulated	SIRT1	Mice/BMSCs/BMMs	Promotes osteogenesis and inhibits osteoclasts	(159)
WTAP	YTHDC1	Upregulation	miR-181/SFRP1	Human bone/mice/ BMSCs	Promotes osteogenesis and inhibits osteoclasts	(160)
WTAP	Not reported	Upregulation	miR-29b-3p/ HDAC4	Human bone/mice/ BMSCs	Promotes osteogenesis and inhibits osteoclasts	(161)

PTHR1, parathyroid hormone 1 receptor; pre-, precursor; miR, microRNA; RUNX2, runt-related transcription factor 2; LINC, long intergenic non-protein coding; BMPR1B, bone morphogenetic protein receptor type 1B; NFATC1, nuclear factor of activated T cells; TCF1, T cell factor 1; SIRT1, sirtuin 1; SFRP1, secreted frizzled-related protein 1; HDAC4, histone deacetylase 4; BMSC, bone marrow mesenchymal stem cell; METTL, methyltransferase-like; WTAP, Wilms' tumor 1-associated protein; YTHDF, YTH domain family; IGF2BP, insulin-like growth factor 2 mRNA-binding protein; YTHDC, YTH domain-containing; m^{6}A, N6-methyladenosine; BMMs, bone marrow mononuclear macrophages.

Functionally, IGFBP7-OT overexpression inhibits chondrocyte viability, promotes apoptosis, and reduces the expression of extracellular matrix components, including collagen II and aggrecan, whereas its silencing exerts the opposite effects. Mechanistically, the upregulation of IGFBP7-OT is partially controlled by METTL3-mediated m^{6}A modification. Increased IGFBP7-OT, in turn, suppresses the occupancy of DNMT1 and DNMT3a on the IGFBP7 promoter, reduces promoter methylation, and thereby upregulates IGFBP7 expression, ultimately promoting OA progression (181). Similarly, in IL-1β-stimulated chondrocytes, METTL3 increases the m^{6}A modification and stability of LINC00680. LINC00680 interacts with the m^{6}A-containing 3'-UTR of SIRT1 mRNA and enhances its stability. Functionally, silencing LINC00680 partially rescues chondrocyte proliferation and attenuates ECM degradation under inflammatory conditions (182). m^{6}A regulation alters OA progression through miRNA-dependent control of macrophage NLRP3 inflammasome signaling. Activation of the NLRP3 inflammasome is a key source of IL-1β- and IL-18-mediated inflammatory responses in OA. EVs derived from MSCs inhibit m^{6}A modification of NLRP3 mRNA by decreasing METTL3 expression, with miR-1208 serving as a central upstream regulator. This indicates that miRNAs modulate m^{6}A modification from an upstream position (183).

WTAP is upregulated in OA and promotes chondrocyte apoptosis while impairing ECM homeostasis by inhibiting ECM synthesis and accelerating ECM degradation. Mechanistically, WTAP-mediated m^{6}A modification enhances the processing of pri-miR-92b into mature miR-92b-5p, which directly suppresses TIMP4; in addition, WTAP facilitates YTHDF2-dependent degradation of m^{6}A-modified TIMP4 mRNA, leading to markedly reduced TIMP4 expression in OA chondrocytes (184). As a demethylase, FTO inhibits OA progression. By reducing the m^{6}A level of pri-miR-515-5p, FTO suppresses the toll-like receptor 4/myeloid differentiation primary response 88/NF-κB signaling pathway, thereby exerting anti-inflammatory effects in OA (185).

Collectively, m^{6}A modification contributes to OA primarily by orchestrating inflammatory signaling, regulating autophagy and apoptosis in FLSs and chondrocytes and disrupting cartilage matrix homeostasis. Rather than acting through isolated regulators, m^{6}A-dependent networks integrate mRNA and ncRNA regulation to drive OA progression. The mechanism

