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Review

Colorectal Cancer Puzzle: m6A Modification and Its Intricate Relationship With Drug Resistance

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Abstract

Colorectal cancer (CRC) is a globally prevalent malignancy with rising incidence and mortality rates over the past decades. N6-methyladenosine (m6A) is the most abundant internal RNA modification in eukaryotes, and plays a pivotal role in post-transcriptional regulation. m6A is dynamically modulated by three core components, namely methyltransferases (writers), demethylases (erasers), and binding proteins (readers), which together govern the transcription, processing, translation, decay, and stability of mRNA. There has been accumulating evidence for the association of dysregulated m6A modification with CRC pathogenesis, metastasis, and therapeutic resistance. This review summarizes the biogenesis of m6A modification and its regulatory mechanisms, and discusses the dysregulation of m6A-related factors in CRC and the functional impacts. Most importantly, the review highlights the key roles of m6A modification in mediating CRC resistance to chemotherapy, targeted therapy, and immunotherapy. These insights may facilitate the development of novel therapeutic strategies for CRC.

Keywords: colorectal neoplasms; N6-methyladenosine; drug resistance; RNA methyltransferases; RNA demethylases

1. Introduction

Colorectal cancer (CRC) is one of the most prevalent malignancies of the digestive system, ranking the third most common cancer only next to breast and lung cancers, which poses significant threats to public health [1]. Early-stage CRC is primarily characterized by non-specific gastrointestinal symptoms, such as abdominal distension and dyspepsia, which are easily overlooked in clinical practice, and therefore many patients miss the window of opportunity for early intervention. Furthermore, management of advanced CRC is associated with high healthcare costs and suboptimal therapeutic efficacy, which further exacerbates the burden on public health systems [2–4].

The pathogenesis, progression, metastasis, and drug resistance of CRC involve multilevel changes spanning genetics, epigenetics, and transcriptomics. Among these changes, epigenetic changes, particularly N6-methyladenosine (m6A) modification, which is the most prevalent internal transcript modification in eukaryotic mRNA, have attracted considerable research interests in the past decade. There has been emerging evidence indicating the pivotal regulatory role of m6A modification in CRC, demonstrating its dual impacts on malignant behaviors (such as proliferation, invasion, and metastasis) and therapeutic resistance to chemotherapeutic agents, targeted drugs, and immunotherapies [5]. Dysregulation of m6A modification can affect the processing, degradation, and translation of mRNA, thereby activating oncogenes while silencing tumor suppressors. These processes are intricately linked to malignant progression. In summary, the m6A regulatory network serves as an 'epitranscriptomic switch' governing CRC initiation and progression, and more importantly it represents a critical bottleneck in overcoming therapeutic resistance in CRC.

In this review, we comprehensively summarize the functions of m6A modification in regulating CRC and drug resistance, providing novel perspectives for clinical translation. In addition, we contend that while current research has sufficiently elucidated the mechanistic diversity, future efforts should prioritize clinical validation of druggable targets rather than remaining mired in repetitive mechanistic characterization.

2. m6A RNA Modification and Its Regulation on mRNA

m6A modification is recognized as the most prevalent internal modification of RNA in eukaryotes among the over 170 identified chemical modifications, and is extensively distributed across a variety of RNA species, including mRNA, lncRNA, and circRNA [6]. Since the first discovery in mammalian mRNA in 1974, m6A modification has been detected in over 7000 human genes and demonstrated to be evolutionarily conserved across viruses, bacteria, yeast, plants, and vertebrates [7]. m6A modification

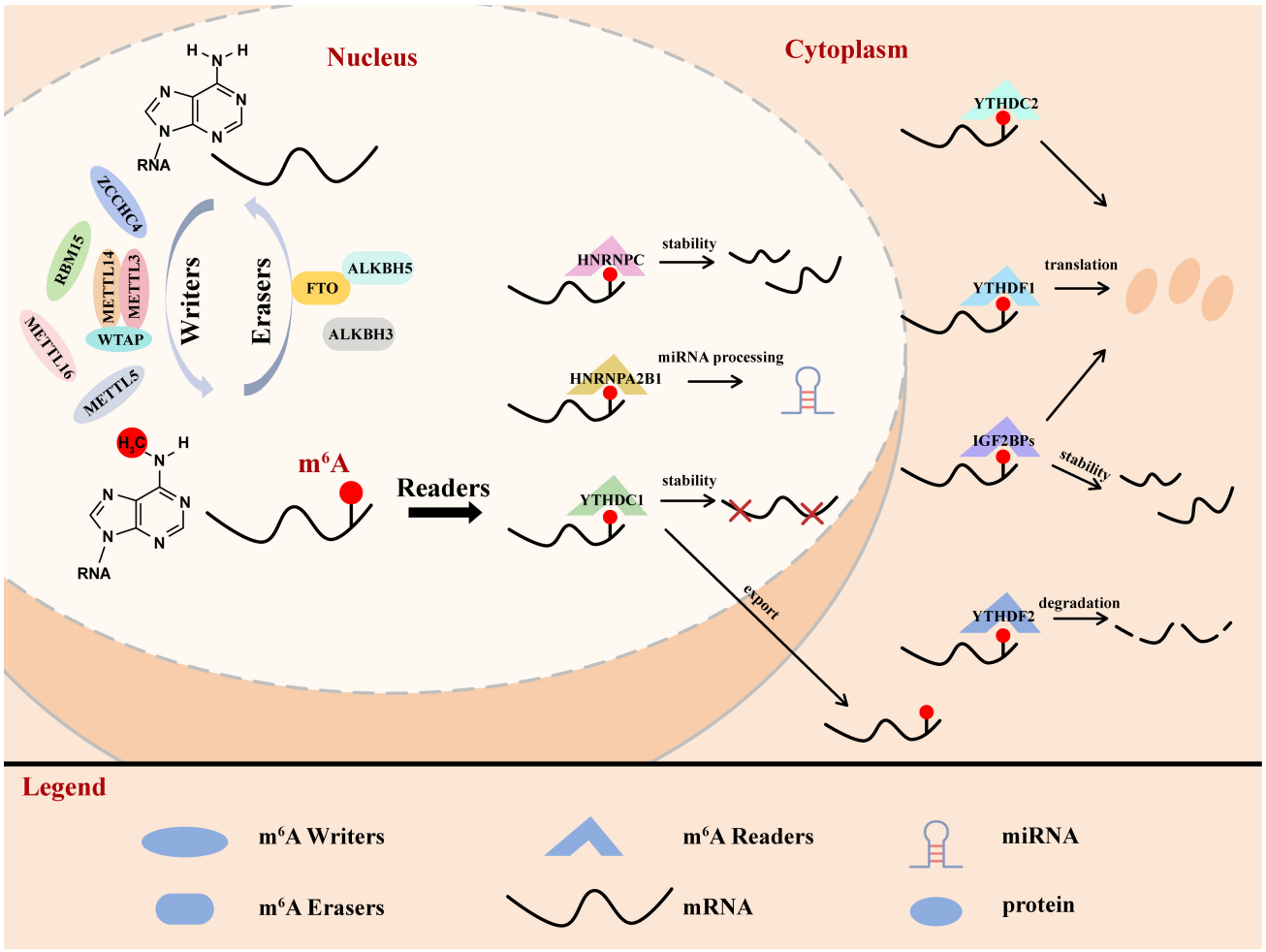

Fig. 1. Roles of different m6A modification proteins. The m6A modification of mRNA is mainly catalyzed by the core methylase complex methyltransferase-like 3 (METTL3)- wilms tumor 1-associated protein (WTAP) - methyltransferase-like 14 (METTL14). The m6A modification is 'erased' by fat mass and obesity-associated protein (FTO), alkB homolog H5 (ALKBH5), and ALKBH3. The readers recognize m6A and affect various functions of the RNA, and mainly include members in the YT521-B homology (YTH) domain-containing family, the insulin-like growth factor 2 mRNA-binding protein (IGF2BP) family, and the heterogeneous nuclear ribonucleoproteins (hnRNPs) family. m6A, N6-methyladenosine; METTL3, methyltransferase-like 3; FTO, fat mass and obesity-associated protein; YTH, YT521-B homology; ALKBH, AlkB homolog H.

involves methylation at the nitrogen-6 position of adenosine, and predominantly occurs at RRACH motifs (where R = G/A and H = A/C/U) and is enriched in regions such as those near stop codons, within 3′ untranslated regions (3′UTRs), and in long internal exons [8].

Generally, the m6A regulatory system functions via three coordinated protein classes, namely methyltransferases (writers), demethylases (erasers), and binding proteins (readers). It is a multi-dimensional, dynamic, and precise process centered around the core "methylation writing-erasing-reading" pathway, and is simultaneously regulated by intracellular and extracellular signals. Ultimately, it achieves precise control over the post-transcriptional fate of RNA, thereby influencing gene expression and cellular functions. The mechanism for the action of m6A modification proteins is illustrated in Fig. 1.

2.1 Methyltransferase Complex: m6A "Writers"

The core function of writers is to transfer the methyl group (-CH_3) from S-adenosylmethionine (SAM, the methyl donor) to the N^6 position of adenine (A) in RNA molecules, a process executed by a multi-subunit complex [9], where the methyltransferase-like 3/14 (METTL3/14) heterodimer serves as the core catalyst. Both METTL3 and METTL14 contain SAM-binding domains, though only METTL3 possesses direct methyltransferase activity. Although METTL14 is lack of intrinsic catalytic activity, it can facilitate substrate recognition by assisting METTL3 to identify specific RNA sequence motifs [10]. Wilms' tumor 1 associated protein (WTAP) does not directly participate in catalytic reactions; instead, it primarily functions to recruit the METTL3/14 heterodimer to the transcriptional sites in cell nucleus (such as the spliceosome-associated regions)

and regulate their subcellular localization [11]. Moreover, VIRMA recruits the METTL3/METTL14/WTAP complex to preferentially modify 3′UTRs and regions near the stop codons. Some other regulatory proteins including vir-like m6A methyltransferase associate (KIAA1429) and RNA-binding motif protein15 (RBM15) contribute to complex assembly and functional regulation, thereby ensuring precise m6A deposition. Notably, METTL5 and ZC3H13 govern the m6A modification of 18S and 28S ribosomal RNAs, while METTL16 regulates m6A on non-coding RNAs [12].

2.2 Demethylases: m6A "Erasers"

The dynamic reversibility of m6A is mediated by fat mass and obesity-associated protein (FTO) and alkB homolog H5 (ALKBH5), two α-ketoglutarate (α-KG) and Fe (II)-dependent dioxygenases. FTO catalyzes stepwise oxidative demethylation of m6A via N6-hydroxymethyladenosine (hm6A) and N6-formyladenosine (f6A) intermediates, and finally converts m6A to adenosine [13]. It exhibits broad substrate specificity, targeting m6A-modified mRNAs, tRNAs, snRNAs, as well as other RNA modifications such as m6Am and m1A. ALKBH5 directly converts m6A to adenosine. It primarily targets spliceosome-associated regions in the cell nucleus, and preferentially acts on nuclear RNAs (such as pre-mRNA), thereby regulating RNA alternative splicing and nuclear export [14].

2.3 Reader Proteins: m6A Effectors

m6A-modified RNAs are decoded by specialized "reader" proteins that trigger downstream biological responses, and the key families include YT521-B homology (YTH) domain proteins, insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs), and heterogeneous nuclear ribonucleoproteins (HNRNPs). YTH m6A RNA-binding protein (YTHDF)1/2/3 are mainly located in the cytoplasm, targeting the 3′UTR region of mRNA at the m6A site. YTHDF1 recruits ribosomes or translation initiation factors (such as eukaryotic initiation factors 3 (eIF3)) to enhance the mRNA translation efficiency. In contrast, YTHDF2 facilitates mRNA decay by recruiting the CCR4-NOT deadenylase complex and other regulatory proteins. YT521-B homology domain-containing family 3 (YTHDF3) exerts dual functions: it synergizes with YTHDF1 to promote mRNA translation, and also interacts with YTHDF2 to enhance YTHDF2-mediated RNA degradation. YTHDC1/2 are mainly located in the cytoplasm, and YTHDC1 regulates pre-mRNA splicing and nuclear export [15]. The IGF2BP family proteins (IGF2BP1–3) recognize m6A modification sites through their unique domains. After binding to mRNA, they can enhance its stability and promote its translation process [16]. Heterogeneous nuclear ribonucleoprotein A2B1 (HNRNPA2B1) regulates pre-miRNA processing, whereas HNRNPC and HNRNPG mediate alternative splicing of m6A-containing transcripts [17]. In addition, HUR, eIF3, and Prrc2a further expand the functional repertoire of m6A recognition, linking RNA methylation to diverse cellular processes from translation control to epigenetic memory. The roles of different regulatory factors in m6A modification are summarized in Table 1.

2.4 Detection of m6A Modification

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) and colorimetric assays are commonly employed for global m6A quantification. LC-MS/MS involves the enzymatic digestion of RNA, followed by chromatographic separation and mass spectrometric analysis to accurately determine the ratio of m6A to total adenosine [18]. Colorimetric assays, which are based on ELISA-like principles, utilize m6A antibodies and color development for semi-quantitative detection [19]. Both approaches are straightforward and appropriate for assessing the overall m6A level. However, they cannot provide transcript- or site-specific information, which limits their utility in mechanistic studies.

MeRIP-seq (m6A-seq) remains the most widely used method for m6A detection, which combines m6A-specific antibody-based immunoprecipitation of methylated RNA fragments with high-throughput sequencing for transcriptome-wide mapping of m6A modifications [20]. This approach benefits from a well-established workflow and commercially available reagents, but suffers from several limitations, including low resolutions (typically spanning 100–200 nucleotides), inability to pinpoint the modified adenosines at single-base resolution, low reproducibility, and requirement for a substantial amount of input RNA. To overcome the resolution constraint, a m6A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP) method was developed [21], which employs UV cross-linking to covalently link antibodies to m6A sites, introducing mutations or truncations during reverse transcription to allow base-resolution mapping. However, the miCLIP method is still highly dependent on antibody quality and cross-linking efficiency, and struggles in resolving closely adjacent m6A sites.

Advancements in epitranscriptomics have led to the emergence of several novel m6A detection technologies. In 2019, Meyer [22] developed DART-seq, which employs a fusion protein of the YTH domain and APOBEC1 enzyme to identify m6A sites in an antibody-free manner. In the same year, researchers introduced MAZTER-seq and m6A-REF-seq, both leveraging the m6A-sensitive MazF ribonuclease for site-specific cleavage within defined motifs [23]. Subsequent innovations include m6A-SEAL-seq, which employs the demethylase FTO for selective chemical labeling [24], and m6A-SAC-seq based on selective allyl chemical labeling [25]. Beyond these next-generation sequencing-based methods, nanopore direct RNA sequencing represents a third-generation strategy that enables the

Table 1. Roles of different regulatory factors in m6A modification of RNA.

Type	Regulator	Mechanism
Methyltransferase (Writers)	METTL3	Transfers methyl from SAM to adenine bases
	METTL14	Recognizes the m6A-specific sequence
	WTAP	Facilitates m6A modification by guiding METTL3/14 heterodimer localization to nuclear spots
	RBM15	Binds to u-rich region and guide METTL3/METTL14/WTAP complex to m6A-specific motifs
	ZC3H13	Combines with WTAP and enhances m6A modification
	ZCCHC4	Participates in translation biology
	METTL16	Targets U6 small nuclear RNA and recognizes the splice site during pre-mRNA splicing
	METTL5	Catalyzes m6A installation for 18s rRNA to increase metabolic stability
Demethylases (Erasers)	FTO	Mediates the demethylation of m6A in RNA
	ALKBH5	Preferentially recognizes the m6A for demethylation
	ALKBH3	Mediates the demethylation of m6A in tRNA
RNA-binding Proteins (Readers)	YTHDC1	Regulates mRNA splicing and export of m6A-containing mRNA from the nucleus to the cytoplasm
	YTHDC2	Enhances translation efficiency
	YTHDF1	Mediates translation promotion via the interaction with translation initiation complex
	YTHDF2	Induces the instability and accelerates the degradation of m6A-methylated mRNA
	YTHDF3	Facilitates translation and affects the decay of m6A-containing mRNA in synergy with YTHDF1 and YTHDF2, respectively
	HNRNPA2B1	Binds m6A-bearing RNAs to elicit alternative splicing effects and promotes primary miRNA processing
	HNRNPC	Affects the abundance and alternative splicing of target RNAs
	IGF2BP1/2/3	Promotes mRNA stability and translation
	eIF3	Initiates translation in a cap-independent manner

Abbreviations: METTL, methyltransferase-like; WTAP, wilms' tumor 1 associated protein; ALKBH, AlkB homolog H; FTO, fat mass and obesity-associated protein; ZC3H, zinc finger CCCH-type containing; IGF2BP, insulin-like growth factor 2 mRNA-binding proteins; HNRNP, heterogeneous nuclear ribonucleoprotein; YTHDF, YTH m6A RNA-binding protein; HNRNPA2B1, heterogeneous nuclear ribonucleoprotein A2B1; RBM15, RNA-binding motif protein15; ZCCHC4, zinc finger CCHC-type containing 4; eIF3, eukaryotic initiation factors 3; YTHDF3, YT521-B homology domain-containing family 3.

detection of modifications in native RNA, and does not require reverse transcription, antibodies, or enzymatic steps [26]. This method provides single-molecule resolution and can simultaneously identify a broad spectrum of RNA modifications beyond m6A.

