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Center Molecular Mechanisms of Non-coding RNAs and RNA Modifications in Colorectal Cancer

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Abstract:

Colorectal cancer (CRC) is a malignant tumor that occurs in the mucosal epithelial cells of the colon or rectum, with high mortality and morbidity. Therefore, the study of its molecular principle is more important. In recent years, more attention has been paid to the regulation of CRC by epigenetic factors, especially the regulation of non-coding RNAs (ncRNAs) and RNA modifications. ncRNAs include miRNA, circRNA, and lncRNA. Among them, miRNAs regulate MAPKs, Wnt, and TGF-β signaling pathways and are involved in the tumor process. CircRNAs, as regulators of gene expression, can participate in various biological processes. lncRNA interacts with DNA, RNA, and proteins to regulate signaling pathways in various ways, potentially acting as biomarkers in non-invasive examinations. RNA modifications, especially N6-methyladenosine (m6A), affect CRC development. m6A consists of three proteins, writers, erasers, and readers, which maintain the stability of RNA modifications. Different levels of expression of these three proteins in CRC can lead to changes in the tumor microenvironment, gene expression, and other factors, and thus play an important role in CRC. ncRNAs and RNA modifications comprise the CRC epigenetic regulatory network, providing a new direction for the diagnosis and treatment of CRC in the future.

Keywords: Colorectal cancer, ncRNAs, RNA modifications, m6A.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer death [1]. Cancer cases account for about 10% of new cancer cases worldwide each year [2]. Due to the high incidence and mortality of CRC, its complex gene regulatory network has also become a research focus. Non-coding RNAs (ncRNAs) can affect CRC by regulating the tumor microenvironment, signaling pathways and gene expression. At the same time, RNA modification can further participate in the

process of cancer by affecting the stability, splicing and translation efficiency of RNA. This paper mainly focuses on some epigenetic variables such as ncRNA and RNA modifications, which provides a new perspective for in-depth understanding of the molecular pathological mechanism of CRC and the precision treatment strategies.

2. ncRNA

ncRNAs are RNA molecules that are not translated into proteins and have functions such as controlling chromosome dynamics, splicing, RNA editing, translation repression and mRNA destruction [3]. More than 90% of the RNA in the human genome is ncRNA. Most of the ncRNAs have only been discovered in the past 15 years but remain unstudied. Nevertheless, many studies have shown that small ncRNAs can survive in the blood, thus becoming ideal biomarkers for tumor detection [4].

2.1 miRNA

miRNA typically denotes endogenous small ncRNAs ranging from 18 to 25 nucleotides in length [5,6]. In CRC, miRNAs act upon signaling pathways such as MAPKs, Wnt and TGF-β, participating in tumor initiation, progression, and drug resistance processes [7].

MAPKs are primarily categorized into three classical pathways: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38. These pathways are chiefly involved in cellular processes such as growth, proliferation, differentiation, inflammation, and stress responses. Research on ERK has been most extensively explored. For instance, in CRC, downregulation of miR-143/145 impairs its ability to inhibit ERK5, thereby promoting tumor progression. However, its dual pro- and anti-tumor effects depend on the regulated target genes and their signaling pathways [7]. For instance, the regulation of cancer-associated genes such as EGFR and KRAS by miR-143/145 has been demonstrated to correlate with CRC initiation and progression, while also inhibiting CRC cell proliferation and carcinogenicity [8].

Under normal circumstances, β-catenin is degraded by a destructive complex comprising Axin, APC, CK1, and GSK3, thereby maintaining stable levels. However, in CRC, abnormal activation of the Wnt pathway leads to nuclear accumulation of β-catenin, which drives oncogene expression. miR-155 regulates cell survival and growth in CRC cells by targeting Axin1 and TCF4, thereby sustaining prolonged Wnt/β-Catenin activation. TGF-β typically exhibits dual roles in CRC: its early downregulation aids tumor initiation, whilst its subsequent low expression exerts tumor-suppressive effects [7].