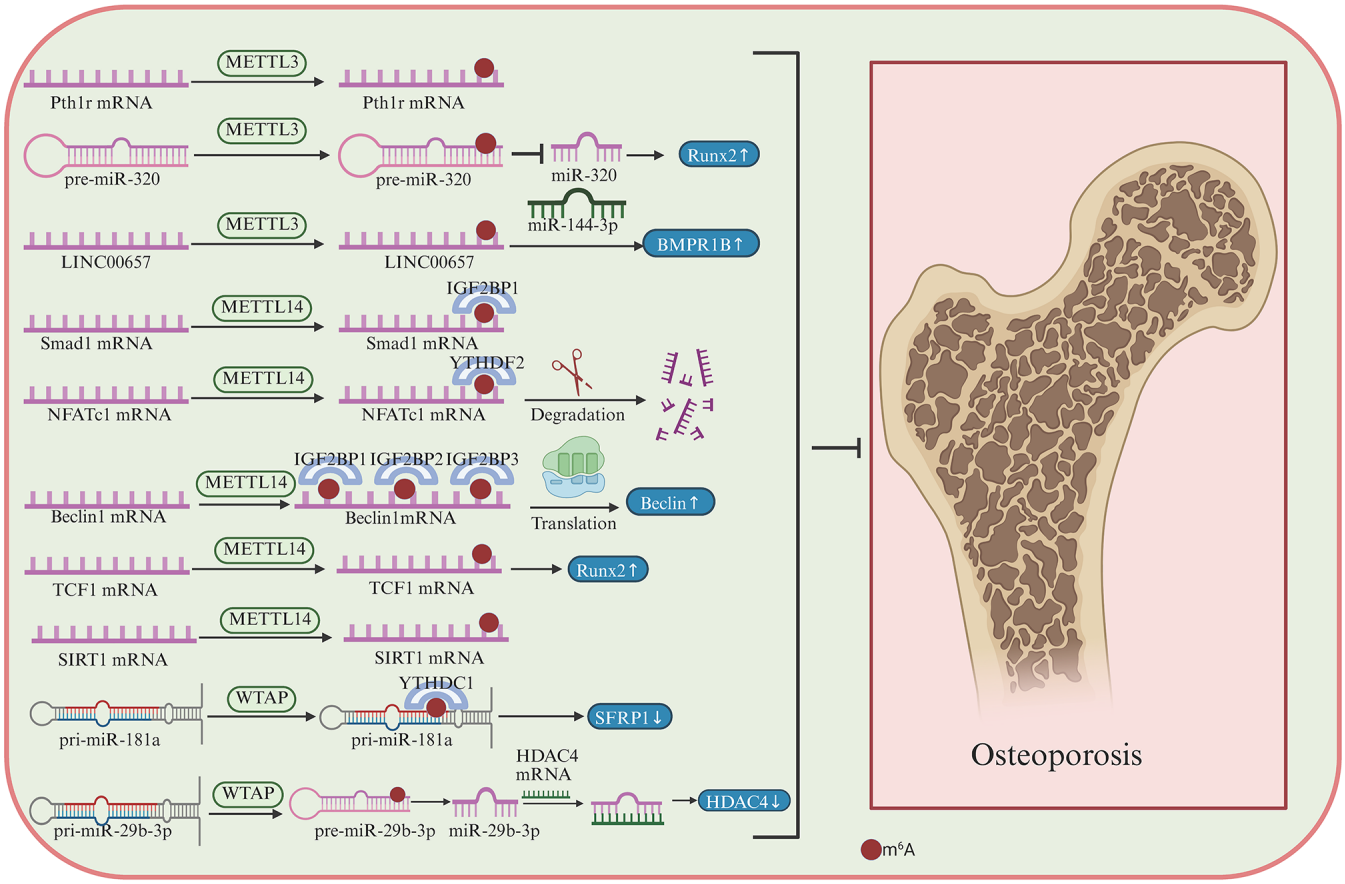

Figure 2. Association between m^{6}A RNA methylation and osteoporotic bone loss. The m^{6}A writers METTL3, METTL14 and WTAP install m^{6}A marks (circles) on selected mRNAs, lncRNAs and pri-/pre-miRs, modulating RNA processing, stability, degradation or translation through m^{6}A readers (IGF2BP1/2/3, YTHDF2 and YTHDC1) and downstream osteogenic/osteoclastic programs. Examples include METTL3-dependent m^{6}A regulation of PTH1R mRNA and pre-miR-320 maturation with subsequent RUNX2 induction, as well as METTL3-mediated m^{6}A on LINC00657, which influences the miR-144-3p/BMPR1B pathway. METTL14-dependent m^{6}A promotes SMAD1 mRNA stability through IGF2BP1, facilitates NFATC1 mRNA turnover through YTHDF2 and enhances beclin 1 mRNA translation through IGF2BP1/2/3, increasing beclin 1 protein expression. METTL14 is associated with m^{6}A regulation of TCF1 mRNA with downstream RUNX2 induction and m^{6}A marking of SIRT1 mRNA. WTAP-dependent m^{6}A facilitates pri-miR processing, including pri-miR-181a in association with YTHDC1 with downstream SFRP1 reduction and pri-miR-29b-3p maturation to miR-29b-3p, which suppresses HDAC4. Collectively, these m^{6}A-regulated axes converge on pathways controlling osteogenesis, osteoclastogenesis, autophagy and epigenetic regulation, contributing to osteoporosis. PTH1R, parathyroid hormone receptor-1; RUNX2, runt-related transcription factor 2; m^{6}A, N6-methyladenosine; METTL, methyltransferase-like; WTAP, Wilms tumor 1-associated protein; YTHDF, YTH domain family; miR, microRNA; IGF2BP, insulin-like growth factor 2 mRNA-binding protein; pri-, primary; lnc, long non-coding; pre-, precursor; YTHDC, YTH domain-containing; NFATC1, nuclear factor of activated T cells 1; TCF1, T cell factor 1; SIRT1, sirtuin 1; SFRP1, secreted frizzled-related protein 1; HDAC4, histone deacetylase 4; BMPR1B, bone morphogenetic protein receptor type 1B.

by which m^{6}A regulates osteoarthritis is summarized in Table II and Fig. 3.