Despite these advancements, m6A detection is still faced with multiple challenges, including limited resolution and specificity, antibody dependency, sample throughput restrictions, lack of data standardization, difficulties in detecting isoform-specific modifications, and high sequencing costs [27]. Looking forward, the field is moving toward the integration of multiple technologies, single-cell multi-omics analyses, and clinical translation. In parallel, the expansion of public datasets and development in deep learning are expected to yield more accurate and efficient algorithms for m6A identification and quantification.

2.5 Functions of m6A Modification

The m6A modification has been reported to participate in nearly all aspects of RNA metabolic regulation, including mRNA polyadenylation, pre-mRNA splicing, nuclear export of mRNA, as well as mRNA stability and translation under normal physiological conditions [28]. m6A modification plays a pivotal role in regulating critical biological processes such as cell proliferation, differentiation, development, and senescence [29]. Dysregulation of m6A modification is closely linked to the pathogenesis and progression of multiple diseases, particularly in cancers. During tumorigenesis, aberrant expression or functional perturbation of m6A regulatory enzymes, including "writers" (such as METTL3 and METTL14), "erasers" (such as FTO and ALKBH5), and their associated regulators, disrupts global and transcript-specific m6A methylation landscapes. This dysregulation subsequently modulates a broad spectrum of malignant phenotypes, encompassing cancer initiation, progression, metastasis, metabolic reprogramming (such as glycolysis), therapy resistance, immune evasion, cancer stem cell self-renewal, and remodeling of the tumor microenvironment. These findings collectively underscore the significant therapeutic potential of targeting the dysregulated m6A machinery. The role of m6A modification in other types of cancer has been reviewed in detail by other studies [30,31].

Aberrant m6A modification frequently occurs under CRC, and is closely associated with tumor initiation, progression, metastasis, and drug resistance. Therefore, comprehensive insights into the mechanistic roles of m6A modification in CRC hold significant theoretical and clinical importance in elucidating the disease pathogenesis and identifying novel therapeutic targets.

3. m6A Modification During CRC Progression

3.1 Abnormal Expression of m6A Modification Proteins Under CRC

Numerous studies have demonstrated aberrant expression of m6A modification proteins in both CRC tissues and cell lines, and their dysregulation is mechanistically linked to CRC and disease progression. It has been shown that most m6A-related genes such as *METTL3*, *WATP*, and *YTHDF1* are significantly up-regulated in tumor tissues, while *METTL14*, *YTHDF3*, and *ALKBH5* were downregulated in CRC as determined with multiple databases (The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), Human Protein Atlas (HPA) and TMA) [32,33]. An analysis of MeRIP-seq data revealed the presence of m6A peaks in most mRNAs in CRC patients, and CRC samples had 1343 out-of-balance m6A peaks (625 significantly up-regulated and 718 significantly down-regulated) [34]. Wang *et al*. [35] further demonstrated that elevated m6A modification levels and expression of *METTL3*, *METTL16*, and *WTAP* are significantly associated with poor clinical outcomes in CRC patients, suggesting their potential as prognostic biomarkers. The dysregulation of m6A modification proteins confer their oncogenic or anti-oncogenic functions in CRC pathogenesis and progression.

Consistently, it has been demonstrated that METTL3 and WTAP are overexpressed in CRC tissues and strongly correlated with poor prognosis in CRC patients. From a functional perspective, these m6A writers act as oncogene by promoting CRC cell invasion, migration, tumor progression, cancer stem cell maintenance, and drug resistance [36–38]. In contrast to METTL3, METTL14 shows opposite expression patterns and functions in CRC. METTL14 is downregulated and regarded as an independent risk factor in CRC. It has been shown that KDM5C-mediated demethylation of H3K4me3 in the METTL14 promoter reduces METTL14 transcription. Moreover, METTL14 increases the m6A methylation of SOX4 mRNA in a YTHDF2-dependent manner, thereby inhibiting SOX4-mediated EMT process and the PI3K/AKT pathway, ultimately suppressing CRC tumorigenesis [39].

FTO is significantly upregulated in CRC tissues and cells, where it promotes tumor cell proliferation while suppressing apoptosis [40,41]. In contrast, the role of another demethylase ALKBH5 in CRC remains controversial: one study proposed that ALKBH5 downregulation is significantly associated with poor prognosis in CRC patients [42], while another study demonstrated that elevated ALKBH5 expression represents unfavorable prognosis in CRC [43].

3.2 m6A Modification Proteins Function as Oncogene or Tumor Suppressors

m6A modification plays an important role in the occurrence and development of CRC by modifying the mRNA of some key CRC genes, affecting their stability, translation efficiency, splicing, and other processes, thereby regulating their expression levels (Table 2, Ref. [41–57]). METTL3 affects the m6A modification level of Myc proto-oncogene protein (MYC), influences the stability of MYC mRNA in an IGF2BP1-dependent manner, and promotes CRC cell proliferation, migration, and invasion [44]. Moreover, it can increase the transcripts of Sry-related HMG box (SOX)2 and maintain the mRNA stability of SOX2 together with IGF2BP2, thereby affecting the expression of downstream target genes *MYC*, *CCND1*, and *POU5F1*, and further influencing tumorigenesis and tumor metastasis [45]. METTL3 further drives CRC progression by regulating proliferation-related genes. Overexpression of METTL3 was found to enhance m6A modification in the 5′UTR region of cyclin E1 (CCNE1) mRNA, thereby increasing its stability and ultimately promoting the proliferation and colony formation capabilities of CRC cells [46]. METTL3 also promotes m6A modification on lncRNA hepatocyte nuclear factor 1-α (HNF1A)-AS1 and YPEL5 mRNA with IGF2BP2 and YTHDF2 to regulate HNF1A-AS1 stability and YPEL5 translation. Subsequently, HNF1A-AS1 and YPEL5 regulate CCNB1 and PCNA expression, thereby stimulating the expansion of CRC cells [47,58]. Stromal antigen 3 (STAG3) is also regulated by m6A modification. METTL3 knockdown reduces m6A methylation and regulates STAG3 protein expression together with the reader protein IGF2BP2, thereby inhibiting CRC cell proliferation and migration while promoting apoptosis [48]. However, it has been identified that METTL14 regulates the expression of the tumor suppressor Krüppel-like factor 4 (KLF4) by altering its m6A methylation [49].

Zhou *et al*. [50] showed that silencing of WTAP potently restrained the CRC tumorigenesis *in virto* and *in vivo*. Mechanically, WTAP regulates SOD2 m6A modification in an IGF2BP3-dependent manner to maintain its mRNA stability. Notably, SOD2 overexpression could rescue the tumor-suppressive effects induced by WTAP knockdown. The m6A methyltransferase KIAA1429 acts as an oncogenic factor in CRC. It has been suggested that KIAA1429 promotes the proliferation, colony formation, and growth of CRC cells by regulating the level of m6A and expression of Wee1-like protein kinase 1 (WEE1), SIRT1, and NFκB1 [51,52]. Another methyltransferase RBM15 can bind to E2F transcription factor 2 (E2F2) to increase its m6A binding capability and stabilize the corresponding E2F2 mRNA formation to result in malignant cellular processes in CRC cells [53].

Numerous studies have demonstrated that FTO plays pivotal roles in regulating cell cycle progression and apoptosis in CRC. Mechanistically, FTO reduces the m6A modification level of the key oncogenes *MYC* and myeloid zinc finger 1 (*MZF1*), thereby increasing the proportion of S-phase CRC cells [41]. Downregulation of ALKBH5 is significantly associated with poor prognosis in CRC patients, and ALKBH5 has been revealed to suppress the proliferation, migration, and invasion capacities of LOVO and RKO cells. In terms of mechanism, ALKBH5 may destabilize PHF20 mRNA by removing m6A modification in the 3′UTR region [42]. In contrast, another study demonstrated that elevated ALKBH5 expression generally represents unfavorable prognosis in CRC [43]. Shen *et al*. [54] found that ALKBH5 promotes the proliferation, migration, and invasion abilities of CRC cells *in vitro* and enhances subcutaneous tumor growth *in vivo*. Mechanistically, ALKBH5 can perform post-transcriptional activation of RAB5A by m6A demethylation through a YTHDF2-mediated pathway.

Reader proteins regulate RNA metabolic processes through m6A and are involved in the regulation of CRC. YTHDF1 directly binds to m6A-modified SH3TC2 mRNA and enhances its expression, thereby accelerating cell cycle progression and promoting tumor growth in CRC [55]. Yang *et al*. [56] demonstrated that IGF2BP3 promotes the cell cycle progression and proliferation of CRC cells by recognizing m6A modification in CCND1 and enhancing its mRNA expression.

3.3 m6A Modification Orchestrates CRC Progression Through Master Signaling Pathways

Extensive research has demonstrated that m6A modification regulates CRC progression through multiple signaling pathways (Table 3, Ref. [59–68]). METTL3 modulates the m6A modification level of crumbs protein homolog 3 (*CRB3*) mRNA in CRC cells. The m6A-YTHDF2 axis suppresses CRB3 protein translation efficiency, ultimately reducing the phosphorylation levels of key Hippo pathway components (MST1, LATS1, MOB1, and YAP) [59]. METTL3 upregulates plasminogen activator (PLAU) mRNA in an m6A-dependent manner, and then participates in the MAPK/ Extracellular signal-regulated kinase (ERK) pathway to promote angiogenesis and metastasis in CRC [60]. METTL3 also regulates the m6A modification of Membrane-bound erythropoietin-producing hepatocellular receptor tyrosine kinase class A2 (EphA2) and vascular endothelial growth factor A (VEGFA), and stabilizes their transcripts through IGF2BP2/3 to prevent their degradation, thereby activating both the PI3K/AKT and ERK1/2 signaling pathways and promoting vasculogenic mimicry formation in CRC [61]. Notably, METTL3 upregulates JAK1 and Signal transducer and activator of transcription 3 (STAT3) expression through both m6A-dependent and -independent mechanisms, and cooperates with NF-κB to enhance STAT3 transcription, thereby activating the p-STAT3 signaling pathway and driving CRC progression [62]. These findings underscore the mechanistic complexity of m6A-mediated regulation in CRC. However, some conflicting results demonstrate that METTL3 downregulation activates the p-p38 and p-ERK signaling pathways, and inhibition of p38 or ERK significantly reverses the enhanced migration and invasion capacities of CRC cells induced by *METTL3* knockdown [63].

YTHDF1 is widely involved in the regulation of signaling pathways. It can promote the expression of ARHGEF2, TCF7L2, T-cell factor 4 (TCF4), and Glucocorticoid modulatory element-binding proteins 2 (GMEB2) in an m6A-dependent manner, thereby activating multiple oncogenic signaling pathways including RhoA, Wnt/β-catenin, and NF-κB, and ultimately promoting tumor cell growth, migration, invasion in CRC [64–66]. YTHDF2 is also involved in regulating the Wnt/β-catenin signaling pathway. It can regulate the expression of Glycogen synthase kinase 3β (GSK3β) and six-transmembrane epithelial antigen of the prostate family member 3 (STEAP3), which influence Wnt/β-catenin to regulate CRC progression. Intriguingly, YTHDF2 is regulated by miR-6125 and interacts with lncRNA STEAP3-AS1 to modulate Wnt/β-catenin signaling [67,68].

3.4 m6A Modification Regulates CRC Progression Through Non-coding RNA

There have been emerging studies revealing the dual substrate specificity of m6A modification in CRC, as it mediates both protein-coding transcripts and non-coding RNAs to orchestrate oncogenic programs (Table 4, Ref. [58,69–82]). It has been revealed that porcine retina-derived POU domain factor 1 (POU6F2)-AS1 may function as a novel oncogenic lncRNA in CRC with potential diagnostic and therapeutic value. *In vitro* studies have demonstrated that POU6F2-AS1 promotes the growth of both CRC cells and patient-derived organoids (PDOs). METTL3 induces the modification of m6A and stability of POU6F2-AS1 through IGF2BP2, and upregulation of POU6F2-AS1 could tether YBX1 to the FASN promoter to induce its transcriptional activation, thus facilitating the growth and lipogenesis of CRC cells [69]. Similarly, the methyltransferase KIAA1429 regulates POU6F2-AS1 to modulate CRC cell survival, migration, and metastasis, underscoring the diversity of m6A-mediated lncRNA regulation in cancer [70]. METTL3-mediated m6A modification can induce abnormal LINC02418 expression in CRC, where LINC02418 regulates the proliferation and migration of CRC cells by interacting with YBX1 to activate *CTNNB1* transcription, and promoting the interaction between IGF2BP1 and CTNNB1 mRNA to enhance CTNNB1 stability [71]. It has also been confirmed that METTL3 is critical for maintaining the RNA stability of alpha/beta hydrolase domain-containing protein 11-antisense

Table 2. Dysregulation of m6A modification proteins as oncogene or tumor suppressors.