2.2 circRNA

circRNA represents a unique class of covalently closed ncRNA molecules characterized by the absence of free 5‘ and 3‘ ends, formed through reverse splicing. Exon circRNAs (ecircRNAs) primarily reside in the cytoplasm and regulate mRNA stability, whereas intron circRNAs (ciRNAs) and exon-intron circRNAs (EIciRNAs) predominantly function in the nucleus to modulate transcription [9].

circRNA, as a regulator of gene expression, participates in several biological processes, including acting as miRNA sponges, regulating transcription and translation, and interacting with ribosomal binding proteins (RBPs). The miRNA sponge function of circRNA arises from its ability to competitively bind the miRNA, thereby weakening its inhibitory effect on target mRNA. For example, circSHKBP1 promotes CRC progress by acting as a sponge for miR-328-5p, and in this way reverses its inhibition of E2F1 [10].

Transcription is the process of converting DNA into mRNA, and circRNAs can directly or indirectly regulate transcribed genes and interact with RNA polymerase II complexes. For example, the interaction between circEIF3J and circPAIP2, RNA polymerase II and U1 snRNP promotes the transcription of PAIP2 and EIF3J. circMEMO1 promotes the progression of hepatocellular carcinoma (HCC) by regulating the methylation of TCF21 promoter and the transcriptional expression of subsequent genes. Translation is the process by which mRNA is translated into protein by ribosomes. circVAMP3 reduces Myc protein levels by inhibiting c-Myc translation, thereby inhibiting tumor growth in HCC. This mechanism has been extensively studied in HCC, and it is speculated that a similar role may exist in CRC [10].

circRNAs interact with miRNAs and RNA-binding proteins (RBPs) and act as antagonists. In the interaction with RBPs, circRNAs act as „scaffolding“ molecules to bind to multiple RBPs, promote the formation of RNA-protein complexes, and affect their stability and function [10]. In addition, due to high stability, low immunogenicity, and tissue specificity expression, naturally occurring circRNAs are considered promising alternatives to existing RNA-based therapeutics [11].

2.3 lncRNA

lncRNAs are defined as non-coding transcripts over 200 nucleotides in length [12]. As key regulators of gene expression, they can alter susceptibility to various diseases through a variety of mechanisms, including gene-environment interactions [13].

lncRNAs mainly interact with DNA, RNA and proteins.

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The binding to DNA can recruit epigenetic complexes (PRC2, DNMT1) that alter chromatin modification and gene transcription. When interacting with RNA, they act as ceRNAs to sequester miRNAs or regulate mRNA stability. Their binding to proteins enables them to act as molecular scaffolds or decoys to assist in the assembly and functional regulation of protein complexes [12].

lncRNAs contribute to various stages of carcinogenesis and metastasis in CRC by regulating multiple signaling pathways including MAPK, PI3K/AKT/mTOR, Wnt/β-catenin, JAK1/STAT3, and TGF-β/Smad. This occurs through mechanisms such as acting as miRNA sponges, modulating mRNA stability, or recruiting epigenetic complexes [13]. Research indicates that lncRNA HOTAIR promotes CRC progression and mediates chemotherapy resistance by regulating miR-203a-3p expression levels and Wnt/β-catenin pathway activity [14]. Furthermore, lncRNA-APC1, induced by APC expression, inhibits exosome assembly and suppresses CRC cell growth, metastasis, and angiogenesis by reducing Rab5b stability. As a member of the Rab small GTPase family, Rab5b participates in early endosome fusion and receptor endocytosis; its abnormal upregulation drives tumor cell growth and metastasis [13].

lncRNAs may also serve as biomarkers in blood or bodily fluids, offering the potential for non-invasive detection of CRC. For instance, CCAT1 can be detected in peripheral blood and fecal samples from CRC patients. As a long ncRNA located in the 8q24 chromosomal region adjacent to the MYC gene, CCAT1 exhibits widespread overexpression in both CRC tissue and bodily fluid samples. Its expression promotes tumor cell proliferation and metastasis. However, research in this field remains in its infancy, necessitating further investigation and validation in the future [13].