m^{6}A in RA. RA is a chronic autoimmune-inflammatory disease that primarily affects the joints and may involve numerous organs and tissue (186). Its hallmark pathological features include persistent synovitis with progressive cartilage destruction and bone erosion. In RA, protein citrullination generates neoepitopes that are recognized by ACPAs, thereby triggering autoimmune inflammation (187). This response promotes synovial fibroblasts and macrophages to produce pro-inflammatory mediators, particularly TNF-α, IL-1β, IL-6, IL-8, IL-17, and GM-CSF, which drive joint inflammation and subsequent cartilage and bone damage (188). Sustained autoimmune and vascular inflammation contributes to systemic complications, including small vessel vasculitis, interstitial lung disease, pleuritis/pericarditis, cardiovascular disease, secondary amyloidosis, and lymphoma (189). In RA, FLSs serve as major effector cells in synovial hyperplasia and joint destruction. Activated RA-FLSs exhibit a hyperplastic and aggressive phenotype characterized by increased proliferation, migration, and invasion, and they produce pro-inflammatory mediators, including TNF-α, IL-1β, IL-6, and IL-8. Through these pathogenic properties, RA-FLSs contribute to pannus formation and promote cartilage and bone destruction (190,191). METTL3 expression is elevated in RA synovial tissue and RA-FLSs (192). METTL3 silencing decreases IL-6 production, downregulates MMP-3 and MMP-9, and suppresses FLS proliferation, migration, and invasion, whereas METTL3 overexpression exerts the opposite effects. Mechanistically, METTL3 may regulate FLS activation and inflammatory responses via the NF-κB signaling pathway, thereby contributing to RA progression m^{6}A (192).

Beyond inflammation, m^{6}A modification promotes the invasive behavior of RA-FLSs via regulation of epithelial-mesenchymal transition (EMT). EMT, known for its roles in tumor invasion and fibrosis, also contributes to FLS migration and joint invasion in RA (193). The transcriptional

Table II. Roles of m^{6}A methylation-associated regulators in osteoarthritis.

Writer	Reader	Expression of m^{6}A regulators	Target genes or pathways	Research objects	Biological function	(Refs.)
METTL3	YTHDF2	Upregulated	ATG7	Human synovial tissue/ mice/FLS	Promotes chondrocyte apoptosis	(171)
METTL3	Not reported	Upregulated	DNMT1/DNMT3A/ IGFBP7	Human synovial tissue/ mice/FLS	Promotes chondrocyte apoptosis	(181)
METTL3	Not reported	Upregulated	LINC00680/SIRT1	Human synovial tissue/ FLS	Promotes chondrocyte apoptosis	(182)
METTL3	Not reported	Downregulated	miR-1208/NLRP3	Mice/FLS	Promotes chondrocyte apoptosis	(183)
WTAP	YTHDF2	Upregulated	miR92b-5p/TIMP4	Human synovial tissue/ mice/FLS	Promotes chondrocyte apoptosis	(184)
FTO	Not reported	Upregulated	miR-515-5p/TLR4/ MyD88/NF-κB	Rats/FLS	Inhibits chondrocyte apoptosis	(185)

ATG7, autophagy-related 7; DNMT, DNA methyltransferase; IGFBP, insulin-like growth factor-binding protein; LINC, long intergenic non-protein coding; SIRT1, sirtuin 1; miR, microRNA; NLRP3, NLR family pyrin domain containing 3; TIMP4, tissue inhibitor of metalloproteinases 4; TLR4, toll-like receptor 4; MyD88, myeloid differentiation primary response 88; WTAP, Wilms' tumor 1-associated protein; YTHDF, YTH domain family; METTL, methyltransferase-like; FTO, fat mass and obesity-associated protein; FLS, fibroblast-like synoviocyte; NLRP3, NLR family pyrin domain containing 3; m^{6}A, N6-methyladenosine.