Gene	Expression	Target genes	Reader	Function	Sample source	Detection method	Ref.
METTL3	Up	*MYC*	IGF2BP1	Promotes MYC and cell proliferation, migration, and invasion	20 pairs of CRC patient tissues; the TCGA database (434 cases)	IHC, flow cytometry, RIP	[44]
METTL3	Up	*SOX2*	IGF2BP2	Promotes SOX2 and CRC cell stemness and metastasis	432 CRC specimens (primary, lymph node, liver metastases); TCGA database	IHC, MeRIP-seq, RNA-seq, RNA pull-down, RIP	[45]
METTL3	Up	*CCNE1*	-	Stabilizes CCNE1 and facilitates CRC progression	Human CRC tissues (32 pairs)	MeRIP; Luciferase	[46]
METTL3	Up	*YPEL5*	YTHDF2	Represses YPEL5 to accelerate CRC formation and metastasis	Human CRC tissues	MeRIP; RIP; Luciferase	[47]
METTL3	Up	*STAG3*	IGF2BP2	Promotes STAG3 and promotes cell proliferation and migration while inhibiting apoptosis	CRC tissues (30 cases) and adjacent normal tissues (30 cases)	MeRIP, flow cytometry, RIP, pull-down assays	[48]
METTL14	-	*KLF4*	IGF2BP2	MeCP2 binds to METTL14 to co-regulate KLF4 and promote metastasis in CRC cells	216 paired human CRC and normal tissues; TCGA data	IHC; Co-IP; RNA-seq; MeRIP; Dual-luciferase reporter assay	[49]
WTAP	Up	*SOD2*	IGF2BP3	Elevates SOD2 and promotes the CRC tumorigenesis	30 paired human CRC and normal tissues	MeRIP-qPCR, RIP, Bioinformatics	[50]
KIAA1429	Up	*WEE1*	-	Inhibits WEE1 and promotes CRC cell proliferation	43 paired human CRC tissues; 111 CRC tissues	IHC, RNA-seq, RIP-seq, MeRIP, Luciferase Reporter Assay	[51]
KIAA1429	Up	*SIRT1*	-	Increases SIRT1 and promotes tumor progression	Human CRC and paired normal tissues, TCGA/GEPIA database	IHC, qRT-PCR, RIP, m6A-RIP	[52]
RBM15	Down	*E2F2*	-	RBM15 stabilizes E2F2 and promotes malignant cellular processes in CRC cells.	Human CRC tissues (n = 57 pairs)	m6A RNA Methylation Quantification, RIP	[53]
FTO	Up	*MZF1*	-	GSK3β reduces FTO expression, inhibits MZF1 expression by mediating FTO and then decreases CRC cell proliferation	Human CRC tissues (n = 57)	MeRIP, PAR-CLIP	[41]
FTO	-	*KCTD15*	YTHDF2	Prevents KCTD15 mRNA degradation, inhibits CRC cell growth and triggers apoptosis	Human CRC tissues (n = 125)	RIP, Me-RIP, Dual-luciferase reporter assay	[57]
ALKBH5	Down	*PHF20*	-	Decreases PHF20 to suppress CRC progression	Human CRC tissues (n = 57)	MeRIP, PAR-CLIP	[42]
ALKBH5	-	*AXIN2*	IGF2BP1	ALKBH5 inhibits AXIN2 to foster an immunosuppressive tumor microenvironment. ALKBH5 knockdown enhances the efficacy of anti-PD-1 therapy	-	MeRIP-qPCR, MeRIP-seq, RIP	[43]
ALKBH5	Up	*RAB5A*	YTHDF2	Promotes RAB5A and the proliferation, migration and invasion abilities of CRC cells	Human CRC tissues (n = 35)	MeRIP-seq, MeRIP-qPCR, Luciferase reporter assay, RIP	[54]
YTHDF1	UP	*SH3TC2*		Enhances SH3TC2 and promotes proliferation and tumor growth	12 paired tumour and adjacent normal tissues	MeRIP-qPCR, RIP	[55]
IGF2BP3	Up	*CCND1*		Enhances CCND1 to promote proliferation and cell cycle progression	Colon cancer specimens and paired non-tumor bowel tissues	MeRIP-qPCR, RIP	[56]

CRC, Colorectal cancer; CCNE1, Cyclin E1; CCND1, cyclin D1; SH3TC2, SH3 domain and tetratricopeptide repeats 2; AXIN2, axis inhibition protein 2; KCTD15, potassium channel tetramerization domain containing 15; PHF20, plant homeodomain finger protein 20; SIRT1, Sirtuin 1; WEE1, Wee1-like protein kinase 1; YTHDF2, YTH domain family 2; RIP, RNA Immunoprecipitation; PAR-CLIP, Photoactivatable-ribonucleoside-enhanced cross-linking and immunoprecipitation; Co-IP, Co-Immunoprecipitation; AMPKα2, AMP-activated protein kinase-alpha2; SOX2, sex-determining region Y-box 2; MeCP2, methyl-CpG binding protein 2; IHC, immunocytochemistry; MZF1, Myeloid zinc finger 1; STAG3, Stromal antigen 3; KLF4, Krüppel-like factor 4; SOD2, Superoxide dismutase 2; TCGA, The Cancer Genome Atlas; WTAP, Wilms tumor 1-associated protein.

Table 3. Effects of m6A modification on CRC through different signaling pathways.

Gene	Expression	Target genes	Reader	Function	Sample source	Detection method	Ref.
METTL3	Up	*CRB3*	YTHDF2	Inhibits CRB3 expression and Hippo pathway, promotes the proliferation, migration, and invasion of CRC cells	Human CRC, adenoma, and normal tissues	m6A epitranscriptomic microarray, MeRIP-qPCR, RIP, Luciferase reporter	[59]
METTL3	Up	*PLAU*	-	Promotes PLAU and MAPK/ERK pathway, promotes angiogenesis and metastasis	CRC tissues (32 paired) and adjacent normal tissues	RNA-seq, MeRIP-seq, MeRIP-PCR, luciferase reporter assay	[60]
METTL3	Up	*EphA2 VEGFA*	IGF2BP2/3	Promotes EphA2 and VEGFA vasculogenic mimicry via PI3K/AKT and ERK1/2 signaling	Human CRC and paired adjacent tissues	RNA-seq, MeRIP-qPCR, Luciferase reporter	[61]
METTL3	Up	*JAK1*	YTHDF1	Promotes JAK1 and interacts with NF-κB to enhance STAT3 and enhances CRC cell proliferation and metastasis	Human CRC and paired normal tissue	MeRIP-seq, ChIP, RIP, Luciferase reporter	[62]
METTL3	-	*p38* and *ERK*	-	Inhibits p38/ERK pathways and proliferation, migration and invasion in CRC cells	CRC tissues (tissue microarray, 181 patients)		[63]
YTHDF1	Up	*GMEB2*		Promotes GMEB2, thereby activating NF-κB signaling to promote proliferation	Clinical samples. The expression data of GMEB2, ADRM1 and YTHDF1 were downloaded from the TCGA and GEO	MeRIP-qPCR, RIP, SRAMP website predict the m6A modification sites	[64]
YTHDF1	-	*TCF7L2*, *TCF4*		Promotes TCF7L2/TCF4, thereby activating Wnt/β-catenin signaling to promote stemness and tumor growth	-	MeRIP-qPCR, m6A-seq, RIP-qPCR, Ribo-seq, Luciferase reporter assay	[65]
YTHDF1	Up	*ARHGEF2*		Enhances ARHGEF2, thereby activating RhoA signaling to promote cell growth and lung and liver metastasis	Human CRC tissues (n = 151), 208 CRC TMA	MeRIP-qPCR, RIP-qPCR, MeRIP-seq, RIP-seq, Ribo-seq	[66]
YTHDF2	Up	*GSK3β*		MiR-6125 downregulates YTHDF2, thereby increasing GSK3β, which inhibits Wnt/β-catenin signaling to inhibit the proliferation of CRC cells	Human CRC tissues (n = 150)	MeRIP-qPCR, RIP-qPCR, Luciferase reporter assay	[67]
YTHDF2	-	*STEAP3*		STEAP3-AS1 interacts with YTHDF2 to increase STEAP3, subsequently activating Wnt signaling to support CRC progression	-	Me-RIP, RIP, RNA pulldown SRAMP website predict the m6A modification sites	[68]

GSK3β, Glycogen synthase kinase 3β; VEGFA, Vascular endothelial growth factor A; STEAP3, six-transmembrane epithelial antigen of the prostate family member 3; ERK, Extracellular signal-regulated kinase; TCF4, T-cell factor 4; ARHGEF2, Rho guanine nucleotide exchange factor 2; GMEB2, Glucocorticoid modulatory element-binding proteins 2; JAK1, Janus kinase 1; PLAU, Plasminogen activator; EphA2, Membrane-bound erythropoietin-producing hepatocellular receptor tyrosine kinase class A2; CRB3, crumbs protein homolog 3; STAT3, Signal transducer and activator of transcription; GEO, Gene Expression Omnibus.

Table 4. Regulation of M6A modification on CRC through non-coding RNA.

Gene	Expression	Target genes	Reader	Function	Sample source	Detection method	Ref.
METTL3	Up	*lncRNA HNF1A-AS1*	IGF2BP3	Upregulates HNF1A-AS1and promotes proliferation and cell cycle progression	Human CRC tissues (52 pairs)	RIP; RNA Pull-down; Luciferase; MeRIP; FISH	[58]
METTL3	Up	*lncRNA POU6F2*	IGF2BP2	Upregulates POU6F2 to promote CRC cell lipogenesis and growth	CRC tissues (84 paired fresh frozen, 60 paired paraffin-embedded for TMA)	RNA-seq, RNA pull-down, RIP, immunofluorescence, luciferase reporter assay, ChIP, MeRIP-qPCR	[69]
METTL3	-	*LINC02418/CTNNB1*	IGF2BP1	METTL3 induces LINC02418-mediated CTNNB1 transcription, while LINC02418 enhances CTNNB1 stability via IGF2BP1, collectively promoting CRC cell proliferation and metastasis	10 pairs of human CRC/adjacent tissues; TCGA/GEO datasets	RNA-FISH; RNA pull-down; RIP; ChIP; MeRIP-qPCR; Dual-luciferase	[71]
METTL3	-	*lncRNA FAM83H-AS1*	IGF2BP2/IGFBP3	Promotes FAM83H-AS1 to promotes CRC progression	Human CRC tissues	RNA-seq; RIP; MeRIP; MeRIP-qPCR	[72]
METTL3	-	*lncRNA ABHD11-AS1*	IGF2BP2	Upregulates ABHD11-AS1 to promote colorectal cancer progression and inhibit ferroptosis	Human CRC tissues	RNA-seq; RIP; Ubiquitination assay; Ferroptosis assays	[73]
METTL14	Down	*lncRNA XIST*	YTHDF2	Downregulates XIST to suppress proliferation and metastasis	37 paired CRC/normal tissues	RNA-seq; Me-RIP; RIP	[74]
KIAA1429	Up	*lncRNA POU6F2-AS1*	-	Promotes POU6F2-AS1 to facilitate CRC cell malignancy	32 paired human CRC and normal tissue	MeRIP, Bioinformatics Analysis	[70]
ZCCHC4	Up	*LncGHRLOS*	-	Downregulates lncGHRLOS to promote CRC cell survival, migration, and metastasis	243 CRC tissues	RNA-seq; MeRIP-seq; RIP; RNA pull-down	[75]
ALKBH5	-	*LncRNA NEAT1*	-	Promotes NEAT1 to enhance proliferation and migration while inhibiting apoptosis	Human CRC tissues (n = 70)	Me-RIP, RIP	[76]
ALKBH5	-	*lncRNA CARMN*	YTHDF2/YTHDF3	Enhances CARMN to suppress CRC cell proliferation, invasion, and migration potential	-	MeRIP, RIP, SRAMP website predict the m6A modification sites	[77]
YTHDF2	-	*circ0003215*		YTHDF2 decreases circ 0003215 to promote proliferation, invasion and migration	100 pairs tumor and paracancerous tissues	MeRIP, RIP, RNA pulldown SRAMP website predict the m6A modification sites	[78]

Table 4. Continued.

Gene	Expression	Target genes	Reader	Function	Sample source	Detection method	Ref.
YTHDF3	-	*circ-YAP*		YTHDF3 and eIF4G2 enhance circ-YAP to promote migration, invasion and liver metastasis	Normal cases, CRC cases, CRC with liver metastasis, cases with lung metastasis and cases with brain metastasis	Me-RIP, RIP, RNA pulldown	[81]
YTHDC1	-	*circFNDC3B*		Facilitates cytoplasmic translocation of circFNDC3B to inhibit CRC stemness and metastasis	Human CRC tissues (n = 58)	MeRIP-qPCR, RIP, RNA pull-down	[79]
IGF2BP2	-	*circEZH2/CREB1*		circEZH2 interacts with IGF2BP2 to block its degradation and sponges miR-133b to upregulate IGF2BP2. The circEZH2/IGF2BP2 axis stabilizes CREB1 mRNA, aggravating CRC progression	Human CRC tissues (n = 124)	MeRIP-seq, RIP, RNA pulldown	[80]
PRRC2A	Up	*CSNK1E*		Promotes CSNK1E to enhance CRC progression	Human CRC tissues (n = 89)	MeRIP-seq, MeRIP-qPCR, RIP-seq, RIP-qPCR, Dual-Luciferase Reporter Assays, RNA pulldown	[82]

CSNK1E, encoding CK1ϵ; CREB1, Cyclic-AMP response element-binding protein 1; FNDC3B, Fibronectin type III domain containing 3B; CARMN, Cardiac mesoderm enhancer-associated non-coding RNA; POU6F2, porcine retina-derived POU domain factor 1; HNF1A, Hepatocyte nuclear factor 1-α; FAM83H, Family with sequence similarity 83 member H; ABHD11-AS1, alpha/beta hydrolase domain-containing protein 11-antisense RNA 1; UHRF2, Ubiquitin-like with PHD and ring finger domains 2; GAS5, Growth arrest-specific transcript 5; GHRLOS, GhrelinOS; NEAT1, Nuclear paraspeckle assembly transcript 1.

RNA 1 (ABHD11-AS1) and Family with sequence similarity 83 member H (FAM83H)-AS1 through IGF2BP2/3, where ABHD11-AS1 enhances FOXM1 stability and FAM83H-AS1 specifically binds to PTBP1 to regulate its phosphorylation and splicing function in CRC cell proliferation and migration and suppress ferroptosis [72,73].

It has been demonstrated that METTL14 significantly suppresses CRC cell proliferation and migration potential through YTHDF2-mediated inhibition of the oncogenic lncRNA XIST [74]. ZCCHC4, a newly identified m6A methyltransferase, has been functionally validated in CRC, where it downregulates the lncRNA GhrelinOS (GHRLOS) to modulate KDM5D expression, thereby promoting CRC cell survival and migration [75].

Guo *et al*. [76] has confirmed that ALKBH5 increases the expression level of lncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) by lowering its m6A enrichment to enhance cell proliferation and tumor growth while inhibit apoptosis in CRC cells. Inversely, another study reported that ALKBH5-mediated demethylation of m6A on lncRNA Cardiac mesoderm enhancer-associated noncoding RNA (CARMN) can enhance its stability through YTHDF2 recognition, thereby sustaining the expression level of CARMN. Functionally, the CARMN-miR-5683-FGF2 axis suppresses CRC cell proliferation, invasion, and migration potential [77].

Circular RNAs (circRNAs) have emerged as pivotal players in deciphering cancer pathogenesis due to their exceptional stability and resistance to RNase R digestion. Circ ubiquitin-like with PHD and ring finger domains 2 (UHRF2), a novel circRNA, plays a critical oncogenic role in CRC progression by facilitating the binding of IGF2BP1 to DDX27 mRNA, thereby enhancing its stability and expression.

Circ_0003215 is significantly downregulated in CRC and demonstrates strong negative correlations with tumor size, TNM stage, and lymph node metastasis. Mechanistically, YTHDF2 binds to circ_0003215 and promotes its RNA degradation. Functionally, circ_0003215 acts as a tumor suppressor by sponging miR-663b to upregulate DLG4 expression and G6PD ubiquitination [78]. Zeng *et al*. [79] demonstrated that YTHDC1 facilitates cytoplasmic translocation of m6A-modified circ fibronectin type III domain containing 3B (FNDC3B), thereby enhancing the stability of RNF41 mRNA to promote ASB6 degradation, and ultimately inhibiting CRC stemness and metastasis. CircEZH2 interacts with IGF2BP2, impeding its ubiquitination-dependent degradation, and also acts as a miR-133b sponge to upregulate IGF2BP2 expression. Taken together, the circEZH2-IGF2BP2 axis stabilizes cyclic-AMP response element-binding protein 1 (CREB1) mRNA and exacerbates CRC cell proliferation and migration [80].

Dysregulation of YAP has significant implications for the pathological biology and progression of CRC. The lncRNA pituitary tumor-transforming 3, pseudogene (PTTG3P) undergoes m6A methylation mediated by METTL3 and IGF2BP2 to enhance its stability, which promotes glycolytic CRC reprogramming and proliferation in cooperation with YAP1 [38]. YTHDF3 has been identified as a novel target of YAP, and their mRNA and protein levels have significant positive correlations. The circ-YAP transcript encodes a novel 220-amino acid truncated protein (YAP-220aa) that functions as a competitive inhibitor of LATS1, which promotes YAP dephosphorylation and nuclear translocation, thereby activating a pro-metastatic transcriptional program. Notably, YTHDF3 can cooperate with eIF4G to enhance the translation of circ-YAP, while the YAP/TEAD transcription factor complex simultaneously upregulates circ-YAP expression, establishing a self-reinforcing positive feedback loop to promote liver metastasis of CRC [81]. It has also been found that the m6A reader protein PRRC2A directly targets CSNK1E (encoding CK1ϵ) to maintain its RNA stability, and high levels of CK1ϵ can simultaneously activate both the Wnt and YAP signaling pathways, thereby promoting the progression of CRC [82].

The regulation of lncRNAs and circRNAs by m6A modification offers novel perspectives for CRC diagnosis. However, its clinical application is faced with two major challenges: the stability of non-coding RNAs and insufficient biomarker specificity due to tumor heterogeneity. Therefore, integration of m6A sequencing with liquid biopsy techniques (such as exosomal RNA analysis) may enhance the sensitivity of early detection in future studies. Notably, although many lncRNAs and circRNAs demonstrate promising potential in mechanistic research, their validity as independent prognostic indicators still requires large-scale validation with collaboration to mitigate sampling bias.

