The Emerging Roles of N6-Methyladenosine Modification in Hepatocellular Carcinoma

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Abstract: In terms of cancer-related deaths, hepatocellular carcinoma (HCC) ranks high on a global scale. The molecular processes that cause HCC are still a mystery, even though many treatments have been developed for the disease in recent years. In mammals, N6-methyladenosine (m6A) methylation is one of the most significant RNA modifications. It is thought to regulate RNA metabolism and gene expression. Numerous regulatory variables govern this procedure. Two examples are methylase and demethylase. ncRNA, including circular RNAs circRNAs, lncRNAs, and miRNAs, are important players in the development and spread of tumours. They also regulate mRNA production, epigenetic alterations, and other biological processes. The diverse roles of m6A regulators in HCC include ferroptosis and metabolic reprogramming. In addition, this review examines inhibitors that target m6A enzymes as potential therapeutic targets for HCC, along with the present research status of m6A gene editing methods.

1 INTRODUCTION

In the several types of liver malignancies, hepatocellular carcinoma (HCC) accounts for 75–85% of all cases and has a high mortality rate. After lung cancer and stomach cancer, it is the third leading cause of cancer-related death globally. At this time, the most effective method for treating early-stage HCC is surgery. Unfortunately, many HCC patients already have advanced disease when they are diagnosed. The first-line chemotherapeutic treatment for advanced HCC is sorafenib, a kinase inhibitor. Still, cancer metastasis and drug resistance affect a small percentage of people. A better understanding of the molecular pathways that cause HCC is crucial for effective diagnosis, therapy, and prevention of the disease. In epigenetics, the DNA sequence is not changed but gene expression is regulated through heritable mechanisms. These processes incorporate chromatin remodeling, non-coding RNA (ncRNA), DNA methylation, and histone modifications. Adenosine undergoes methylation at the sixth position, resulting in N6-methyladenosine (m6A), an epigenetic modification. This epigenetic alteration is found in the vast majority of eukaryotic RNAs. Enzyme complexes known as m6A methyltransferases (writers) and m6A demethylases (erasers) control the reversible and dynamic m6A modification. Readers are proteins that recognise and carry out m6A's biological functions. Not only that, but these regulators have an effect on the expression and function of circRNA, lncRNA, and microRNA. Recent studies have shown that metabolic reprogramming, cancer progression, and m6A and ncRNA interaction dysfunction can all play a role in cancer. Therefore, understanding the etiology, prognosis, and therapy of HCC requires better clarification of the link between m6A alteration and ncRNA. This review will summarize recent findings on the role of m6A modification in the initiation and development of HCC and elucidate the impact of the interaction between ncRNA and m6A in HCC. Lastly, this review will discuss the therapeutic possibilities of m6A modifiers as biomarkers and targets for HCC therapy.

2 THE REGULATORS RELATED TO M6A

An epigenetic alteration known as m6A attaches a methyl group to the N6 position of adenine and is

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quite common. Typically, it is found in the 3'-untranslated regions (UTRs) of mRNA, close to the stop codon regions, as well as in 5'-UTRs. Additionally, m6A is significantly abundant in ncRNAs, including lncRNAs, miRNAs, and circular RNAs (Xu et al. 2024). The regulation of m6A is mediated by a reversible methylation system that involves three types of proteins: the writer, the eraser, and the reader.

2.1 Writers

Writers are primarily responsible for the methylation of m6A with a protein complex. Its main kernel components, are METTL3 and METTL14. METTL3 is regarded as the main enzyme exerting methyltransferase activity in the protein complexes. METTL14 can bind with METTL3 to form a METTL3-METTL14 complex, which can stabilize the structure of METTL3. METTL14 improves the complex's substrate selectivity via binding to RNA. Although WTAP does not have any enzyme activity, it does serve as a junction protein that helps METTL3-METTL14 target RNA. Furthermore, the amount of components of the writers have been found: METTL16, a protein that is related to virilizer-like m6A methyltransferase (KIAA1429; alternatively called VIRMA). In order to identify the 3' hairpin of U6 snRNA and MAT2A pre-mRNA, which encode SAM synthase, METTL16 can act as a catalyst. Specifically, VIRMA directs preferential mRNA methylation at the 3'UTR and close to stop codons by enlisting catalytic core components (Ma et al. 2024).