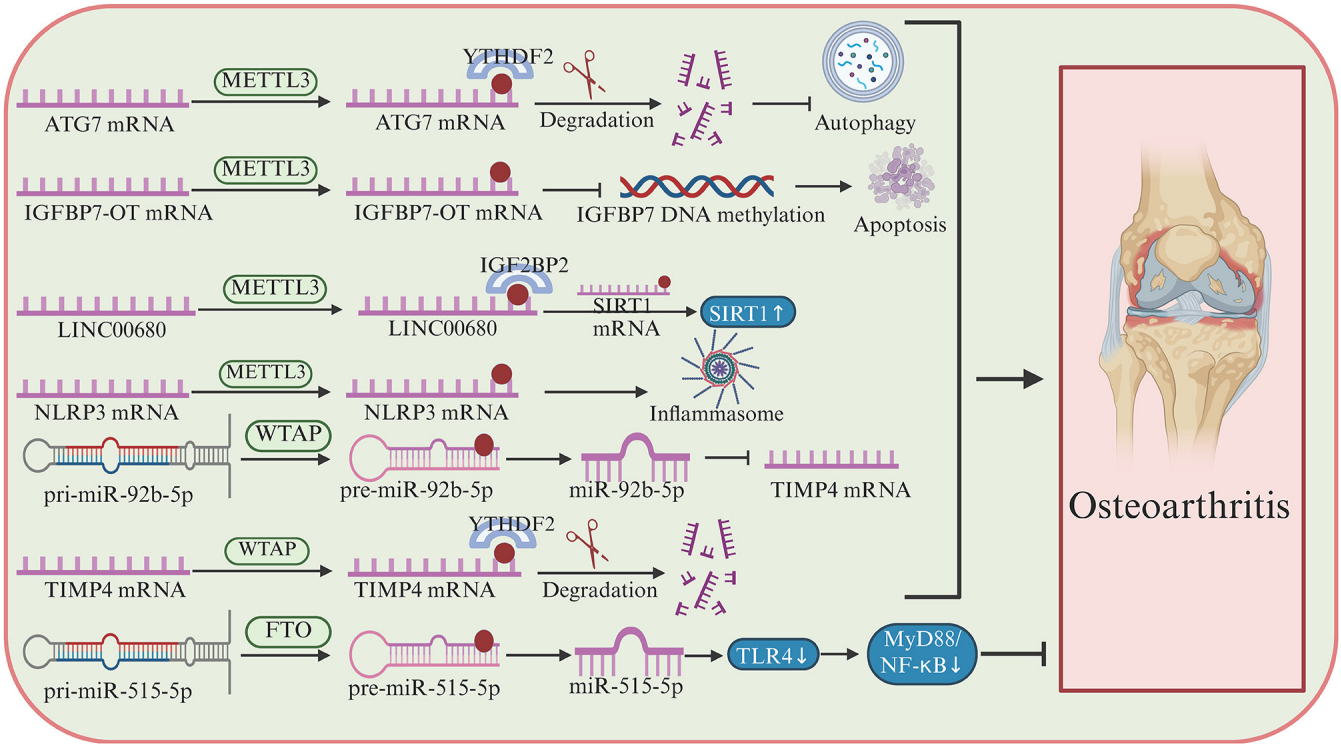

Figure 3. Mechanism by which m^{6}A writers (METTL3 and WTAP), eraser (FTO) and m^{6}A readers (YTHDF2 and IGF2BP2) remodel RNA fate (processing, stability, degradation and translation) to influence chondrocyte homeostasis and osteoarthritis progression. METTL3-installed m^{6}A (circle) on ATG7 mRNA promotes YTHDF2-dependent decay, thereby suppressing autophagy. METTL3-mediated m6A modification of the lncRNA IGFBP7-OT, an overlapping transcript of the IGFBP7 locus, is associated with decreased IGFBP7 promoter methylation and enhanced apoptosis-associated outcomes. METTL3-dependent m^{6}A on LINC00680 facilitates its interaction with SIRT1 mRNA through IGF2BP2, increasing SIRT1 expression. METTL3 regulates NLRP3 mRNA through m^{6}A, promoting inflammasome activation. WTAP-mediated m^{6}A enhances pri-miRNA processing of pri-miR-92b-5p to mature miR-92b-5p, which suppresses TIMP4 mRNA. In parallel, WTAP promotes m^{6}A-dependent, YTHDF2-associated degradation of TIMP4 mRNA, collectively decreasing TIMP4 and favoring matrix catabolism. Conversely, the m^{6}A demethylase FTO alters the processing of pri-miR-515-5p to miR-515-5p, which suppresses TLR4 and attenuates MyD88/NF-κB signaling, thereby dampening inflammatory responses. m^{6}A, N6-methyladenosine; METTL, methyltransferase-like; WTAP, Wilms' tumor 1-associated protein; FTO, fat mass and obesity-associated protein; YTHDF, YTH domain family; miR, microRNA; IGF2BP, insulin-like growth factor 2 mRNA-binding protein; pri-, primary; lncRNA, long non-coding RNA; ATG7, autophagy-related gene 7; SIRT1, sirtuin 1; TIMP4, tissue inhibitor of metalloproteases 4; MyD88, myeloid differentiation primary response 88; NLRP3, NLR family pyrin domain containing 3; TLR4, toll-like receptor 4.

co-activator p300 regulates METTL3 transcription and activation of the PI3K/AKT signaling pathway upregulates p300 expression, thereby increasing METTL3 levels (194,195). METTL3 mediates m^{6}A modification of intercellular adhesion molecule 2 (ICAM2) mRNA (194). Transcriptome-wide m^{6}A-sequencing has identified a prominent m^{6}A peak on ICAM2 mRNA spanning chr17:64002623-64002772, encompassing the predicted m^{6}A sites at chr17:64002634(-) and chr17:64002654(-) (194). MeRIP combined with RT-PCR and RT-qPCR confirms the presence of m6A modification on ICAM2 mRNA in RA-FLSs (194). Functionally, METTL3-mediated m6A methylation of ICAM2 is associated with an aggressive FLS phenotype in RA (194). Moreover, silencing ICAM2 or pharmacologically inhibiting PI3K decreases METTL3 expression, supporting the existence of a METTL3/ICAM2/PI3K/AKT/p300 positive feedback loop that contributes to RA pathogenesis (192,194,196).

LIM and SH3 domain protein 1 (LASP1) is a key epigenetic regulator in RA. LASP1 expression is markedly elevated in cartilage tissue and FLSs of patients with RA (197,198). Loss of LASP1 impairs the invasiveness of FLSs, stabilizes cell-cell contacts and weakens the ability of FLSs to form zipper-like adhesions with the cadherin-11 complex, thereby decreasing bone destruction in RA mouse models (198,199). METTL14 is markedly upregulated in RA rats and its silencing suppresses TNF-α-induced LASP1 expression as well as Src/AKT signaling pathway activation in FLSs, suggesting that METTL14 may promote RA progression through the LASP1/Src/AKT axis (196). In addition, METTL14 may affect the NF-κB pathway to suppress inflammatory responses in RA. Downregulation of METTL14 decreases m^{6}A levels in TNF-α-induced protein-3 mRNA, resulting in activation of NF-κB signaling and subsequent elevation of IL-6 and IL-17 levels (200).

