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Function and Molecular Mechanism of m5C participating Malignant Tumors

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KEYWORDS

5-Methylcytosine (m5C); Epigenetic Modification; RNA; Tumorigenesis; Post Transcriptional; Immune Microenvironment

ABSTRACT

5-methylcytosine (m5C) plays a crucial role in gene expression regulation and RNA metabolism, and it is an important form of RNA epigenetic modification. This article systematically summarizes the molecular mechanisms of m5C regulation, including its dynamic regulatory network composed of methyltransferases, demethylases, and recognition proteins, and explores the function and mechanism of m5C in RNA stability, translation, and nucleocytoplasmic transport. m5C participates in the development of tumors by regulating oncogene expression, immune microenvironment remodeling, and metabolic reprogramming. The correlation between m5C modification levels and tumor patient prognosis is analyzed, and the potential value of m5C as a tumor diagnostic biomarker and therapeutic target is explored. High throughput sequencing and computational biology have driven m5C investigation, but the development and innovation of detection technologies, mechanisms for regulating tissue heterogeneity, and synergistic effects with other modifications such as m6A remain current challenges. In future, it needs to further be analyzed the precise regulatory network of m5C, providing theoretical basis for developing targeted intervention strategies to promote its application in precision medicine.

INTRODUCTION

Epigenetic modification plays a key role in the regulation of gene expression. RNA modification, as an important mechanism of post transcriptional regulation, has received extensive attention in recent years. RNA modifications mainly include various types such as methylation (such as m6A, m5C), pseudouridylation (PSI), and acetylation. These modifications dynamically regulate the stability, localization, splicing, and translation efficiency of RNA, thereby affecting cell differentiation, development, and disease progression[1]. 5-methylcytosine (m5C) is a conserved RNA modification widely distributed in mRNA, tRNA, rRNA, and non coding RNA. Its biological functions involve multiple aspects of RNA metabolism, including nucleocytoplasmic transport, translation regulation, and stress response. The discovery process of m5C can be traced back to the 1970s. Early research mainly focused on DNA methylation, and it was not until the development of high-throughput sequencing technology that its widespread presence and functional diversity in RNA were revealed [3,4]. In recent years, the importance of m5C in RNA epigenetics has become increasingly prominent. Some researches has shown that m5C modification is regulated by methyltransferases (such as NSUN family and DNMT2), demethylases (TET family), and recognition proteins (such as ALYREF, YBX1), forming a dynamically reversible modification network [5,6]. This network plays a crucial role in tumorigenesis, immune regulation, and neural development, for example, NSUN2 mediated m5C modification can promote tumor metastasis by stabilizing oncogene mRNA[7]. However, current research still faces many challenges. Firstly, the detection techniques for m5C, such as bisulfite sequencing, are limited by the difficulty in identify-

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ing RNA degradation and low abundance modification sites [8]; Secondly, the synergistic mechanism between m5C and other modifications (such as m6A) is not yet clear, and its precise function in tissue-specific regulation still needs to be further elucidated [9]. In addition, the clinical translational applications of m5C modification, such as targeted drug development and prognostic model optimization, are still in the exploratory stage [10]. These unsolved problems are urgent issues for future research and highlight the broad research value of m5C in both basic and clinical sciences.

MOLECULAR MECHANISMS OF M5C PARTICIPATING IN TUMORS MALIGNANCE DEVELOPMENT

Dynamic Regulatory Network Modified With m5C

The functional implementation of m5C modification in malignant tumors depends on its dynamic regulatory network, which is composed of three key regulatory factors: writers, erasers, and readers.

Writing Proteins

Methyltransferases mainly include the NSUN family, DNMT2, and TRDMT family, which act as writing proteins. They participate in tumor development by catalyzing methylation modification of the 5th carbon of RNA cytosine. Among writing proteins, members of the NSUN family exhibit significant substrate specificity. NSUN2 mainly targets the 5'UTR and coding region of mRNA, which enhances mRNA stability by methylating cancer-related genes (such as SMOX), thereby promoting the invasive phenotype of esophageal squamous cell carcinoma [5,11]. NSUN4 plays a crucial role in mitochondrial rRNA modification, and its absence can lead to mitochondrial dysfunction and inhibit the proliferation of liver cancer cells[12]; NSUN6 selectively catalyzes mRNA methylation by recognizing UCCA motifs, thereby inhibiting the translation efficiency of target genes [5]. In addition, although DNMT2 and TRDMT1 have similar structures to DNA methyltransferases, they specifically catalyze the m5C modification of tRNA, and their abnormal expression is associated with chemotherapy resistance in gastric cancer and leukemia [13].

