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The Mechanism and Advances of m6A RNA Methylation in Regulating Cuproptosis in Gastric Cancer

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Abstract: Gastric cancer remains one of the leading causes of cancer-related deaths worldwide, with treatment challenges such as drug resistance and metastasis necessitating novel therapeutic strategies. Cuproptosis, a novel form of regulated cell death first elucidated in 2022, is a copper-ion-dependent process originating from mitochondrial metabolic disturbance. Distinct from other known cell death modalities, the regulation of cuproptosis involves various epigenetic modifications. Among these, N6-methyladenosine (m6A), the most abundant internal chemical modification in eukaryotic mRNA, plays a pivotal role in post-transcriptional regulation during cuproptosis. This review begins with the clinical challenges of gastric cancer, systematically discusses the significance of programmed cell death in its treatment, and then delves into the unique molecular mechanisms and complex regulatory networks of cuproptosis. It particularly focuses on analyzing how m6A modification precisely regulates cuproptosis through a "metabolism–epigenetics" crosstalk mechanism. The aim is to provide a new theoretical foundation and potential therapeutic targets for the precise diagnosis, treatment, and drug development in gastric cancer.

Keywords: Gastric cancer; Programmed cell death; Cuproptosis; m6A methylation; Epigenetic regulation

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1. Introduction

Gastric cancer is a common malignant tumor of the digestive tract globally. According to the latest GLOBOCAN 2022 data, its incidence and mortality rank fifth and fourth worldwide, with approximately 968,000 new cases and 660,000 deaths annually[1]. Epidemiological models predict that by 2040, global new cases of gastric cancer will increase to about 1.8 million, and deaths will reach about 1.3 million, representing a 63% and 66% increase from 2020, respectively[2] . In China, the burden of gastric cancer is particularly prominent, with its incidence and mortality ranking third among all cancers, accounting for approximately 44.0% of global new cases and 48.6% of global deaths, posing a significant public health threat. Current clinical diagnosis and treatment of gastric cancer face multiple bottlenecks, including difficulties in early diagnosis, poor prognosis for advanced patients, frequent recurrence and metastasis during treatment, and the tendency of tumor cells to develop multidrug resistance. Therefore, exploring new mechanisms underlying gastric cancer development and progression, and developing novel therapeutic targets and strategies based on these findings, is of great

clinical importance.

Programmed cell death (PCD), an active, genetically regulated process of cellular self-termination, plays a crucial role in maintaining tissue homeostasis and eliminating abnormal cells. Targeting and activating specific PCD pathways within cancer cells is considered a promising anti-cancer strategy that can selectively eradicate cancer cells while minimizing damage to normal tissues. In recent years, besides classic forms such as apoptosis and autophagy, a series of novel PCD modalities, including necroptosis, pyroptosis, and ferroptosis, have been discovered, greatly expanding our understanding of cell death and providing new opportunities for cancer therapy. Among these, cuproptosis, a unique copper-ion-dependent form of PCD first revealed in 2022, has rapidly become a frontier hotspot in tumor research.

Copper is an essential trace element involved in numerous key physiological processes, yet excessive copper ions are cytotoxic. Notably, copper absorption occurs primarily in the stomach and duodenum, suggesting that the gastrointestinal tumor microenvironment may possess unique copper metabolic characteristics and potentially increased sensitivity to cuproptosis. The core mechanism of cuproptosis lies in the direct targeting of lipoylated proteins in the tricarboxylic acid (TCA) cycle by excess copper ions, inducing their oligomerization and leading to the loss of iron-sulfur cluster proteins, ultimately triggering mitochondrial proteotoxic stress and cell death [3]. This process is independent of reactive oxygen species accumulation or caspase activation, distinguishing it from known cell death pathways.