2.2 Erasers

m6A is a vital reversible change regulated by both methylase writers and demethylase erasers. Fat mass and obesity-associated proteins (FTO) can facilitate the demethylation of the m6A site on RNA, resulting in alterations to the structure and function of RNA. Research indicates that FTO significantly influences the proliferation and spread of HCC cells as well as lipid synthesis. AlkB homolog 5(ALKBH5) is predominantly situated in the nucleus and substantially influences mRNA nuclear export and metabolism (Xu et al. 2024, Ma et al. 2024).

2.3 Readers

After RNA has been written by writers and cleared by erasers, methylation modifications of the m6A on it are recognized by readers and mediate RNA fate. A significant number of readers have been found in mammals, such as the YT521-B homology (YTH) domain-containing protein family. Within the YT521-B homology (YTH family, YTHDF1 promotes the translation of m6A-modified mRNAs, YTHDF2 facilitates mRNA degradation, and YTHDF3 collaborates with YTHDF1 and YTHDF2, respectively (Ma et al. 2024).

3 THE NCRNA-MEDIATED REGULATION OF M6A MODIFICATION IN HEPATOCELLULAR CARCINOMA

ncRNAs, which mostly consist of miRNAs, lncRNAs, and circRNAs, are a subset of RNA molecules that are capable of transcription but do not include protein-coding sequences. ncRNAs regulate gene expression and participate in several biological processes, especially in tumor malignancies. Evidence suggests that m6A alterations and ncRNAs interact in ways that contribute to the pathophysiology of numerous illnesses and malignancies. The abnormal expression of ncRNAs can contribute to hepatocellular carcinoma progression by modulating the expression levels of m6A regulators, exhibiting both cancer-promoting and cancer-inhibiting effects. However, the process is intricate, and the precise mechanism remains unclear. Therefore, additional research into the role of ncRNAs and m6A alteration in tumor growth is essential.

3.1 m6A and miRNAs in Hepatocellular Carcinoma

RNA polymerase II produces the primary miRNAs which are small, non-coding RNAs that consist of 20–24 nucleotides. Next, precursor miRNAs (pre-miRNAs) are created when these primary miRNAs are cleaved in the nucleus. Once in the cytoplasm, the pre-miRNA continues its maturation into a fully functional miRNA. In order to degrade or repress the translation of mRNA, this mature miRNA is bound to the RNA-induced silencing complex. Research shows that microRNAs (miRNAs) play a role in the progression of liver cancer by modulating m6A writers. Specifically, miR-362-3p/miR-425-5p is a

miRNA that reduces the expression of the m6A writing enzyme ZC3H13, which has been identified as an oncogene in hepatocellular carcinoma (Cui et al. 2020). The Wnt/β-catenin signaling pathway is inhibited and an aggressive tumor phenotype is caused by miR-186, another microRNA, which reduces METTL3 expression. Furthermore, microRNAs regulate m6A readers to impact HCC progression. One example is the effect of overexpressed miR-145 on hepatocellular carcinoma cells, which causes a rise in m6A levels and a downregulation of YTHDF2 expression. This is because miR-145 targets the 3'-UTR of YTHDF2 mRNA. Hepatocellular carcinoma cells show a marked decrease in miR-145 expression and an increase in m6A levels when 3'-UTR of YTHDF2 mRNA is targeted. The ability of microRNAs to influence m6A writers and readers means that they may influence the advancement of cancer (Cui et al. 2020, Qiu et al. 2024).

3.2 m6A and lncRNA in Hepatocellular Carcinoma

LncRNAs interact with DNA, RNA, and proteins on multiple levels to regulate gene expression. Their length exceeds 200 nucleotides. They can disrupt transcription factor binding, serve as scaffolds for transcriptional regulators, or sequester miRNAs. Moreover, lncRNAs can directly bind to proteins, influencing their activity, stability, and cellular localization, thereby impacting signaling pathways. For instance, the upregulation of LINC01273 in HCC enhances tumor growth and resistance to sorafenib by modulating the miR-600/METTL3 axis to regulate m6A levels. Conversely, the downregulation of the tumor suppressor lncRNA AC1156199 in HCC impedes oncogene expression, cell proliferation, and invasion by disrupting the assembly of the m6A methyltransferase complex through WTAP targeting. Additionally, lncRNA FTO-IT1 stabilizes FTO mRNA, promoting HCC cell proliferation and glycolysis by reducing m6A modification of glycolysis-related genes through recruitment of the ILF2/ILF3 protein complex (Qiu et al. 2024).