Erasing Proteins

Demethylase, as an erasing protein, reverses promoting cancer effect of m5C modification through oxidation reaction. TET1-3 family proteins can gradually oxidize m5C into hydroxymethylation (hm5C), formylation (f5C), and carboxylation (ca5C) intermediates, which exhibit dynamic changes in acute myeloid leukemia and are significantly correlated with disease prognosis[14]. In addition, TET2 gene mutations are frequent in myelodysplastic syndrome, leading to m5C demethylation disorders and malignant transformation of hematopoietic stem cells [15]. ALKBH1, as another potential demethylase, has been shown to affect the metastatic potential of melanoma by regulating tRNA modifications, although its RNA substrate specificity has not been fully elucidated [11].

Identification of Proteins

The recognition proteins directly regulate RNA through binding to m5C modification sites, mainly including ALYREF and YBX1. ALYREF, as a nuclear transport adapter, preferentially recognizes m5C modified mRNA and promotes its nuclear cytoplasmic transport. In hepatocellular carcinoma, high expression of ALYREF leads to abnormal cytoplasmic accumulation of oncogenes (such as CCND1), which in turn drives cell cycle progression [12]. YBX1 forms a ribonucleoprotein complex by binding to the m5C site of the 3'UTR region of mRNA. This cannot only stabilize the transcripts of transfer promoting genes, such as HDGF in bladder cancer, but also shield the nuclease attack site to extend the RNA half-life [16]. It is worth noting that the NSUN2-YBX1-ALYREF axis forms a positive feedback loop in a variety of malignant tumors. For example, in breast cancer, this pathway enhances the stability of HGH1 mRNA through epigenetic regulation, ultimately leading to tumor immune escape and resistance to targeted therapy [17]. This trinity dynamic regulatory network not only reveals the complexity of m5C modification, but also provides multi-level molecular targets for tumors precise intervention.

Distribution Characteristics and Biological Functions of m5C

Distribution Characteristics and Detection Techniques

The distribution of m5C modification in RNA molecules exhibits significant tissue and region specificity. In mRNA, m5C is mainly enriched in the 5'UTR and 3'UTR regions, and modifications in these regions may regulate translation initiation and mRNA stability through affecting the recruitment of RNA binding proteins [18]. In addition, the coding region also contains m5C modifications, but their abundance is relatively low and they often coexist with other modifications such as m6A, jointly regulating the translation extension process[14]. In tRNA, m5C is highly conservatively distributed in key structural domains, such as D-loop and T-loop, maintaining the stability and translation accuracy of tRNA [7]. The m5C in rRNA is mainly concentrated in the catalytic core region of ribosome subunits, such as the C2870 site of 28S rRNA, which is crucial for ribosome assembly and protein synthesis [8]. Non coding RNA is also regulated by m5C modification, for example, m5C modification of lncRNA HOTAIR can affect its interaction with chromatin remodeling complexes, thereby regulating gene expression [7].

The techniques for detecting m5C modifications mainly include Bisulfite sequencing, m5C RIP seq, and nanopore sequencing. Bisulfite sequencing chemically converts unmethylated cytosine to uracil, and combined with high-throughput sequencing, can accurately locate the m5C site, but it is susceptible to RNA degradation [18]. m5C RIP sequencing utilizes specific antibodies to enrich and modify RNA fragments, suitable for whole transcriptome analysis, but antibody cross reactivity may introduce false positives [7]. Nanopore sequencing identifies modifications by directly reading the electrical signal changes of RNA molecules without the need for chemical treatment, but the current resolution still needs to be improved [14].

Biological Functions of m5C

m5C modification regulates RNA metabolism through multiple mechanisms. In mRNA, m5C in 5'UTR and 3'UTR can enhance RNA stability, for example, NSUN6 mediated modifications prolong mRNA half-life by inhibiting nuclease cleavage [5]. Meanwhile, m5C is closely related to transla-

tion efficiency, such as ALYREF protein recognizing m5C modified mRNA and promoting its nuclear output, thereby affecting translation initiation [11]. In tRNA and rRNA, m5C modification maintains its secondary structure to ensure translation accuracy. For example, the absence of m5C72 modification in tRNA can lead to codon recognition errors, while NSUN4 catalyzed mitochondrial rRNA m5C modification is crucial for the assembly of mitochondrial respiratory chain complexes [19]. The m5C modification of non coding RNA regulates its functional diversity, such as the m5C modification of miRNA precursors that can affect Drosha processing efficiency, while the modification of lncRNA may alter its interaction mode with proteins or DNA [11].

