Biological roles of the RNA m6A modification and its implications in cancer

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The N6-Methyladenosine (m6A) modification of RNA transcripts is the most prevalent and abundant internal modification in eukaryotic messenger RNAs (mRNAs) and plays diverse and important roles in normal biological processes. Extensive studies have indicated that dysregulated m6A modification and m6A-associated proteins play critical roles in tumorigenesis and cancer progression. However, m6A-mediated physiological consequences often lead to opposite outcomes in a biological context-dependent manner. Therefore, context-related complexity must be meaningfully considered to obtain a comprehensive understanding of RNA methylation. Recently, it has been reported that m6A-modified RNAs are closely related to the regulation of the DNA damage response and genomic integrity maintenance. Here, we present an overview of the current knowledge on the m6A modification and its function in human cancer, particularly in relation to the DNA damage response and genomic instability.

INTRODUCTION

The RNA m6A modification, that is, the methylation of adenosine at the nitrogen-6 position in an RNA molecule, is considered an epitranscriptomic and posttranscriptional regulatory mark. Among more than 150 posttranscriptional chemical modifications on RNA molecules1–5, m6A is the most abundant internal modification of mRNAs and noncoding RNAs. Since the discovery of m6A6, development of m6A transcriptome-wide mapping technology based on next-generation sequencing (NGS) methods has been used to extensively study this modification, and many related studies have found that this RNA chemical modification exhibits biological significance6–9. Indeed, the m6A modification is closely associated with almost all aspects of RNA-related biological processes, including transcription, pre-mRNA splicing and processing, pri-miRNA processing, nuclear export, translation, RNA stability and decay7–10. In addition to its role in RNA metabolic processes, the m6A modification is involved in other biological processes, such as transcriptional regulation, signal transduction, and the DNA damage response11–26. As the m6A modification and associated factors are significantly dysregulated in cancers, understanding their roles in tumorigenesis and cancer progression will provide in-depth insight into the development of new therapeutic strategies for cancer treatment. In this review, we describe the current understanding of m6A modification and its function in biological processes and cancers, particularly its contribution to the DNA damage response and genomic instability.

THE DYNAMICS OF THE M6A MODIFICATION AND ITS MOLECULAR FUNCTIONS IN RNA METABOLISM

The m6A modification is dynamically deposited and removed by m6A methyltransferase complexes (m6A writers) and demethylases (m6A erasers), respectively (Fig. 1). As the core subunit of the m6A methyltransferase complex, METTL3 and METTL14 form a heterodimer and recognize the consensus sequence motif [G > A][m6A][C/A/C/U], which is preferentially located near stop codons, 3’ untranslated regions (UTRs), and long internal exons16,17,18. The m6A methyltransferase activity of the METTL3-METTL14 heterodimeric complex is modulated by regulatory proteins, including WTAP, VIRMA, RBM15/15B, ZC3H13 and HAKAI19–21,27; these factors are required for nuclear localization as well as the recruitment of the m6A methyltransferase complex to target RNA substrates. The expression levels or activities of the components of the m6A methyltransferase complex can alter the overall level of m6A in cells, and this change significantly affects transcriptome-wide landscape and biological functions. Although extensive studies have identified components of the m6A methyltransferase complex, silencing each component of the current m6A methyltransferase complex via RNA interference (RNAi) or gene knockout (KO) partially reduced the level of m6A in cells but did not abrogate it. Therefore, future studies need to be focused on investigating uncharacterized components of the m6A methyltransferase complex or enzymes that regulate the abundance of the m6A modification in cells.

One of the putative human m6A writer proteins, METTL16, is critical for methylating a few transcripts, such as the U6 small nuclear RNA (snRNA), MALAT1, XIST, and the pre-mRNA MATZLA28–32. However, it has been recently reported that METTL16 preferentially localized to the cytosol and methylated more than 334 mRNA transcripts. In addition, METTL16 KO caused a significant reduction in the rate of m6A deposition on target nascent RNAs compared to poly(A) RNAs. Interestingly, in addition to the previously proposed role involved in RNA splicing, METTL16 interaction with eukaryotic initiation factor...
3a/b (eIF3a/b) and ribosomal RNAs (rRNAs) promotes ribosome assembly, resulting in enhanced translation of more than 4000 target mRNAs. However, this translational regulation by METTL16 is neither methyltransferase activity-dependent nor m6A dependent.