3.5 m6A Modification Governs CRC Progression by Pivotal Biological Events

Cancer cells mainly rely on glycolysis for energy supply, which enables cancer cells to tolerate hypoxic environments, while also increases the lactate level in the microenvironment of cancer cells, thereby stimulating tumor growth and development (Table 5, Ref. [38,83–93]) [94]. There has been accumulating evidence demonstrating that m6A modification regulates tumor glycolysis through multiple pathways, thereby exerting critical impacts on cancer proliferation, metastasis, and therapeutic response. In CRC cells, METTL3 directly targets lncRNA PTTG3P, *HK2*, glucose transport protein type 1 (*GLUT1*) and *PNN* genes, enhancing their mRNA stability by m6A modification through IGF2BP2/3 or YTHDF1, ultimately promoting glycolysis and proliferation in CRC cells [38,83,84]. The methyltransferase KIAA1429 also enhances HK2 mRNA methylation in an m6A-dependent manner, leading to increased transcription level and stability of HK2. This

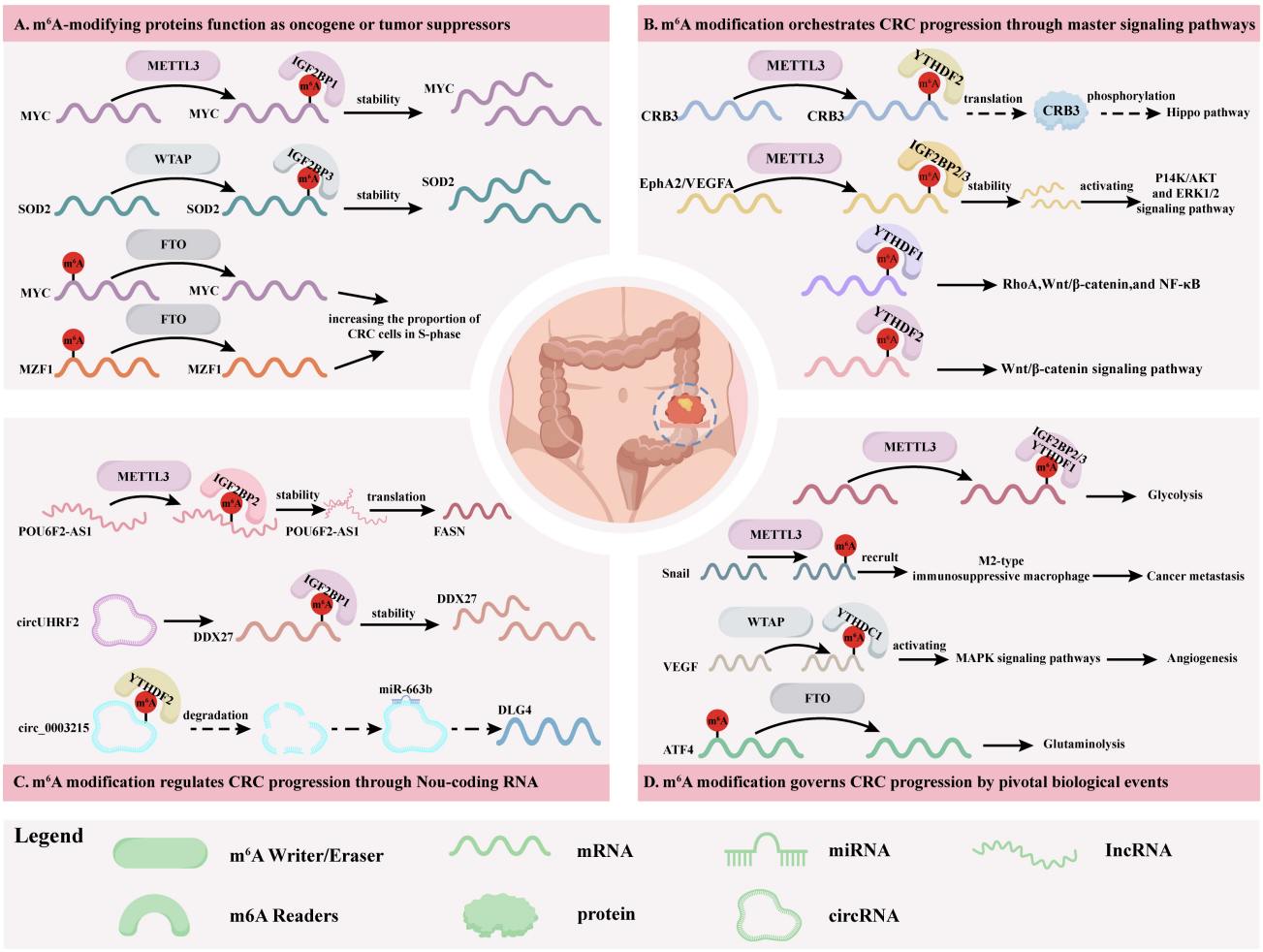

Fig. 2. Roles of m6A modification in CRC progression. (A) m6A modification proteins function as oncogene or tumor suppressors. (B) m6A modification orchestrates CRC progression through master signaling pathways. (C) m6A modification regulates CRC progression through non-coding RNA. (D) m6A modification governs CRC progression by pivotal biological events.

metabolic reprogramming accelerates aerobic glycolysis in CRC cells, thereby promoting tumor aggressiveness [85]. METTL14 inhibits glycolysis, and wild-type p53 activates its transcription, which promotes the biogenesis of pri-miR-6769b and pri-miR-499a. Ultimately, the miR-6769b-3p-GLUT3 and miR-499a-3p-PGAM1 axes suppress aerobic glycolysis and malignant phenotypes in p53 WT CRC cells [86]. Wu *et al.* [87] proposed that ALKBH5/IGF2BPs bind to mjC domain-containing protein 8 (JMJD8) and modulate its stability in an m6A-dependent manner, increasing glycolysis and accelerating the development of CRC by enhancing the enzymatic activity of PKM2. Interestingly, the synthesized ALKBH5 mRNA-loaded folic acid-modified exosome–liposome hybrid nanoparticles were found to significantly inhibit the progression of CRC.

In CRC patients, cancer metastasis is the primary cause of deaths, with the liver and lungs being the most common target organs for metastasis. A previous study has shown that METTL3 facilitates epithelial-mesenchymal transition of pulmonary metastasis by targeting the m6A-Snail- CXC motif chemokine ligand (CXCL)2 axis to recruit M2-type immunosuppressive macrophages [88]. LINC00460 promotes EMT, CRC cell proliferation, migration, invasion *in vitro*, and tumor growth and metastasis *in vivo*. Mechanistically, METTL3 elevates high mobility group A1 (HMGA1) m6A methylation, while LINC00460 directly interacts with IGF2BP2 and DHX9 to bind HMGA1 mRNA, thereby enhancing its stability [89]. High mobility group A2 (HMGA2) (a member of the HMGA1 protein family) has been confirmed to play an oncogenic role in CRC liver metastasis. Mechanistic studies have revealed that circNSUN2 forms a complex with IGF2BP2 to directly bind and stabilize HMGA2 mRNA, thereby driving liver metastasis in CRC [90]. IGF2BP2 also binds to two m6A motifs on LncRNA β-secretase (BACE1)-AS for its stabilization, promoting TUFT1 expression via a ceRNA network involving miR-214-3p, which in turn facilitates CRC liver metastasis [91]. Overexpression of lncRNA SLERT was found to promote liver metastasis in CRC. Mechanistically, SLERT binds to the

methyltransferase RBM15, indicating its ability to stabilize hormonally up-regulated neu-associated kinase (HUNK) mRNA, which will lead to reduced HUNK expression and enhanced metastatic potential in CRC [95].

Tumor-associated macrophages (TAMs) can reduce the antitumor activity of T cells.

Angiogenesis plays an important role in the occurrence and development of CRC. Ye *et al*. [92] found that WTAP overexpression exacerbates CRC cell proliferation, migration, invasion, and angiogenesis. WTAP upregulates VEGF expression in a YTHDC1-dependent manner, which subsequently activates the MAPK signaling pathway to drive angiogenesis.

Upregulation of glutaminolysis facilitates energy generation and biomass accumulation, thereby providing energy for CRC cell proliferation. Han *et al*. [93] demonstrated that FTO promotes ATF4 expression through m6A-dependent stabilization of its mRNA, which can counteract YTHDF2-mediated decay under glutaminolysis inhibition. Functionally, ATF4 activates DDIT4 transcription to inhibit mTOR signaling, thereby triggering pro-survival autophagy in CRC cells. Fig. 2 shows the schematic diagram illustrating the role of m6A modification in CRC.

3.6 Paradoxical Roles of m6A Modification Proteins in CRC

METTL3 and METTL14 are core components of methyltransferases, and can form a heterodimer to exert consistent regulatory functions to control other biological events. However, they play opposite roles in CRC: METTL3 generally acts as a promoter, while METTL14 mostly functions as a suppressor, which may be attributed to the differences in their specific upstream and downstream mechanisms within cells. Methyl-CpG binding protein 2 (MeCP2) is a methylated DNA-binding protein, and has been reported to have oncogenic functions in gastric cancer, CRC, hepatocellular carcinoma, and osteosarcoma. It was identified that MeCP2 can bind to METTL14 to occupy the interaction interface between METTL3 and METTL14, indicating a competitive relationship between MeCP2 and METTL3 for METTL14. Furthermore, MeCP2 acts as an oncogene in CRC progression by interacting with METTL14 and reducing m6A modification [49]. Some studies have shown that METTL3 can promote glycolysis by affecting the downstream target genes such as *lncRNA PTTG3P*, *HK2*, *GLUT1*, and *PNN*, thereby facilitating the proliferation of CRC cells [38,83,84]. In contrast, METTL14 inhibits glycolysis via pri-miR-6769b and pri-miR-499a, thus suppressing the progression of CRC [86]. Therefore, the contrasting roles of METTL3 and METTL14 in CRC may be ascribed to their distinct upstream binding factors and downstream regulatory pathways.

In addition, the demethylases FTO and ALKBH5 exhibit paradoxical roles in CRC. Many studies have demonstrated that FTO plays a promoting role in CRC progression. However, another study showed that FTO may act as anti-tumor factor in CRC progression. FTO expression was found to prevent YTHDF2-mediated degradation of the anti-proliferative and pro-apoptotic factor potassium channel tetramerization domain containing 15 (KCTD15) mRNA. Furthermore, KCTD15 overexpression downregulates HDAC1 expression and enhances p53 acetylation, thereby establishing a tumor-suppressive signaling cascade [57]. We hypothesize that these paradoxical roles may be attributed to two factors. On the one hand, it may be caused by differences in experimental samples; on the other hand, it may be associated with the microenvironment for the CRC cells. Specifically, in tumors with abundant immune cell infiltration, FTO tends to regulate apoptosis-associated genes (such as KCTD15).

ALKBH5 plays even more complex bidirectional roles in CRC. First, different studies have reported inconsistent expression patterns of ALKBH5 in CRC, with some showing its upregulation while others displaying its downregulation. Second, numerous studies have documented both pro-tumorigenic and anti-tumorigenic effects of ALKBH5 in CRC. It remains challenging to elucidate the mechanism underlying such paradoxical roles. We suspect that one possible reason may be the variations in clinical stages across different studies. For instance, in early-stage CRC, ALKBH5 appears to suppress cell proliferation by inhibiting PHF20 [42]; in contrast, in advanced metastatic lesions, it seems to promote tumor invasion by activating RAB5A [54]. This stage-dependent effect suggests that the clinical utility of ALKBH5 as a therapeutic target must be evaluated in the context of tumor progression.

4. m6A Modification and Drug Resistance in CRC

4.1 Therapeutic Agents for CRC Treatment

The main treatment for CRC is usually surgery combined with radiotherapy, chemotherapy, targeted therapy, and immunotherapy [96]. Chemotherapy is key for advanced/metastatic CRC, and the first-line drugs such as 5-fluorouracil (5-FU) and oxaliplatin (OX) form the FOLFOX regimen (OX inhibits DNA replication) [97]. Immunotherapy, especially the use of immune checkpoint inhibitors (ICIs) such as PD-1/PD-L1 inhibitors (approved by the FDA in 2014), shows promise for metastatic CRC by activating T cells in the tumor microenvironment [98]. Although immunotherapy has advanced significantly in CRC treatment, its clinical use is limited by low response rate, unique toxicity, and acquired resistance. Thus, targeted therapy has become the optimal option for personalized and comprehensive CRC treatment [99]. It targets dysregulated pathways (such as EGFR and Wnt/β-catenin) with drugs such as cetuximab (for mCRC with wild-type RAS/BRAF, blocking EGFR signaling) and bevacizumab (inhibiting angiogenesis). The treatment of CRC has been reviewed in

Table 5. Regulation of M6A modification on CRC through pivotal biological events.

Gene	Expression	Target genes	Reader	Function	Sample source	Detection method	Ref.
METTL3	-	*lncRNA PTTG3P*	IGF2BP2	Increases PTTG3P to facilitate CRC glycolysis and proliferation	Human CRC tissues (n = 120)	flow cytometry, MeRIP-qPCR	[38]
METTL3	-	*HK2/GLUT1*	IGF2BP2/3	Stabilizes HK2/GLUT1 to induce CRC tumorigenesis depends on cell glycolysis	CRC patient tissues (n = 47)	MeRIP-seq, MeRIP-qPCR, RIP	[83]
METTL3	Up	*Pinin*	YTHDF1	Enhances PNN to induce glycolysis, proliferation and metastasis	Human COAD tissues	RIP, m6A assay, IHC	[84]
METTL3	Up	*SNAIL*	YTHDF1	Promotes SNAIL to promote colorectal cancer pulmonary metastasis	Clinical CRC and lung metastasis specimens	ChIP, RNA-seq, MeRIP-qPCR, IHC, metabolite assays	[88]
METTL3	-	*HMGA1*	IGF2BP2	Elevates HMGA1 to promote CRC proliferation and metastasis	498 paired human CRC and adjacent normal tissues	RNA pull-down, RIP, MeRIP	[89]
METTL14	Down	*pri-miR-6769b/pri-miR-499a*	YTHDF2	Promotes pri-miR-6769b/499a to suppress aerobic glycolysis and malignant phenotypes in p53 WT colorectal cancer cells	Human p53-wild-type and p53-mutant colorectal cancer tissues and cell lines	ChIP-qPCR, RNA-seq, miRNA microarray, MeRIP-qPCR, Co-IP, IHC, ISH	[86]
WTAP	Up	*VEGFA*	YTHDC1	Upregulates VEGF to exacerbate cell proliferation, migration, invasion, and angiogenesis	Human CRC tissues	RNA-seq, MeRIP-seq, RIP	[92]
KIAA1429	Up	*HK2*		Increases HK2 to accelerate the aerobic glycolysis and malignant phenotype of CRC cells	48 paired human CRC and adjacent normal tissues	RIP, MeRIP	[85]
RBM15	-	*HUNK*	-	LncSLERT binds to RBM15 to decrease HUNK and enhance liver metastasis in CRC	Human colorectal cancer tissues	RNA-FISH, qRT-PCR, RNA-seq, Western Blot, RIP-qPCR, Luciferase reporter assay	[87]
FTO	-	*ATF4*	YTHDF2	Promotes ATF4 to inhibit tumor cells autophagy while promoting cell survival	-	RIP, ChIP	[93]
ALKBH5	Down	*JMJD8*	IGF2BPs	Inhibits JMJD8 to suppress glycolysis and accelerate the development of CRC	8 pairs of CRC tissues and adjacent normal tissues; 192 pairs of CRC tissues and matched nontumor tissues 1078 CRC tumor and matched normal tissues	MeRIP-seq, MeRIP-qPCR, RNA pull-down, Luciferase reporter assay	[87]
IGF2BP2	-	*HMGA2*		circNSUN2 and IGF2BP2 stabilize HMGA2 to promote hepatic metastasis in CRC	-	Me-RIP, RIP, RNA-EMSA assay	[90]
IGF2BP2	Up	*LncRNA BACE1-AS*		Stabilizes BACE1-AS to facilitate CRC liver metastasis	Human CRC tissues (n = 4)	RIP, S1m-tagged immunoprecipitation	[91]

HUNK, Hormonally up-regulated neu-associated kinase; HMGA2, High mobility group A2; JMJD8, mjC domain-containing protein 8; BACE1, β-secretase; PTTG3P, Pituitary tumor-transforming 3, pseudogene; GLUT1, Glucose transport protein type 1; HMGA1, High mobility group A1.