m^{6}A demethylases exert context-dependent and sometimes opposing effects in RA. Recent clinical studies have shown that FTO expression is notably increased in FLSs and synovial tissue from patients with RA (201,202). FTO knockdown suppresses the invasiveness of RA-FLSs and reduces IL-1β and MMP-13 expression (201,203). Mechanistically, FTO mediates these effects by decreasing the m^{6}A modification of ADAMTS15 mRNA, which is recognized by the reader protein IGF2BP1 (201,203). However, conflicting findings have also been reported (204), suggesting FTO may ameliorate RA by inhibiting nucleolar protein/sun domain family member 2, thereby blocking the Wnt/β-catenin signaling pathway. These results indicate FTO may exert context-dependent effects in RA through distinct mechanisms, warranting further investigation. ALKBH5, the second identified m^{6}A demethylase, has recently been reported to exhibit markedly lower expression in patients with RA compared with healthy controls (201). Decreased ALKBH5 mRNA expression in peripheral blood neutrophils is associated with enhanced autophagy in RA, with m^{6}A-modified ATG7 mRNA identified as a functional target, suggesting ALKBH5 may serve as a biomarker for RA diagnosis and disease activity assessment (205).

Collectively, m^{6}A modification contributes to RA pathogenesis by integrating inflammatory signaling, FLS invasiveness, EMT and immune-associated pathways. Rather than acting through a single linear mechanism, m^{6}A regulators exert context-dependent effects across cell and molecular processes, underscoring the complexity of epitranscriptomic regulation in autoimmune arthritis. The key m^{6}A-dependent mechanisms involved in RA are summarized in Table III.

m^{6}A in IVDD. IVDD is a notable contributor to spinal pain, particularly lower back pain, and is also implicated in neck pain (206,207). Globally, low back pain affected 619 million people in 2020; this is projected to increase to 843 million by 2050, while neck pain affected 203 million people in 2020 and is projected to rise to 269 million by 2050 (208,209). IVDD is associated with inflammation (210), autophagy (211) and oxidative stress-induced damage (212,213). Global dysregulation of m^{6}A machinery has been observed during IVDD progression (214). In a bipedal standing-induced mouse model of IVDD, the expression of m^{6}A writers, including METTL3, METTL14 and WTAP, is elevated in nucleus pulposus (NP) tissue compared with controls, indicating a positive association between enhanced m^{6}A modification and disc degeneration (214).

m^{6}A modification contributes to IVDD by regulating transcription factors involved in cartilage and disc matrix integrity. SOX5 (a SOXD family member) and SOX9 (a SOXE family member) are key transcription factors in the chondrogenic gene program; with SOX6, they form the SOX trio, in which SOX5/6 cooperatively potentiate SOX9-driven expression of cartilage-like ECM genes such as aggrecan (215). Notably, SOX5 function is context- and dosage-dependent in degenerative settings, as SOX5 overexpression has been reported to exacerbate cartilage damage in OA mice (216). In IVDD, SOX-factor regulation is compartment- and context-dependent, varying across disc regions and degenerative stimuli rather than reflecting a true contradiction between studies (217-219). In a TNF-α-induced *in vitro* IVDD model, METTL3-mediated m^{6}A modification promotes the maturation of miRNA-143-3p, which is associated with decreased SOX5 transcription and accelerated degenerative progression (220). In parallel, m^{6}A-dependent post-transcriptional regulation also contributes NP senescence, as ncRNA activated by DNA damage (NORAD), an lncRNA, exhibits elevated m^{6}A modification in senescent NP cells, where WTAP facilitates its interaction with the methyltransferase complex and YTHDF2 promotes NORAD decay, resulting in decreased transcript stability (221).

Endplate cartilage is a key disc component and apoptosis of endplate chondrocytes is a key driver of IVDD (222), with iron overload-mediated oxidative stress contributing to this process (223). Consistent with a mechanotransduction-driven m^{6}A-transcription factor axis in endplate degeneration, mechanical loading increases both METTL3 expression and global m^{6}A levels in endplate chondrocytes (223). SOX9 is an m^{6}A target and METTL3 overexpression enhances m^{6}A modification of SOX9 precursor mRNA, decreases SOX9 RNA abundance and promotes IVDD, whereas METTL3 inhibition alleviates disease severity (224). Collectively, these findings indicate that SOX5- and SOX9-associated observations in IVDD reflect distinct, context-dependent m^{6}A regulatory programs, namely an inflammatory NP-associated pathway impacting SOX5 and lncRNA stability, as well as a mechanically driven endplate pathway suppressing SOX9, both converging on ECM dysregulation and disc degeneration.

Table III. Roles of m^{6}A methylation related regulators in rheumatoid arthritis.

