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Review Article

METTL3 plays multiple functions in biological processes

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Abstract: N^6-methyladenosine (m^6A) is the most common internal modification of mRNAs in higher eukaryotic. This process is performed by methyltransferase. Methyltransferase-like 3 (METTL3) is the best known m^6A methyltransferase that functions in the reversible epi-transcriptome modulation of m^6A modification. Besides acting as a m^6A methyltransferase, METTL3 also regulates mRNA translation and other biological processes. In recent years, studies have identified numerous roles and molecular mechanisms associated with METTL3 in multiple biological processes. However, these findings have not been summarized. In this review, we have systematically summarized the most recent important roles of METTL3 in various biological processes, including cell cycle progression, cell proliferation, cell apoptosis, cell migration and invasion, cell differentiation and inflammatory response. In addition, we discuss the prospect of using a METTL3 as a new diagnostic biomarker and therapeutic target for human cancers.

Keywords: m^6A modification, METTL3, biological process, diagnostic biomarker, cancer therapy

Introduction

N^6-methyladenosine (m^6A) modification is the most common internal modification of mammalian RNAs such as mRNA, tRNA, rRNA, small nuclear RNA, microRNA precursor and long non-coding RNA [1, 2]. m^6A methylation is performed by methyltransferases, erased by demethylases, and bound by reader proteins that recognize the m^6A methylation site. It therefore regulates RNA stability or translation [3]. Some of the methyltransferases involved in m^6A methylation include methyltransferase-like 3 (METTL3), METTL5 [4], METTL14, METTL16 [5], RBM15 [6], Wilms' tumor 1-associated protein (WTAP), VIRMA [6], and ZCCHC4 [7]. METTL3 is a key component of the m^6A methyltransferase complex which is made up of METTL3, METTL14, WTAP and KIAA1429 [8]. Extraordinarily, apart from its eminent methyltransferase activity, METTL3 has also been reported to function as a m^6A reader. It directly enhances the translation of several oncogene mRNAs such as epidermal growth factor receptor (EGFR) and the Hippo pathway effector TAZ in cancer cells. It promotes the translation of m^6A subset containing mRNAs by interacting with translation initiation machinery independent of its methyltransferase and m^6A reader activity [3]. Thus METTL3 is a transferase that methylates mRNA, identifies methylated mRNA, and directly promotes mRNA translation.

METTL3 plays a role in m^6A modification of its target RNAs. The activity of its m^6A methyltransferase can be regulated by post-translational modification such as SUMOylation. Du et al. reported the characteristics of METTL3 in activities and post-translational modification. Besides, METTL3 can be modified by SUMO1 at several sites (lysine residues K^{177}, K^{211}, K^{212} and K^{215}). It was revealed that SUMOylation of METTL3 does not affect its stability, subcellular localization and WTAP interaction. However, it significantly represses METTL3 m^6A methyltransferase activity. This alternation of the m^6A modification level in mRNAs and subsequent effect on its expression may directly influence

soft-agar colony formation and xenograft tumor growth in human non-small cell lung carcinoma (NSCLC) cells [1].

Currently, there is growing interest on the study of the potential role and mechanism of METTL3 in various biological processes. These studies have yielded interesting findings. For instance, it was shown that METTL3 regulates hematopoietic stem cells (HSCs) differentiation by targeting myelocytomatosis viral oncogene (Myc). Moreover, deletion of METTL3 lead to cell-intrinsic HSC reconstitution, differentiation defects, and had no impact on the maintenance or function of the myeloid cell [9]. In ovarian carcinoma, METTL3 is normally upregulated and its high expression is significantly associated with overall survival rate of cancer patients. METTL3 acts an oncogene in ovarian carcinoma by stimulating receptor tyrosine kinase AXL translation and epithelial-mesenchymal transition (EMT). Therefore, it is a potential novel prognostic or therapeutic target in ovarian cancer [10]. In NSCLC, MELLT3 functions as an oncogene by methylating none coding RNAs. It promotes m^6A modification of the lncRNA MALAT1 and thus increases its stability [11]. In this review, we first introduce the characteristics and catalytic activities of METTL3 such as methyltransferase and translation activity. Meanwhile, to better understand the multiple roles of METTL3 and their corresponding signal transduction pathways, we have summarized the most important functions of METTL3 in cell cycle progression, cell proliferation, cell apoptosis, cell migration and invasion, cell differentiation, and inflammatory response. In addition, we have discussed the prospects of using METTL3 as a new cancer diagnostic biomarker or therapeutic target.

Characteristics and catalytic activities of METTL3

Apart from its m^6A methylation activities, some studies have revealed that METTL3 can directly target methylated mRNA, and subsequently promote translation of the mRNAs such as EGFR and TAZ [3]. The following are the characteristics and catalytic activities of METTL3.

The methyltransferase complex and methylation catalytic activity of METTL3

M^6A is installed by a methyltransferase complex consisting of METTL3, METTL14, WTAP and KIAA1429 [8]. Among them, METTL3 (also known as MT-A70) belongs to a large and conserved family of methyltransferases [12, 13]. It has a MT-A70 domain (also referred to as methyltransferase domain, MTD) which catalyzes methyl transfer to adenosine. Together with METTL14, METTL3 forms a heterodimeric enzyme complex METTL3/METTL14 in the nucleus where it is restricted to nuclear speckles and interacts with the WTAP [14]. In the absence of WTAP, the RNA-binding capacity of METTL3 is highly reduced. Therefore, WTAP potentially regulates the recruitment of m^6A methyltransferase complex to mRNA targets [15]. In the m^6A methyltransferase complex, METTL3 primarily functions as the catalytic core while METTL14 serves as the RNA-binding platform [16]. The helical structure at the N terminus of METTL3 also known as the leader helix (LH) is necessary for the interaction of METTL3 and WTAP. The binding site of METTL3 is within the first 150 amino acids of WTAP, and main interaction of METTL3 and METTL14 is mediated by MTD. The catalytic activity of the complex is solely provided by METTL3 and favorably modifies substrate RNAs conforming to GGACU consensus sequence. In addition it has sequence specificity but displays less structural preference to RNA substrates [17]. It is noteworthy that, METTL14 cannot be expressed and purified in soluble form in the absence of METTL3 thus METTL3 is required to stabilize METTL14 [18]. Similarly, knockout of either METTL3 or METTL14 in mouse embryonic stem cells results in complete loss of m^6A modification in mRNA [19].