m5C modification plays a central role in cellular physiological processes. Mitochondrial function depends on NSUN4-mediated 12S rRNA m5C modification, and its absence can lead to mitochondrial translation defects and energy metabolism disorders [13]. During neural development, tRNA modification catalyzed by NSUN2 is essential for neuronal differentiation, and NSUN2 mutations can cause intellectual disabilities and neurodegenerative disorders [20]. In addition, m5C is involved in stress response, such as TET enzyme mediated m5C demethylation under oxidative stress, which can activate protective gene expression [7]. During embryonic development, the dynamic changes of m5C modification regulate the degradation of maternal mRNA and activation of the zygote genome. Some studies have shown that knocking out mouse NSUN2 can lead to embryonic lethality [19]. These findings highlight the irreplaceable role of m5C in basic cellular activity and higher biological development.

Functions and Mechanisms of m5C

Mechanism of m5C Promoting Tumorigenesis

M5C modification promotes the tumorigenesis and development through various pathways. In terms of gene expression dysregulation, methyltransferase NSUN2 enhances the stability of oncogene mRNA by catalyzing m5C modification, thereby promoting the malignant phenotype of tumor cells. In addition, m5C modification of long non coding RNA (lncRNA) HOTAIR can enhance its binding ability to chromatin modification complexes, thereby activating cancer signaling pathways, such as Wnt/β-catenin, further driving tumor progression [19]. m5C modification also promotes immune escape by reshaping tumor immune microenvironment. Some researches have shown that m5C modification can upregulate the expression of immune checkpoint molecule PD-L1, inhibit the anti-tumor activity of CD8+T cells, and help tumor cells evade immune surveillance [10]. In melanoma, high levels of m5C modification are associated with increased infiltration of immunosuppressive cells, such as regulatory T cells and myeloid derived suppressor cells, leading to immune therapy resistance [21]. In addition, m5C modification regulates the expression of immune related genes, affects the polarization state of immune cells in the tumor microenvironment, and further promotes the formation of an immunosuppressive microenvironment [11]. Genomic instability is another important mechanism by which m5C modification promotes cancer. In colorectal cancer, mutations in the TET1 gene lead to the loss of its demethylase activity, which in turn affects the expression of DNA damage repair related genes and exacerbates genomic instability [19]. In addition, the abnormal distribution of m5C modifications may lead to changes in RNA secondary structure, affecting DNA replication and repair processes, thereby increasing the accumulation of mutations in tumor cells [12].

Anti Cancer Mechanism

Although m5C modification exhibits a promoting cancer effect in most cases, it may also exert anti-cancer functions under specific conditions. For example, m5C modification can inhibit tumor growth and metastasis by stabilizing the mRNA of tumor suppressor genes. In hepatocellular carcinoma, m5C modified TP53 mRNA exhibits higher stability, thereby enhancing the expression of p53 protein and inhibiting tumor cell proliferation [7]. In addition, m5C modification can induce cell cycle arrest and inhibit tumor progression by regulating the expression of cell cycle related genes such as CDKN1A (p21) [8]. m5C modification also plays an important role in maintaining cellular homeostasis. For example, NSUN4 mediated mitochondrial rRNA m5C modification is crucial for maintaining mitochondrial function, and its absence can lead to energy metabolism disorders and increased cell apoptosis [19]. In neuroblastoma, m5C modification exerts anticancer effects by regulating the expression of neuronal differentiation related genes, inhibiting the dedifferentiation process of tumor cells [20]. In addition, m5C modification can regulate the expression of stress-related proteins by affecting the nuclear cytoplasmic transport and translation efficiency of RNA, helping cells cope with adverse conditions such as oxidative stress, and maintaining genomic stability [7].