3.3 m6A and circRNAs in Hepatocellular Carcinoma

CircRNA is a circular, single-stranded RNA molecule that ranges in length from 100 nucleotides to over 4 kilobases. Its circular shape enhances its stability, making it highly resistant to degradation by nucleic acid exonucleases. CircRNA functions as a molecular sponge, effectively sequestering miRNAs, and also interacts with a variety of RNA-binding proteins, potentially altering their typical functions. Lin et al discovered that RERE overexpression boosts cell viability and invasiveness while decreasing apoptosis. RERE facilitates the progression of hepatocellular carcinoma by increasing the expression of ZC3H133, which subsequently mediates the m6A modification of GBX2. 2) . Liu et al noted a marked reduction in circRNA-GPR137B expression in hepatocellular carcinoma tissues. GPR137B can suppress cancer cell proliferation by adsorbing miR-4739 to enhance FTO (Lin et al. 2022, Qiu et al. 2024).

4 THE ROLE AND SIGNIFICANCE OF M6A METHYLATION IN HEPATOCELLULAR CARCINOMA

In order to meet the needs of survival, HCCs have evolved a variety of mechanisms such as metabolic reprogramming and anti-ferroptosis. Metabolic reprogramming enables HCC to better adapt to the tumor's special microenvironment. Anti-ferroptosis can reduce cell death caused by abnormal iron ions and lipid-reactive oxygen species. Innovative approaches to treating HCC can be derived from a more thorough comprehension of these pathways.

4.1 m6A Modification in Metabolic Reprogramming

One of the significant features of tumor cells is their metabolic reprogramming, which fulfills their biosynthetic, bioenergetic, and redox requirements. The metabolism of glucose has been most intensively studied. To promote tumor growth, cancer cells frequently utilize glycolysis to break down glucose and maintain their metabolic energy, referred to as the Warburg effect (Warburg, 1925). By shifting glucose synthesis from oxidative phosphorylation to aerobic glycolysis, the Warburg effect promotes cancer growth via the MTORC1 pathway. Overexpression of METTL3 enhances glycolysis in HCC cells, which speeds up cancer progression, according to the current study. Two mechanisms have been identified so far: (1) METTL3 can cause metabolic

reprogramming by way of overexpression of pyruvate dehydrogenase kinase 4 (PDK4). METTL3 can accelerate the translation of PDK4 as well as increase the sexual stabilization of mRNAs by recruiting the YTHDF1/eEF-2 complex and IGF2BP3 by adding an m6A modification to the 5'UTR of PDK4. PDK4 has a critical role in glycolysis. Overexpression of PDK4 shifts carbon flow from oxidative phosphorylation to glycolysis. (2) The METTL3 m6A subunit methylates HIF-1α to improve glycolysis, while the Hepatitis B Virus X Protein Interacting Protein (HBXIP) acts as a positive regulator of METTL3. Additionally, it is crucial for the progression of lipid metabolism HCC. Scientists have found that METTL5 is essential for the progression of HCC in an in vitro model. Reduced translation of genes implicated in fatty acid metabolism and defective 80S ribosome assembly is observed in the absence of METTL5-m6A-methylated 18S rRNA (Peng et al. 2022).

4.2 m6A Modification in Ferroptosis

The buildup of intracellular iron-dependent lipid peroxides—linked to iron ions, lipid reactive oxygen species (L-ROS), and glutathione peroxidase 4 (GPX4)—defines ferroptosis, a non-apoptotic kind of cell death. In order to keep up with the demands of fast growth, tumor cells typically have a higher metabolic activity and iron need. For example, increased synthesis of PUFA-PL (unsaturated ether phospholipids) in cancer cells leads to destabilization of the iron pool, thereby increasing susceptibility to Ferroptosis. In addition, disturbances in lipid metabolism in tumor cells can also trigger Ferroptosis. However, hepatocellular carcinoma cells usually show some resistance to Ferroptosis. Some recent studies have shown that the m6A mechanism affects the susceptibility of hepatocellular carcinoma cells to Ferroptosis. The researchers found that hepatocellular carcinoma tissues exhibiting elevated expression of METTL16 and SENP3 correlated with worse prognostic outcomes. The m6A mutation of METTL16 resulted in elevated production of SENP3, which, by stabilising LTF proteins by de-SUMOylation, augmented their ability to sequester free iron. The METTL16-SENP3-LTF axis is implicated in modulating ferroptosis and hepatocellular carcinoma progression, as indicated by these studies (Wang et al. 2024). Shuwei Chen and colleagues discovered that WTAP can m6A-modify circCMTM3 to facilitate iron mortality in hepatocellular carcinoma (HCC). CircCMTM3 reduces ferroptosis by engaging IGF2BP1 to enhance the stability of PARK7, a crucial antioxidant protein, in hepatocellular carcinoma (HCC). A study by Z. Fan et al. revealed that METTL14 specifically targets m6A methylation of the SLC7A11 mRNA 5'UTR, and the methylation-altered SLC7A11 mRNA is subsequently recognised by YTHDF2 (readers) for destruction. The depletion of SLC7A11 ultimately enhances ROS production and triggers Ferroptosis (Chen et al. 2023).