Translation activity of METTL3

In addition to methyltransferase activity, METTL3 enhances the translation of target mRNAs independent of its methyltransferase catalytic subunit. Firstly, independent of m^6A readers METTL3 promotes translation. For example, in human lung cancer, METTL3 directly promotes the translation of oncogenes such as EGFR and the Hippo pathway effector TAZ. Here, METTL3 recruits eIF3 to the translation initiation complex independent of methyltransferase activity or downstream m^6A reader proteins [3]. Similarly, another study showed that METTL3 can promotes the translation of a large subset of mRNAs without m^6A readers. In the cytoplasm, METTL3 recognizes 3'-UTR m^6A sites on mRNA and promotes protein translation from the transcript by facilitating translation loop for-

mation via interaction with the eukaryotic translation initiation factor 3 subunit h (eIF3h). The METTL3-eIF3h interaction is crucial for promoting mRNA translation and polysome conformation, hence important for METTL3 oncogenic function [20].

Secondly, METTL3 can enhance its target gene translation by interacting with m^{6}A readers. For instance, METTL3 promotes YAP mRNA translation by recruiting YTHDF1/3 and eIF3b into the translation initiation complex [11]. In immune regulation studies *in vivo* and *in vitro,* it was shown that METTL3 can promote maturation and activation of dendritic cell by upregulating translation of the key regulators such as CD40, CD80, and the TLR signaling adaptor Tirap. Notably, enhancing of CD40 and CD80 m^{6}A-dependent translation is positively associated with the expression of YTHDF1 [21]. In acute myeloid leukaemia, METTL3 binds to the transcriptional initiation site of the target genes through CAATT-box binding protein CEBPZ increasing the translation of the corresponding mRNA by relieving ribosome stalling [22]. A study by Yang F et al. revealed that METTL3 and YTHDF1 but not YTHDF2 and YTHDF3 bind to the 3'-UTR of CDCP1 mRNA promoting its translation. Ultimately, this plays an important role in cell proliferation, migration, as well as the invasion of the bladder cancer (BCa) cells and the chemical-transformed uroepithelial cells [23]. Overall, many research studies propose that METTL3 can directly regulate the translation of target mRNAs either independently or by interacting with m^{6}A readers.

The emergent roles and mechanism of METTL3 in biological processes

Recent studies have shown that METTL3 plays some significant roles in various biological processes. These include; cell cycle, cell proliferation, cell apoptosis, cell migration and invasion, cell differentiation, inflammation response, metabolism, and innate immunity (**Table 1**). These biological processes are closely associated with several diseases such as various types of cancer, inflammatory diseases, ischemic heart diseases, immunological diseases, and metabolic diseases (**Table 1**). For instance, METTL3 serves as an oncogene in several cancers and stabilizes balance in biological processes *in vivo* such as spermatogenesis [24] (**Table 1**). Mechanistically, METTL3 modulates biological processes by regulating some important signaling pathways. These pathways include JAK/STAT [25], PI3K/AKT [26], Wnt/β-catenin [27] and MAPK/NF-κB [28] pathway.

METTL3 regulates cell cycle

Recently, several studies have revealed that METTL3 is involved in the regulation of cell cycle, the underlying process of cell duplication. In the process of adipogenesis, depletion of ZFP217 increases the expression of METTL3 leading to increased m^{6}A level in cyclin D1 mRNA. YTHDF2 recognizes and degrades the methylated cyclin D1 mRNA, inhibiting cell-cycle progression and adipogenesis process [64] (**Figure 1**). Similarly, combined with WTAP and METTL14, METTL3 coordinates cell cycle and promotes mitotic clonal expansion (MCE) during adipocyte differentiation by regulating cyclin A2. The knockdown of these three proteins leads to cell cycle arrest and impaired adipogenesis which is associated with suppression of cyclin A2 upregulation during MCE [60] (**Figure 1**). In renal cell carcinoma, METTL3 knockdown significantly reduces G0/G1 arrest by lowering p21 protein expression, while increased levels of METTL3 induces G0/G1 arrest [42] (**Figure 1**).

METTL3 regulates cell proliferation

Several studies have revealed that METTL3 plays an important role during tumor cell proliferation in a m^{6}A-dependent manner. METTL3 was first reported to be involved in promoting cell proliferation in lung cancer [3]. Subsequently, the capacity of METTL3 in promoting cell growth has been discovered in various cancers such as cutaneous squamous cell carcinoma [38], BCa [23, 41], ovarian carcinoma [10], breast cancer [43], gastric cancer [30-32], NSCLC [48], prostate cancer [56], pancreatic cancer [57], leukemia [45] and osteosarcoma [27]. A study on the mechanism of different pathways involved in cell proliferation such as Wnt/β-catenin, PI3K/AKT/mTOR and p38/ERK pathways has revealed that they are regulated by METTL3. Moreover, silencing METTL3 was demonstrated to inhibit cell proliferation in osteosarcoma by lowering the m^{6}A methylation and the total mRNA levels of lymphoid enhancer-binding factor 1 (LEF1), leading to inhibition of Wnt/β-catenin signaling pathway [27] (**Figure 2**). Similarly, another study showed that METTL3 regulates cell proliferation by modulating