APPLICATION OF M5C MODIFICATION IN TUMOR DIAGNOSIS AND THERAPY

In recent years, significant progress has been made in the application of m5C modification in tumor molecular typing, therapeutic target, and prognosis evaluation. In terms of molecular typing, based on the expression profile of m5C regulatory genes, pancreatic cancer can be divided into three subtypes, of which Cluster 2 subtype shows significant immune cell infiltration and good prognosis, while Cluster 1 is related to chemotherapy resistance, suggesting that m5C modification mode can be used as the basis for personalized treatment stratification [22]. In addition, the expression characteristics of m5C related genes (such as HIPK3 and DPP8) also demonstrate subtyping potential in colorectal cancer and gastric cancer, providing new molecular markers for precision medicine [19]. In the development of therapeutic targets, key molecules in the m5C regulatory network have become an important direction for drug research and development. Methyltransferase inhibitors, such as the small molecule compound BRD-K41404348 targeting NSUN2/NSUN3, can significantly reduce the proliferation ability of cancer cells in vitro experiments [23]. The studies in melanoma have found that, the patients with high m5C modification scores exhibit stronger therapeutic responses to PD-1 inhibitors, while low score tumors are more sensitive to chemotherapy, suggesting that m5C patterns can guide the selection of immunotherapy strategies [21]. In the binding protein intervention strategy, YBX1 inhibitor can effectively inhibit the invasive metastasis of bladder cancer by blocking its binding with m5C modified mRNA (such as HDGF) [17]. The reactivation of demethylase TET1/2 can reverse the methylation status of oncogenic

RNA, restore the expression of tumor suppressor genes, and provide new ideas for epigenetic therapy [11]. In addition, the combination of m5C and DNA methylation inhibitors (such as 5-azacytidine) can synergistically enhance anti-tumor effects, while integrating m6A modified regulatory networks (such as METTL3 inhibitors) can further optimize the combination therapy regimen [12]. In the field of prognostic evaluation, the expression pattern of m5C related genes has become an independent prognostic indicator for various cancers. For example, the high expression of NSUN4 and ALYREF in hepatocellular carcinoma is significantly associated with shortened overall survival of patients, and its mechanism may be related to promoting tumor immune escape [7]. The scoring system based on m5C regulatory gene shows high prediction efficiency in many cancers. For example, the AUC value of pancreatic cancer model exceeds 0.8, which can accurately distinguish high-risk patients [24]. In addition, in triple negative breast cancer, high m5C score is related to rich immune cell infiltration (such as CD8+T cells and NK cells) and better prognosis, while low score patients may benefit from platinum chemotherapy, highlighting the clinical transformation value of m5C score [25]. These advances have laid an important foundation for the transition of m5C modification from basic research to clinical applications, but its tissue-specific mechanisms and drug toxicity control remain key challenges for future research.

PROSPECTIVE

In the future study on m5C, it needs to be breakthroughs in mechanisms, technologies, and clinical translation. In regulating mechanism of m5C, the analysis of tissue-specific regulatory networks remains a key challenge. At present, there is a preliminary understanding to the tissue distribution of m5C methyltransferases (such as NSUN family), but there is still a lack of direct evidence for the activity verification of demethylases (such as TET family) in RNA [7]. In the future, it is necessary to clarify the substrate specificity and other characteristics of erasure proteins, and reveal their dynamic patterns in development or disease. The breakthrough of technological bottlenecks will drive research towards higher precision. The existing m5C detection methods are difficult to analyze heterogeneity at the single-cell level, and cannot distinguish multiple modifications coexisting on the same RNA molecule [8]. The development of dynamic detection technology for m5C at the single-cell level will play an important role in revealing the intercellular communication mechanism of m5C in the tumor microenvironment. In addition, clinical application translation needs to address two core issues. On the one hand, small molecule inhibitors targeting m5C regulatory proteins (such as NSUN2 antagonists) are still in the preclinical research stage, and their off target effects and long-term toxicity urgently need to be systematically evaluated [23]; On the other hand, personalized treatment strategies based on m5C modification mode need to be validated through multi center clinical trials. The integration of multiple omics is the ultimate path to understanding the biological significance of m5C. By coupling genomic variations (such as TET1 mutations), transcriptome dysregulation (such as ALYREF overexpression), and epigenetic profiling (such as m5C/m6A co modification), an "m5C disease" regulatory network can be constructed [11]. This type of network can identify key node molecules (such as the role of NSUN3 in mitochondrial RNA modification [13]) and provide a unified framework for pan cancer analysis [16]. Ultimately, this systematic research paradigm is expected to drive the comprehensive transformation of m5C from basic discovery to precision medicine. M5C modification, as the core of RNA epigenetic regulation, has significant potential in the study and analysis of tumor mechanisms and precision medicine. Although there are still limitations in current research, with the advancement of technology and relentless exploration of its mechanisms and applications, m5C modification will demonstrate a powerful role in clinical medicine.

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