5 THE CLINICAL APPLICATIONS OF M6A MODIFICATION IN HEPATOCELLULAR CARCINOMA

Considering the various pathways outlined in several research related to hepatocellular carcinoma, the modification of m6A RNA is anticipated to serve as a significant target for prognosis and treatment of the disease. There are many aberrant m6A regulators that have been identified as oncogenic factors, and targeting these factors for the treatment of HCC has been given high hopes. Small molecule inhibitors, m6A gene editing systems, and other approaches can all be utilized as targeting strategies.

5.1 m6A Small Molecule Inhibitors

m6A small molecule inhibitors are compounds that inhibit the catalytic function or interfere with protein-RNA interactions by specifically binding to the active sites of m6A-related regulatory enzymes (writers, erasers, and readers). These inhibitors regulate the expression of downstream oncogenes or tumor suppressor genes by reducing or enhancing the level of m6A modification. Several small molecule inhibitors have been developed to target m6A enzymes (METTL3, ALKBH5, and IGF2BP1) by disrupting their catalytic function or structure. Quercetin, an inhibitor of METTL3, blocks the adenosine pocket of SAM, leading to enzyme inhibition and suppression of HCC cell proliferation (Du et al. 2022). Cucurbitacin B (CuB), an inhibitor of IGF2BP1, induces apoptosis in HCC cells through a metastable inhibitory effect, enhances immune cell infiltration, and reduces PD-L1 expression (Ma et al. 2024).

5.2 m6A Gene Editing Systems

In addition, m6A gene editing systems have received widespread attention. The traditional CRISPR system achieves gene editing by targeting DNA, while the m6A editing system uses catalytically inactive Cas proteins (such as dCas9 or dCas13) as positioning modules, and m6A regulatory factors such as writers and erasers as action modules, which are guided to the target RNA sequence through sgRNA to methylate the RNA. The CRISPR/Cas9 m6A editing system allows for site-specific m6A modification through the modification of the m6A "writer" complex. A dCas9 mutant with the RNA-targeted catalytic domains of METTL3 and METT14 (M3-M14) can have a fusion protein with the M3 and M14 domains linked to its N-terminus. By doing so, particular RNA sequences can be targeted by the dCas9-M3-M14 complex. Using this technique, Liu et al were able to cause RNA degradation by installing an m6A alteration at the 3′UTR of ACTB mRNA and a m6A modification at the 5′UTR of Hsp70, both of which enhance protein translation (Liu et al. 2019). A different study created dCas13b-YTHDF1 and dCas13b-YTHDF2 proteins by joining the N-terminal part of YTHDF1 or YTHDF2 with inactivated dCas13b. Regardless of the target RNA's m6A modification state, these proteins can attach to particular RNA targets through complementary sequences on the gRNA (Rauch et al. 2018, Chen & Wong 2020).

6 CONCLUSION

m6A is among the most prevalent RNA epigenetic alterations. Its impact on HCC has been examined in recent years. This review examines the controls and roles of m6A alteration in hepatocellular carcinoma (HCC). Writers, erasers, and readers are the primary determinants of m6A alterations; the aberrant expression of m6A enzymes precipitates hepatocellular carcinoma (HCC). Nonetheless, the functions of certain m6A regulators, such as FTO, remain contentious. Researchers believe that the inconsistency may stem from cancer heterogeneity and cellular context. ncRNA also can effects through the interaction of the m6A. ncRNA through interaction with the erasers and writers, which affects m6A modification and the readers in regulating signaling pathways and metabolic processes downstream of ncRNAs. Also, m6A modification can affect ncRNA expression. This would complicate the effect of m6A on HCC. The fact that Azza and other medications that target DNA methylases or histone-modifying enzymes have received clinical approval for the treatment of cancer is well-known. Despite various attempts, treatments targeting m6A alteration in HCC have not been evaluated. In addition, gene editing systems targeting m6A are a potential therapy that can directly target RNA to modify it. Overall, therapies targeting m6A have potential clinical applications, and combining them with immunotherapy is essential to improve clinical outcomes in HCC. However, the current understanding of m6A modifications is still in its infancy, and more valuable evidence on the effects of m6A modulation patterns on ncRNA biosynthesis and function deserves further investigation in future studies.

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