**REVIEW**

*N*°-Methyladenosine modification: a novel pharmacological target for anti-cancer drug development

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**Abstract**

*N*°-Methyladenosine (m\(^6\)A) modification is the most pervasive modification of human mRNA molecules. It is reversible via regulation of m\(^6\)A modification methyltransferase, demethylase and proteins that preferentially recognize m\(^6\)A modification as “writers”, “erasers” and “readers”, respectively. Altered expression levels of the m\(^6\)A modification key regulators substantially affect their function, leading to significant phenotype changes in the cell and organism. Recent studies have proved that the m\(^6\)A modification plays significant roles in regulation of metabolism, stem cell self-renewal, and metastasis in a variety of human cancers. In this review, we describe the potential roles of m\(^6\)A modification in human cancers and summarize their underlying molecular mechanisms. Moreover, we will highlight potential therapeutic approaches by targeting the key m\(^6\)A modification regulators for cancer drug development.

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1. Introduction

As the delivery vehicle of genome-encoded information to the functional protein, message RNA (mRNA) plays an important role in life processes in eukaryotes. A bulk of evidence suggested that abnormal transcription or expression of mRNA was closely related to various human diseases\(^1\)–\(^10\). A high volume of drugs has been designed to target mRNA maturational, transportation, location and expression in the past decades\(^11\). In recent years, with development of gene examination technology, more than 100 types of chemical modifications of mRNA have been explored successively\(^12\)–\(^20\), such as m\(^1\)A\(^9\)–\(^10\), m\(^5\)C\(^1\)–\(^2\), m\(^6\)A\(^1\)–\(^2\), 5hmC\(^1\)–\(^2\), pseudouridine, \(\Psi\)\(^5\)–\(^10\), 2'-O-methylation\(^1\)–\(^2\), etc. These modifications join in regulating RNA splicing, translation and stability, thus effecting gene expression in diverse physiological processes\(^12\)–\(^14\). Studies on mRNA modification have identified the pathology, development and prognosis of various diseases, and provided relative therapeutics and numerous new targets for drug development. N\(^6\)methyladenosine (m\(^6\)A) is the most pervasive internal modification of mRNA in the human cell, which is the methylation of the adenosine base at the nitrogen-6 position of mRNA (Fig. 1). m\(^6\)A modification is widely distributed (on average, approximately 3–5 m\(^6\)A modification sites per mRNA molecule\(^22\)–\(^25\)) with high abundance (>25%) in the transcripts in human cells\(^12\)–\(^13\). m\(^6\)A modification was first reported in poly (A) RNA fractions in 1970s\(^26\)–\(^27\). Due to the lack of specific technique for detecting the m\(^6\)A modification sites in mRNAs, the research on m\(^6\)A modification has been stagnant for decades until the gene FTO (the first m\(^6\)A modification demethylase) was found to revive the field of RNA methylation. This discovery suggested that RNA modification, analogous to the well-studied reversible DNA and histone modifications, might also impact biological regulation\(^28\). N\(^6\)-Methyladenosine often locates in the 5' untranslated regions (5'-UTRs), near the stop codons, in the 3'-UTRs and within internal long exons\(^22\)–\(^29\),\(^30\). Unlike other mRNA modification, the m\(^6\)A modification is dynamic and versatile, which can be regulated by its formation as well as its removal. The m\(^6\)A modification modulators include the m\(^6\)A modification methyltransferase, demethylase and proteins that preferentially recognized m\(^6\)A modification as “writers”, “erasers” and “readers”, respectively.