Two other putative m6A writer proteins are METTL5 and ZCCHC4, which are critical for the m6A modification of 18S rRNA and 28S rRNA, respectively. METTL5, not ZCCHC4, forms a heterodimeric complex with TRMT112, a methyltransferase activator. Their biological function on 18S and 28S rRNAs remains unknown because knocking out Mettl5 or Zcchc4 negligibly affected human colon cancer HCT116 cell growth or mature rRNA processing and production. However, a study showed that the proliferation of Zcchc4 KO HepG2 cells, derived from the liver tissue of a patient with hepatocellular carcinoma (HCC), was significantly inhibited, which was consistent with the defective translation of specific mRNAs in regulating tumorigenesis. This phenotypical discrepancy of Zcchc4 KO might be cell-type specific or context dependent. Nevertheless, Mettl5 and Zcchc4 clearly methylate 18S and 28S rRNAs. Thus, the biological significance of Mettl5- or Zcchc4-mediated rRNA methylation in tumorigenesis should be addressed in different contexts.

Increasing evidence indicates that the m6A modification is deposited cotranscriptionally on nascent transcripts. In acute myeloid leukemia (AML), CAATT-box binding protein (CEBPZ) directs METTL3 at the transcriptional start site (TSS) in a METTL14-independent manner. METTL3 recruitment by CEBPZ promotes the m6A modification of target mRNA transcripts and enhances their translation. In mouse embryonic stem cells (mESCs), m6A deposition by Mettl3 occurs cotranscriptionally at Mettl3-bound chromatin regions, as indicated by both the genomic binding of Mettl3 and m6A modification are mainly enriched in the 3′ UTR. Moreover, m6A modification depends upon the transcriptional dynamics of RNA polymerase II. Attenuated transcriptional activity of RNA polymerase II induced an increase in m6A modification abundance mediated through the physical interaction between RNA PolII and METTL3, resulting in inefficient translation. These studies suggest that the m6A modification is, at least, a cotranscriptional event and is one of the factors linking transcription to translation. However, H3 trimethylation at Lys36 (H3K36me3), is associated with transcript...
elongation, which recruits the m^6^A methyltransferase complex to chromatin through the physical interaction between H3K36me3 and METTL14 to deposit m^6^A cotranscriptionally on nascent transcripts\(^3^9\). Furthermore, several study groups have independently shown that nascent RNAs labeled with 4-thiouridine (4SU) in a short time (5–20 min) were deposited with m^6^A25, and chromatin-associated regulatory RNAs (carRNAs) were highly modified with m^6^A marks, revealing it to be among the major substrates for the methyltransferase of m^6^A modification40,41.

Overall, the data obtained to date suggest that m^6^A deposition occurs cotranscriptionally on nascent- and chromatin-associated RNA molecules via chromatin association with the m^6^A methyltransferase complex.

The identification of two m^6^A demethylases, namely, the m^6^A erasers α-ketoglutarate-dependent dioxygenase alk B homolog 5 (ALKBH5) and fat mass and obesity-associated protein (FTO), has suggested that m^6^A deposition is dynamically reversible23,24. ALKBH5 selectively removes the methyl group from N^6^-adenosine in target mRNAs, whereas FTO demethylates both internal m^6^A marks and N^6,2′-O-dimethyladenosine in the 5′ cap (m^6^Am)10, suggesting that ALKBH5 is a primary m^6^A demethylase (Fig. 2b).

Interestingly, notable phenotypes in mammalian development...
have indicated that these two mA demethylases play a broad regulatory role in developmental processes. Fto-KO mice display partial embryonic lethality, postnatal growth retardation, and increased postnatal lethality. Recently, it has been reported that RNA transcribed from long-interpersed element 1 (LINE1) is a physiological substrate of FTO that regulates the chromatin state in mammalian tissues and during development. Alkbh5 KO led to impaired fertility through a dysregulated splicing process in sperm development, although Alkbh5-KO mice were viable and reached adulthood. Indeed, ALKBH5 biological processes, adding a layer to posttranscriptional modification of mRNAs, which plays critical roles in fundamental biological processes, adding a layer to posttranscriptional regulatory mechanisms.