Within the complex regulatory network of cuproptosis, epigenetic regulation, particularly RNA-level chemical modifications, is increasingly recognized for its importance. N6-methyladenosine (m6A) is the most prevalent and abundant internal chemical modification in eukaryotic mRNA. Through the dynamic and reversible regulation by “writers” (methyltransferases), “erasers” (demethylases), and “readers” (recognition proteins), m6A precisely controls mRNA splicing, nuclear export, stability, and translation efficiency at the post-transcriptional level, thereby extensively participating in cellular proliferation, differentiation, metabolism, and death[4,5]. Recent studies indicate a profound functional connection between m6A modification and cuproptosis. Specifically, in gastric cancer, the m6A methyltransferase METTL16 has been shown to directly regulate the mRNA stability of the core cuproptosis gene FDX1 through its lactylation modification, forming a novel “metabolism–epigenetics–cell death” regulatory axis.

Based on this, this review will focus on the molecular mechanisms of cuproptosis and its m6A epigenetic regulatory network in gastric cancer. It will systematically outline the core molecular events from copper ion homeostasis maintenance to cuproptosis execution, deeply analyze the key role and molecular mechanism of m6A modification, particularly the METTL16-FDX1 axis, and discuss the translational prospects and challenges of related targeted therapies. The aim is to provide new insights for developing precise therapeutic strategies for gastric cancer.

2. Molecular Mechanisms and Regulatory Network of Cuproptosis in Gastric Cancer

2.1. Core Molecular Mechanisms of Cuproptosis: From Copper Ion Activation to Mitochondrial Collapse

The molecular pathway of cuproptosis primarily revolves around the mitochondrial respiratory chain and the TCA cycle. Its core mechanism can be summarized into three key steps: FDX1-mediated copper ion activation, abnormal aggregation of lipoylated proteins, and loss of iron-sulfur cluster proteins. These three processes act synergistically, ultimately leading to irreversible mitochondrial proteotoxic stress and cell death[6].

The primary and most critical upstream event is the activation of copper ions. Ferredoxin 1 (FDX1) has been identified as an indispensable positive regulator and core initiator of cuproptosis[3]. FDX1 encodes a small iron-sulfur cluster protein that acts as a reductase, capable of reducing intracellular divalent copper ions (Cu^{2+}) to the more toxic monovalent form (Cu^{+}). This reduction process is crucial for the subsequent effective binding of copper ions to target proteins, greatly enhancing the bioactivity and toxicity release of copper ions within the mitochondrial matrix. Further research revealed that FDX1 is functionally highly associated with lipoic acid synthase (LIAS). FDX1 can directly bind to LIAS, catalyzing the generation of lipoyl groups essential for protein lipoylation modification, thereby tightly linking

copper metabolism with key mitochondrial enzyme modification processes[7].

The activated Cu^+ ions then directly target the hub of mitochondrial metabolism—the TCA cycle. Cu^+ specifically binds to lipoylation sites on key TCA cycle enzymes (such as the pyruvate dehydrogenase complex component DLAT), inducing the formation of intermolecular disulfide bonds and leading to abnormal oligomerization and insoluble aggregation of these lipoylated proteins[3]. This protein aggregation is not a random event but strictly depends on the aforementioned FDX1-mediated protein lipoylation process. Experimental evidence shows that genetic knockout of FDX1 or pharmacological inhibition of lipoylation-related enzymes can completely block copper ion-induced protein aggregation, thereby effectively preventing cuproptosis[8]. This clearly indicates that lipoylation is a prerequisite for cuproptosis execution.

In addition to the aggregation of lipoylated proteins, another hallmark of cuproptosis is the dramatic loss of iron-sulfur cluster (Fe-S) proteins within mitochondria. Studies show that the rapid degradation of Fe-S cluster proteins in cells treated with the copper ionophore elesclomol also depends on the presence of FDX1. Fe-S clusters are core cofactors for numerous mitochondrial enzymes and electron transport chain complexes; their massive loss inevitably leads to oxidative phosphorylation dysfunction and cellular energy metabolism collapse.

In summary, the abnormal aggregation of lipoylated proteins and the functional loss of Fe-S cluster proteins together constitute the unique "proteotoxic stress" characteristic of cuproptosis. These two pathological changes act synergistically, severely disrupting mitochondrial structural integrity and metabolic function, ultimately resulting in cell death[3]. This unique death mechanism endows cuproptosis with significant therapeutic potential for cancer treatment, especially against tumor cells with highly active metabolism.