3. RNA Modifications

N6-methyladenosine (m6A) is the most prevalent, abundant, and conserved internal post-transcriptional modification in eukaryotic RNA, particularly in higher eukaryotes [15]. It is widely recognized as a major form of RNA modification and has been found to play a significant role in multiple cancers, including CRC [16]. m6A modification is dynamically regulated by the „Writers“ proteins METTL3/METTL14/VIRMA, the „Erasers“ proteins FTO and ALKBH5, and the „Readers“ proteins YTHDC1/2, YTHDF1/2/3 [17].

3.1 “Writers” Proteins

Research indicates that METTL3, a member of the „Writers“ protein family [7]. In CRC, it impairs its capacity to drive the accumulation and suppressive efficacy of myeloid-derived suppressor cells (MDSCs), particularly G-MDSCs. Specifically, METTL3 enhances the stability and activity of the transcription factor BHLHE41 through m6A modification, thereby upregulating CXCL1 expression and activating the CXCL1/CXCR2 axis to recruit MDSCs into the tumor microenvironment. This process leads to heightened immune suppression and impaired $CD8^+$ T cell function in CRC, thereby accelerating tumor progression [18].

METTL14, acting as a CRC inhibitor, exhibits markedly lower expression in epithelial tissues of CRC patients compared to healthy individuals [19]. Its downregulation correlates with poor patient prognosis. METTL14 reduces NANOG mRNA stability through m6A modification, thereby suppressing cancer stem cell (CSC) properties. Concurrently, METTL14 regulates both the transcription and translation of β-catenin, thereby influencing NANOG expression and consequently inhibiting the proliferation, metastasis, and drug resistance of CSCs in CRC [20].

VIRMA (also known as KIAA1429) is essential for mRNA methylation and, along with WTAP, localizes to the nuclear dots. VIRMA constitutes a necessary component of the methyltransferase complex within the m6A modification pathway. It mediates methylation near the 3‘-UTR and stop codon regions of mRNA [21]. Research indicates that KIAA1429 upregulation may serve as a prognostic biomarker in CRC patients. Furthermore, KIAA1429 overexpression promotes proliferation and colony-forming capacity in CRC cells. Mechanistically, KIAA1429 binds to the third segment of the 3′ UTR in WEE1 mRNA, reducing mRNA stability and thereby suppressing WEE1 expression, though this occurs independently of m6A. This study further demonstrates the tumor-suppressive role of butyrate in CRC. NFκB1 (p50), a member of the NFκB protein family, participates in CRC initiation and progression. Sodium butyrate has been shown to inhibit KIAA1429 expression by downregulating NFκB1 [22].

3.2 “Erasers” Proteins

FTO and ALKBH5, acting as „Erasers“ proteins, play a crucial role in the proliferation and drug resistance of CRC tumors by reversibly regulating m6A levels on RNA, thereby altering mRNA stability and translation efficiency. As m6A demethylases, FTO and ALKBH5 are commonly downregulated in CRC, with their low expression closely associated with poor patient prognosis [23].

Mechanistically, FTO inhibits CRC cell proliferation by reducing MTA1 mRNA stability and regulating the m6A-GLUT1–mTORC1 axis, whilst also participating in BHL-

HE41–CXCL1/CXCR2-mediated immune regulation [24]. Ferroptotic cell death, has demonstrated significant potential in cancer therapy and enhances the efficacy of chemotherapy and radiotherapy. FTO protects CRC cells from ferroptotic cell death. Studies indicate that FTO knockout elevates m6A modification levels on SLC7A11/GPX4 mRNA, enhancing YTHDF2 recognition and thereby reducing its stability or translational efficiency. This subsequently decreases SLC7A11/GPX4 expression, ultimately rendering CRC cells more susceptible to ferroptotic cell death [25].

ALKBH5 inhibits the growth, migration and invasion of CRC cells by reducing the stability of PHF20 mRNA and regulating the expression of SOX4, HK2 [24]. Meanwhile, demethylation of ALKBH5 modulates the expression of circAFF2, which binds to CAND1 and promotes its interaction with Cullin1. This inhibits the neddylation modification of CRC cells, thereby enhancing their radiosensitivity. In contrast, ALKBH5 knockdown resulted in increased circAFF2 m6A modification and YTHDF2-mediated degradation, thereby reducing its radiation density effect. Clinical evidence further confirmed that the expression of ALKBH5 and circAFF2 is increased in the tissues of radiosensitive CRC patients, which is closely related to the improved prognosis of patients. This suggests that ALKBH5 not only plays a tumor suppressor role in CRC development but may also serve as an important biomarker for predicting radiotherapy response and a potential therapeutic target [26].