Table 1. The role of METTL3 in various biological processes

Cases	Roles of METTL3	Biological process	Ref
1	Promote proliferation, invasion and migration	Lung cancer	[3]
2	Maintain hematopoietic stem cell differentiation	Hematopoietic stem cell	[9]
3	Promote proliferation, invasion and migration	Ovarian carcinoma	[10]
4	Promotes oncogenesis	Lung cancer	[20]
5	Promotes dendritic cell activation and function	Innate immunity	[21]
6	Promote proliferation, invasion and migration	Bladder cancer	[23]
7	Regulate proliferation and differentiation	Spermatogenesis	[24]
8	Promote proliferation, invasion and migration	Osteosarcoma	[27, 29]
9	Promotes proliferation	Gastric cancer	[30, 31]
10	Apoptosis	Gastric cancer	[31]
11	Promote proliferation and migration	Gastric cancer	[32-34]
12	Inhibit proliferation and migration	Colorectal cancer	[35]
13	Promote proliferation, migration and metastasis	Colorectal cancer	[36, 37]
14	Promote proliferation, inhibit differentiation	Cutaneous squamous cell carcinoma	[38]
15	Promote proliferation and migration	Lung cancer	[39]
16	Promote migration	Melanoma	[40]
17	Promote proliferation and invasion	Bladder cancer	[41]
18	Inhibit proliferation, invasion and migration	Renal cell carcinoma	[42]
19	Promote proliferation	Breast cancer	[43, 44]
20	Promote proliferation and inhibit differentiation	Leukemia	[45]
21	Critical regulator for skeletal muscle differentiation	Skeletal myoblasts	[46]
22	Maintain spermatocyte differentiation	Spermatocyte	[47]
23	Promote proliferation	NSCLC	[11, 48]
24	Promote proliferation	Bladder cancer	[49]
25	Crucial for embryo development	Embryonic development	[50]
26	Regulate differentiation	Osteoblast	[26, 51]
27	Lack can lead to osteoporosis	Mesenchymal stem cells	[52]
28	Maintain the nerve ball and inhibit differentiation	Glioma	[53]
29	Promote cardiomyocyte hypertrophy	Apoptosis	[54]
31	Maintain the stasis of hematopoietic stem cells	Hematopoietic stem cell	[55]
30	Promote proliferation and inhibit differentiation	Leukemia	[22]
31	Promote proliferation and metastasis	Prostate cancer	[56]
32	Promotes proliferation and invasion	Pancreatic cancer	[57]
33	Inhibiting apoptosis	Nasopharyngeal carcinoma	[58]
34	Enhances long-term memory consolidation	Long-term memory formation	[59]
35	Regulates mitotic clonal expansion in adipogenesis	Obesity	[60]
36	Inhibits Hepatic Insulin Sensitivity	Type II diabetes	[61]
37	Drives M1 macrophage polarization	Inflammatory response	[62]
38	Negative regulator of autophagy	Ischemic Heart Disease	[63]

the ATPase family AAA domain-containing 2 (ATAD2) in osteosarcoma [29] (**Figure 2**). The ATAD2 further activates transcription factors such as E2F family members, regulating gene expressions or chromatin modifications involved in cancer cell proliferation [65, 66]. In addition, METTL3 expression is elevated in BCa tissue. Decreasing the levels of METTL3 inhibits cell proliferation by directly lowering the expression of MYC and AFF4. Remarkably, AFF4 can also directly regulates the expression of MYC, which leads to BCa progression [41] (**Figure 2**). These studies suggest that METTL3 acts as an oncogene and promotes cell proliferation.

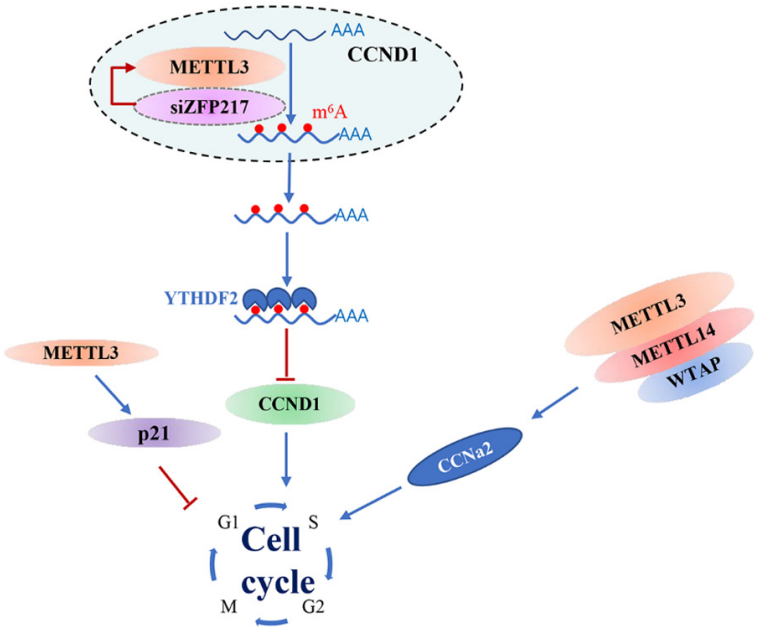

Figure 1. METTL3 regulates cell cycle. METTL3 regulates cell cycle by targeting p21, cyclin A2, and cyclin D1.

Nevertheless, METTL3 has also been reported to be a tumor-suppressor gene in colorectal cancer (CRC), and inhibits cell proliferation by modulating p-p38/p-ERK pathways [35] (**Figure 2**). A similar role of METTL3 is found in renal cancer (RCC). The down-regulation of METTL3 significantly increases the expressions of p-PI3K, p-AKT, p-mTOR, and p-p70, while overexpression of METTL3 lowers their expression. Therefore, METTL3 regulates cell proliferation by affecting PI3K/AKT/mTOR signaling pathway in RCC [42]. In addition to regulating mRNAs, METTL3 regulates cell proliferation by promoting noncoding miRNAs maturation. In BCa, METTL3 acts as an oncogene and positively regulates cell proliferation via m^{6}A-dependent pathway. Mechanistically, by interacting with the microprocessor protein DiGeorge critical region 8 (DGCR8), METTL3 positively modulates the maturation of pri-miR221/222. This decreases the expression of phosphatase and tensin homolog deleted on chromosome 10 (PTEN). Promoting tumor cell proliferation [49] (**Figure 2**).

In contrast, METTL3 is a potential miRNA target in cancer cell proliferation. For instance, in breast cancer progression, HBXIP increases the expression levels of METTL3 by inhibiting miRNA let-7g expression inhibiting the expression of METTL3. Extraordinarily, METTL3 promotes the expression of oncoprotein HBXIP by promoting m^{6}A modification. Thus, it forms a positive feedback loop of HBXIP/let-7g/METTL3/HBXIP which accelerates cell proliferation in breast cancer [43] (**Figure 2**). Similar to let-7g, miR-600 [39] and miR-33a [48] inhibits NSCLC cell proliferation by directly targeting METTL3. Knocking down METTL3 significantly inhibits the expression of EGFR. Subsequently, this inhibits PI3K and lowers the levels of the phosphorylated form of AKT leading to inhibition of NSCLC cell growth [39] (**Figure 2**).