1.1. m\(^6\)A modification writers: a complex of METTL3, METTL14, and WTAP

The m\(^6\)A modification “writers” is a methyltransferase complex comprising of methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14) and Wilms' tumor 1-associating protein (WTAP). METTL3 is the primary component of this complex. Knockout of METTL3 resulted in almost complete loss of the m\(^6\)A modification activity in polyadenylated RNAs\(^22\)–\(^25\),\(^31\). METTL14, another important component in the m\(^6\)A modification methyltransferase complex, forms a stable heterodimer complex with METTL3 in a stoichiometric ratio of 1:1\(^32\). METTL4 is a methyltransferase, but an adaptor required for enhancing the METTL3 activity by binding substrate RNAs and positioning the methyl group towards adenosine\(^33\)–\(^35\). As a binding partner of the methyltransferase, WTAP is essential for RNA methylation. WTAP facilitates the METTL3-METTL14 complex to locate into nuclear speckles\(^36\). Genetic depletion of WTAP causes the m\(^6\)A modification of mRNA occurs in methyl group at nitrogen atoms of adenosine.

Fat mass and obesity-associated protein (FTO) and alkylated DNA repair protein AlkB homolog 5 (ALKB5), referred as m\(^6\)A modification “erasers”, are both the members of the AlkB family\(^37\)–\(^39\). FTO was the first m\(^6\)A modification demethylase found in cells and it is able to demethylate m\(^6\)A modification in mRNA both in vitro and in vivo\(^11\). The demethylation of m\(^6\)A modification in nucleic acids by FTO relies on oxidative function of FTO in a Fe(II)- and \(\alpha\)-KG-dependent manner. ALKB5, another m\(^6\)A modification demethylase found in the nucleus, regulates export and metabolism of mRNA by m\(^6\)A modification demethylation\(^40\). Deletion of ALKB5 affects 9% of total m\(^6\)A modification sites, indicating that ALKB5 may target specific m\(^6\)A modification sites in mRNAs\(^40\).

1.2. m\(^6\)A modification erasers: mRNA demethylation by FTO and ALKB5

Functions of m\(^6\)A-containing RNAs were achieved by recruiting m\(^6\)A-binding proteins (termed as m\(^6\)A modification readers) that can preferentially recognize m\(^6\)A modification sites in methylated RNA and facilitate downstream processes. The “m\(^6\)A readers” and the “m\(^6\)A switch readers” include YTH domain family (YTS21-B homology), HNRNP (Heterogeneous nuclear ribonucleoproteins) and insulin-like growth factor-2 mRNA-binding proteins 1, 2 and 3 (IGF2BP1–3). Mammalian genomes contain five YTH domain-containing proteins: YTHDF1–3 and YTHDC1–2. YTHDF1–3, the m\(^6\)A modification readers in cytoplasm, prefer to bind methylated RNA with concentration ranging from 180 to 520 nmol/L\(^46\). Previous study suggested that YTHDF1 promoted the translation of m\(^6\)A-containing transcripts and YTHDF2 mediated mRNA decay\(^41\). YTHDC1, another member of the YTH domain family, was identified as the major readers of nuclear m\(^6\)A modification\(^42\). It was reported that YTHDC1 took part in the m\(^6\)A-regulated splicing\(^3\). Originally, HNRNPA2B1 was shown to bind RG m\(^6\)A C-containing sites on nuclear RNAs in vivo and in vitro, which regulated the alternative splicing of exons in a set of transcripts, and facilitated the processing of pri-miRNAs\(^43\). Subsequent structure analysis showed that HNRNPA2B1 functioned as the “m\(^6\)A switch readers” instead of the “m\(^6\)A readers”\(^45\). IGF2BP1–3 were the latest m\(^6\)A readers as recently determined. Their K homology (KH) domains recognize m\(^6\)A-containing RNAs selectively and promote their translation and stability\(^46\) (Fig. 2).
2. m6A modification are potential targets for cancer therapy

The foundation of drug development was to identify and prove the association between specific pharmacological target and disease.

Drugs were designed to recover the body through regulating the diseases-relevant molecular targets. m6A modification has been found to play significant roles in cancers and has provided a series of new pharmacological targets, such as m6A modification “writers”, “erasers” or “readers” for drug development. Their mechanisms referring as the molecular target of cancers will be summarized as follows.