2.2. Copper Homeostasis Regulatory Network

Intracellular copper ion concentration is maintained within an extremely narrow physiological range through a complex network encompassing uptake, intracellular transport, utilization, and efflux. Dysregulation at any point in this network may lead to abnormal copper accumulation, potentially triggering cuproptosis.

The first step for copper ions to enter cells is high-affinity uptake. The high-affinity copper transporter 1 (CTR1, SLC31A1) located on the plasma membrane is the primary protein responsible for this process. It exists as a homotrimer and specifically mediates the transmembrane influx of Cu^{2+}[9]. Once in the cytosol, copper ions are not present in a free form but are immediately captured and transported by specific copper chaperone proteins (e.g., ATOX1) and precisely delivered to specific organelles or copper-dependent enzymes (e.g., superoxide dismutase SOD1) for utilization[10].

When intracellular copper levels are excessive, cells initiate efflux mechanisms to maintain homeostasis. ATP7A and ATP7B are two structurally similar P-type ATPases that are core executors of copper efflux. ATP7A is primarily expressed in tissues like the intestine, responsible for pumping copper ions from enterocytes into the blood. ATP7B is mainly expressed in the liver, responsible for loading copper onto ceruloplasmin and excreting copper via bile[10,11]. The intracellular localization of these transporters is dynamic: under normal copper levels, they reside in the trans-Golgi network, participating in the maturation of cuproproteins; when copper levels rise, they translocate to the plasma membrane or vesicular membranes to directly mediate copper efflux.

Beyond these specific transport systems, cells possess non-specific buffering mechanisms. Glutathione (GSH), as the most abundant non-protein thiol in cells, is a significant copper ion chelator, capable of binding free Cu^+ and preventing harmful reactions. Studies show that inhibiting GSH synthesis with buthionine sulfoximine (BSO) reduces the cell's buffering capacity for free copper ions, thereby sensitizing cells to cuproptosis inducers. Additionally, metallothioneins (MTs) and other metal-regulating factors participate in the fine-tuning of copper homeostasis at both transcriptional and protein levels, collectively forming a multi-layered, comprehensive copper homeostasis defense network.

3. RNA Epigenetic Modification: An Emerging Mechanism in Cuproptosis Regulation

3.1. Overview of the m6A Modification System and Its Central Role in Tumors

Epigenetic regulation has expanded from traditional DNA methylation and histone modification to the emerging frontier of RNA chemical modifications. Among these, N6-methyladenosine (m6A) is the most prevalent internal modification in eukaryotic mRNA. Through its dynamic and reversible regulation, it functions as an "RNA code" at the post-transcriptional level, widely involved in tumor initiation, progression, metastasis, and drug resistance[5].

The establishment, erasure, and recognition of m6A modifications are carried out by three classes of functional proteins, constituting a precise regulatory system:

Writers: The core complex consists of methyltransferase-like 3 (METTL3) and methyltransferase-like 14 (METTL14) as the catalytic core. METTL3 is the primary catalytic subunit, while METTL14 serves a structural scaffold and substrate recognition role. Other auxiliary proteins such as WTAP, RBM15/RBM15B, and VIRMA (KIAA1429) participate in complex assembly, localization, and substrate specificity selection. This complex preferentially recognizes the classical RRACH (R=G/A; H=A/C/U) sequence motif and tends to methylate mRNAs in the 3›-untranslated region (3›-UTR), coding sequence (CDS), and particularly near the stop codon.

Erasers: Fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5) are the two known m6A demethylases. Both belong to the α-ketoglutarate-dependent dioxygenase superfamily and dynamically remove m6A modifications via oxidation reactions, maintaining the dynamic balance of modification.

Readers: These proteins specifically recognize and bind m6A modifications, mediating downstream biological effects. The YTH domain family proteins (including cytoplasmic YTHDF1, YTHDF2, YTHDF3, and nuclear YTHDC1, YTHDC2) are the primary readers. YTHDF1 promotes mRNA translation, YTHDF2 primarily accelerates mRNA degradation, YTHDF3 has dual functions in promoting both translation and degradation, and YTHDC1 is mainly involved in mRNA alternative splicing and nuclear export regulation. Another important class of readers is the IGF2BP family (IGF2BP1/2/3), which, upon recognizing m6A modifications, enhances mRNA stability and translation efficiency.