Furthermore, miR-4429 targets METTL3 which promotes GC cell proliferation by upregulating the expression of SEC62. Mechanistically, METTL3 promotes the m^{6}A modification on SEC62 mRNA and stabilizes it through IGF2BP1, leading to a high expression of SEC62 [30] (**Figure 2**). Generally, METTL3 acts as an oncogene by promoting cell proliferation in most types of cancer, but also acts as a tumor suppressor by inhibiting cell proliferation in RCC and CRC. The study on its process reveals that it not only regulates cell proliferation through mRNA expression but also modulates the maturation of none coding RNAs such as miRNA. Contrary, METTL3 is also regulated by miRNA such as let-7g, miR-600, miR-33a and miR-4429.

METTL3 regulates cell apoptosis

Cell apoptosis is the spontaneous and orderly death of cells controlled by genes [67]. It is mainly triggered by either the caspase-mediated extrinsic or intrinsic pathways [68]. Several studies indicate that knocking down METTL3 promotes cancer cell apoptosis by regulating the Bcl-2 signal pathway, SHH/GLI pathway, and ZNF750-FGF14 pathway. For instance, Lin et al. found that downregulating METTL3 decreases the expression of pro-survival Bcl-2 and increases the pro-apoptosis regulator Bax as well as active caspase-3 promoting GC cell apoptosis [31]. In lung cancer, knockdown of METTL3 too induces mitochondrial apoptotic

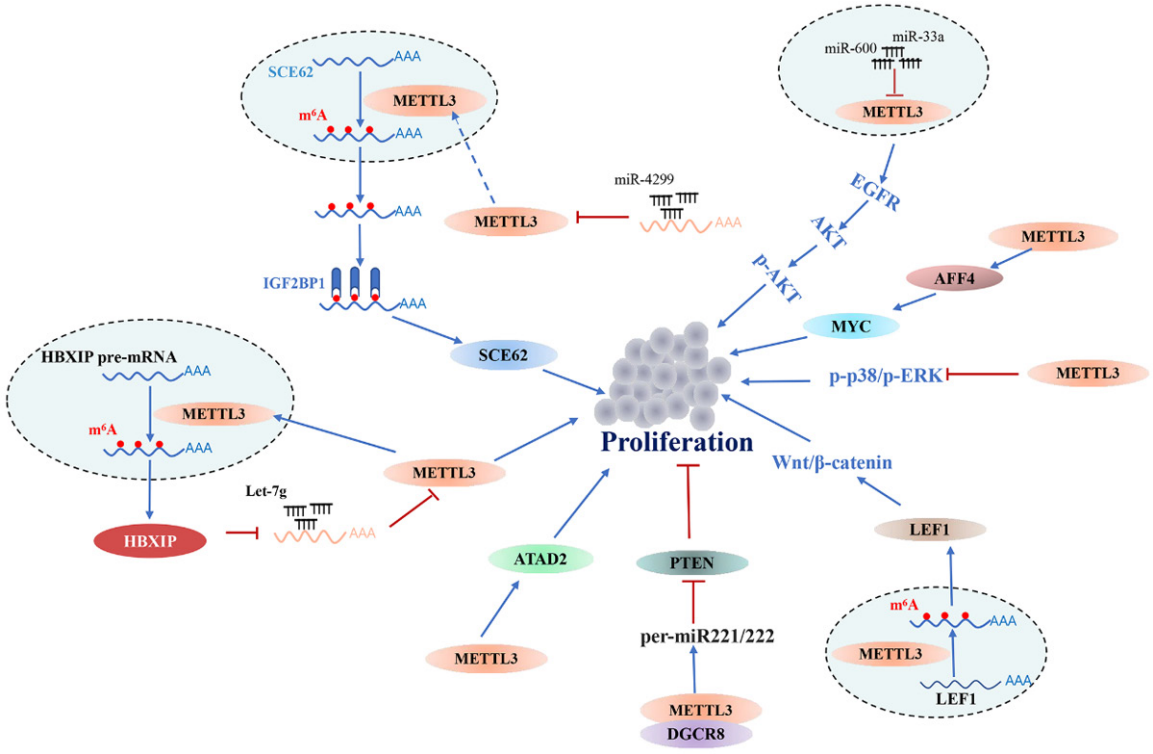

Figure 2. METTL3 regulates cell proliferation. METTL3 promotes the proliferation of tumor cells by targeting SEC62, LEF1, ATAD2, Wnt/β-catenin, AFF4/MYC, and EGFR/AKT signaling pathways. METTL3 promotes cell proliferation by modulating miRNA221/222 maturation via DGCR8. METTL3 regulates cell proliferation by regulating miRNAs, such as miR-4299, miR-600, miR-33a and Let-7g. METTL3 decreases tumor cell proliferation by inhibiting the p-p38/p-ERK pathway.

pathways by lowering the expression of Bcl-2 and increasing the expression of Bax and caspaes-3 [39]. Similarly, METTL3 can regulate cell apoptosis by regulating Bcl-2 in breast cancer (**Figure 3**) [44] and acute myeloid leukemia (AML) [45]. It does so by promoting m^{6}A methylation in Bcl-2 and its translation. In prostate cancer, METTL3 regulates cell apoptosis through SHH/GLI pathway (**Figure 3**) in an m^{6}A catalytic activity-dependent manner [56]. In Nasopharyngeal carcinoma (NPC), METTL3 induces cell apoptosis by modulating ZNF750-FGF14 signaling axis.

Studies shows that METTL3 promotes m^{6}A modification of ZNF750 mRNA and reduces its expression [58] (**Figure 3**). In chondroprogenitor cells, silencing METTL3 protects against IL-1β-induced ATDC5 cell apoptosis [69]. Moreover, many studies indicate a close relationship between cell apoptosis and autophagy. For instance, hypoxia/reoxygenation (H/R) treatment enhances cell apoptosis by inducing the expression of METTL3 in cardiomyocytes. Mechanistically, METTL3 methylates TFEB (the transcription factor EB) at the two m^{6}A residues in its 3'-UTR region. Consequently, this promotes the association of HNRNPD with TFEB pre-mRNA, leading to reduced expression of TFEB mRNA hence inhibiting TFEB activity. Downregulation of TFEB, a key regulator of lysosomal biogenesis and autophagy genes, inhibits cell autophagy maturation consequently promoting apoptosis in H/R-treated cardiomyocytes [63] (**Figure 3**).