2.1. The function of m6A modification methyltransferase in human cancers

METTL3 was the major RNA N6-adenosine methyltransferase, which was reported to be closely associated with the genesis and development of cancers. Chen et al. showed that METTL3 was significantly upregulated in human hepatocellular carcinoma (HCC) and multiple solid tumors. Clinically, overexpression of METTL3 was associated with poor prognosis of HCC patients. Knockdown or knockout of METTL3 would drastically reduce HCC cell proliferation, migration, colony formation in vitro and suppress HCC progression and lung metastasis in vivo in a mechanism of augmenting tumor suppressor gene SOCS2 expression post-transcriptionally. In acute myeloid leukemia (AML), METTL3 mRNA and protein were highly expressed. METTL3 played as an essential gene for growth of AML cells. Downregulation of METTL3 resulted in cell cycle arrest, differentiation of leukemic cells and failure to establish leukemia in immune-deficient mice. In this regard, METTL3 could be recruited by the CAATT-box binding protein CEBPZ to the transcriptional start sites and initiate transcription of some target genes. METTL3 bound to the promoter and induced m6A modification within the coding region of the associated mRNA transcript, enhancing its translation by relieving ribosome stalling. Likewise, METTL3 expression was elevated in lung adenocarcinoma. Loss- and gain-of function studies showed that METTL3 promotes growth, survival, and invasion of human lung cancer cells. Mechanistically, METTL3 could enhance translation of certain mRNAs including epidermal growth factor receptor (EGFR) and the Hippo pathway effector TAZ though interacting with the translation initiation machinery, which was independent of methyltransferase activity.
and downstream m6A modification readers proteins. Mammalian hepatitis B X-interacting protein (HBXIP) was originally discovered for its binding to the C terminus of the hepatitis B virus X protein, which was documented as an oncoprotein with high expression in breast cancer. Cai et al. showed that expression of METTL3 was positively related to that of HBXIP in clinical breast cancer tissues. HBXIP could up-regulate METTL3 by inhibiting miRNA let-7g which down-regulated the expression of METTL3 by targeting its 3' UTR. As a feedback, METTL3 promoted the expression of HBXIP through m6A modification, forming a positive feedback loop of HBXIP/let-7g/METTL3/HBXIP and leading to acceleration of cell proliferation in breast cancer.

Similarly, Weng et al. showed that METTL14 was highly expressed in acute myeloid leukemia (AML) cells. Silencing of METTL14 promoted terminal myeloid differentiation of normal HSPCs and AML cells, and thus inhibiting AML cell survival and proliferation. Mechanistically, METTL14 exerted its oncogenic role by regulating its mRNA targets (MYB and MYC) through m6A modification, while the protein itself was negatively regulated by SPI1. While some studies have shown that METTL3 and METTL14 may play oncogenic roles in cancers, which were essential for growth of tumors. Controversially, Cui et al. found that m6A modification functioned as a tumor suppressor for glioblastoma stem cell (GSC) self-renewal and tumorigenesis. Knockdown of METTL3 or METTL14 would dramatically upregulate expression of some oncogenes, such as ADAM19, EPHA3 and KLF4, which promote human GSC growth, self-renewal and tumorigenesis. Similarly, Li et al. found that METTL3 might have a suppressive role in cell proliferation, migration, invasion and cell cycle of renal cell carcinoma (RCC). They found METTL3 mRNA and protein expression were downregulated in RCC samples and RCC cell lines. Up-regulation of METTL3 could obviously inhibit RCC cell proliferation, migration and invasion, and induce G0/G1 arrest thus significantly suppressed tumor growth in vivo.

2.2. Oncogenic role of m6A modification demethylase in human cancers

As the first identified RNA demethylase that regulates demethylation of target mRNAs, FTO was reported to play critical roles in...
cancer development and progression. Previously, Li et al. showed that FTO expression was increased in acute myeloid leukemia (AML). Overexpression of FTO could promote cell proliferation and viability in two AML cell lines, MONOMAC-6 and MV4–11, while knockdown of FTO expression led to the opposite effects. Mechanically, FTO exerted its oncogenic role via targeting and suppressing expression of a set of critical transcripts, such as ASB2 and RARA. Specifically, FTO decreased stability of ASB2 and RARA mRNA transcripts upon FTO-mediated demethylation of the m6A modification level in their mRNA transcripts.