The mA modification determines the fate of RNAs via selective mA-recognition factors. The YTH domain-containing proteins YTHDC1-2 (YTHDC1 and YTHDC2) and YTHDF1-3 (YTHDF1, YTHDF2, and YTHDF3) are direct mA readers that modulate the fate of mA-modified RNAs in cells. First, YTHDC1 binds mA-modified target RNAs and regulates RNA splicing, nuclear export, exosome-mediated RNA decay and stability of carRNAs in the nucleus. YTHDF2 also regulates the stability of mA-modified RNAs and promotes translation efficiency in the cytoplasm. YTHDF1-3 bind mA-modified mRNAs and facilitate cytosolic RNA decay. In particular, YTHDF1 and YTHDF3 enhance the translation of mA-modified mRNAs with translation initiation complexes. In addition to YTH domain-containing proteins, insulin-like growth factor 2 mRNA-binding proteins, IGFBP1-3, recognize the mA mark on mRNAs and thus regulate their stability and translation. Finally, heterogeneous nuclear ribonucleoproteins (HNRNPs), namely, hnRNPA2B1, hnRNPG, and hnRNPC, are potential mA readers. However, since they bind to mA-modified RNAs and change the structure of target RNAs, they seem to play roles as “mA switches” not as direct mA readers. Nevertheless, hnRNPA2B1 mediates the processing of mA-modified primary mRNAs (pri-mRNAs) through the recruitment of DGCR8, a component of the microprocessor complex, and regulates the alternative splicing process. The mA modification determines the fate of RNAs via selective mA-recognition factors (mA readers). The YTH domain-containing proteins YTHDC1-2 (YTHDC1 and YTHDC2) and YTHDF1-3 (YTHDF1, YTHDF2, and YTHDF3) are direct mA readers that modulate the fate of mA-modified RNAs in cells. First, YTHDC1 binds mA-modified target RNAs and regulates RNA splicing, nuclear export, exosome-mediated RNA decay and stability of carRNAs in the nucleus. YTHDF2 also regulates the stability of mA-modified RNAs and promotes translation efficiency in the cytoplasm. YTHDF1-3 bind mA-modified mRNAs and facilitate cytosolic RNA decay. In particular, YTHDF1 and YTHDF3 enhance the translation of mA-modified mRNAs with translation initiation complexes. In addition to YTH domain-containing proteins, insulin-like growth factor 2 mRNA-binding proteins, IGFBP1-3, recognize the mA mark on mRNAs and thus regulate their stability and translation. Finally, heterogeneous nuclear ribonucleoproteins (HNRNPs), namely, hnRNPA2B1, hnRNPG, and hnRNPC, are potential mA readers. However, since they bind to mA-modified RNAs and change the structure of target RNAs, they seem to play roles as “mA switches” not as direct mA readers. Nevertheless, hnRNPA2B1 mediates the processing of mA-modified primary mRNAs (pri-mRNAs) through the recruitment of DGCR8, a component of the microprocessor complex, and regulates the alternative splicing process.

CELLULAR AND MOLECULAR FUNCTIONS OF THE mA MODIFICATION

mRNA instability by the mA modification

One of the best characterized functions of mA modification causes the destabilization of mA-modified mRNAs. Although all YTHDF1-3 proteins contribute to the destabilization of mA on target mRNAs, recent studies have implied that YTHDF2 is the major mA reader involved in the decay of mA-modified RNAs. Increasing evidence has revealed that YTHDF2 is required for directing mA-modified mRNAs to processing bodies (P-bodies), where mA decay-associated proteins accumulate. All three YTHDF1-3 proteins facilitate phase-separation with mA-modified mRNAs to induce the accumulation of transcripts at P-bodies, stress granules and neuronal RNA granules. Independent of P-bodies, YTHDF2 directly interacts with CNOT1 and recruits the CCR4/NOT deadenylase complex to mA-modified mRNAs, leading to the deadenylation and degradation of mRNAs. Furthermore, YTHDF2 associates with RNase P/MRP, an endoribonuclease, through direct interaction with heat-responsive protein 12 (HRSP12). The depletion of any of these three proteins abrogated mA-mediated degradation of mRNAs, suggesting that YTHDF2 recognition of mA-modified mRNAs mediates mRNA decay mediated via HRSP12 and RNase P/MRP. A transcriptome-wide analyses of HRSP12-binding sites and cleavage sites of RNase P/MRP further supported the finding that HRSP12 binds upstream of YTHDF2-binding sites and that RNase P/MRP endoribonucleolytically cleaves downstream YTHDF2-binding sites within target mA-modified mRNAs, demonstrating that the mA modification promotes the degradation of target mRNAs through the recruitment of RNase P/MRP mediated via HRSP12.

Alternative splicing by the mA modification

Several lines of evidence show the role played by the mA modification in mRNA splicing. Previous reports showed that the overall levels of mA on mRNAs were significantly enriched in the early onset of embryogenesis in Drosophila and were rapidly decreased during embryogenesis. Ime4, a Drosophila METTL3 homolog, regulates the female-specific splicing of the Sex- lethal (Sxl) gene. The mA demethylases FTO and ALKBH5 are also involved in splicing machinery, regulating alternative splicing of long 3′UTRs containing pre-mRNAs or a subset of adipogenesis-associated mRNAs, respectively. Transcriptome-wide mapping of RNAs bound by FTO showed significant overlap with previously reported mA locations within intronic regions of pre-mRNAs, and depletion of FTO led to the inclusion of alternatively spliced exons. ALKBH5 also regulates the proper splicing of longer 3′UTR transcripts, particularly in mitotic and meiotic male germ cells. Even though mA may affect the splicing process in only a subset of genes, these mA-mediated splicing events might be functionally important. Although the abundance of mA marks is related to modulated alternative splicing, the mA reader protein YTHDC1 interacts with splicing regulators, including SAM68, SC35, SRSF1 and SRSF3, suggesting that the mA reader plays a role in mRNA splicing. However, it remains unclear whether YTHDC1 activity is coordinated with these splicing regulators in a mA-dependent or mA-independent manner.

