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REVIEW ARTICLE

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RNA methylation in inflammatory bowel disease

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Abstract

RNA modifications, including the renowned m6A, have recently garnered significant attention. This chemical alteration, present in mRNA, exerts a profound influence on protein expression levels by affecting splicing, nuclear export, stability, translation, and other critical processes. Although the role of RNA methylation in the pathogenesis and progression of IBD and colorectal cancer has been reported, many aspects remain unresolved. In this comprehensive review, we present recent studies on RNA methylation in IBD and colorectal cancer, with a particular focus on m6A and its regulators. We highlight the pivotal role of m6A in the pathogenesis of IBD and colorectal cancer and explore the potential applications of m6A modifications in the diagnosis and treatment of these diseases.

KEYWORDS
colorectal cancer, inflammatory bowel disease, m6A, methylation, RNA

Abbreviations: 2OG, 2-oxoglutarate; 5-ASA, 5-aminosalicylic acid; 6-MP, 6-mercaptopurine; AKT, AKT serine/threonine kinase 1; ALKBH1, AlkB homolog 1, histone H2A dioxygenase; ALKBH3, AlkB homolog 3, alpha-ketoglutarate dependent dioxygenase; ALKBH5, AlkB homolog 5, RNA demethylase; ARHGEF2, Rho/Rac guanine nucleotide exchange factor 2; AZA, azathioprine; CBX8, chromobox 8; CCNE1, cyclin E1; CD, Crohn's disease; CRC, colorectal cancer; DART seq, deletion adjacent to RNA modification targets; DNMT2, DNA (Cytosine-5)-methyltransferase-like protein 2; EMT, epithelial-mesenchymal transition; FOXO3, forkhead box O3; FTO, FTO alpha-ketoglutarate dependent dioxygenase; GAS5, growth arrest specific 5; GATA3, GATA binding protein 3; GLUT1, glucose transporter type 1; HBP1, HMG box transcription protein1; HIF-1α, hypoxia inducible factor 1 subunit alpha; HK2, hexokinase 2; HNRNPA2B1, heterogeneous nuclear ribonucleoprotein A2/B1; HSF1, heat shock transcription factor 1; IBD, inflammatory bowel disease; IFN, Interferon; IGF2BP1/2/3, insulin like growth factor 2 mRNA binding protein 1/2/3; IL-23, interleukin-23; JAK, Janus kinase; m5C, 5-methylcytidine; m7G, N7-methylguanosine; MA, meclofenamic acid; MeRIP-seq, methylated RNA immunoprecipitation sequencing; METTL1, methyltransferase 1, tRNA methylguanosine; METTL14, methyltransferase 14, N6-adenosine-methyltransferase subunit; METTL3, methyltransferase 3, N6-adenosine-methyltransferase complex catalytic subunit; METTL8, methyltransferase 8, TRNA N3-cytidine; MYC, MYC proto-oncogene, BHLH transcription factor; ncRNA, non-coding RNA; NF-κB, nuclear factor kappa B subunit 1; Nsun2, NOP2/Sun RNA methyltransferase 2; NSun4, NOP2/Sun RNA methyltransferase 4; NSun5, NOP2/Sun RNA methyltransferase 5; NSun6, NOP2/Sun RNA methyltransferase 6; PI3K, phosphatidylinositol-3 kinase; RBM15, RNA binding motif protein 15; scRNAseq, single-cell RNA-seq; Sec62, SEC62 homolog, preprotein translocation factor; SOCS2, suppressor of cytokine signaling; SOX2, SRY-Box transcription factor 2; TGB, thromboglobulin, beta-1; TNF-α, tumor necrosis factor-α; TOB1, transducer of ERBB2, 1; Treg, regulatory T cell; TRM61, tRNA methyltransferase 61A; TRMT10C, tRNA methyltransferase 10C; TRMT6, tRNA methyltransferase 6 non-catalytic subunit; TRMT61B, tRNA methyltransferase 61B; UC, ulcerative colitis; VIRMA, Vir like m6A methyltransferase associated; WDR4, WD repeat domain 4; XIST, X inactive specific transcript; YPEL5, Yippee Like 5; YTHDC1, YTH N6-Methyladenosine RNA Binding Protein C1; YTHDF1, YTH N6-methyladenosine RNA binding protein F1; ZC3H13, zinc finger CCCH-type containing 13; ZCCHC4, zinc finger CCHC-type containing 4.

1 | INTRODUCTION

IBD is a chronic and debilitating condition characterized by two major phenotypes: CD and UC.[1,2] Patients with IBD experience persistent symptoms, including abdominal pain, diarrhea, bloody stools, and fever, which substantially impair their quality of life. Moreover, IBD patients have a heightened risk of developing colorectal cancer, underscoring the urgent need to develop effective treatment methods and gain a deeper understanding of its pathogenesis.