In gastric cancer, m6A regulatory factors are commonly dysregulated. Analysis of databases like TCGA reveals that compared to normal tissues, gastric adenocarcinoma shows significant upregulation of "writer" expressions such as METTL14, METTL16, WTAP, RBM15, and RBM15B, while expressions of VIRMA, ZC3H13, and METTL3 are downregulated. This disordered regulatory network profoundly influences the malignant progression of gastric cancer.

3.2. Key Regulatory Role of m6A Modification in Cuproptosis

Due to its high dynamism and comprehensive impact on RNA metabolism, m6A modification occupies a special position in cuproptosis regulation. Its unique advantage lies in the ability to rapidly respond to intracellular and extracellular signals (such as metabolic status, metal ion concentration changes) and directly and precisely control the fate of key gene mRNAs in the cuproptosis pathway, thereby achieving spatiotemporal-specific control of this death modality.

Pioneering research first established a direct link between copper metabolism and RNA epigenetic modification. Sun et al. found a significant positive correlation between copper concentration and total m6A modification levels in tumor tissues through analysis of 48 paired gastric cancer specimens. In vitro experiments using different concentrations of $CuCl_2$ to treat HGC-27 gastric cancer cells confirmed that copper ions could upregulate the overall m6A modification level in a dose-dependent manner, providing direct experimental evidence for the role of the "copper-m6A modification axis" in gastric cancer[12].

4. Molecular Mechanism and Regulatory Network of the m6A-Cuproptosis Axis in Gastric Cancer

4.1 The METTL16-FDX1 Axis: The Core Hub of Cuproptosis Regulation in Gastric Cancer

Among the numerous m6A regulatory factors, the atypical methyltransferase METTL16 stands out as the core molecule

connecting m6A modification to cuproptosis. METTL16 is generally highly expressed in gastric cancer tissues and cell lines, and its high expression is significantly associated with poor patient prognosis[13]. Loss-of-function experiments show that knocking down METTL16 significantly enhances the resistance of gastric cancer cells (e.g., HGC-27 and AGS) to cuproptosis inducers (e.g., Elesclomol-Cu), suggesting METTL16 is a key determinant of cuproptosis sensitivity in gastric cancer cells[12].

How does METTL16 exert its effect? Further mechanistic investigation focused on the core executor of cuproptosis, FDX1. Tissue microarray analysis revealed that FDX1 expression was significantly higher in gastric cancer tissues compared to adjacent normal tissues. Using methylated RNA immunoprecipitation sequencing (MeRIP-seq) technology, researchers found that in gastric cancer cells with METTL16 knockdown, the genome-wide m6A modification level was generally decreased, with the m6A modification level and expression of FDX1 mRNA being the most significantly downregulated. This strongly suggested FDX1 is one of the most critical downstream targets of METTL16[12].

Gene-specific MeRIP-qPCR experiments confirmed that the m6A modification level on FDX1 mRNA significantly decreased upon METTL16 loss and increased upon METTL16 overexpression. To explore the functional consequence of this m6A modification, RNA stability experiments were conducted. After blocking new RNA synthesis with the transcriptional inhibitor actinomycin D, it was found that the degradation rate of FDX1 mRNA was significantly faster in the METTL16 knockdown group compared to the control group. This indicates that METTL16-mediated m6A modification primarily maintains high FDX1 expression levels by enhancing FDX1 mRNA stability. Using bioinformatics tools (e.g., SRAMP) combined with experimental validation, the research team ultimately identified a specific site (adenosine 602) within the coding sequence (CDS) of the FDX1 mRNA transcript as the primary functional site for METTL16-mediated m6A modification[12]. Thus, a clear "METTL16-m6A-FDX1" regulatory axis was revealed: METTL16 catalyzes m6A methylation at a specific site on FDX1 mRNA, preventing its degradation, thereby stabilizing and upregulating FDX1 expression. High levels of FDX1 protein then drive a more intense cuproptosis process.