Writer	Reader	Expression of m^{6}A regulators	Target genes or pathways	Research objects	Biological function	(Refs.)
METTL3	Not reported	Upregulated	NF-κB	Human synovial tissue/ rats	Promotes the proliferation of FLSCs	(192)
METTL3	Not reported	Upregulated	ICAM2/PI3K/AKT/ EP300	Human synovial tissue/ mice	Promotes the proliferation of FLSCs	(194)
METTL14	Not reported	Upregulated	LASP1/SRC/AKT	Rats/rat synovial tissue	Promotes the proliferation of FLSCs	(196)
METTL14	Not reported	Downregulated	TNFAIP3/NF-κB	Mice/peripheral blood mononuclear cells	Promotes inflammation	(200)
FTO	IGF2BP1	Downregulated	ADAMTS15	Human synovial tissue/ mice/rats	Inhibits the proliferation of FLSCs	(201)
FTO	Not reported	Upregulated	NSUN2/Wnt/ β-catenin	Human synovial tissue/ rats	Inhibits the proliferation of FLSCs	(204)

ICAM2, intercellular adhesion molecule 2; EP300, E1A binding protein p300; LASP1, LIM and SH3 protein 1; TNFAIP3, TNF-α-induced protein 3; ADAMTS15, a disintegrin and metalloproteinase with thrombospondin motifs 15; NSUN2, NOP2/Sun RNA methyltransferase 2; METTL, methyltransferase-like; m^{6}A, N6-methyladenosine; FTO, fat mass and obesity-associated protein; IGF2BP, insulin-like growth factor 2 mRNA-binding protein; FLSCs, fibroblast-like synoviocytes.

Table IV. Roles of m^{6}A methylation-associated regulators in intervertebral disc degeneration.

Writer	Reader	Expression of m^{6}A regulators	Target genes or pathways	Research objects	Biological function	(Refs.)
METTL3	Not reported	Upregulated	miR-143-3p/SOX5	Human nucleus pulposus cells/rat	Promotes apoptosis of nucleus pulposus cells	(220)
WTAP	YTHDF2	Upregulated	NORAD/PUMILIO/ E2F3	Human nucleus pulposus tissue/mice	Promotes apoptosis of nucleus pulposus cells	(221)
METTL3	Not reported	Upregulated	SOX9	Human endplate cartilage tissue/human endplate chondrocytes/ rat	Promotes apoptosis of nucleus pulposus cells	(224)
METTL14	Not reported	Upregulated	NLRP3/SIRT1	Human nucleus pulposus tissue/cells	Promotes apoptosis of nucleus pulposus cells	(225)
METTL14	Not reported	Upregulated	miR-34a-5p/SIRT1	Human nucleus pulposus tissue/cells	Promotes apoptosis of nucleus pulposus cells	(228)
ALKBH5	Not reported	Upregulated	DNMT3B/E4F1	Human nucleus pulposus tissue/cells	Promotes apoptosis of nucleus pulposus cells	(231)

miR, microRNA; NORAD, non-coding RNA activated by DNA damage; PUMILIO, Pumilio RNA-binding family; E2F3, E2F transcription factor 3; NLRP3, NLR family pyrin domain containing 3; SIRT1, sirtuin 1; DNMT3B, DNA methyltransferase 3-β; E4F1, E4F transcription factor 1. METTL, methyltransferase-like; WTAP, Wilms' tumor 1-associated protein; ALKBH5, AlkB homolog 5, RNA demethylase; m^{6}A, N6-methyladenosine.

Inflammation and apoptosis of disc cells are affected by m^{6}A signaling. NLRP3 contributes to IVDD by inducing inflammatory responses. EVs derived from human umbilical cord MSCs enhance the activity of NP cells in IVDD by downregulating METTL14 levels. miRNA-26a-5p serves as an intermediary in this process, binding complementarily to METTL14 mRNA. NLRP3 is a downstream target of METTL14, with METTL14-mediated upregulation of NLRP3 mRNA m^{6}A levels promoting apoptosis of NP cells (225).

SIRT1 expression is decreased in degenerated IVD tissue. METTL14 functions as an upstream regulator of SIRT1, mediating the m^{6}A modification of miRNA-34a-5p, which suppresses SIRT1 expression and induces senescence