Nuclear export mediated via the mA modification

The mA modification influences the nuclear export of mA-modified mRNAs. ALKBH5 loss induced the nuclear accumulation of mA-modified mRNAs, suggesting that the mA mark mediated mRNA export. Another study demonstrated that YTHDC1 was involved in the nuclear export of mA-modified mRNAs via its interaction with splicing factor SRSF3 and nuclear RNA export factor 1 (NXF1). The nuclear export of mRNAs is cotranscriptionally coupled with the capping, splicing and 3′ end processing of primary transcripts. However, although the mA modification of pre-mRNAs has been associated with the splicing process, it has been recently reported that the role of mA modification in pre-mRNA splicing is limited to a small number of pre-mRNA groups, suggesting that the mA-modified pre-mRNA splicing process is not closely related to the nuclear export process. The nuclear export of mRNAs is also regulated by the nuclear export machinery, involving the nuclear export factor 1 (NXF1) and the CRM1 (CRANsomal exportin). The nuclear export of mRNAs is mediated by the CRM1 (CRANsomal exportin) and the CRM1 (CRANsomal exportin) regulates the nuclear export of mRNAs through the recruitment of CRM1 (CRANsomal exportin) to the CRM1 (CRANsomal exportin). The nuclear export of mRNAs is mediated by the CRM1 (CRANsomal exportin) and the CRM1 (CRANsomal exportin) regulates the nuclear export of mRNAs through the recruitment of CRM1 (CRANsomal exportin) to the CRM1 (CRANsomal exportin).
YTH domain-containing m6A readers, including YTHDF1, YTHDF3, and YTHDC2, have been reported to enhance the translation of m6A-modified mRNAs in a cap-independent translation. In particular, YTHDF1 promotes the translation of m6A-modified mRNAs through its interaction with the eukaryotic translation initiation factor eIF3 complex. YTHDF1 binds to m6A sites that are located around a stop codon and in the 3′ UTR and facilitates translation. However, it remains to be investigated how eIF3 regulates the translation of m6A-modified mRNAs, since eIF3 is recruited to the 5′ UTR or upstream of the translation start site for translation initiation. Another study reported that eIF3 binds directly to 5′UTR-m6A sites, leading to the recruitment of the ribosomal 43S preinitiation complex. Translation initiation by eIF3 binding to 5′UTR-m6A sites does not require the cap-binding factor eIF4E, suggesting cap-independent translation. In addition, recent studies demonstrate that eIF3H at the 5′UTR directly binds to METTL3 at the 3′ UTR-m6A sites, promoting mRNA circularization and increasing cap-dependent or cap-independent ribosome translation efficiency. It is still unclear how METTL3 binds to m6A-modified mRNA and contributes to translation efficiency. Future studies should address the molecular details of how METTL3 and readers recognize m6A marks and how the m6A modification contributes to each step of translation.

RNA M6A MODIFICATIONS IN CANCER

Although the current knowledge of the precise mechanism by which m6A modification regulates diverse biological processes remains to be further explored, an increasing number of studies examined the effects of m6A modification in various types of cancer. In this section, we summarize the recent findings of these studies with respect to the most common types of human cancer.

Acute myeloid leukemia (AML)