Table 6. Role of m6A modification in drug resistance.

Gene	Expression	Target genes	Reader	Function	Sample source	Detection method	Ref.
METTL3	-	*TRAF5*	-	M2-polarized TAMs enable the OX resistance via elevating METTL3-mediated TRAF5	20 human CRC tissues (OX-sensitive vs. OX-resistant	Flow cytometry; Dot blot; MeRIP	[103]
METTL3	-	*LDHA*	YTHDF1	Triggers LDHA to promote glycolysis and inducing resistance to 5-FU	Using colorectal cancer cell lines and their drug-resistant variants, public database data	Metabolic profiles	[104]
METTL3	Up	*RAD51AP1*	-	Promotes RAD51AP1 to enhance 5-FU resistance in CRC cells by attenuating DNA damage accumulation and cell apoptosis	Human CRC cell lines (HCT-8, 5-FU-resistant HCT-8R)	Flow Cytometry, Immunofluorescence, Comet Assay	[105]
METTL3	-	*Sec62*	IGF2BP1	Upregulates Sec62 to promote the stemness and chemoresistance of CRC by enhancing Wnt signaling	102 CRC patient tissue	Co-IP, GST Pull-down, IHC, Immunofluorescence, m6A-MeRIP, Flow Cytometry	[108]
METTL3/14	-	*Stat1* and *Irf1*	YTHDF2	Deficiency of METTL3/METTL14 promotes IFN-γ-Stat1-Irf1 signaling to increase the infiltration of CD8+ T cells and enhance response to anti-PD-1 therapy	Human pMMR-MSI-L CRC tissues (n = 59).	RNA-seq, MeRIP-seq, flow cytometry	[113]
METTL3	-	*CircQSOX1*	IGF2BP2	Improves CircQSOX1 to facilitate immune escape of CRC, which reduces the therapeutic effect of anti-CTLA-4	Human CRC tissues (n = 60)	RNA pull-down, MeRIP, flow cytometry, glycolysis assays	[114]
METTL14	-	*SLC7A11/HOXA13*	YTHDF2	Curdione induces ferroptosis in CRC by upregulating METTL14	-	Flow Cytometry, Dot Blot (global m6A), MeRIP-qPCR	[119]
METTL16	Down	*PD-L1*	-	Decrease PD-L1 to enhance the tumor suppressive effect of anti-PD-1 antibody	Human CRC tissues (n = 20)	IHC, RIP, MeRIP-qPCR, flow cytometry	[115]
FTO	Up	*NUPR1*	YTHDF2	Prevents degradation of NUPR1 to facilitate CRC chemoresistance	-	RNA m6A dot blot assay, MeRIP-qPCR, RIP	[106]
FTO	-	*G6PD/PARP1*	YTFDF2	Promotes G6PD/PARP1 to enhance CRC progression and chemotherapy resistance	Human CRC tissues	RNA m6A dot blot assay, Me-RIP, MeRIP-qPCR	[107]
FTO	-	*GPX4*	YTHDF2	AKT inhibition downregulates FTO, decreasing GPX4 to activate ferroptosis and suppress CRC progression	-	RNA m6A dot blot assay, MeRIP-qPCR	[120]
ALKBH5	Down	*SLC7A11*	-	Reduces SLC7A11 to promote ferroptosis of CRC	Human CRC tissues (n = 82)	m6A RNA methylation quantification, MeRIP-qPCR	[121]
YTHDF1	Up	*GLS1*		Promotes GLS1, which finally decreases the cisplatin sensitivity of CRC through the GLS1-glutamine metabolism	-	RIP, RNA pulldown, Luciferase reporter assay	[109]
YTHDF3	-	*ATP7A*, *DYRK1B*, *ERCC1*		Promotes ATP7A, DYRK1B and ERCC1 via eIF3A and eIF2AK2 to promote oxaliplatin-resistant	-	Me-RIP, LC-MS/MS, RNA pulldown assay, Co-IP	[110]
IGF2BP3	-	*ABCB1*		Enhances ABCB1 mRNA, which leads to DOX resistance in CRC cells	-	RIP-qPCR, m6A RIP-qPCR	[111]
IGF2BP3	Up	*EGFR*		Enhances EGFR to increase CRC tumorigenesis, progression and drug resistance to cetuximab	-	RNA m6A dot blot assay, MeRIP-qPCR, RNA-EMSA assay	[117]
hnRNPA2B1	Up	*TCF7L2*		HNRNPA2B1 interacts with MIR100HG to maintain TCF7L2, sustains cetuximab resistance and facilitates invasion and metastasis	12 paired tumor specimens and 14 paired specimens of primary CRC tissues	MeRIP-seq, SRAMP website predict, S1m aptamer-based pull-down assay, RIP	[116]

TRAF5, Tumor necrosis factor receptor associated factor 5; DYRK1B, Dual-specificity tyrosine-regulated kinase 1B; ABCB1, ATP-binding cassette sub-family B member 1; eIF2AK2, EIF2 α kinase; SLC7A11, light chain subunit solute carrier family 7 member 11; ERCC1, Somatic excision repair cross-complementing rodent repair deficiency, complementation group 1; PARP1, Poly(ADP-ribose) polymerase 1; RAD51AP1, RAD51-associated protein 1; QSOX1, Quiescin Q6 sulfhydryl oxidase 1; HOXA13, Human class I homeobox A13; LDHA, Lactate dehydrogenase A; NUPR1, Nuclear protein 1; ATP7A, ATPase 1.

detail by several papers [100–102]. While these drugs have demonstrated clinical efficacy and can improve patient survival, their therapeutic potential is significantly compromised by the frequent emergence of drug resistance in practice.

4.2 Roles of m6A Modification Proteins in Drug Resistance

Chemoresistance to OX and 5-FU agents in CRC cells represents a major therapeutic challenge in advanced CRC, and is the primary cause of treatment failure and poor patient prognosis. Lan *et al.* [103] demonstrated that OX-resistant CRC tissues exhibit elevated total m6A RNA levels and increased expression of METTL3. Mechanistically, M2-polarized TAMs enhance the OX resistance via elevating METTL3-mediated m6A modification of tumor necrosis factor receptor associated factor 5 (TRAF5) in cells [103]. Zhang *et al.* [104] and Li *et al.* [105] observed upregulation of both METTL3 expression and global m6A level in 5-FU-resistant CRC cell lines. METTL3 increases lactate dehydrogenase A (LDHA) transcription by stabilizing HIF-1α and triggers the translation of LDHA mRNA via YTHDF1. LDHA catalyzes the conversion of pyruvate to lactate, thereby promoting glycolysis and inducing resistance to 5-FU. This pathway concurrently involves metabolic reprogramming (lactate accumulation) and DNA damage repair. Targeting METTL3 can simultaneously reverse both the metabolic phenotype and drug resistance, which has been supported by early-phase clinical data on METTL3 inhibitors in solid tumors. METTL3 also regulates the expression of RAD51-associated protein 1 (RAD51AP1), promoting 5-FU resistance in CRC cells by attenuating DNA damage accumulation and cell apoptosis. FTO also enhances chemoresistance in CRC. It has been shown that FTO promotes 5-FU resistance in CRC cells by participating in DNA damage, apoptosis, and iron homeostasis through the demethylation of G6PD/ Poly (ADP-ribose) polymerase 1 (PARP1), SIVA1, and nuclear protein 1 (NUPR1) [40,106,107].

Wnt/β-catenin signaling plays an important role in maintaining CRC, and Sec62 upregulation is associated with the chemoresistance of CRC and poor outcomes of CRC patients. Furthermore, upregulation of Sec62 by METTL3-mediated m6A modification promotes the stemness and chemoresistance of CRC by binding to β-catenin and enhancing Wnt signaling. Thus, the m6A modification-Sec62-β-catenin molecular axis may act as a therapeutic target in improving the treatment of CRC [108]. YTHDF1 is positively associated with cisplatin resistance of CRC cells, and binds to the 3′UTR of GLS1 to promote the synthesis of GLS1 protein, which finally mediates cisplatin resistance through the GLS1-glutamine metabolism axis [109]. Another study found that YTHDF3 is highly expressed in OX-resistant (OXAR) CRC tissues and cells. YTHDF3 is enriched in the 5′UTR of RNA from m6A-methylated tumor resistance genes (ATPase 1 (*ATP7A*), dual-specificity tyrosine-regulated kinase 1B (*DYRK1B*), Somatic excision repair cross-complementing rodent repair deficiency (*ERCC1*)), and promotes the translation of target genes by recruiting eIF3A. EIF2 α kinase (eIF2AK2) enhances the stability of the YTHDF3/eIF3A complex in OXAR CRC cells, which collaboratively regulate the translation process of target genes in oxaliplatin-resistant CRC [110]. YTHDF3 is specifically overexpressed in resistant cells, and its interaction with eIF3A can be disrupted by small molecules, providing a well-defined molecular interface for precision drug design. IGF2BP3 is upregulated in HCT8/T cells and affects doxorubicin (Dox) sensitivity. It can recognize m6A-modified ATP-binding cassette subfamily B member 1 (ABCB1) mRNA, enhance its stability, and promote its expression. Upregulation of ABCB1 leads to DOX resistance in CRC cells [111].

The TAMs, which consist of extracellular matrix, myofibroblasts, cytokines, fibroblasts, neuroendocrine cells, adipocytes, immune-related cells, and blood vessels, induce phenotypic changes in cancer cells and immune cells through complex molecular mechanisms to promote immune escape [112]. In CRC cells, deletion of METTL3/METTL14 causes dysfunction of CD8+ T cells, thereby restricting the anti-tumor T cell response in the tumor immune microenvironment. Wang *et al.* [113] found that deficiency of METTL3/METTL14 in CRC significantly increases the infiltration of CD8+ T cells and promotes the secretion of cytokines such as IFN-γ, CXCL9, and CXCL10, thereby enhancing the response of patients to anti-PD-1 therapy. T regulatory (Treg) cells express the immune checkpoint receptor of CTLA-4 and promote tumor immunological tolerance. In CRC, Circ Quiescin Q6 sulfhydryl oxidase 1 (QSOX1) is methylated under the action of METTL3, and its stability is improved after binding to IGF2BP2. The m6A-modified CircQSOX1 can recruit miR-326 and miR-330-5p to increase the expression of PGAM1 and enhance glycolysis to promote lactate production, thereby facilitating immune escape of CRC, which weakens the therapeutic effect of anti-CTLA-4 [114]. In a CRC cell/T cell co-culture system, METTL16 can regulate immune evasion by controlling PD-L1 RNA stability. Furthermore, METTL16 overexpression in CRC cells induces a decrease in the proportion of PD-1 positive T cells [115].

Zhai *et al.* [43] reported that elevated ALKBH5 expression represents unfavorable prognosis in CRC. ALKBH5 demethylates the m6A of axis inhibition protein 2 (AXIN2) mRNA, leading to its dissociation from IGF2BP1 and subsequent degradation, which upregulates the downstream Wnt/β-catenin target gene dickkopf-1 (*DKK1*) and recruits myeloid-derived suppressor cells (*MDSCs*), thereby fostering an immunosuppressive tumor microenvironment. Finally, vesicle-like nanoparticles encapsulating ALKBH5-siRNA or anti-DKK1 antibodies were found to

significantly enhance the efficacy of anti-PD-1 therapy in suppressing CRC growth by potentiating anti-tumor immunity [43].

In terms of targeted therapy drugs and resistance to them, lncRNA MIR100HG sustains cetuximab resistance and facilitates invasion and migration of CRC cells both *in vitro* and *in vivo*. HNRNPA2B1 interacts with MIR100HG to maintain the mRNA stability of TCF7L2, which activates Wnt/β-catenin signaling to confer resistance to cetuximab [116]. IGF2BP3 acts as an m6A reader, and collaborates with METTL14 to enhance the mRNA stability and translation of EGFR, thereby activating the EGFR signaling pathway. Moreover, IGF2BP3 contributes to the resistance of CRC cells to the EGFR-targeted antibody cetuximab [117].

Notably, drug-resistant cancer cells, particularly those in a mesenchymal state with high metastatic potential, generally exhibit marked vulnerability to ferroptosis. This unique vulnerability makes pharmacological induction of iron oxidation a promising therapeutic strategy against refractory malignancies [118]. Curdione can induce ferroptosis to trigger necrotic death of CRC cells. Mechanistically, it promotes the expression of METTL14 and YTHDF2, which in turn elevates m6A methylation and upregulates the expression of *light chain subunit solute carrier family 7 member 11* (*SLC7A11*) and Human class I homeobox A13 (*HOXA13*) genes [119].

Zhang *et al*. [120] found that targeting AKT could significantly induce GPX4-dependent ferroptosis and suppress CRC growth both *in vitro* and *in vivo*. AKT inhibition downregulates FTO expression, increasing the m6A modification of GPX4 mRNA. The m6A-marked GPX4 transcripts are subsequently recognized and degraded by YTHDF2, which subsequently suppresses CRC progression. The demethylase ALKBH5 also exhibits pro-ferroptotic effects on CRC cells. ALKBH5 is downregulated in CRC and its overexpression reduces SLC7A11 transcription by erasing m6A modification, thus promoting ROS release and ferroptosis [121]. The schematic diagram for the role of m6A modification in CRC drug resistance is shown in Table 6 (Ref. [103–111,113–117,119–121]) and Fig. 3.

4.3 Therapeutic Potential of Targeting m6A Modification in CRC

While the role of the m6A regulatory axis in drug resistance has been well-established, its clinical translation potential remains not fully explored. To date, inhibitors targeting m6A modification have been extensively reported [122]. It has been shown that STM2457 has great impacts on cell growth suppression and apoptosis of CRC cells *in vitro* and subcutaneous xenograft growth *in vivo* mediated by the METTL3/ASNS axis [123]. In 2019, a new, specific, and cell active FTO inhibitor compound FB23/FB23-2 was reported. Compared with FB23, FB23-2 has a slightly lower inhibitory activity but a significantly higher cell uptake rate [124]. Lin *et al*. [40] demonstrated that FB23-2 could increase the sensitivity of CRC cells to 5-FU both *in vitro* and *in vivo* by inhibiting FTO. Therefore, targeting m6A regulators with small molecules or modulating their expression represents a promising therapeutic strategy for cancer treatment and is worth of significant scientific interests.

In the treatment of certain cancers (such as oral squamous cell carcinoma), STM2457 has shown promising outcomes. The combination of STM2457 and anlotinib (targeting EGFR) can exert a multifaceted antitumor effect [125]. Despite the promising prospects of m6A-targeted drugs, their clinical translation is still confronted with significant challenges in terms of toxicity, bioavailability, and patient stratification.

The primary concern is "on-target/off-tumor" toxicity. Small-molecule inhibitors targeting m6A modification proteins may exert certain off-target effects on the enzymes with similar structures. Moreover, since m6A modification plays a fundamental role in normal physiology, such inhibitors may trigger systemic toxicity. For example, inhibition of METTL3 can impair postnatal liver regeneration in mice [126], while knockout of YTHDF2 causes hematopoietic system failure [127], indicating the risk of non-target tissue damage. Future studies should prioritize the development of tissue-specific delivery systems to mitigate such risks. In addition, utilization of well-established drugs with existing safety certifications can bypass the "de novo" development of small molecules, which is expected to accelerate clinical application.

In terms of bioavailability, although small-molecule inhibitors (such as FB23-2) exhibit robust activity *in vitro*, their *in vivo* efficacy is often compromised by poor solubility, low metabolic stability, and insufficient tumor penetration. Encouragingly, Huang *et al*. [128] recently proposed a novel strategy to circumvent this bottleneck. They found that homoharringtonine, a clinically approved drug, does not directly inhibit FTO enzymatic activity; instead, it promotes FTO proteasomal degradation, thereby upregulating the global m6A level in acute monocytic leukemia.