METTL3 regulates cell migration and invasion

After EMT, cell motility and migration are stimulated. Therefore, invasiveness is enhanced in many cancers. During the EMT process, cells lose their epithelial features such as epithelial polarity and cell-cell contact structures but acquire a mesenchymal phenotype. Thus, they attain migratory behavior which allows them to migrate away from epithelial cell neighbors and invade surrounding tissues [70]. Many studies indicate that METTL3 contributes to cancer cell

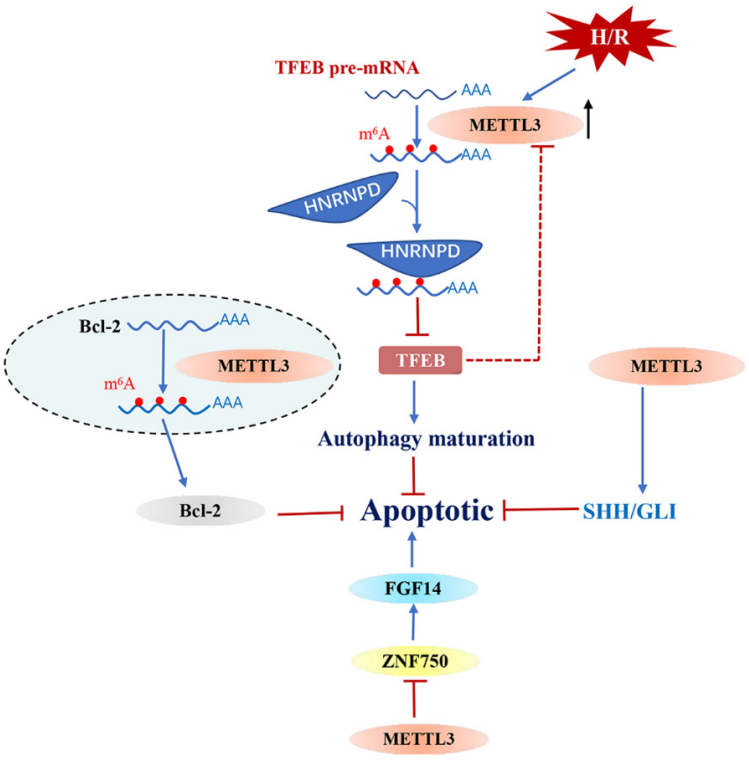

Figure 3. METTL3 regulates cell apoptosis. METTL3 inhibits cell apoptosis by regulating Bcl-2, SHH/GLI and ZNF750/FGF14 signaling pathways. METTL3 promotes cell apoptosis via TFEB-signaling pathway, SHH, Sonic Hedgehog.

migration and invasion by promoting EMT [10, 23, 32, 33, 39, 40, 42]. For example, melanoma cells express higher levels of METTL3 than normal melanocytes. Besides, METTL3 promotes aggregation of MMP2 in melanoma cells depending on its catalytic activity. Therefore, it plays an important function in cell invasion and migration [40] (**Figure 4**). In ovarian cancer, METTL3 induces EMT by promoting the expression of AXL leading to cell invasion and metastasis [10] (**Figure 4**). Similarly, METTL3 transforms growth factor-beta-induced EMT of lung cancer cells through m^{6}A methylation of JUNB mRNA promoting its stabilization and expression [71] (**Figure 4**). Furthermore, another study revealed that METTL3 promotes GC cell invasion and metastasis *in vitro* and *in vivo*. Mechanistically, in an m^{6}A-HuR-dependent manner, METTL3 promotes the mRNA expression of ZMYM1 which represses E-cadherin transcription by interacting with the CtBP/LSD1/CoREST complex. Here, HuR recognizes ZMYM1 mRNA m^{6}A modification as an m^{6}A reader [33] (**Figure 4**). However, increasing the levels of METTL3 reverses EMT progression in RCC [42].

Besides regulating the EMT pathway, METTL3 can regulate cell migration by directly regulating the expression of its target genes such as HDGF, YAP and SOX2. For example, increasing METTL3 levels promotes liver metastasis of GC cells *in vitro* and *in vivo*. Mechanistically, P300 mediates H3K27, acetylates the promoter of METTL3 and enhances its transcription. METTL3 increases m^{6}A modification of HDGF mRNA and maintains its stability through m^{6}A reader IGF2BP3. Besides, HDGF promotes liver metastasis of GC cells via two pathways. On one hand, secreted HDGF promotes tumor angiogenesis. On the other hand, nuclear HDGF promotes glycolysis in GC cells by stimulating the expression of GLUT4 and ENO2. Moreover, HDGF is a novel growth factor that has been reported to be involved in several cancer progressions such as cancer metastasis, apoptosis, angiogenesis and growth [34] (**Figure 4**). In NSCLC, m^{6}A mRNA methylation initiated by METTL3 induces cell metastasis. Mechanistically, aberrant METTL3 activation increases m^{6}A modification in YAP mRNA and increases its expression through YTHDF3. YAP interacts with transcription factors TEA domain family members (TEADs) to activate YAP's target genes CTGF and Cyr61 promoting NSCLC metastasis. Notably, METTL3/YTHDF3 complex increases the stability of MALAT1 in an m^{6}A dependent manner, the latter sponges miR-1914-3p which directly targets the 3'-UTR of YAP and decreases YAP expression in A549 and H1299 cells, finally promoting the YAP expression in NSCLC [11] (**Figure 4**). In CRC, METTL3 is highly expressed in CRC metastatic tissues and facilitates CRC metastasis *in vivo*. Mechanistically, METTL3 increases m^{6}A methylation level of SOX2 transcripts and maintains its mRNA stability and expression through m^{6}A reader IGF2BP2. Finally, increasing SOX2 ex-