AML is the most common hematopoietic malignant leukemia in adults. Recurring chromosomal aberration and genetic mutations as well as aberrant alteration of epigenetic modifications, including DNA methylation and histone modification, contribute to hematopoietic malignancies such as AML. The m6A methyltransferase METTL3 is more abundant in AML cells than in CD34-positive stem and hematopoietic progenitor cells (HSPCs) and is required for the differentiation of AML cells. METTL3 binds to promoters associated with the differentiation of AML in a METTL14-independent manner, leading to direct transcriptional activation. METTL3 is recruited by CEBPZ at a transcriptional start site, and promoter-bound METTL3 adds the m6A mark to a cognate mRNAs, enhancing its ribosomal translation. A study revealed that METTL3 deposited the m6A mark to pro-oncogenes such as the MYC proto-oncogene (c-MYC), B-cell lymphoma 2 (BCL2), and phosphatase and tensin homolog (PTEN), activating phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB) signaling pathways, which regulate cell differentiation and self-renewal. METTL14, a key component of the m6A methyltransferase complex, is highly expressed in HSPCs and AML cells. Although negatively regulated by SP1, METTL14 is involved in the m6A modification of target mRNAs of MYB and MYC, promoting translation and inhibiting myeloid differentiation. Interestingly, FTO, a m6A eraser, also plays a critical oncogenic role in AML, promoting leukemic oncogene-mediated transformation and leukemogenesis by regulating target mRNAs, such as ASB2 and RARA mRNAs, through the removal of methyl group from N6-adenosine. Moreover, YTHDC1 and YTHDC2, m6A readers, play important roles in the survival and differentiation of AML cells. YTHDC1 undergoes liquid–liquid phase separation with m6A-modified mRNAs and forms nuclear YTHDC1-m6A condensates (nYACs). Abundant nYACs in AML cells protect m6A-modified mRNAs (i.e., MYC and others) from the polyA tail exosome targeting complex (PAXT) and exosome-associated RNA degradation. YTHDF2 destabilizes m6A-modified mRNAs (i.e., tumor necrosis factor receptor Tnfrsf2 and others) that are associated with the function of self-renewing leukemic stem cells (LSCs), contributing to the initiation of AML.

Hepatocellular carcinoma (HCC)

Liver cancer is a highly progressive and the second most life-threatening tumor. It comprises hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) in accordance with histological features. Recent reports have shown that METTL3 is upregulated in human HCC, leading to m6A hypermethylation of the tumor suppressor SOCS2 (Suppressor Of Cytokine Signaling 2). The m6A reader protein YTHDF2-dependent RNA degradation pathway mediates the degradation of SOCS2 mRNAs, suggesting that METTL3 represses the expression and stability of critical tumor suppressor genes at the posttranscriptional level. In addition, METTL3 regulates the expression of USP7 (Ubiquitin Specific Peptidase 7) through an increase in oncogenic activities of HCC cells. However, YTHDF2 functioned as a tumor suppressor in HCC via destabilization of epidermal growth factor receptor (EGFR) mRNA, leading to the inhibition of the ERK/MEK signaling pathway in HCC. These results suggest that m6A modification has a dual role in HCC by promoting HCC tumor progression or inhibiting oncogenic pathways. Thus, the role of m6A modification in HCC remains to be further explored.

Glioblastoma (GBM)

Glioblastoma (GBM) is the most aggressive and common primary brain and central nervous system (CNS) malignancy in adults. GBMs are characterized by heterogeneity: that is, they contain glioblastoma stem-like cell (GSC) populations with stem-like properties, contributing to tumor initiation and therapeutic resistance. The METTL3 level is highly elevated in GSCs and is required for the maintenance of GSCs and the dedifferentiation of glioma cells through an m6A modification in the 3′ UTR of sex-determining region Y (SRY)-Box 2 (SOX2) mRNA. The m6A modification of SOX2 mRNAs and recruitment of human antigen R (HUr), which is also highly expressed in GBMs, are essential for SOX2 mRNA stabilization, which leads to the maintenance of GSCs. Most GBM cases are refractory to radiotherapy and the chemotherapy drug temozolomide (TMZ) via rapid DNA repair by O-6-methylguanine-DNA methyltransferase (MGMT). Both METTL3 and SOX2 regulate DNA repair genes and partially mediate m6A-dependent radioresistance. Therefore, further studies are required to examine the potential of SOX2 as a predictor for the outcome of and benefit from TMZ chemotherapy, in addition to MGMT. Interestingly, ALKBH5 is highly expressed in GSCs and demethylates FOXM1 nascent transcripts with a long noncoding RNA antisense strand (FOXM1-AS), leading to enhanced FOXM1 expression, GSC proliferation, and tumorigenesis. A study showed that m6A modification functions as a tumor suppressor for GSC self-renewal and tumorigenesis. The abundance of m6A marks after METTL3 or METTL14 knockdown promoted the tumorigenesis of GSCs. In contrast, the inhibition of FTO by the ethyl ester form of meclofenamic acid (MA2) suppressed the progression of GSC-grafted tumors. Taken together, these results suggest that m6A-mediated tumor formation can lead to opposite results, even in the same context. Thus, future studies should address the context- or experimental condition-based comprehensive interpretation to understand the precise role of m6A modification in GBMs.