Precise patient stratification remains a critical bottleneck. The functional roles of m6A regulators are highly context-dependent, showing significant variations across different cancer types and even subtypes [129]. Currently, there is still a lack of validated biomarkers for prediction of the treatment response or drug resistance, making it difficult to identify patients who would truly benefit from m6A-targeted therapies. The treatment of CRC is developing toward greater precision and individualization, and biomarker-driven trial design has become a focus of current research. Molecular biomarkers such as MSI/dMMR, BRAF, KRAS, and HER-2 have been widely used to guide treatment decisions, while the development of liquid biopsy technologies such as ctDNA has provided new tools for CRC management [130]. Therefore, combining m6A ex-

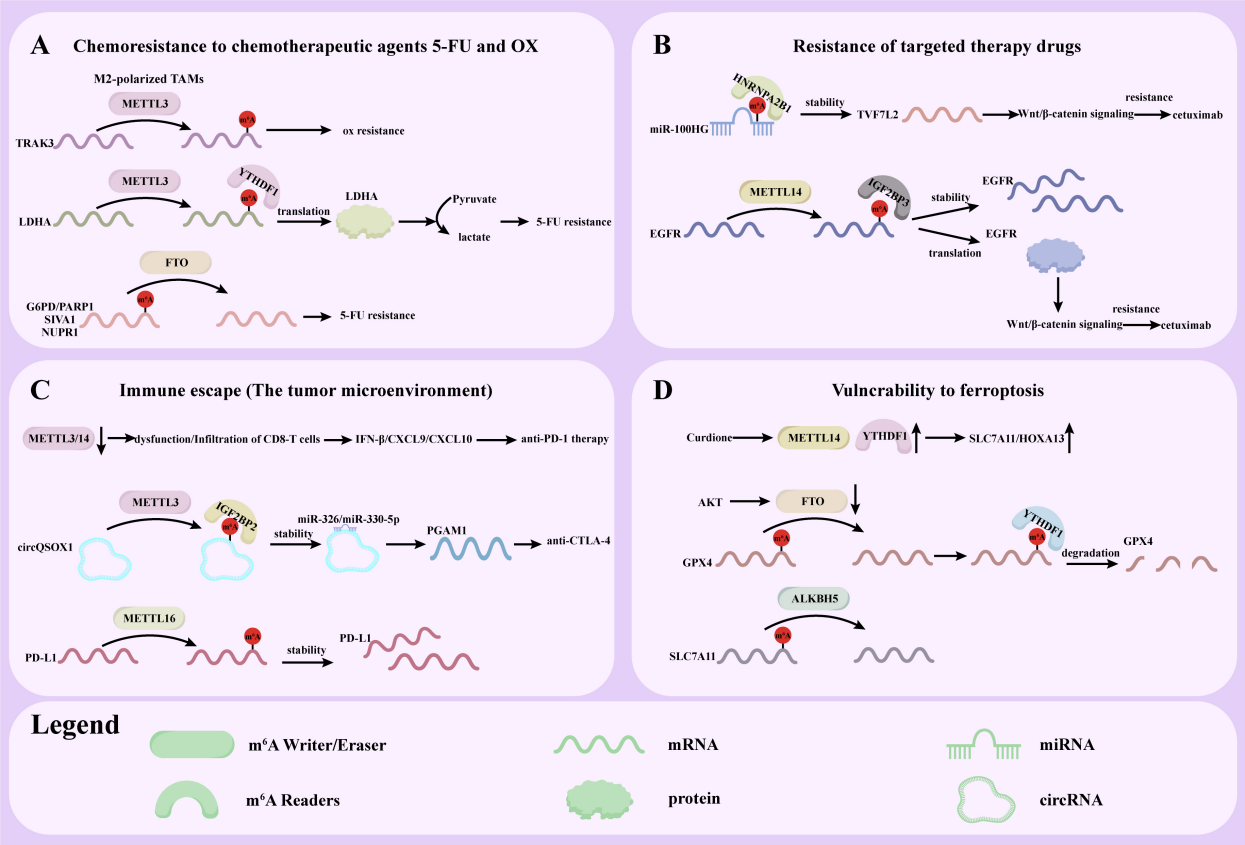

Fig. 3. Role of m6A modification in CRC drug resistance. (A) Chemoresistance to chemotherapeutic agents 5-FU and OX. (B) Resistance to targeted therapy drugs. (C) Immune escape (tumor microenvironment). (D) Vulnerability to ferroptosis.

pression profiles with these biomarkers may facilitate the clinical application of m6A modification.

Finally, in current CRC treatment strategies, single therapeutic approaches usually have certain limitations, and the optimization of combined therapies will become a future hotspot. Multiple strategies, including immunotherapy combinations, chemotherapy combined with targeted therapy, and novel ADC drugs, have shown significant efficacies [131]. For example, regarding microsatellite instability-high (MSI-H) and microsatellite stable (MSS) subtypes, a key research direction is to determine whether further combining PD-1 antibodies with chemotherapy plus bevacizumab can improve the efficacy. The complexity of the m6A regulatory network, its functional redundancy, and the dynamic evolution of the epitranscriptome under therapeutic pressure all make it necessary to develop combination therapies. For instance, YTHDF1-mediated chemoresistance is closely associated with glutamine metabolism, suggesting the potential of combining its inhibitors with GLS1 blockers. Similarly, the immunosuppressive microenvironment induced by METTL14 deficiency may be reversed through combination with PD-1 inhibitors. Nevertheless, combination regimens may also introduce new complexities regarding optimal dosage determination and additive toxicity [132].

In our view, the field of m6A research in CRC is at a critical juncture. While preclinical data are robust, clinical translation largely lags behind due to insufficient understanding of the dose-dependent effects and long-term safety. To successfully integrate m6A-targeted strategies into the therapeutic arsenal for CRC, these challenges must be addressed by developing more specific inhibitors, advanced drug delivery systems, and reliable predictive biomarkers, as well as by better understanding the biological mechanisms through which m6A regulates CRC.

5. Conclusions and Perspectives

Through the coordinated actions of methyltransferases, demethylases, and reader proteins, m6A modification dynamically regulates the metabolic processes of RNA. This process maintains the molecular equilibrium within cells under normal physiological conditions. The abnormal expression of m6A modification-related proteins is closely associated with the proliferation, migration, invasion, apoptosis, and drug resistance of CRC. In this review, we summarize the roles of several key m6A modification proteins in CRC. Their mechanisms involve the regulation of multiple key genes, signaling pathways, and specific biological processes of non-coding RNAs. It can be concluded that certain methyltransferases, demethylases, and reader pro-

teins can serve as potential biomarkers for the diagnosis of CRC and crucial targets for CRC treatment.

Despite the significant progress achieved in the research on m6A modification in CRC, numerous gaps still exist. In the future, in-depth research on the mechanism of m6A modification in CRC, especially its interactions with other epigenetic modifications and roles in the tumor microenvironment, holds great theoretical significance. Moreover, it is of great practical value to develop drugs targeting m6A modification-related proteins, establish m6A modification-based diagnostic and prognostic assessment models for CRC, and explore m6A modification-based immunotherapeutic strategies.

Notably, while existing evidence strongly indicates that m6A modification is closely associated with CRC drug resistance, there are still several challenges for the clinical translation. Future research must go beyond the single-gene approach and instead establish a clinically actionable integrated framework, specifically by combining m6A expression profiles with well-established biomarkers and developing real-time clinical m6A monitoring systems. Furthermore, although combinatorial strategies may overcome chemoresistance, they require rigorous safety assessment. These efforts will provide new theoretical basis and treatment approaches for the prevention and treatment of CRC, offering greater opportunities to improve the prognosis and enhance the life quality of CRC patients.

Author Contributions

MY, ZD, YH, YS, JW carried out the design, the acquisition, analysis and interpretation of the data; MY wrote the draft manuscript; YS, JW critically reviewed the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, we used ChatGpt to check spelling and grammar. After using this tool, we reviewed and edited the content as needed and took full responsibility for the content of the publication.

References

[1] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, *et al*. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a Cancer Journal for Clinicians. 2024; 74: 229–263. https://doi.org/10.3322/caac.21834.

[2] Qiao H, Liu L, Chen J, Shang B, Wang L. The functions of N6-methyladenosine (m6A) RNA modifications in colorectal cancer. Medical Oncology (Northwood, London, England). 2022; 39: 235. https://doi.org/10.1007/s12032-022-01827-4.

[3] Otani K, Kawai K, Hata K, Tanaka T, Nishikawa T, Sasaki K, *et al*. Colon cancer with perforation. Surgery Today. 2019; 49: 15–20. https://doi.org/10.1007/s00595-018-1661-8.

[4] Kanth P, Inadomi JM. Screening and prevention of colorectal cancer. BMJ (Clinical Research Ed.). 2021; 374: n1855. https://doi.org/10.1136/bmj.n1855.

[5] Jiang X, Jin Z, Yang Y, Zheng X, Chen S, Wang S, *et al*. m6A modification on the fate of colorectal cancer: functions and mechanisms of cell proliferation and tumorigenesis. Frontiers in Oncology. 2023; 13: 1162300. https://doi.org/10.3389/fonc.2023.1162300.

[6] Wang X, Wu R, Liu Y, Zhao Y, Bi Z, Yao Y, *et al*. m^{6}A mRNA methylation controls autophagy and adipogenesis by targeting *Atg5* and *Atg7*. Autophagy. 2020; 16: 1221–1235. https://doi.org/10.1080/15548627.2019.1659617.

[7] Zhang Y, Chen W, Zheng X, Guo Y, Cao J, Zhang Y, *et al*. Regulatory role and mechanism of m^{6}A RNA modification in human metabolic diseases. Molecular Therapy Oncolytics. 2021; 22: 52–63. https://doi.org/10.1016/j.omto.2021.05.003.

[8] Sun H, Li K, Liu C, Yi C. Regulation and functions of non-m^{6}A mRNA modifications. Nature Reviews. Molecular Cell Biology. 2023; 24: 714–731. https://doi.org/10.1038/s41580-023-00622-x.

[9] Jiang X, Liu B, Nie Z, Duan L, Xiong Q, Jin Z, *et al*. The role of m6A modification in the biological functions and diseases. Signal Transduction and Targeted Therapy. 2021; 6: 74. https://doi.org/10.1038/s41392-020-00450-x.

[10] Wang X, Feng J, Xue Y, Guan Z, Zhang D, Liu Z, *et al*. Structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex. Nature. 2016; 534: 575–578. https://doi.org/10.1038/nature18298.

[11] Ping XL, Sun BF, Wang L, Xiao W, Yang X, Wang WJ, *et al*. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Research. 2014; 24: 177–189. https://doi.org/10.1038/cr.2014.3.

[12] Warda AS, Kretschmer J, Hackert P, Lenz C, Urlaub H, Höbartner C, *et al*. Human METTL16 is a N^6-methyladenosine (m^{6}A) methyltransferase that targets pre-mRNAs and various non-coding RNAs. EMBO Reports. 2017; 18: 2004–2014. https://doi.org/10.15252/embr.201744940.

[13] Fu Y, Jia G, Pang X, Wang RN, Wang X, Li CJ, *et al*. FTO-mediated formation of N6-hydroxymethyladenosine and N6-formyladenosine in mammalian RNA. Nature Communications. 2013; 4: 1798. https://doi.org/10.1038/ncomms2822.

[14] Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, *et al*. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Molecular Cell. 2013; 49: 18–

29. https://doi.org/10.1016/j.molcel.2012.10.015.
[15] Sikorski V, Selberg S, Lalowski M, Karelson M, Kankuri E. The structure and function of YTHDF epitranscriptomic m^{6}A readers. Trends in Pharmacological Sciences. 2023; 44: 335–353. https://doi.org/10.1016/j.tips.2023.03.004.
[16] Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, *et al*. Recognition of RNA N^6-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. Nature Cell Biology. 2018; 20: 285–295. https://doi.org/10.1038/s41556-018-0045-z.
[17] Xie W, Zhu H, Zhao M, Wang L, Li S, Zhao C, *et al*. Crucial roles of different RNA-binding hnRNP proteins in Stem Cells. International Journal of Biological Sciences. 2021; 17: 807–817. https://doi.org/10.7150/ijbs.55120.
[18] Baek A, Rayhan A, Lee GE, Golconda S, Yu H, Kim S, *et al*. Mapping m^{6}A Sites on HIV-1 RNA Using Oligonucleotide LC-MS/MS. Methods and Protocols. 2024; 7: 7. https://doi.org/10.3390/mps7010007.
[19] Li H, Xie X, Liu X, Wu P, He J, Lin F, *et al*. Ultrasensitive Biosensors Detecting m^{6}A in Blood: Achieving Early Screening and Typing of Tumors. ACS Sensors. 2024; 9: 6011–6021. https://doi.org/10.1021/acssensors.4c01875.
[20] Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, *et al*. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature. 2012; 485: 201–206. https://doi.org/10.1038/nature11112.
[21] Linder B, Grozhik AV, Olarerin-George AO, Meydan C, Mason CE, Jaffrey SR. Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nature Methods. 2015; 12: 767–772. https://doi.org/10.1038/nmeth.3453.
[22] Meyer KD. DART-seq: an antibody-free method for global m^{6}A detection. Nature Methods. 2019; 16: 1275–1280. https://doi.org/10.1038/s41592-019-0570-0.
[23] Garcia-Campos MA, Edelheit S, Toth U, Safra M, Shachar R, Viukov S, *et al*. Deciphering the "m^{6}A Code" via Antibody-Independent Quantitative Profiling. Cell. 2019; 178: 731–747.e16. https://doi.org/10.1016/j.cell.2019.06.013.
[24] Wang Y, Xiao Y, Dong S, Yu Q, Jia G. Antibody-free enzyme-assisted chemical approach for detection of N^6-methyladenosine. Nature Chemical Biology. 2020; 16: 896–903. https://doi.org/10.1038/s41589-020-0525-x.
[25] Ge R, Ye C, Peng Y, Dai Q, Zhao Y, Liu S, *et al*. m^{6}A-SAC-seq for quantitative whole transcriptome m^{6}A profiling. Nature Protocols. 2023; 18: 626–657. https://doi.org/10.1038/s41596-022-00765-9.
[26] Workman RE, Tang AD, Tang PS, Jain M, Tyson JR, Razaghi R, *et al*. Nanopore native RNA sequencing of a human poly(A) transcriptome. Nature Methods. 2019; 16: 1297–1305. https://doi.org/10.1038/s41592-019-0617-2.
[27] Sun K, Li J, Chen C, Zhou X, Ma G, Mao L, *et al*. Advances in nanopore direct RNA sequencing and its impact on biological research. Biotechnology Advances. 2025; 85: 108710. https://doi.org/10.1016/j.biotechadv.2025.108710.
[28] Huo XS, Lu D, Chen DG, Ye M, Wang XW, Shang FS. METTL3 Promotes Osteosarcoma Metastasis via an m6A-dependent Epigenetic Activity of CBX4. Frontiers in Bioscience (Landmark Edition). 2024; 29: 120. https://doi.org/10.31083/j.fbl2903120.
[29] Sendinc E, Shi Y. RNA m6A methylation across the transcriptome. Molecular Cell. 2023; 83: 428–441. https://doi.org/10.1016/j.molcel.2023.01.006.
[30] Deng X, Qing Y, Horne D, Huang H, Chen J. The roles and implications of RNA m^{6}A modification in cancer. Nature Reviews. Clinical Oncology. 2023; 20: 507–526. https://doi.org/10.1038/s41571-023-00774-x.
[31] Wang Y, Wang Y, Patel H, Chen J, Wang J, Chen ZS, *et al*. Epigenetic modification of m^{6}A regulator proteins in cancer. Molecular Cancer. 2023; 22: 102. https://doi.org/10.1186/s12943-023-01810-1.
[32] Sun W, Su Y, Zhang Z. Characterizing m6A modification factors and their interactions in colorectal cancer: implications for tumor subtypes and clinical outcomes. Discover Oncology. 2024; 15: 457. https://doi.org/10.1007/s12672-024-01298-1.
[33] Li W, Gao Y, Jin X, Wang H, Lan T, Wei M, *et al*. Comprehensive analysis of N6-methylandenosine regulators and m6A-related RNAs as prognosis factors in colorectal cancer. Molecular Therapy. Nucleic Acids. 2022; 27: 598–610. https://doi.org/10.1016/j.omtn.2021.12.007.
[34] Zhang Z, Wang Q, Zhang M, Zhang W, Zhao L, Yang C, *et al*. Comprehensive analysis of the transcriptome-wide m6A methylome in colorectal cancer by MeRIP sequencing. Epigenetics. 2021; 16: 425–435. https://doi.org/10.1080/15592294.2020.1805684.
[35] Wang S, Fan X, Zhu J, Xu D, Li R, Chen R, *et al*. The differentiation of colorectal cancer is closely relevant to m6A modification. Biochemical and Biophysical Research Communications. 2021; 546: 65–73. https://doi.org/10.1016/j.bbrc.2021.02.001.
[36] Chen H, Yao J, Bao R, Dong Y, Zhang T, Du Y, *et al*. Cross-talk of four types of RNA modification writers defines tumor microenvironment and pharmacogenomic landscape in colorectal cancer. Molecular Cancer. 2021; 20: 29. https://doi.org/10.1186/s12943-021-01322-w.
[37] Huang L, Zhu J, Kong W, Li P, Zhu S. Expression and Prognostic Characteristics of m6A RNA Methylation Regulators in Colon Cancer. International Journal of Molecular Sciences. 2021; 22: 2134. https://doi.org/10.3390/ijms22042134.
[38] Zheng Y, Wang Y, Liu Y, Xie L, Ge J, Yu G, *et al*. N6-Methyladenosine Modification of PTTG3P Contributes to Colorectal Cancer Proliferation *via* YAP1. Frontiers in Oncology. 2021; 11: 669731. https://doi.org/10.3389/fonc.2021.669731.
[39] Chen X, Xu M, Xu X, Zeng K, Liu X, Pan B, *et al*. METTL14-mediated N6-methyladenosine modification of SOX4 mRNA inhibits tumor metastasis in colorectal cancer. Molecular Cancer. 2020; 19: 106. https://doi.org/10.1186/s12943-020-01220-7.
[40] Lin Z, Wan AH, Sun L, Liang H, Niu Y, Deng Y, *et al*. N6-methyladenosine demethylase FTO enhances chemo-resistance in colorectal cancer through SIVA1-mediated apoptosis. Molecular Therapy: the Journal of the American Society of Gene Therapy. 2023; 31: 517–534. https://doi.org/10.1016/j.ymthe.2022.10.012.
[41] Zhang Z, Gao Q, Wang S. Kinase GSK3β functions as a suppressor in colorectal carcinoma through the FTO-mediated MZF1/c-Myc axis. Journal of Cellular and Molecular Medicine. 2021; 25: 2655–2665. https://doi.org/10.1111/jcmm.16291.
[42] Zhang Z, Wang L, Zhao L, Wang Q, Yang C, Zhang M, *et al*. N6-methyladenosine demethylase ALKBH5 suppresses colorectal cancer progression potentially by decreasing PHF20 mRNA methylation. Clinical and Translational Medicine. 2022; 12: e940. https://doi.org/10.1002/ctm2.940.
[43] Zhai J, Chen H, Wong CC, Peng Y, Gou H, Zhang J, *et al*. ALKBH5 Drives Immune Suppression Via Targeting AXIN2 to Promote Colorectal Cancer and Is a Target for Boosting Immunotherapy. Gastroenterology. 2023; 165: 445–462. https://doi.org/10.1053/j.gastro.2023.04.032.
[44] Xiang S, Liang X, Yin S, Liu J, Xiang Z. N6-methyladenosine methyltransferase METTL3 promotes colorectal cancer cell proliferation through enhancing MYC expression. American Journal of Translational Research. 2020; 12: 1789–1806.
[45] Li T, Hu PS, Zuo Z, Lin JF, Li X, Wu QN, *et al*. METTL3 facilitates tumor progression via an m^{6}A-IGF2BP2-dependent mechanism in colorectal carcinoma. Molecular Cancer. 2019; 18: 112. https://doi.org/10.1186/s12943-019-1038-7.
[46] Zhu W, Si Y, Xu J, Lin Y, Wang JZ, Cao M, *et al*. Methyl-