FTO was proved to be a direct target of R-2-hydroxyglutarate (R-2HG). R-2HG was reported to exhibit growth-suppressive activity and glycolysis-inhibitory function in gliomas. A recent study demonstrated that R-2HG showed growth-suppressive activity in leukemia and significantly inhibited progression of sensitive AMLs in vivo. While FTO could inhibit accumulation of m6A modification on MYC transcripts, leading to the enhancement of MYC mRNA stability and upregulation of MYC signaling and contributing to tumor progression in many cancers. R-2HG exerted its anti-tumor effect largely through inhibiting the enzymatic activity of FTO. Additionally, Cui et al. showed that FTO inhibitor MA2, the ethyl ester form of meclofenamic acid (MA), could increase mRNA m6A modification levels in glioblastoma stem cell (GSCs) and suppress GSC growth. Moreover, treatment of GSCs with the FTO inhibitor MA2 suppressed GSC-initiated tumorigenesis and prolonged the lifespan of GSC-engrafted mice. In cervical squamous cell carcinoma (CSCC), FTO was found to be elevated in CSCC tissues and promote chemo-radiotherapy resistance of CSCC in vitro and in vivo by decreasing m6A modification and promoting stability of β-catenin (an EMT maker) mRNA. Collectively, these studies suggested a key role of FTO in suppressing stability of the critical factors in cancers by reducing m6A modification process, and further affecting development and prognosis of cancers. It indicates that FTO might be a potential molecular target for cancer therapy and drug development.

Similarly, another m6A modification demethylase ALKBH5 played a critical role in tumor growth. A study conducted on glioblastoma stem-like cells (GSCs) showed that ALKBH5 was highly expressed in GSCs. Silencing ALKBH5 could suppress proliferation and tumorigenesis of patient-derived GSCs. Mechanistically, ALKBH5 demethylated the nascent transcripts of transcription factor FOXM1 that was identified as the central molecular mediator of GSC proliferation and enhanced the expression of mature RNA or protein. Knockdown of ALKBH5 would cause 40% reduction of FOXM1 precursor mRNA expression. Further, a long non-coding RNA antisense to FOXM1 (FOXM1-AS) was detected to promote the interaction between ALKBH5 and FOXM1 nascent transcripts. The FOXM1 axis in GSC could be disrupted by depletion of FOXM1-AS. ALKBH5 could exert its oncogenic function under hypoxia as well, and promote EMT in a vary of aggressive cancers, thus causing resistance to cancer therapy. Zhang et al. reported that exposure of breast cancer cells to hypoxia would stimulate a significant increase of hypoxia inducible factor (HIF)-1α- and HIF-2α-dependent ALKBH5 expression. Moreover, the hypoxia induced ALKBH5 expression in HIF-dependent manner could enhance NANOG mRNA stability by catalyzing m6A modification demethylation. Previous studies demonstrated that pluripotency factor NANOG played a critical role in the maintenance and specification of cancer stem cells, which is required for primary tumor formation and metastasis. Therefore, ALKBH5 was proved to enhance BCSC enrichment in the hypoxic tumor microenvironment, and it was verified in immune deficient mice. Despite few studies work on the relationship between ALKBH5 and tumors, m6A modification demethylase ALKBH5 was certainly proved to involve in mechanism of tumor initiation and progression, holding the potential therapeutic role for anti-tumor drugs.
2.3. YTHDF2 links RNA metabolism to cancer progression

The role of m^6^A modification binding proteins in human cancers was poorly documented. YTHDF2 was found to promote tumorigenesis and cell proliferation in hepatocellular carcinoma (HCC)^8^4. It showed that YTHDF2 was closely associated with malignance of HCC. Mechanistically, miR-145 was found to directly target YTHDF2 mRNA in 3' UTR and down-regulate YTHDF2 expression level in HepG2 cells, consequently increasing the m^6^A modification levels of mRNAs and decreasing proliferation of HepG2 cells^8^4.