Despite initial attempts to treat IBD with 5-ASA, corticosteroids, and AZA/6-MP, achieving a complete cure or maintaining remission has proven to be a formidable challenge.[3–6] While several molecular targeted therapies, such as anti-TNF inhibitors, IL-23 inhibitors, sphingosine 1-phosphate receptor 1 modulators, and selective JAK-1 inhibitors have been developed, they have either failed to provide adequate therapeutic efficacy or are still awaiting clinical trial results.[7–10]

To develop superior therapeutic targets, it is imperative to elucidate the pathogenesis of IBD and develop effective therapeutic agents against it. Research has revealed that genes,[11] diet,[12] smoking,[13] intestinal environment,[14] and other factors play a pivotal role in the pathogenesis of IBD. While several studies have shed light on some of the mechanisms underlying IBD, the therapeutic effects of these drugs are not yet fully understood (Figure 1).

Recently, RNA modifications have garnered attention due to their role in RNA structure, function, and turnover.[15] Methylation is the most crucial epigenetic modification in ncRNA and mRNA. RNA methylation influences RNA splicing, translation, and other biological processes (Table 1).[16] However, this information is often lacking in RNA sequencing data and has received scant attention. In this review, we explore the role of RNA methylation in IBD and its potential as a future therapeutic target.

2 | TYPES OF RNA METHYLATION AND THEIR MECHANISMS

To date, approximately 150 types of RNA modifications have been discovered in various species.[15–19] These modifications are present in all RNAs and are necessary for RNA function. These modifications include methylation of bases and ribose, acetylation, hydroxylation, sulfation, selenation, reduction, isomerization, dehydration, cyclization, addition of amino acids and sugars, and many other chemically diverse modifications. Although m6A is one of the most frequently observed RNA modifications, other modifications such as m1A, m3A, m5C, m7G, 2′-*O*-methylation, and other methylations have also been discovered. Each of these modifications will be discussed further below. The biochemical significance of m6A modification has been studied extensively (Figure 2).

2.1 | m1A

For m1A in RNA, the methyltransferases are TRMT10C, TRMT61B, TRMT6, and TRM61; demethylases are ALKBH1/3; and readers are YTHDF1/2/3. However, no research has been conducted on IBD regarding m1A.[15–17]

2.2 | m3A

3-Methylcytosine is formed when the third position of cytosine is methylated. METTL8 is the RNA m3A methyltransferase, and ALKBH3 is the reader. However, no studies on IBD have been conducted regarding m3A.[15–17]

2.3 | m6A

The N6 position of adenosine is methylated to produce m6A. It is the most common modification in mammalian mRNAs[17] and is found primarily in RNA exons, stop codons, and 3′UTRs. In RNA, methylation enzymes such as METTL3 and METTL14; demethylation enzymes such as FTO and ALKBH5; and readers such as IGF2BP1/2/3, YTHDC1/2, and YTHDF1/2/3 are involved in m6A methylation. m6A regulates a wide range of biological functions, including processing,[18] translation,[19] stability,[20] and stem cell differentiation.[21]

2.4 | m5C

m5C is the methylated fifth N of cytosine. DNMT2, Nsun2, NSun4, NSun5, and NSun6 are m5C methyltransferases.[15–17,22,23] However, no research has been conducted on this aspect of IBD.

2.5 | m7G

m7G is a methyl group modification of the seventh N of guanine in RNA. METTL1 and WDR4 are components of m7G methylation complexes that can regulate cell differentiation.[24] However, no research has been conducted on this aspect of IBD.

2.6 | 2′-*O*-methylation

The methylation of RNA 2′-OH results in 2′-*O*-methylation.[1] Pre-mRNA splicing and small RNA stability are regulated by 2′-*O*-methylation.[25] However, no research has been conducted on this aspect of IBD.

There are no papers on m^1A, m^3A, m5C, m7G, or 2-*O*-methylation regarding IBD, but we hope to see them in the future. The function of m6A in IBD, which has already been reported,[26] will be discussed further below. Following that, we will note the significance of CRC.