transferase like 3 promotes colorectal cancer proliferation by stabilizing CCNE1 mRNA in an m6A-dependent manner. Journal of Cellular and Molecular Medicine. 2020; 24: 3521–3533. https://doi.org/10.1111/jcmm.15042.

[47] Zhou D, Tang W, Xu Y, Xu Y, Xu B, Fu S, *et al*. METTL3/YTHDF2 m6A axis accelerates colorectal carcinogenesis through epigenetically suppressing YPEL5. Molecular Oncology. 2021; 15: 2172–2184. https://doi.org/10.1002/1878-0261.12898.

[48] Yi J, Peng F, Zhao J, Gong X. METTL3/IGF2BP2 axis affects the progression of colorectal cancer by regulating m6A modification of STAG3. Scientific Reports. 2023; 13: 17292. https://doi.org/10.1038/s41598-023-44379-x.

[49] Wang S, Gan M, Chen C, Zhang Y, Kong J, Zhang H, *et al*. Methyl CpG binding protein 2 promotes colorectal cancer metastasis by regulating N^6 -methyladenosine methylation through methyltransferase-like 14. Cancer Science. 2021; 112: 3243–3254. https://doi.org/10.1111/cas.15011.

[50] Zhou C, Wang M, Du X, Xue L, Zhu X, Li X, *et al*. WTAP/IGF2BP3 Mediated m6A Modification of SOD2 mRNA Aggravates the Tumourigenesis of Colorectal Cancer. Journal of Biochemical and Molecular Toxicology. 2025; 39: e70117. https://doi.org/10.1002/jbt.70117.

[51] Ma L, Lin Y, Sun SW, Xu J, Yu T, Chen WL, *et al*. KIAA1429 is a potential prognostic marker in colorectal cancer by promoting the proliferation via downregulating WEE1 expression in an m6A-independent manner. Oncogene. 2022; 41: 692–703. https://doi.org/10.1038/s41388-021-02066-z.

[52] Zhou Y, Pei Z, Maimaiti A, Zheng L, Zhu Z, Tian M, *et al*. m^6A methyltransferase KIAA1429 acts as an oncogenic factor in colorectal cancer by regulating SIRT1 in an m^6A-dependent manner. Cell Death Discovery. 2022; 8: 83. https://doi.org/10.1038/s41420-022-00878-w.

[53] Zhang H, Li Y, Zhou Y, Xu Q, Liao B, Qiu X, *et al*. RNA methylase RBM15 facilitates malignant progression of colorectal cancer through regulating E2F2 in an m6A modification-dependent manner. Journal of Biochemical and Molecular Toxicology. 2024; 38: e70014. https://doi.org/10.1002/jbt.70014.

[54] Shen D, Lin J, Xie Y, Zhuang Z, Xu G, Peng S, *et al*. RNA demethylase ALKBH5 promotes colorectal cancer progression by posttranscriptional activation of RAB5A in an m6A-YTHDF2-dependent manner. Clinical and Translational Medicine. 2023; 13: e1279. https://doi.org/10.1002/ctm2.1279.

[55] Wu H, Chu F, Li L, Li K, Xiao X, Zhang L, *et al*. Up-Regulation of SH3TC2 Induced by YTHDF1 Predicts Poor Outcome and Facilitates Cell-Cycle Progress in Colorectal Cancer. Journal of Oncology. 2022; 2022: 1600611. https://doi.org/10.1155/2022/1600611.

[56] Yang Z, Wang T, Wu D, Min Z, Tan J, Yu B. RNA N6-methyladenosine reader IGF2BP3 regulates cell cycle and angiogenesis in colon cancer. Journal of Experimental & Clinical Cancer Research: CR. 2020; 39: 203. https://doi.org/10.1186/s13046-020-01714-8.

[57] Zhang FY, Wu L, Zhang TN, Chen HH. KCTD15 acts as an anti-tumor factor in colorectal cancer cells downstream of the demethylase FTO and the m6A reader YTHDF2. Communications Biology. 2024; 7: 262. https://doi.org/10.1038/s42003-024-05880-9.

[58] Bian Y, Wang Y, Xu S, Gao Z, Li C, Fan Z, *et al*. m^6A Modification of Long Non-Coding RNA HNF1A-AS1 Facilitates Cell Cycle Progression in Colorectal Cancer via IGF2BP2-Mediated CCND1 mRNA Stabilization. Cells. 2022; 11: 3008. https://doi.org/10.3390/cells11193008.

[59] Pan J, Liu F, Xiao X, Xu R, Dai L, Zhu M, *et al*. METTL3 promotes colorectal carcinoma progression by regulating the m6A-CRB3-Hippo axis. Journal of Experimental & Clinical Cancer Research: CR. 2022; 41: 19. https://doi.org/10.1186/s13046-021-02227-8.

[60] Yu T, Liu J, Wang Y, Chen W, Liu Z, Zhu L, *et al*. METTL3 promotes colorectal cancer metastasis by stabilizing PLAU mRNA in an m6A-dependent manner. Biochemical and Biophysical Research Communications. 2022; 614: 9–16. https://doi.org/10.1016/j.bbrc.2022.04.141.

[61] Liu X, He H, Zhang F, Hu X, Bi F, Li K, *et al*. m6A methylated EphA2 and VEGFA through IGF2BP2/3 regulation promotes vasculogenic mimicry in colorectal cancer via PI3K/AKT and ERK1/2 signaling. Cell Death & Disease. 2022; 13: 483. https://doi.org/10.1038/s41419-022-04950-2.

[62] Sun Y, Gong W, Zhang S. METTL3 promotes colorectal cancer progression through activating JAK1/STAT3 signaling pathway. Cell Death & Disease. 2023; 14: 765. https://doi.org/10.1038/s41419-023-06287-w.

[63] Deng R, Cheng Y, Ye S, Zhang J, Huang R, Li P, *et al*. m^6A methyltransferase METTL3 suppresses colorectal cancer proliferation and migration through p38/ERK pathways. OncoTargets and Therapy. 2019; 12: 4391–4402. https://doi.org/10.2147/OTT.S201052.

[64] Ning Z, Wu Z, Zhang F, Yang M, Lu Z, Yu B, *et al*. GMEB2 Promotes the Growth of Colorectal Cancer by Activating ADRM1 Transcription and NF-κB Signalling and Is Positively Regulated by the m^6A Reader YTHDF1. Cancers. 2022; 14: 6046. https://doi.org/10.3390/cancers14246046.

[65] Han B, Yan S, Wei S, Xiang J, Liu K, Chen Z, *et al*. YTHDF1-mediated translation amplifies Wnt-driven intestinal stemness. EMBO Reports. 2020; 21: e49229. https://doi.org/10.15252/embr.201949229.

[66] Wang S, Gao S, Zeng Y, Zhu L, Mo Y, Wong CC, *et al*. N6-Methyladenosine Reader YTHDF1 Promotes ARHGEF2 Translation and RhoA Signaling in Colorectal Cancer. Gastroenterology. 2022; 162: 1183–1196. https://doi.org/10.1053/j.gastro.2021.12.269.

[67] Li H, Zhang N, Jiao X, Wang C, Sun W, He Y, *et al*. Downregulation of microRNA-6125 promotes colorectal cancer growth through YTHDF2-dependent recognition of N6-methyladenosine-modified GSK3β. Clinical and Translational Medicine. 2021; 11: e602. https://doi.org/10.1002/ctm2.602.

[68] Zhou L, Jiang J, Huang Z, Jin P, Peng L, Luo M, *et al*. Hypoxia-induced lncRNA STEAP3-AS1 activates Wnt/β-catenin signaling to promote colorectal cancer progression by preventing m^6A-mediated degradation of STEAP3 mRNA. Molecular Cancer. 2022; 21: 168. https://doi.org/10.1186/s12943-022-01638-1.

[69] Jiang T, Qi J, Xue Z, Liu B, Liu J, Hu Q, *et al*. The m^6A modification mediated-lncRNA POU6F2-AS1 reprograms fatty acid metabolism and facilitates the growth of colorectal cancer via upregulation of FASN. Molecular Cancer. 2024; 23: 55. https://doi.org/10.1186/s12943-024-01962-8.

[70] Lu D, Chen A. lncRNA POU6F2-AS1 Regulated by KIAA1429 Contributes to Colorectal Cancer Progression in an m^6A Modification Manner. Molecular Biotechnology. 2025; 67: 115–122. https://doi.org/10.1007/s12033-023-00986-7.

[71] Zhang H, Han Y, Wu C, Wang S, Chen M, Xu Q, *et al*. m6A-modified LINC02418 induces transcriptional and post-transcriptional modification of CTNNB1 via interacting with YBX1 and IGF2BP1 in colorectal cancer. Cell Death Discovery. 2025; 11: 101. https://doi.org/10.1038/s41420-025-02365-4.

[72] Luo XJ, Lu YX, Wang Y, Huang R, Liu J, Jin Y, *et al*. M6A-modified lncRNA FAM83H-AS1 promotes colorectal cancer progression through PTBP1. Cancer Letters. 2024; 598: 217085. https://doi.org/10.1016/j.canlet.2024.217085.

[73] Bian Y, Xu S, Gao Z, Ding J, Li C, Cui Z, *et al*. m^6A modification of lncRNA ABHD11-AS1 promotes colorectal cancer

progression and inhibits ferroptosis through TRIM21/IGF2BP2/FOXM1 positive feedback loop. Cancer Letters. 2024; 596: 217004. https://doi.org/10.1016/j.canlet.2024.217004.

[74] Yang X, Zhang S, He C, Xue P, Zhang L, He Z, *et al*. METTL14 suppresses proliferation and metastasis of colorectal cancer by down-regulating oncogenic long non-coding RNA XIST. Molecular Cancer. 2020; 19: 46. https://doi.org/10.1186/s12943-020-1146-4.

[75] Chen K, Zhang J, Meng L, Kong L, Lu M, Wang Z, *et al*. The epigenetic downregulation of LncGHRLOS mediated by RNA m6A methylase ZCCHC4 promotes colorectal cancer tumorigenesis. Journal of Experimental & Clinical Cancer Research: CR. 2024; 43: 44. https://doi.org/10.1186/s13046-024-02965-5.

[76] Guo T, Liu DF, Peng SH, Xu AM. ALKBH5 promotes colon cancer progression by decreasing methylation of the lncRNA NEAT1. American Journal of Translational Research. 2020; 12: 4542–4549.

[77] Liu N, Jiang X, Zhang G, Long S, Li J, Jiang M, *et al*. LncRNA CARMN m6A demethylation by ALKBH5 inhibits mutant p53-driven tumour progression through miR-5683/FGF2. Clinical and Translational Medicine. 2024; 14: e1777. https://doi.org/10.1002/ctm2.1777.

[78] Chen B, Hong Y, Gui R, Zheng H, Tian S, Zhai X, *et al*. N6-methyladenosine modification of circ_0003215 suppresses the pentose phosphate pathway and malignancy of colorectal cancer through the miR-663b/DLG4/G6PD axis. Cell Death & Disease. 2022; 13: 804. https://doi.org/10.1038/s41419-022-05245-2.

[79] Zeng W, Zhu JF, Guo J, Huang GJ, Ai LS, Zeng Y, *et al*. m^6A-modified circFNDC3B inhibits colorectal cancer stemness and metastasis via RNF41-dependent ASB6 degradation. Cell Death & Disease. 2022; 13: 1008. https://doi.org/10.1038/s41419-022-05451-y.

[80] Yao B, Zhang Q, Yang Z, An F, Nie H, Wang H, *et al*. CircEZH2/miR-133b/IGF2BP2 aggravates colorectal cancer progression via enhancing the stability of m^6A-modified CREB1 mRNA. Molecular Cancer. 2022; 21: 140. https://doi.org/10.1186/s12943-022-01608-7.

[81] Zeng K, Peng J, Xing Y, Zhang L, Zeng P, Li W, *et al*. A positive feedback circuit driven by m^6A-modified circular RNA facilitates colorectal cancer liver metastasis. Molecular Cancer. 2023; 22: 202. https://doi.org/10.1186/s12943-023-01848-1.