In all, the impact of m^6^A modification in human cancers was mainly through three ways. Firstly, m^6^A modification regulates stabilities of various oncogene mRNAs. In this scenario, methylation would promote the mature mRNA decay, and inhibit cancer procession, which was evidenced in many studies. Therefore, the m^6^A modification erasers and readers play oncogenic roles in cancers and could be therapeutic targets accordingly. Secondly, the m^6^A modification readers METTL3 was found to bind to the transcriptional start site of some genes that are essential for cancer cell surviving. Thus, inducing m^6^A modification by upregulating METTL3 could increase mRNA transcripts to promote cancer growth. Lastly, it was notable that m^6^A modification could influence cancers by regulating immune system, providing clues to the link between m^6^A modification and cancer immunotherapy. Targeting such m^6^A modification could facilitate patient's own immune system to fight against the progressive cancers^8^5.

Collectively, these findings suggested that the m^6^A modification writers, erasers and readers could play significant roles in regulation of RNA metabolism, stem cell self-renewal, and metastasis in various cancers, and it indicates that m^6^A modification could be targeted for prevention and treatment of human cancers. Therefore, the key

| Inhibitor | Structure | Target | IC_{50} (μmol/L) |
|----------|-----------|--------|-----------------|
| Compound 1 | ![structure](image1) | FTO | 3.3±1.1 |
| Compound 2 | ![structure](image2) | FTO | 2.8±0.9 |
| Compound 3 | ![structure](image3) | FTO | 4.9 |
| Compound 4 | ![structure](image4) | FTO | 8.7 |
| Compound 5 | ![structure](image5) | ALKB | 0.5 |
| Compound 6 | ![structure](image6) | ALKB | 5.3 |
| Compound 7 | ![structure](image7) | ALKB | 5.4 |
| IOX3 | ![structure](image8) | FTO | 2.8 |
regulators of m6A modification could be theoretically served as the pharmacological targets for anti-cancer drug development.

3. Targeting m6A modification regulators in human cancers

As we know, the m6A modification is involved in cancer initiation, progression and prognosis. The key regulator genes of m6A modification become crucial to regulating the downstream targets. Targeting m6A modification regulators by small molecules has been proposed as a potential treatment for human cancers. Here, we focus on those small molecules, and discuss their potential applications in cancer treatment.

3.1. Inhibitors of 2-oxoglutarate (2OG) and iron-dependent oxygenases via suppressing m6A modification demethylation

2-Oxoglutarate and iron-dependent oxygenases (2OGX) are widely distributed in human beings, and their function relies on Fe(II) as a co-factor, 2OG and molecular oxygen as co-substrates to catalyze a broad range of biochemical reactions. ALKBH5 and FTO belong to 2OGX-dependent nucleic acid oxygenase (NAOX) family that catalyzed demethylation of N6-methyladenine in RNA. Existing inhibitors of 2OGX could be served as unspecific inhibitors of m6A modification demethylations, including 2OG competitor (such as N-oxalylglycine and its cell-penetrating derivative dimethyl oxalylglycine, succinate, fumarate, 2-hydroxyglutarate, etc.), metal chelators (hydroxamic acids, flavonoids), divalent transition metal ions and endogenous inhibitors that regulate the activity of 2OGX (succinate dehydrogenase, fumarate hydratase and isocitrate dehydrogenase). We have summarized those inhibitors that were examined in ALKB family (Table 1). Later on, based on FTO and ALKBH5 domains, more and more 2OGX inhibitors have been developed to inhibit the demethylations in m6A modification. There are 3 main different 2OGX inhibitor types, and they are 2OG competitor, substrate competitor or substrate and 2OG competitor (Fig. 3). In this scenario, Aik et al. screened a set of 2OG analogues and related compounds using differential scanning fluorometry- and liquid chromatography-based assays. Sets of both cyclic and acyclic 2OG analogues had been identified as FTO inhibitors, including the well-characterized 2OG oxygenase inhibitors N-oxalylglycine and pyridine-2,4-dicarboxylate, as well as hydroxyquinoline-, pyridyl-, and isoquinoline-based compounds. Crystal structure analysis further showed that two compounds (compound 1 and 2) were able to closely bind with active site of FTO and show comparatively good inhibitory effects, with the IC50 at 3.37 ± 1.1 and 2.87 ± 0.9 μmol/L respectively. In a subsequent study, Zheng et al. had designed a new class of compounds to mimic ascorbic acid and inhibit 2-oxoglutarate-dependent hydroxylases. The compound 3 and 4 have been shown to inhibit the 2-oxoglutarate dependent hydroxylase FTO with IC50 of 4.9 and 8.7 μmol/L, respectively. To assess their cellular effect of FTO inhibition, the level of m6A modification in a cell-based model was examined and quantified. For example, treatment with compound 4 with a concentration of 25 μmol/L could result in a 9.3% increase in m6A modification in cells. Additionally, this compound 4 showed anticonvulsant activity in vivo and modulated various microRNAs. By adopting dynamic combinatorial mass spectrometry, Woon et al. had identified the N-oxalyl-l-cysteine derivatives compound 5, 6 and 7 as potent inhibitors of AlkB. The researchers used a capillary electrophoresis-based assay to measure IC50 values of the compounds against AlkB, and they showed the IC50s of compounds 5, 6 and 7 were 0.5, 5.2 and 5.4 μmol/L.