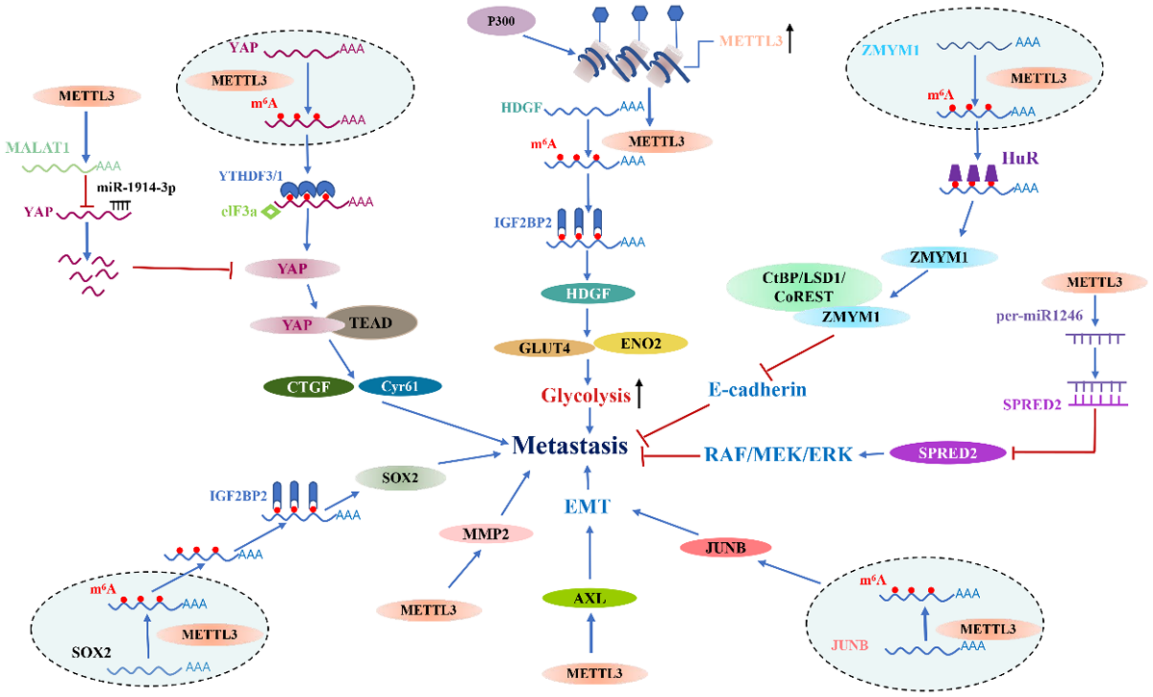

Figure 4. METTL3 regulates cell migration and invasion. METTL3 promotes cell metastasis and invasion by regulating SOX2, MMP2, AXL, JUNB, ZMYM1/E-cadherin, HDGF, YAP and miR-1246/SPRED2/MAPK signaling pathways.

pression promotes CRC cell metastasis by regulating its downstream targets [36] (**Figure 4**). Meanwhile, METTL3 can also regulate cell metastasis by targeting non-coding RNAs. For instance, METTL3 has been shown to promote cell metastasis by promoting the expression of miR-1246 which directly binds to SPRED2 reducing its expression. Consequently into RAF/MEK/ERK pathway is activated [37] (**Figure 4**). In summary, METTL3 promotes cell metastasis by targeting mRNA and non-coding RNAs.

METTL3 regulates cell differentiation

METTL3 plays some crucial roles in cell differentiation processes such as cancer stem cell differentiation, mammalian spermatogenesis, osteogenic differentiation, hematopoietic stem cells differentiation, and skeletal muscle differentiation. For instance, in AML, METTL3 regulates c-MYC, Bcl-2, and PTEN genes promoting leukemia progression and inhibiting myeloid differentiation [45] (**Figure 5A**). Similarly, during the process of cutaneous squamous cell carcinoma differentiation, knockdown of METTL3 promotes cell differentiation *in vitro* and *in vivo*. The study of its mechanism reveals that knocking down METTL3 reduces the expression of the undifferentiated marker K14 and enhances the expression of the early-stage differentiation marker K10 in A431 and HSC-1 cells [38]. From the above two examples, it is clear that METTL3 inhibits cell differentiation in leukemia and cutaneous squamous cell carcinoma.

However, METTL3 plays a positive role in mammalian spermatogenesis. Loss of m^{6}A methylation due to METTL3 deficiency disrupts homeostasis of spermatogonial stem cell (SSC)/progenitor cell. This is because the loss of m^{6}A methylation dysregulates key regulators of SSC proliferation and differentiation such as Plzf, Id4, Dnmt3b, and Sohlh2 [24]. It is noteworthy that METTL3 plays a crucial role in skeletal health related to cell differentiation. In osteogenically differentiated bone mesenchymal stem cells (BMSCs) METTL3 is highly expressed. Loss of METTL3 suppresses PI3K-AKT signaling and the osteogenic differentiation potential of BMSCs.

In addition, METTL3 knockdown lowers the expression of Vegfa-164 and Vegfa-188 mRNA in BMSCs which are positively associated with osteogenic differentiation [26]. In another