[82] Wu X, Wang S, Pan Y, Li M, Song M, Zhang H, *et al*. m^6A Reader PRRC2A Promotes Colorectal Cancer Progression via CK1ε-Mediated Activation of Wnt and YAP Signaling Pathways. Advanced Science (Weinheim, Baden-Wurttemberg, Germany). 2025; 12: e2406935. https://doi.org/10.1002/advs.202406935.

[83] Shen C, Xuan B, Yan T, Ma Y, Xu P, Tian X, *et al*. m^6A-dependent glycolysis enhances colorectal cancer progression. Molecular Cancer. 2020; 19: 72. https://doi.org/10.1186/s12943-020-01190-w.

[84] He M, Jiang D, Xun A, Yang J, Luo Q, Wu H. Methyltransferase like 3 enhances pinin mRNA stability through N^6 -methyladenosine modification to augment tumourigenesis of colon adenocarcinoma. Experimental Physiology. 2022; 107: 1283–1297. https://doi.org/10.1113/EP090273.

[85] Li Y, He L, Wang Y, Tan Y, Zhang F. N^6-methyladenosine methyltransferase KIAA1429 elevates colorectal cancer aerobic glycolysis via HK2-dependent manner. Bioengineered. 2022; 13: 11923–11932. https://doi.org/10.1080/21655979.2022.2065952.

[86] Hou Y, Zhang X, Yao H, Hou L, Zhang Q, Tao E, *et al*. METTL14 modulates glycolysis to inhibit colorectal tumorigenesis in p53-wild-type cells. EMBO Reports. 2023; 24: e56325. https://doi.org/10.15252/embr.202256325.

[87] Wu S, Yun J, Tang W, Familiari G, Relucenti M, Wu J, *et al*. Therapeutic m^6A Eraser ALKBH5 mRNA-Loaded Exosome-Liposome Hybrid Nanoparticles Inhibit Progression of Colorectal Cancer in Preclinical Tumor Models. ACS Nano. 2023; 17: 11838–11854. https://doi.org/10.1021/acsnano.3c03050.

[88] Ouyang P, Li K, Xu W, Chen C, Shi Y, Tian Y, *et al*. METTL3 recruiting M2-type immunosuppressed macrophages by targeting m6A-SNAIL-CXCL2 axis to promote colorectal cancer pulmonary metastasis. Journal of Experimental & Clinical Cancer Research: CR. 2024; 43: 111. https://doi.org/10.1186/s13046-024-03035-6.

[89] Hou P, Meng S, Li M, Lin T, Chu S, Li Z, *et al*. LINC00460/DHX9/IGF2BP2 complex promotes colorectal cancer proliferation and metastasis by mediating HMGA1 mRNA stability depending on m6A modification. Journal of Experimental & Clinical Cancer Research: CR. 2021; 40: 52. https://doi.org/10.1186/s13046-021-01857-2.

[90] Chen RX, Chen X, Xia LP, Zhang JX, Pan ZZ, Ma XD, *et al*. N^6-methyladenosine modification of circNSUN2 facilitates cytoplasmic export and stabilizes HMGA2 to promote colorectal liver metastasis. Nature Communications. 2019; 10: 4695. https://doi.org/10.1038/s41467-019-12651-2.

[91] Wang X, Liu Y, Zhou M, Yu L, Si Z. m6A modified BACE1-AS contributes to liver metastasis and stemness-like properties in colorectal cancer through TUFT1 dependent activation of Wnt signaling. Journal of Experimental & Clinical Cancer Research: CR. 2023; 42: 306. https://doi.org/10.1186/s13046-023-02881-0.

[92] Ye M, Chen J, Yu P, Hu C, Wang B, Bao J, *et al*. WTAP activates MAPK signaling through m6A methylation in VEGFA mRNA-mediated by YTHDC1 to promote colorectal cancer development. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2023; 37: e23090. https://doi.org/10.1096/fj.202300344RRR.

[93] Han S, Zhu L, Zhu Y, Meng Y, Li J, Song P, *et al*. Targeting ATF4-dependent pro-survival autophagy to synergize glutaminolysis inhibition. Theranostics. 2021; 11: 8464–8479. https://doi.org/10.7150/thno.60028.

[94] Yue SW, Liu HL, Su HF, Luo C, Liang HF, Zhang BX, *et al*. m6A-regulated tumor glycolysis: new advances in epigenetics and metabolism. Molecular Cancer. 2023; 22: 137. https://doi.org/10.1186/s12943-023-01841-8.

[95] Wang L, Zhao L, Liu J, Cheng P, Han M, Zheng Z. lncSLERT Promotes Liver Metastasis in Colorectal Cancer by Down-Regulating HUNK Expression via RBM15-Mediated m6A Modification. OncoTargets and Therapy. 2025; 18: 631–646. https://doi.org/10.2147/OTT.S514001.

[96] Ciardiello F, Ciardiello D, Martini G, Napolitano S, Tabernero J, Cervantes A. Clinical management of metastatic colorectal cancer in the era of precision medicine. CA: a Cancer Journal for Clinicians. 2022; 72: 372–401. https://doi.org/10.3322/caac.21728.

[97] Zeng K, Li W, Wang Y, Zhang Z, Zhang L, Zhang W, *et al*. Inhibition of CDK1 Overcomes Oxaliplatin Resistance by Regulating ACSL4-mediated Ferroptosis in Colorectal Cancer. Advanced Science (Weinheim, Baden-Wurttemberg, Germany). 2023; 10: e2301088. https://doi.org/10.1002/advs.202301088.

[98] Lu J, Wu J, Mao L, Xu H, Wang S. Revisiting PD-1/PD-L pathway in T and B cell response: Beyond immunosuppression. Cytokine & Growth Factor Reviews. 2022; 67: 58–65. https://doi.org/10.1016/j.cytogfr.2022.07.003.

[99] Fan A, Wang B, Wang X, Nie Y, Fan D, Zhao X, *et al*. Immunotherapy in colorectal cancer: current achievements and future perspective. International Journal of Biological Sciences. 2021; 17: 3837–3849. https://doi.org/10.7150/ijbs.64077.

[100] Xie YH, Chen YX, Fang JY. Comprehensive review of targeted therapy for colorectal cancer. Signal Transduction

and Targeted Therapy. 2020; 5: 22. https://doi.org/10.1038/s41392-020-0116-z.

[101] Li R, Liang M, Liang X, Yang L, Su M, Lai KP. Chemotherapeutic Effectiveness of Combining Cetuximab for Metastatic Colorectal Cancer Treatment: A System Review and Meta-Analysis. Frontiers in Oncology. 2020; 10: 868. https://doi.org/10.3389/fonc.2020.00868.

[102] Biller LH, Schrag D. Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. JAMA. 2021; 325: 669–685. https://doi.org/10.1001/jama.2021.0106.

[103] Lan H, Liu Y, Liu J, Wang X, Guan Z, Du J, *et al*. Tumor-Associated Macrophages Promote Oxaliplatin Resistance *via* METTL3-Mediated m^{6}A of TRAF5 and Necroptosis in Colorectal Cancer. Molecular Pharmaceutics. 2021; 18: 1026–1037. https://doi.org/10.1021/acs.molpharmaceut.0c00961.

[104] Zhang K, Zhang T, Yang Y, Tu W, Huang H, Wang Y, *et al*. N^6-methyladenosine-mediated LDHA induction potentiates chemoresistance of colorectal cancer cells through metabolic reprogramming. Theranostics. 2022; 12: 4802–4817. https://doi.org/10.7150/thno.73746.

[105] Li M, Xia M, Zhang Z, Tan Y, Li E, Guo Z, *et al*. METTL3 antagonizes 5 FU chemotherapy and confers drug resistance in colorectal carcinoma. International Journal of Oncology. 2023; 63: 101. https://doi.org/10.3892/ijo.2023.5549.

[106] Xu C, Shen T, Feng L, Wang L, Li S, Ding R, *et al*. FTO facilitates colorectal cancer chemoresistance via regulation of NUPR1-dependent iron homeostasis. Redox Biology. 2025; 83: 103647. https://doi.org/10.1016/j.redox.2025.103647.

[107] Wang J, Qiao Y, Sun M, Sun H, Xie F, Chang H, *et al*. FTO promotes colorectal cancer progression and chemotherapy resistance via demethylating G6PD/PARP1. Clinical and Translational Medicine. 2022; 12: e772. https://doi.org/10.1002/ctm2.772.

[108] Liu X, Su K, Sun X, Jiang Y, Wang L, Hu C, *et al*. Sec62 promotes stemness and chemoresistance of human colorectal cancer through activating Wnt/β-catenin pathway. Journal of Experimental & Clinical Cancer Research: CR. 2021; 40: 132. https://doi.org/10.1186/s13046-021-01934-6.

[109] Chen P, Liu XQ, Lin X, Gao LY, Zhang S, Huang X. Targeting YTHDF1 effectively re-sensitizes cisplatin-resistant colon cancer cells by modulating GLS-mediated glutamine metabolism. Molecular Therapy Oncolytics. 2021; 20: 228–239. https://doi.org/10.1016/j.omto.2021.01.001.

[110] Zhao Y, Zhao H, Zhang D, Quan Q, Ge Y, Li L, *et al*. YTHDF3 Facilitates eIF2AK2 and eIF3A Recruitment on mRNAs to Regulate Translational Processes in Oxaliplatin-Resistant Colorectal Cancer. ACS Chemical Biology. 2022; 17: 1778–1788. https://doi.org/10.1021/acschembio.2c00131.

[111] Yang Z, Zhao F, Gu X, Feng L, Xu M, Li T, *et al*. Binding of RNA m6A by IGF2BP3 triggers chemoresistance of HCT8 cells via upregulation of ABCB1. American Journal of Cancer Research. 2021; 11: 1428–1445.

[112] Zhou Y, Zhou H, Shi J, Guan A, Zhu Y, Hou Z, *et al*. Decreased m6A Modification of CD34/CD276(B7-H3) Leads to Immune Escape in Colon Cancer. Frontiers in Cell and Developmental Biology. 2021; 9: 715674. https://doi.org/10.3389/fcell.2021.715674.

[113] Wang L, Hui H, Agrawal K, Kang Y, Li N, Tang R, *et al*. m^6 A RNA methyltransferases METTL3/14 regulate immune responses to anti-PD-1 therapy. The EMBO Journal. 2020; 39: e104514. https://doi.org/10.15252/embj.2020104514.

[114] Liu Z, Zheng N, Li J, Li C, Zheng D, Jiang X, *et al*. N6-methyladenosine-modified circular RNA QSOX1 promotes colorectal cancer resistance to anti-CTLA-4 therapy through induction of intratumoral regulatory T cells. Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy. 2022; 65: 100886. https://doi.org/10.1016/j.drup.2022.100886.

[115] Wang A, Sun Y, Wang X, Yan Z, Wang D, Zeng L, *et al*. m^{6}A methyltransferase METTL16 mediates immune evasion of colorectal cancer cells via epigenetically regulating PD-L1 expression. Aging. 2023; 15: 8444–8457. https://doi.org/10.18632/aging.204980.

[116] Liu H, Li D, Sun L, Qin H, Fan A, Meng L, *et al*. Interaction of lncRNA MIR100HG with hnRNPA2B1 facilitates m^{6}A-dependent stabilization of TCF7L2 mRNA and colorectal cancer progression. Molecular Cancer. 2022; 21: 74. https://doi.org/10.1186/s12943-022-01555-3.

[117] Chen LJ, Liu HY, Xiao ZY, Qiu T, Zhang D, Zhang LJ, *et al*. IGF2BP3 promotes the progression of colorectal cancer and mediates cetuximab resistance by stabilizing EGFR mRNA in an m^{6}A-dependent manner. Cell Death & Disease. 2023; 14: 581. https://doi.org/10.1038/s41419-023-06099-y.

[118] Hangauer MJ, Viswanathan VS, Ryan MJ, Bole D, Eaton JK, Matov A, *et al*. Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. Nature. 2017; 551: 247–250. https://doi.org/10.1038/nature24297.

[119] Wang F, Sun Z, Zhang Q, Yang H, Yang G, Yang Q, *et al*. Curdione induces ferroptosis mediated by m6A methylation via METTL14 and YTHDF2 in colorectal cancer. Chinese Medicine. 2023; 18: 122. https://doi.org/10.1186/s13020-023-00820-x.

[120] Zhang G, Mi W, Wang C, Li J, Zhang Y, Liu N, *et al*. Targeting AKT induced Ferroptosis through FTO/YTHDF2-dependent GPX4 m6A methylation up-regulating and degradating in colorectal cancer. Cell Death Discovery. 2023; 9: 457. https://doi.org/10.1038/s41420-023-01746-x.

[121] Luo J, Yu H, Yuan Z, Ye T, Hu B. ALKBH5 decreases SLC7A11 expression by erasing m6A modification and promotes the ferroptosis of colorectal cancer cells. Clinical & Translational Oncology: Official Publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico. 2023; 25: 2265–2276. https://doi.org/10.1007/s12094-023-03116-6.

[122] Feng G, Wu Y, Hu Y, Shuai W, Yang X, Li Y, *et al*. Small molecule inhibitors targeting m^{6}A regulators. Journal of Hematology & Oncology. 2024; 17: 30. https://doi.org/10.1186/s13045-024-01546-5.

[123] Yu Y, Hai Y, Zhou H, Bao W, Hu X, Gao Y, *et al*. METTL3 Inhibition Suppresses Cell Growth and Survival in Colorectal Cancer via ASNS Downregulation. Journal of Cancer. 2024; 15: 4853–4865. https://doi.org/10.7150/jca.96760.

[124] Huang Y, Su R, Sheng Y, Dong L, Dong Z, Xu H, *et al*. Small-Molecule Targeting of Oncogenic FTO Demethylase in Acute Myeloid Leukemia. Cancer Cell. 2019; 35: 677–691.e10. https://doi.org/10.1016/j.ccell.2019.03.006.

[125] Liu L, Zhao T, Zheng S, Tang D, Han H, Yang C, *et al*. METTL3 inhibitor STM2457 impairs tumor progression and enhances sensitivity to anlotinib in OSCC. Oral Diseases. 2024; 30: 4243–4254. https://doi.org/10.1111/odi.14864.

[126] Xu Y, Zhou Z, Kang X, Pan L, Liu C, Liang X, *et al*. Mettl3-mediated mRNA m^{6}A modification controls postnatal liver development by modulating the transcription factor Hnf4a. Nature Communications. 2022; 13: 4555. https://doi.org/10.1038/s41467-022-32169-4.

[127] Mapperley C, van de Lagemaat LN, Lawson H, Tavosanis A, Paris J, Campos J, *et al*. The mRNA m6A reader YTHDF2 suppresses proinflammatory pathways and sustains hematopoietic stem cell function. The Journal of Experimental Medicine. 2021; 218: e20200829. https://doi.org/10.1084/jem.20200829.

[128] Huang F, Luo X, Zhang M, Jin L, Sun W, Chen P, *et al*. Homoharringtonine Promotes FTO Degradation to Suppress LILRB4-

Mediated Immune Evasion in Acute Monocytic Leukaemia. Cell Proliferation. 2025; e70090. https://doi.org/10.1111/cpr.70090.

[129] Hua X, Xu Q, Wu R, Sun W, Gu Y, Zhu S, *et al*. ALKBH5 promotes non-small cell lung cancer progression and susceptibility to anti-PD-L1 therapy by modulating interactions between tumor and macrophages. Journal of Experimental & Clinical Cancer Research: CR. 2024; 43: 164. https://doi.org/10.1186/s13046-024-03073-0.

[130] Sveen A, Kopetz S, Lothe RA. Biomarker-guided therapy for colorectal cancer: strength in complexity. Nature Reviews. Clinical Oncology. 2020; 17: 11–32. https://doi.org/10.1038/s41571-019-0241-1.

[131] Singh M, Morris VK, Bandey IN, Hong DS, Kopetz S. Advancements in combining targeted therapy and immunotherapy for colorectal cancer. Trends in Cancer. 2024; 10: 598–609. https://doi.org/10.1016/j.trecan.2024.05.001.

[132] Zhuang H, Yu B, Tao D, Xu X, Xu Y, Wang J, *et al*. The role of m6A methylation in therapy resistance in cancer. Molecular Cancer. 2023; 22: 91. https://doi.org/10.1186/s12943-023-01782-2.