| Table 3 | Crystal structure based m6A modification demethylase inhibitors. |
|---------|---------------------------------------------------------------|
| Inhibitor | Structure | Target | IC50 (μmol/L) |
| Rhein | ![structure](image1) | FTO | 21 |
| MA | ![structure](image2) | FTO | 7 |
| MA2 | ![structure](image3) | FTO | - |
| Compound 8 | ![structure](image4) | FTO | 0.81 |
| Compound 9 | ![structure](image5) | FTO | 2.11 |
respectively. Further, 2 physiologically important human 2OG oxygenases PHD2 and PHF8 had been tested to be inhibited by these 3 compounds and found that their IC50 were all 4.1 mmol/L for both PHD2 and PHF8, representing significant selectivity towards AlkB. IOX3 was a known inhibitor of hypoxia inducible factor prolyl-hydroxylases (PHDs), and was proved to bind at the active site of both FTO and PHDs. IOX3 was able to occupy both the 2OG and the nucleotide binding sites. McMurray et al. proved that the IC50 value of IOX3 for FTO was 2.8 μmol/L and it could decrease the protein expression of FTO, PHDs and other 2OG oxygenases in C2C12 mouse muscle myoblast cells in vitro. This in vivo experiment suggested that IOX3 might fail to alter FTO protein level of mice at the dose of 60 mg/kg, but it could significantly reduce bone mineral density and content, and alter adipose tissue distribution, which indicating IOX3 might function via affecting the enzyme activity of FTO. Nonetheless, these inhibitors (Table 2) were not selective and they could suppress all the Fe (II)- and 2OG-dependent oxygenases. Therefore, endogenous 2OG or substrate might compete with them and weaken their inhibitory effects. More highly selective and potent inhibitors of m6A modification demethylase were required (Table 3).