Breast cancer

Breast cancer is the most frequently diagnosed cancer in women worldwide. Breast cancer is heterogeneous and classified by the expression of hormone receptors (estrogen receptor and...
progesterone receptor) and human epidermal growth factor receptor 2 (HER2)\textsuperscript{68}. The molecular subtypes of breast cancer are luminal A, luminal B, HER2-positive, and basal-like triple-negative breast cancer (TNBC), which are considered to show similar clinical behaviors prior to the treatment of breast cancer. In breast cancer, increasing evidence has shown that the m^6^A modification is critical for tumorigenesis and progression. Silencing of METTL14 and ALKBH5 significantly inhibited breast cancer cell growth and invasive activity\textsuperscript{99}. METTL14 and ALKBH5 regulate m^6^A-modified mRNAs involved in the cell cycle, the epithelial-mesenchymal transition (EMT), and angiogenesis through the HuR-mediated stabilization of target mRNAs. In particular, a study demonstrated the specific role of m^6^A modification in regulating the TGFβ signaling pathway in the tumorigenesis of breast cancer. In contrast, another study showed that METTL14 overexpression increased the abundance of m^6^A marks and increased oncogenic activity\textsuperscript{100}. In TNBC, a lower level of METTL3 and a higher level of FTO were associated with poor prognosis, indicating that a lower m^6^A mark level contributes to the progression of TNBC\textsuperscript{101}. In addition, under hypoxic stress conditions, elevated ALKBH5 reduces the abundance of the m^6^A mark on pluripotency marker NANOG and KLF4 mRNAs, leading to increased NANOG and KLF4 expression and an enhanced breast cancer stem cell phenotype\textsuperscript{62}.

**Lung cancer**

Lung cancer is one of the most common cancers in the world, and small-cell lung cancers (SCLCs) and non-small cell lung cancer (NSCLC) are the two major histologic subtypes\textsuperscript{103,104}. Despite our understanding of the biology of this disease and mechanisms of lung tumor progression, the overall cure and survival rates for lung cancer patients remain very low, particularly for patients with metastatic disease. Increasing evidence indicates that METTL3 is highly expressed in NSCLC cells and related to oncogenic activity in lung cancer\textsuperscript{77,78,105–107}. Although METTL3 is localized mainly in the nucleus, one study demonstrated that cytosolic METTL3 functioned as a m^6^A reader that bound to a 3′-UTR near a stop codon. m^6^A-modified mRNA-bound METTL3 directly interacted with eIF3h in eIF3 complex, facilitating mRNA looping to induce the recycling of polyribosomes\textsuperscript{77,78}. Transcriptome-wide analyses demonstrated that METTL3 regulated a large subset of oncogenic mRNAs through the METTL3-eIF3h axis without affecting mRNA abundance, facilitating oncogenic activity\textsuperscript{77,78}. In addition, several groups supported findings showing that downregulation of METTL3 expression levels through miR-600, miR-33a or RNAi clearly inhibited the oncogenic activities of lung cancer cells\textsuperscript{105–107}. miR-600 and miR-33a bound to the 3′-UTR of METTL3 mRNAs, leading to the degradation of METTL3 mRNAs and subsequently inducing apoptosis in lung cancer cells. The level of METTL3 was also controlled posttranslationally. Indeed, METTL3 is SUMOylated by small ubiquitin-related modifier 1 (SUMO1), leading to a reduction in m^6^A methyltransferase activity. Thus, SUMOylation of METTL3 decreases the global cellular abundance of the m^6^A mark and subsequently alters the transcriptome of m^6^A-modified RNAs in cells, facilitating the development of NSCLC.

**THE M^6^A MODIFICATION AND GENOMIC INSTABILITY**

Several studies have found that METTL3-mediated m^6^A modification plays a critical role in the DNA damage response (DDR) to regulate the DNA repair pathway. RNA m^6^A modification rapidly occurs in ultraviolet (UV)-irradiated chromatin, indicating that METTL3 is specifically recruited to the UV-damaged chromatin region\textsuperscript{108} (Fig. 3a). This extensive recruitment of METTL3 depends on ADP-ribose polymerase 1 (PARP1). This DNA repair pathway may be mediated by trans-lesion DNA polymerase κ (Pol κ), which has been implicated in both nucleotide excision repair (NER) and trans-lesion synthesis (TLS)\textsuperscript{108–110}. The m^6^A-mediated recruitment of Pol κ differentially regulates the UV-induced DNA damage response mediated by the canonical NER pathway and the Rad18/PCNA-regulated TLS pathway. However, the precise role of m^6^A modification and Pol κ in the UV damage response remains to be further investigated. Another study supported these findings by showing that nucleoplasmic fractions of m^6^A RNAs were immediately concentrated in UV-irradiated DNA lesions without affecting the level of METTL3, METTL14 or FTO\textsuperscript{111} (Fig. 3a). The authors showed that UV radiation reduced the levels of 2,2,7-methylguanosine (m^5^G/TMG) and N^\prime^-methyladenosine (m^6^A) in RNA as results of DNA damage. These results suggest that METTL3 rapidly localizes to UV-irradiated genomic regions and methylates RNAs and that these concentrated m^6^A-modified RNAs regulate the downstream DNA damage repair pathway to promote cell survival.