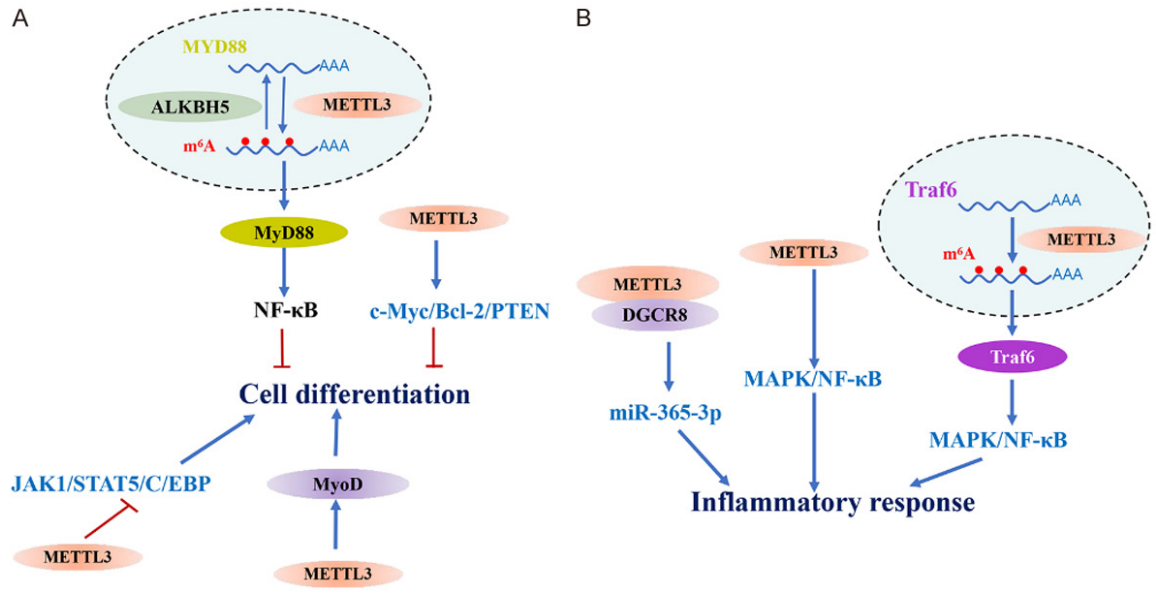

Figure 5. The molecular mechanism of METTL3 in cell differentiation and inflammatory response. A. METTL3 modulates cell differentiation by regulating MyoD, c-MYC/Bcl-2/PTEN, MYD88/NF-κB, and JAK1/STAT5/C/EBP signaling pathways. B. METTL3 promotes inflammatory response by targeting miR-365-3p, and MAPK/NF-κB signaling pathways.

study, the efficient and specific regulation of m^{6}A on bone marrow mesenchymal stem cells (MSCs) has been revealed. Here, the deletion of METTL3 in MSCs disrupted the fate of the cell in mice and resulted in osteoporosis pathological phenotypes such as reduced bone mass with incompetent osteogenic potential, and improved marrow adiposity with enhanced adipogenic potential. In contrast, METTL3 gain-of-function prevents estrogen deficiency-induced postmenopausal osteoporosis. This demonstrates that METTL3 regulates the fate of bone marrow MSCs and hence skeletal health. It is evident that the downregulation of METTL3 reduces the translation efficiency of parathyroid hormone receptor-1 (Pth1r), and disrupts the PTH-induced osteogenic and adipogenic responses *in vivo* [52]. However, different from the BMSCs and MSCs, METTL3 is negatively correlated with adipose differentiation of bone mesenchymal stem cells (pBMSCs). Knockout of METTL3 promotes the differentiation of pBMSCs into adipocytes. Mechanistically, downregulating METTL3 reduces the m^{6}A levels of JAK1 mRNA and promotes its expression. JAK1 activates STAT5 by regulating its phosphorylation. Subsequently, STAT5 binds to the promoter of C/EBP beta activating the JAK1/STAT5/C/EBP beta pathway [25] (**Figure 5A**). In addition, METTL3 shows different regulatory functions in osteogenesis differentiation. A study by Yu et al. found that METTL3 inhibited osteogenesis differentiation. Together with demethylase ALKBH5, METTL3 positively regulates the expression of MYD88 by facilitating its m^{6}A methylation modification. Subsequently, it induces the activation of NF-κB suppressing osteogenic differentiation [51] (**Figure 5A**). In another study, METTL3 acted as a positive regulator of osteoblast differentiation and mineralization in physiological and inflammatory conditions [72]. Moreover, METTL3 can promote skeletal muscle differentiation by promoting MyoD mRNA maintenance via METTL3-mediated m^{6}A modifications [46] (**Figure 5A**). In summary, METTL3 plays several roles in regulating cell differentiation. In leukemia and cutaneous squamous cell carcinoma, METTL3 inhibits cell differentiation. Conversely, it plays a positive role by promoting mammalian spermatogenesis. Remarkably, METTL3 either promotes or inhibits the process of BMSCs (pBMSCs) and osteogenesis depending on the conditions.

METTL3 regulates inflammatory response

Studies show that m^{6}A plays an important role in inflammation-responses. On one hand, several studies have shown that METTL3 promo-

tes inflammation. For instance, deficiency of METTL3 inhibits inflammation response mediated by LPS, exerting antimalabsorption of LCFA activity *in vitro*. Mechanistically, depletion of METTL3 decreases the m^{6}A levels of Traf6 mRNA entrapping its transcripts in the nucleus. Subsequently, of Traf6 expression reduces leading to suppression of NF-κB and MAPK signaling pathway. Consequently, the inflammation response is suppressed resulting in sustained absorption of LCFA [73] (**Figure 5B**).

Besides, METTL3 promotes experimental osteoarthritis development. Essentially, knocking down METTL3 inhibits IL-1β-induced inflammatory response and extracellular matrix (ECM) synthesis. One of the main causes of cartilage degeneration is imbalanced ECM synthesis and degradation of articular chondrocytes [69]. Feng Z et al. reported that the levels of m^{6}A methylation and METTL3 expression were upregulated in LPS-stimulated human dental pulp cells (HDPCs). Moreover, it was clear that the depletion of METTL3 reduced the accumulation of inflammatory cytokines and suppressed the activation of the NF-κB and MAPK signaling pathways [28] (**Figure 5B**). Meanwhile, in a mouse model of Complete Freund's Adjuvant (CFA)-induced chronic inflammatory pain there was a significant increase in the level of spinal m^{6}A modification accompanied by augmentation of METTL3 expression in the spinal cord. The mechanism of inflammatory pain is METTL3 modulates the pain sensitization by regulating m^{6}A modification which modulates the pri-miR-65-3p processing in a microprocessor protein DGCR8-dependent manner [74] (**Figure 5B**). miR-365-3p, which is highly expressed in spinal cord neurons, plays an important role in the regulation of inflammatory-associated pain [75]. Furthermore, METTL3 can inhibit inflammation responses. Accordingly, when METTL3 is depleted it enhances proinflammatory cytokine expression by inducing phosphorylation of ERK, p38, JNK, and p65 in the MAPK and NF-κB signaling pathways in LPS-treated osteoblasts [72]. In addition, METTL3 inhibits the inflammatory functions of pTHP-1 macrophages through NF-κB [76]. Overall, METTL3 either promotes or inhibits inflammation.