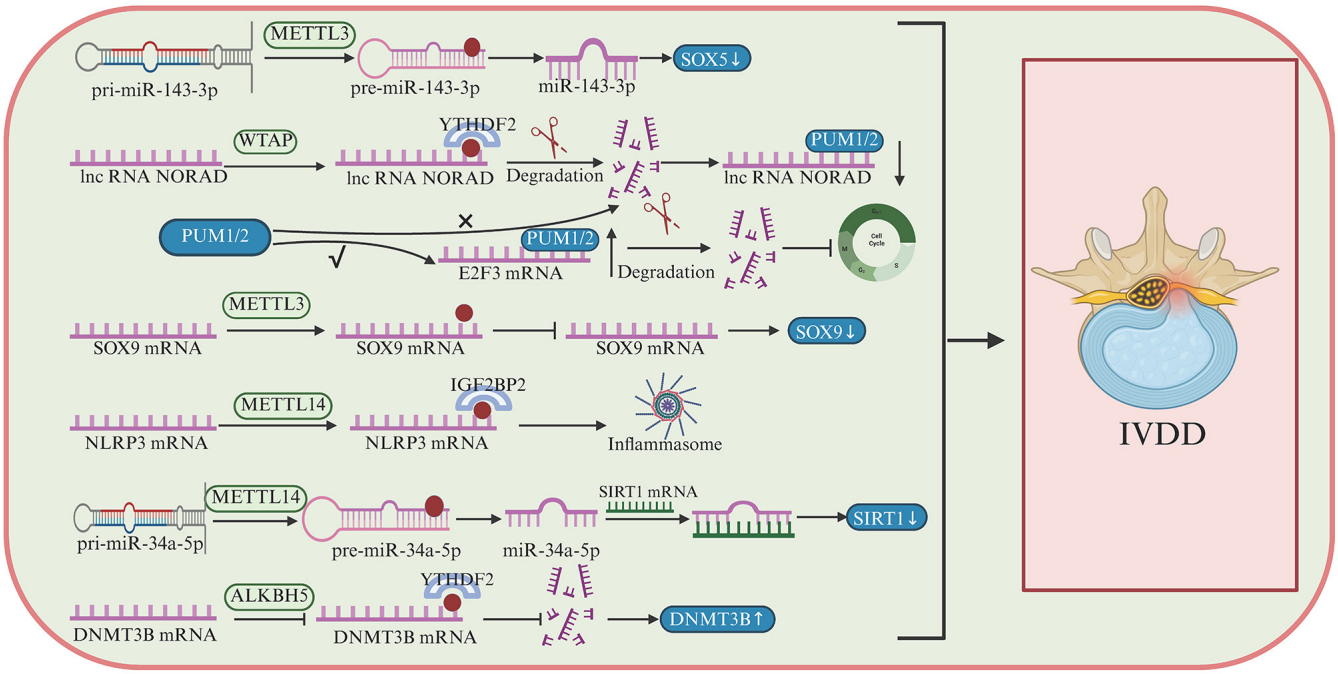

Figure 4. Representative m^{6}A-regulated pathways involved in NPC dysfunction and IVDD progression. The m^{6}A (circle) writer METTL3 promotes m^{6}A-dependent processing of pri-miR-143-3p to mature miR-143-3p, which suppresses SOX5. WTAP installs m^{6}A on the lncRNA NORAD, facilitating YTHDF2 recognition and NORAD decay; decreased NORAD diminishes its sequestration of PUM1/2, thereby enhancing PUM1/2-mediated degradation of E2F3 mRNA and perturbing cell cycle control. METTL3-mediated m^{6}A modification of SOX9 mRNA decreases SOX9 expression, consistent with impaired chondrogenic/ECM programs in disc cells. The writer METTL14 methylates NLRP3 mRNA and, through the reader IGF2BP2, stabilizes NLRP3 transcripts to promote inflammasome activation. METTL14 enhances pri-miRNA processing of pri-miR-34a-5p, increasing miR-34a-5p, which targets SIRT1 mRNA and decreases SIRT1 expression. Conversely, the m^{6}A demethylase ALKBH5 reduces m^{6}A on DNMT3B mRNA, weakening YTHDF2-associated decay and increasing DNMT3B levels. m^{6}A, N6-methyladenosine; METTL, methyltransferase-like; WTAP, Wilms' tumor 1-associated protein; ALKBH5, AlkB homolog 5, RNA demethylase; YTHDF, YTH domain family; miR, microRNA; IGF2BP, insulin-like growth factor 2 mRNA-binding protein; pri-, primary; lnc, long non-coding; SIRT1, sirtuin 1; NLRP3, NLR family pyrin domain-containing 3; ECM, extracellular matrix; IVDD, intervertebral disc degeneration; DNMT3B, DNA methyltransferase 3-β; NORAD, non-coding RNA activated by DNA damage; E2F3, E2F transcription factor 3; PUM, Pumilio RNA-binding family 1; NPC, nucleus pulposus cell.

in NP cells (225-228). ALKBH5 expression is increased in aging IVD tissues, and that its silencing partially alleviates age-related degeneration (229,230). Mechanistically, reduced H3K9me3 enrichment at the ALKBH5 promoter contributes to its upregulation, whereas ALKBH5 promotes IVD cell senescence by reducing m6A modification of its downstream target DNMT3B m6A (231). m6AFTO and YAP1 are downregulated in degenerative nucleus pulposus tissues from patients with IVDD and in rat models, and this is associated with increased m6A modification of YAP1 transcripts; however, the reader proteins mediating this effect remain unclear and may be related to mRNA stability or degradation pathways(232,233).

Collectively, m^{6}A modification contributes to IVDD through coordinated regulation of disc cell survival, inflammation, senescence and ECM homeostasis. Rather than acting through isolated molecular events, m^{6}A-dependent regulatory networks integrate transcription factors, ncRNAs, inflammatory signaling and aging-associated pathways to drive disc degeneration. The key mechanisms of m^{6}A-mediated regulation in IVDD are summarized in Table IV and Fig. 4.

6. Conclusion

With the advancement of epigenetics, m^{6}A modification has become as a notable focus of research in skeletal system disease (35,234). Studies regarding m^{6}A modification have primarily concentrated on OP, OA, RA and IVDD, all of which are prevalent skeletal disorders (132,235). The primary focus has been on the three core m^{6}A regulators-writers, readers, and erasers, which regulate coding and non-coding RNAs, affect downstream signaling pathways and ultimately modulate the functions of disease-relevant cells, such as osteoblast-lineage cells and osteoclasts, chondrocytes, fibroblast-like synoviocytes, macrophages, and nucleus pulposus cells (132,236).