METTL3-mediated m^6^A modification also mediates homologous recombination (HR)-mediated double-strand DNA (dsDNA) break repair\textsuperscript{112} (Fig. 3b). METTL3 is phosphorylated at Ser43 by ataxia telangiectasia mutated (ATM) in response to double-strand breaks (DSBs). Phosphorylated METTL3 can be localized at DSB regions, leading to the m^6^A modification of nascent RNAs derived from damaged chromatin regions. These m^6^A-modified RNAs are recognized by YTHDC1, resulting in the formation of DNA–RNA hybrids at DSBs. Subsequently, the formation of DNA–RNA hybrids induces the recruitment of repair-related proteins, including RAD51 and BRCA1, to promote or HR-mediated repair, preventing genomic instability\textsuperscript{113,114}. Depletion of METTL3 significantly enhanced the sensitivity of cancer cells and murine xenograft models to DNA damage-based therapies, such as chemotherapy drugs or radiation. Furthermore, a higher level of METTL3 predicted a poor survival probability for head and neck squamous carcinoma (HNSC) patients who had been treated with cisplatin or radiation. These results suggest that m^6^A modification in DSB repair is a potential target for cancer therapy. However, since METTL3 can increase the efficiency of DSB repair, it may also contribute to drug resistance in DNA damage-based treatment\textsuperscript{89,112}.

RNA m^6^A modification also occurs in the majority of DNA–RNA hybrids (R-loops) in human pluripotent stem cells\textsuperscript{115} (Fig. 3b). The m^6^A modification of RNA in R-loops is increased during the G2/M phase and disappears in the G0/G1 phase of the cell cycle, indicating cell cycle-dependent regulation of this modification. YTHDF2 binds to m^6^A-modified RNAs in R-loops, leading to the degradation of RNAs and the reduction in the number of R-loops. Inhibition of METTL3 or YTHDF2 results in the accumulation of R-loops and YHAX, a DSB marker, and subsequently induces cell growth retardation. Thus, the regulation of METTL3- and YTHDF2-mediated RNA–DNA hybrids may represent a critical process in preventing genomic instability caused by the accumulation of cotranscriptional R-loops during mitosis. Furthermore, m^6^A can also resolve R-loops induced by DNA damage via UV or camptothecin (CPT) through toxicity-responsive enhancer-binding protein (TonEBP)\textsuperscript{116}. TonEBP directly binds to R-loops and recruits METTL3, leading to m^6^A modification on an RNA strand of the R-loop. TonEBP also recruits RNase H1 to resolve R-loops. However, in different studies and cellular contexts, the m^6^A modification promoted R-loop formation to facilitate transcription termination\textsuperscript{117}, suggesting context-dependent regulation. Nevertheless, the studies demonstrate that the m^6^A modification plays a critical role in regulating R-loops, the DNA damage response, and genomic stability.

It has been reported that FTO is important for the maintenance of bone mass and functions because it protects osteoblasts from genotoxic damage\textsuperscript{45}. Previously, large-scale genome-wide association studies (GWAS) showed that FTO was closely linked to obesity and body composition in multiple human populations\textsuperscript{118–120}. FTO specifically removes a methyl group from the
N6-adenoside on the mRNAs of DNA repair genes (e.g. Hspa1, Cdk9, Kdm2a, and Ube2v1), leading to increased mRNA stability. Subsequently, upregulation of Hspa1a and DNA repair genes protects osteoblasts from genotoxic agent (UV and H2O2)-mediated apoptosis. In addition, FTO protects osteoblasts from genotoxic damage induced by metabolic stress caused by the loss of Fto, which exacerbated osteoblast DNA damage in mice fed a high-fat diet. ALKBH5 also plays a role in the regulation of the DNA damage response and repair by N6-methyladenosine (m6A). DNA damage response and repair. a Upon genotoxic stress, ATM phosphorylates METTL3, which binds to a DNA lesion, and m6A-modified RNAs direct PARP1 and Pol K for nucleotide excision repair (NER). b The m6A-modified RNAs are recognized by YTHDC1 or YTHDF2, which recruits RAD51 to the damaged region for homologous recombination repair (HRR). c Reactive oxygen species (ROS) activate the ERK-JNK pathway, which phosphorylates ALKBH5. UBC9 binds to phosphorylated ALKBH5, inducing its SUMOylation. Inhibition of the demethylase activity of ALKBH5 by SUMOylation increases the level of m6A-modified RNAs that are related to DNA damage repair. Transcriptional regulation of DNA damage response and repair. d METTL3 stabilizes the p53 protein in a m6A-independent manner. METTL3 deposits an m6A mark on p53 target mRNAs for regulating the DNA damage response and tumor suppression. This image was created with BioRender (https://biorender.com/).
damage response and apoptosis mediated via reactive oxygen species (ROS) (Fig. 3c). ROS activate the ERK/JNK signaling pathway, which phosphorylates ALKBH5 at Ser87 and Ser321. Phosphorylated ALKBH5 interacts with UBC9, a SUMO E2 conjugating enzyme, leading to SUMOylation of ALKBH5 at Lys86 and Lys321. The enzymatic activity of SUMOylated ALKBH5 was thus inhibited, and then, the abundance of m6A on the mRNA of DNA repair genes increased, protecting cells from ROS-induced DNA damage response.