### 3.2. Crystal structures based inhibitors for m6A modification demethylase

Crystal structures of FTO and ALKBH5 proteins have been studied, which provide a basis for understanding FTO and ALKBH5 substrate-specificity, and facilitate the rational design of FTO and ALKBH5 inhibitors. Following this strategy, structure-based FTO and ALKBH5 specific inhibitors had been developed extensively. For instance, Xu et al. have presented the crystal structures of the ALKBH5 catalytic domain. A citrate molecule was observed in the active site of ALKBH5 instead of 2OG and Mn(II) (Fig. 4A and B). Most of the residues involved in the citrate binding are involved in binding 2OG and Mn(II), that participated in regulation of the enzyme activity. Studies have showed that the IC50 of citrate for ALKBH5 was at 488 μmol/L, which is comparable to that for human FTO (300 μmol/L). Rhein was the first potent FTO m6A modification demethylase inhibitor, which was neither a structural mimic of 2-oxoglutarate nor a chelator of metal ion. It was shown that Rhein reversibly bound to FTO catalytic domain and competitively prevented the recognition of m6A modification substrates. The details of interaction between FTO and Rhein were shown in Fig. 4C. The IC50 value of Rhein against FTO was 21 μmol/L, and its off-target selectivity analyses has proved that Rhein did not show inhibitory activity against other 2OG-dependent hydroxylases such as prolyl-4-hydroxylase, HDAC3 histone deacetylase and APOBEC3 DNA deaminases, which belong to transition metal-dependent histone and nucleic acid modifying enzymes. Additionally, Rhein was found with low cytotoxicity and was capable of increasing the modification level of m6A modification in mRNA in cells. In another study, meclofenamic acid (MA) was identified as a potent inhibitor of FTO, and belonged to a non-steroidal anti-inflammatory drug. In this study, MA was shown to selectively and efficiently inhibit FTO demethylation in a dose dependent manner by competition on m6A-containing substrate binding in HeLa cells. It was notable that a β-hairpin motif, a part of the FTO nucleotide recognition lid (NRL), provides hydrophobic interactions between FTO and MA. However, ALKBH5 lacks such region of the part of NRL and causes leakage when binding to MA, making MA a selective inhibition of FTO over ALKBH5 (Fig. 4D). MA2 was the ester form of MA, which completely lost its inhibitory activity in vitro. The ester modification could...
facilitate penetration of the inhibitors in cells and further be hydrolyzed to yield active MA. Treatment with MA2 had led to the elevated levels of m^6A modification in mRNA in HeLa cells. Previous study has shown that the substrate specificity of the AlkB enzymes could partly arise from structural differences within their nucleotide-binding sites. Glu234^_FTO_ was likely a key residue that determined the affinity and specificity of FTO for its substrates. In this thread, Toh et al. had identified compound 8 (Fig. 4E) as a potent and subfamily-selective inhibitor of FTO that could selectively interacted with Glu234^_FTO_. Further cell-based assays were shown that compound 9 was able to inhibit m^6A modification demethylase activity in cells (Fig. 4F).

Collectively, emerging studies have worked on m^6A modification in various diseases. Targeting m^6A modification regulators have become a hot spot in drug design and development. As FTO was the first and most robust obesity-risk gene discovered in genome-wide association studies, its inhibitors are currently the focus among other m^6A modification regulators. Though many kinds of inhibitors targeting m^6A modification demethylases were successfully identified, their pharmaceutical effects in vivo were rarely verified yet. Therefore, discovery of potent and selective inhibitors for m^6A modification modulators is as important as design of pharmacological experiments for those identified inhibitors in clinical research.

4. Conclusions and perspectives

A large body of researches has confirmed that N^6-methyladenosine modification was involved broadly in multiple types of human cancers. The m^6A modification “writers” “erasers” and “readers” are certainly set at the important position of many biological pathways involved in cell metabolism, growth, proliferation and stem cell self-renewal. The m^6A modification methyltransferases METTL3 and METTL14 are known to play diverse roles in specific tumors by affecting pre- or post-transcription of onco-genes, while the m^6A modification demethylases and m^6A modification recognition proteins sustain tumorigenicity of various cancers. Theoretically, these m^6A modification regulators can be recognized as the bona fide targets in diagnosis and drug discovery of human cancers.

For the oncocgenic roles of FTO and ALKBH5 being identified, small molecule inhibitors are served as the candidates for anti-cancer drug development. Up to now, FTO and ALKBH5 inhibitors are divided into two kinds of categories including broadly 2-oxoglutarate (2OG) and ferrous iron depressors, such as N-oxalylglycine and pyridine-2,4-dicarboxylate, and structure-based selective inhibitors like Rhein, MA and IOX3. All these inhibitors had been proved to inhibit tumor growth through depressing the m^6A modification levels in cancer cells. Although these inhibitors have not yet been verified in vivo and in clinical trials, it provides clues to development of m^6A-specific regulators and paves the way for the treatment of human cancers, providing novel pharmacological targets for anti-cancer drug development.

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