However, a number of studies have not identified specific m^{6}A modification sites, focusing on overall changes in m^{6}A levels in cells or tissues and their effects on downstream signaling (237-239). Regulation of cell physiological functions is rarely attributable to a single RNA modification and typically involves numerous layers of transcriptional and translational control (35,237,239,240). Despite this, research targeting specific m6A sites is valuable in elucidating the precise mechanisms underlying m6A-mediated regulation. Research places emphasis on methyltransferases, with METTL3, METTL14 and WTAP being more extensively studied in skeletal disease, whereas demethylases have received comparatively little attention (132,241). Since m6A modification is a dynamic and reversible process, maintaining the balance between writers and erasers is key in disease development. Investigating whether the interplay between these regulators changes at different stages of disease represents an important avenue for future research. Furthermore, studies regarding m6A in skeletal system disease

remain largely limited to animal models; to the best of our knowledge, there are no clinical investigations into its potential diagnostic or therapeutic applications. Addressing this gap represents a key direction for future research (132,134).

The primary method for detecting m^{6}A levels is MeRIP sequencing (seq), the earliest high-throughput sequencing technique developed using m^{6}A-specific antibodies (47,52). MeRIP-seq offers simple operation, provides a transcriptome-wide view, supports numerous RNA types and features high throughput, making it suitable for multi-sample screening. Consequently, it is the preferred approach for m^{6}A sequencing (47,237,242). However, MeRIP-seq has limitations. It typically identifies broad m^{6}A-enriched regions rather than precise nucleotide-resolution sites, requires relatively large amounts of input RNA and does not provide absolute quantification. In addition, it is susceptible to antibody-associated biases, including nonspecific binding, cross-reactivity with structurally related modifications such as m^{6}Am, and batch- or protocol-dependent variability, all of which can increase background noise, generate false-positive signals, and reduce inter-study reproducibility. Therefore, MeRIP-seq is most suitable for transcriptome-wide screening and typically requires orthogonal validation for precise site identification and quantitative analysis (51,243,244). To overcome these limitations, antibody-independent methods have emerged. For example, FTO-assisted selective chemical labeling of m^{6}A uses the demethylase FTO to label m^{6}A *in vitro*, theoretically achieving near single-nucleotide resolution and enabling more precise site localization (245). Selective acryloyl chemical labeling seq directly labels m^{6}A, covering nearly all classical m^{6}A motifs and quantitatively analyzes captured m^{6}A sites at single-base resolution (246). Requiring only minimal RNA input, this method simultaneously enables whole-transcriptome m^{6}A modification profiling and gene expression analysis, providing both high-resolution localization and the ability to reveal m^{6}A heterogeneity among cell subpopulations in complex tissue.

Although high-throughput sequencing technology rapidly generates data on thousands of m^{6}A sites, sequencing biases and technical limitations may still result in false positives or negatives. Therefore, methods capable of detecting m^{6}A at single-gene resolution are key. The single-base extension and ligation-based quantitative PCR amplification method leverages m^{6}A inhibition of DNA polymerase and ligase activity, enabling rapid, convenient detection and quantification of site-specific m^{6}A modification (247).

Future studies should delineate the regulatory networks of m^{6}A writers, erasers, and readers, including how these factors are recruited to specific RNA substrates and cellular contexts. Particular attention should be given to the identification of novel reader proteins and to the crosstalk between m^{6}A and other RNA modifications, especially in ncRNAs. Among these, m5C (5-methylcytidine) is a cytosine methylation mark that has been implicated in RNA stability, nuclear export, and translational regulation, whereas ac4C (N4-acetylcytidine), an acetylation mark mainly installed by NAT10, is generally associated with enhanced RNA stability and translation efficiency. Clarifying how these modifications cooperate or compete with m^{6}A in ncRNAs may provide a more comprehensive understanding of epitranscriptomic regulation in skeletal disease (248). Such multi-modification interactions may arise from co-occurring marks on the same transcript, RNA structural remodeling or altered recruitment of RNA-binding proteins, converging on shared RNA processing and decay pathways; however, direct mechanistic evidence associating these processes with skeletal system disease remains limited (132). Accordingly, methodological innovation is needed, including single-base-resolution, single-cell and spatially resolved sequencing to resolve cell heterogeneity in m^{6}A regulation, as well as live-cell imaging tools to monitor dynamic m^{6}A changes in real-time. From a translational perspective, the majority of m^{6}A-associated findings in musculoskeletal disorder remain preclinical and patient-level validation of candidate biomarkers is scarce (21,132,134); to the best of our knowledge, no publicly registered interventional clinical trials specifically targeting m^{6}A regulators have been initiated for skeletal system disease, with central barriers including tissue-specific delivery, on-target safety and context-dependent effects of writers, readers or erasers. m^{6}A modification holds promise for clinical applications in diagnosis and therapy A representative example is STC-15, an orally administered METTL3 inhibitor that has entered first-in-human phase I evaluation in patients with advanced malignancies (NCT05584111) (249). This provides proof of principle that m^{6}A regulators are pharmacologically tractable targets, that is, drug-like small-molecule modulators can be developed against them and advanced into human clinical testing (250). Extending this strategy to skeletal indications will still require tissue-selective delivery, rigorous assessment of on-target safety, and validation in disease-specific contexts. Ultimately, m^{6}A-targeted approaches may offer future opportunities for biomarker development and precision therapy in skeletal system diseases (132).

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Authors' contributions

XX, CL, HTZ, XG and GG analyzed the data and constructed the figures. XG conceived and designed the study and wrote and reviewed the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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