METTL3 regulates other biological functions

Besides regulating cell cycle progression, cell proliferation, cell apoptosis, cell migration and invasion, cell differentiation and inflammatory response, METTL3 has important functions in the following biological process: oocyte maturation early embryogenesis, long-term memory formation, metabolism, innate immunity etc. In mammalian oocyte maturation and pre-implantation embryonic development processes, knocking down METTL3 in female germ cells highly inhibits oocyte maturation by lowering mRNA translation efficiency. Consequently, this leads to defects in maternal-to-zygotic transition [77]. Regarding the role of MEETL3 in early embryogenesis, METTL3 is important in regulating the expression of hnRNPA2/B1 in an m^{6}A-dependent manner. hnRNPA2/B1 regulates transcription-related factors and determines the fate of cell transition [50]. In addition, Zhang et al. reported that abundant METTL3 correlates with learning efficacy and promotes hippocampus-dependent long-term memory. This is probably achieved by enhanced translation efficacy of activity-induced immediate early genes (IEGs) whose deficiency in mice lead to dysfunctions of synaptic plasticity and/or long-term meory [78]. In Type II diabetes mellitus (T2D), METTL3 promotes fatty acid metabolism by inhibiting hepatic insulin sensitivity via m^{6}A methylation of the Fasn mRNA. Studies have revealed that the level of m^{6}A methylation and METTL3 are consistently higher in the liver tissues of patients with T2D. Hepatocyte-specific knockout of METTL3 in mice fed with HFD improved glucose tolerance and insulin sensitivity, decreased fatty acid synthesis, and prevented obesity-induced metabolic complications [61]. Moreover, METTL3 can drive M1 macrophage polarization. Mechanically, METTL3-mediated methylation increases the stability and expression of STAT1 mRNA, which controls M1 macrophage polarization [62]. As the fast-growing number of articles about METTL3 rapidly increases, additional functions of METTL3 in biological processes and their corresponding molecular mechanisms will emerge.

Conclusion and future prospective

m^{6}A methylation on the RNAs is performed by a methyltransferase complex made up of METTL3, METTL14, WTAP, and other helper proteins. METTL3 is a key catalytic component of this complex and has an active methyltransferase domain that catalyzes the conversion of adenosine (A) to m^{6}A. In addition to the methyltransferase activity, METTL3 regulates transla-

Table 2. METTL3 is associated with cancer survival

Types of cancers	The expression of METTL3 in cancers	Survival rate of patients with cancer	References
Ovarian cancer	High	Low	[10]
Osteosarcoma	High	Low	[27]
Gastric cancer	High	Low	[30, 32-34]
Colorectal cancer	Low	High	[35]
Colorectal cancer	High	Low	[36]
Cutaneous squamous cell carcinoma	High	NA	[38]
Bladder cancer	High	NA	[41]
Renal cell carcinoma	Low	High	[42]
Breast cancer	High	NA	[43]
Leukemia	High	NA	[45]
Bladder cancer	High	Low	[49]
Prostate cancer	High	NA	[56]
Pancreatic cancer	High	Low	[57]
Acute Lymphoblastic leukemia	Low	NA	[79]

NA: Not available.

tion of target mRNAs independent of its catalytic subunit. However, the specific mechanisms remain unclear. For instance, it is not clear whether there are key activity sites that participate in the translation role of METTL3. In addition, the factors required for the translation role of METTL3 remain unknown.

Depending on its activities, METTL3 plays a crucial role in many biological processes especially tumorigenesis and development. Generally, METTL3 acts an oncogene in cancer. Therefore, it causes alterations of mRNA translation and acceleration of tumor progression, and downregulating METTL3 results in tumor inhibition. Accordingly, METTL3 mRNA expression is significantly elevated in cancers tissues compared to normal tissues. Thus, it is associated with poor prognosis hence a potential novel diagnostic and prognostic biomarker in cancer clinics. **Table 2** shows that METTL3 is upregulated in cancer tissues and its higher expression indicates poorer prognostic biomarkers in most cancers such as osteosarcoma [27], GC [30, 32-34], CRC [35], ovarian cancer [10], BCa [49] and pancreatic cancer [57]. However, METTL3 can also act as a tumor suppressor gene in colorectal and renal cell carcinoma. Here, METTL3 is highly expressed and this predicts better prognosis. This may be due to tumor heterogeneity and the complex physiological functions of METTL3. Since METTL3 plays an important role in regulating the progression of many cancers via regulation of translation and multiple signaling pathways, it can be used as a cancer therapeutic target.

Despite the recent discoveries into the role of METTL3 in cancers, the underlying mechanism of METTL3 in cancer remains unknown. It is therefore not known whether METTL3 levels can be potential biomarkers for the diagnosis and prognosis of some cancers. So far, the roles of METTL3 in the same type of cancer have been inconsistent across studies. For instance, it has been revealed that elevated METTL3 promotes the progression of NSCLC [3], while METTL3 also inhibits the tumorigenesis and cancer development of NSCLC [48]. In addition, alteration of m^6A levels in HCC development is discordant across different studies. METTL3 and METTL14 show complete opposing effects on the migration of HCC cells [13, 80]. These results suggest that some functions of METTL3 are independent of m^6A modification and the underlying mechanisms need to be explored.

METTL3 not only regulates the expression of target mRNAs but also regulates the maturation of target miRNAs such as miR-365-3p, pre-miR221/222 and pre-miRNA-1426. Comparing, miRNAs such as miR-600 [39], miRNA-33a [48], miR-4299 [30] and miR-1914-3p [11] can inhibit the expression of METTL3 and reverse its oncogenic effect in cancer progression. Thus, these miRNAs are considered as potent cancer inhibitors. Furthermore, the methyltransferase activity of METTL3 can be regulated by post-translational modifications such as SUMOylation. Since SUMOylation does not affect the stability of METTL3, subcellular localization and WTAP interaction the specific mechanism by which SUMOylation regulates methyltransferase activity is still unclear. Thus, there are other types of modifications involved

in the activity of METTL3 which need to be explored. Therefore, more comprehensive experiments are required to fully understand the functions of METTL3 in biological processes.

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Disclosure of conflict of interest

None.

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