4.2. Metabolism-Epigenetics Convergence: Gastric Cancer-Specific Mechanism of METTL16 Lactylation Modification

The sophistication of this regulatory axis lies further in its responsiveness to cellular metabolic status. A landmark finding by Sun et al. was that copper stress in gastric cancer cells promotes lactylation modification at lysine 229 (K229) of the METTL16 protein[12]. This is a post-translational modification that directly connects metabolic signals to epigenetic regulation.

The molecular mechanism of this process is as follows: the high-copper environment enhances the interaction between specific lactyltransferases (e.g., AARS1 and AARS2) and METTL16, thereby catalyzing lactylation of METTL16-K229. Conversely, the deacetylase SIRT2 was identified as a binding protein of METTL16, capable of reversibly removing the lactylation modification at the K229 site, forming a delicate regulatory balance akin to an "accelerator" and "brake" [12].

The lactylation modification of METTL16 holds significant functional implications. First, lactylation enhances METTL16's own methyltransferase activity, making it more efficient in catalyzing m6A modification of downstream targets like FDX1 mRNA. Second, this establishes a positive feedback loop promoting cuproptosis: tumor cells typically exhibit active glycolysis, leading to increased lactate production; high lactate and high copper together promote METTL16 lactylation; activated METTL16 upregulates FDX1 via m6A, exacerbating cuproptosis; and cuproptosis itself, as a process of mitochondrial metabolic collapse, may further influence cellular metabolic status. SIRT2 acts as a negative regulator in this loop, and its inhibitor AGK2 can enhance cuproptosis by inhibiting delactylation[12].

5. Challenges and Future Perspectives

Despite breakthrough progress in understanding m6A RNA methylation-regulated cuproptosis in gastric cancer and its demonstrated therapeutic potential, translating these findings into clinical practice still faces numerous challenges.

First, drug development targeting the m6A pathway presents technical difficulties and specificity issues. Currently, apart from a few compounds like STC-15 entering preclinical studies, most m6A modulators remain in early development stages. Since m6A writers and readers often lack high substrate specificity, systemic inhibition or activation of their activity may lead to broad off-target effects and unforeseen toxicity. For instance, preclinical studies show that the METTL3 inhibitor STM2457, while inhibiting leukemia, also has observable effects on normal hematopoietic function, particularly erythropoiesis[14]. Therefore, developing strategies that can precisely intervene in m6A modifications of specific genes (e.g., FDX1) without affecting the global m6A methylome is a key future direction.

Second, our understanding of the role of cuproptosis itself in gastric cancer remains incomplete. Most current studies are still at the stage of constructing prognostic models using cuproptosis-related genes or performing bioinformatics analyses[15]. There is a lack of in-depth, systematic experimental research on the specific molecular mechanisms of cuproptosis in gastric cancer initiation, progression, metastasis, and drug resistance, as well as its interaction with the tumor microenvironment.

However, challenges coexist with opportunities. Molecular subtyping based on m6A modification patterns holds promise for providing new tools for precise stratification and prognosis prediction of gastric cancer patients. In terms of technological innovation, developing programmable m6A editing tools or nanoparticles capable of specifically delivering m6A modulators may enable more precise targeted therapy. Regarding therapeutic strategies, exploring combination regimens of m6A-targeting drugs with traditional chemotherapy, radiotherapy, immune checkpoint inhibitors, and even other cell death inducers has the potential to overcome resistance issues in current treatments and improve efficacy.

6. Conclusion

In summary, cuproptosis, as a novel form of cell death, with its unique copper-ion dependence and mitochondrial metabolism-targeting characteristics, brings new hope for overcoming therapeutic challenges in gastric cancer. m6A RNA methylation modification, particularly through the METTL16-FDX1 axis and its upstream lactylation modification, constitutes a precise and metabolically responsive regulatory network, playing a central role in regulating cuproptosis in gastric cancer. This “metabolism–epigenetics–cell death” pathway not only deepens our understanding of gastric cancer biology but also reveals numerous potential intervention targets. Although challenges remain in drug development targeting this pathway and in mechanistic exploration, with the deepening understanding of the m6A-cuproptosis regulatory network and the emergence of new technologies and strategies, targeting this axis is poised to open promising new chapters for the precise treatment of gastric cancer.

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