3.3 "Readers" Protein

YTHDF1, a „Readers" protein and highly expressed in colorectal cancer. It promotes colorectal carcinogenesis and impairs anti-tumor immunity via the m6A-p65-CXCL1/CXCR2 axis, thereby compromising the function of $CD4^+$, $CD8^+$ T cells, and NK cells and facilitating tumor immune escape. Moreover, its overexpression correlates closely with poor response to immune checkpoint inhibitors. Targeting YTHDF1 reduces MDSC infiltration, restores anti-tumor immunity, and significantly enhances anti-PD-1 therapy efficacy, particularly in MSS colorectal cancer. Consequently, YTHDF1 is recognized as an immunotherapy target in CRC [27].

Furthermore, studies indicate that another ‚Readers' protein, YTHDC1, is also highly expressed in CRC tissue samples and cells, with its elevated levels closely associated with larger tumor size, more advanced disease stage, and poorer histological grade. LINC00857 interacts with YTHDC1 via RIP and RNA pull-down assays. This interaction recruits YTHDC1 to SLC7A5 mRNA, thereby enhancing its stability and expression levels. Inhibiting YTHDC1 or knocking down LINC00857 reduces the mRNA half-life of SLC7A5, diminishing the migration capabilities of CRC cells [28].

4. Conclusion

CRC ranking among the world's most prevalent and lethal malignancies, occurring and developing under the influence of a complex epigenetic network. miRNAs within ncRNAs exhibit dual roles in gene regulation and expression through signaling pathways such as MAPKs, Wnt and TGF-β, revealing their complex duality in both tumor suppression and promotion. circRNAs play a significant role in CRC initiation and progression through multiple mechanisms. They hold promise as alternative approaches to existing RNA-based therapies. lncRNAs can interact with DNA, RNA and proteins to regulate multiple signaling pathways. In addition, they can also serve as biomarkers for non-invasive diagnostic tests.

Moreover, RNA modifications, particularly m6A, also play a pivotal role in CRC. Among the „Writers" proteins, METTL3 and VIRMA promote the initiation and progression of CRC, whereas METTL14 exerts an inhibitory effect. The „Erasers" proteins FTO and ALKBH5 function as tumor suppressors in CRC, and their downregulation facilitates the onset and advancement of the disease. Elevated expression of the „Readers" proteins YTHDF1 and YTHDC1 in CRC enhances the proliferation and migratory capacity of CRC cells.

Despite the wealth of research findings on ncRNAs and RNA modifications to date, the field currently faces multiple limitations and challenges. Given that ncRNAs and RNA modifications involve multiple levels of regulation, including transcriptional and post-transcriptional mechanisms, their precise role in the development and metastasis of CRC requires further in-depth investigation. Furthermore, CRC exhibits significant heterogeneity, with distinct expression patterns observed across different patients. Current research predominantly focuses on individual molecules or pathways, lacking comprehensive studies on the overall mechanisms. Additionally, while certain ncRNAs and RNA modifications have been implicated in CRC, large-scale clinical trials are still required to validate their clinical applicability.

Overall, ncRNAs and RNA modifications have deepened our understanding of CRC at the molecular mechanism level, while also offering novel perspectives on therapeutic targets. Future research should integrate these findings with clinical big data and single cell sequencing to systematically elucidate their roles across different CRC subtypes, immune responses, and treatment stages, while exploring their potential as drug targets. In clinical transla-

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tion, research into molecular biomarkers such as miRNAs, circRNAs, and lncRNAs can facilitate the development of more advanced non-invasive detection methods for CRC assessment and treatment. Furthermore, combining RNA modifications with immunotherapy strategies may explore ways to enhance immunotherapy efficacy and address issues of drug resistance.

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