The m6A mark engages in crosstalk with the transcription factor, p53, and regulates the p53-mediated transcriptomic program induced by DNA damage stimuli (Fig. 3d). METTL3 has been identified as a p53-interacting partner after treatment with the DS8 inducer doxorubicin. METTL3 stabilizes the p53 protein in a m6A-independent manner. However, METTL3 deposited the m5A mark on p53-targeted mRNAs to regulate the DNA damage response and tumor suppression only in the presence of an intact p53 protein. Therefore, further investigation should address whether RNA is dispensable for the direct interaction between METTL3 and p53 since p53 can bind to other RNA species.

The m6A RNA modification is involved in the regulation of telomere length and genomic integrity in human cancers (Fig. 4). Telomeres are specialized structures at the ends of mammalian linear chromosomes and consist of tandem TTAGGG DNA nucleotide repeats. Shelterin, a protein complex that binds to single- or double-strand telomeres, protects telomeres from being inappropriately recognized as damaged DNA (Fig. 4b). In human cancer cells, telomerase, which consists of the catalytic subunit TERC and RNA template TERC, adds TTGGG repeats during every cell division, preventing gradual telomere shortening due to the end replication problem of semiconservative DNA replication.

In normal somatic cells, TERT is transcriptionally repressed, and therefore, telomere length is gradually shortened with defective telomerase activity and progressive cell divisions, leading to a shortened-telomere-driven crisis point. A shortened telomere crisis is closely linked to the genomic alterations found in cancer-relevant genomes. Homeobox-Containing 1 (HMBOX1, also known as HOT1 or TAH1) is a mammalian telomere-binding protein that is involved in the recruitment of active telomerase and is required for telomere maintenance in the alternative lengthening of telomeres (ALT) in cancer cells. Notably, HMBOX1 mRNA has been identified as a de novo target for m6A modification in cancer cells. The authors of this study found that m6A marks in the 3′ UTR of HMBOX1 mRNA facilitated its degradation through YTHDF2 (Fig. 4a). In line with the function of HMBOX1 in the recruitment of telomerase to telomeres, downregulation of HMBOX1 mediated by the overexpression of METTL3 failed to maintain telomere length, as indicated by the defective recruitment of telomerase to telomeres (Fig. 4b). A previous report showed that HMBOX1 functions as a transcriptional repressor. HMBOX1 suppresses the expression of MDM2 and is essential for the competency of p53 signaling (Fig. 4c). METTL3 upregulation in human cancer cells leads to shortened telomere-driven telomere dysfunction and inactivation of the p53-dependent DNA damage response pathway through MDM2 derepression (Fig. 4b, c). In the cancer-relevant genome, this coordinating environment might contribute to various types of telomere-associated chromosomal aberrations (e.g., translocations, amplifications, and deletions), enhancing the tumorigenicity and aggressiveness of cancer cells (Fig. 4d). Taken together, these results suggest an unexpected regulatory role for m6A marks in telomere biology and genome integrity.

**CONCLUDING REMARKS AND FUTURE DIRECTIONS**

The review discusses our current understanding of the multifaceted roles of the m6A modification in regulating RNA metabolic processes and related biological processes, including the DNA damage response and genomic instability. The role of m6A modification and m6A-associated proteins is cell- or disease-context dependent. Because of the significant role played by m6A in a variety of biological and physiological processes, context-dependent coordinated action among m6A-associated proteins determines the outcomes of m6A modification. The molecular details of the crosstalk between m6A modification and m6A-associated proteins and how this crosstalk affects diverse biological processes still need to be investigated. In particular, understanding how m6A modification and its associated proteins modulate DNA damage responses to maintain genomic integrity may lead to a new therapeutic strategy in cancer.
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**AUTHOR CONTRIBUTIONS**

J.H., K.X., and J.L. designed and wrote the manuscript. J.L. revised and supervised manuscript preparation.

**COMPETING INTERESTS**

The authors declare no competing interests.

**ADDITIONAL INFORMATION**

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