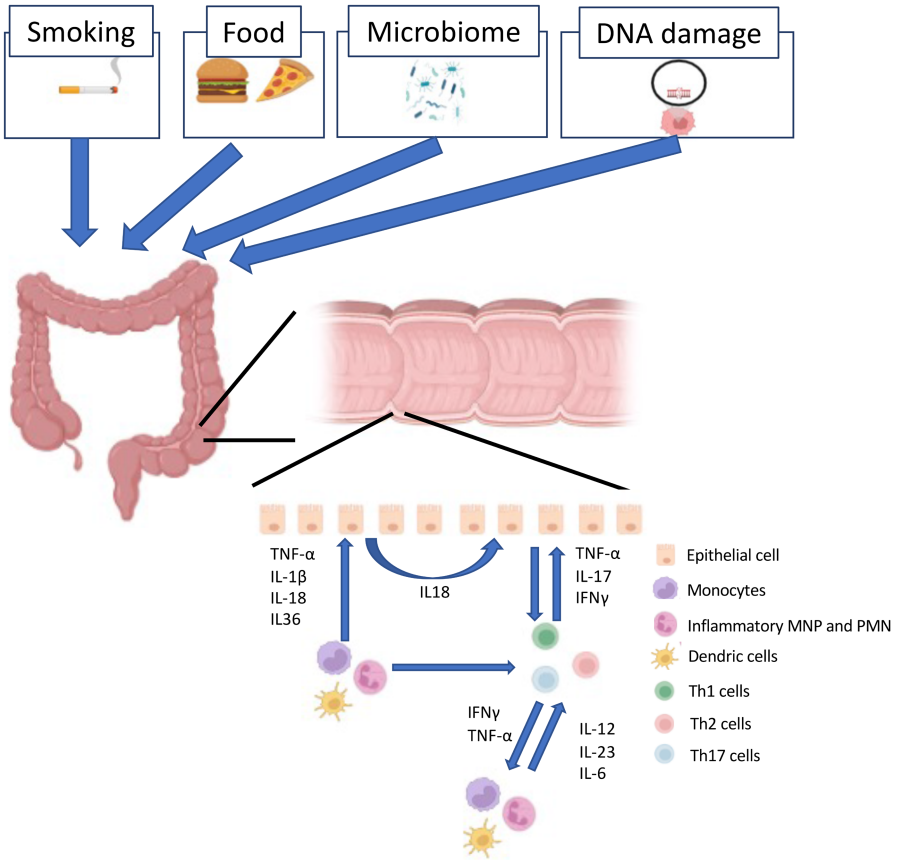

FIGURE 1 Risk factors and molecular mechanisms in IBD development. This figure illustrates the risk factors and underlying molecular mechanisms associated with the development of IBD. Smoking can cause DNA damage affecting both the microbiome and immune cells, leading to an imbalance triggering an immune response involving various immune cell types. Activated immune cells release cytokines and interleukins, exacerbating the inflammatory response within the gut. Damage to epithelial cells in the gastrointestinal tract can also contribute to IBD. In summary, Figure 1 shows how smoking, microbiome alterations, DNA damage, various immune cells, and cytokine secretion play a crucial role in IBD development.

TABLE 1 m6A RNA modifying enzymes.

Type	Enzyme	Function
Writers	METTL14	Colon stem cell apoptosis, mucosal barrier dysfunction, severe colitis RNAs of cytokines involved in IBD are methylated
Readers	IGF2BP1 IGF2BP2	Involved in the development of disease
Readers	IGF2BP2	Involved in the development of disease
Writers	METTL3	Involved in the development of disease
Writers	METTL14	Colitis develops spontaneously due to abnormal RNA methylation in T cells

3 | ROLE OF M6A IN IBD

Despite the limited research on RNA methylation concerning IBD, a published interaction network analysis between IBD risk genes (257 genes) and the *m6A* gene has revealed a significant level of interaction.[26] Consequently, m6A is believed to play a critical role in IBD. As a result, we have compiled a list of relevant studies (Figure 3A; Table 2).

3.1 | The role of m6A in epithelial cells

In the mouse colon, the *METTL14* gene plays a pivotal role in reducing apoptosis of colonic epithelial cells. It achieves this by regulating the stability of *NFKBIA* mRNA and modulating the NF-κB pathway. This regulation leads to colonic stem cell loss and consequent colitis, resulting in mucosal barrier dysfunction and severe colitis.[27] While this phenomenon has been confirmed in mice, it remains uncertain whether the same occurs in humans.

In IBD patients, the expression of *IGF2BP2*, *HNRNPA2B1* (leader), and *ZCCHC4* (writer) is diminished. However, the specific roles of these genes at the single-cell level in IBD are yet to be elucidated. Notably, IGF2BP2 (also known as IMP2), an m6A reader, is a direct mTOR substrate involved in glucose, lipid, protein, and energy metabolism,[28] which is a pivotal event in IBD pathogenesis[29,30] and may exert influence on IBD pathology. HNRNPA2B1 (leader) promotes

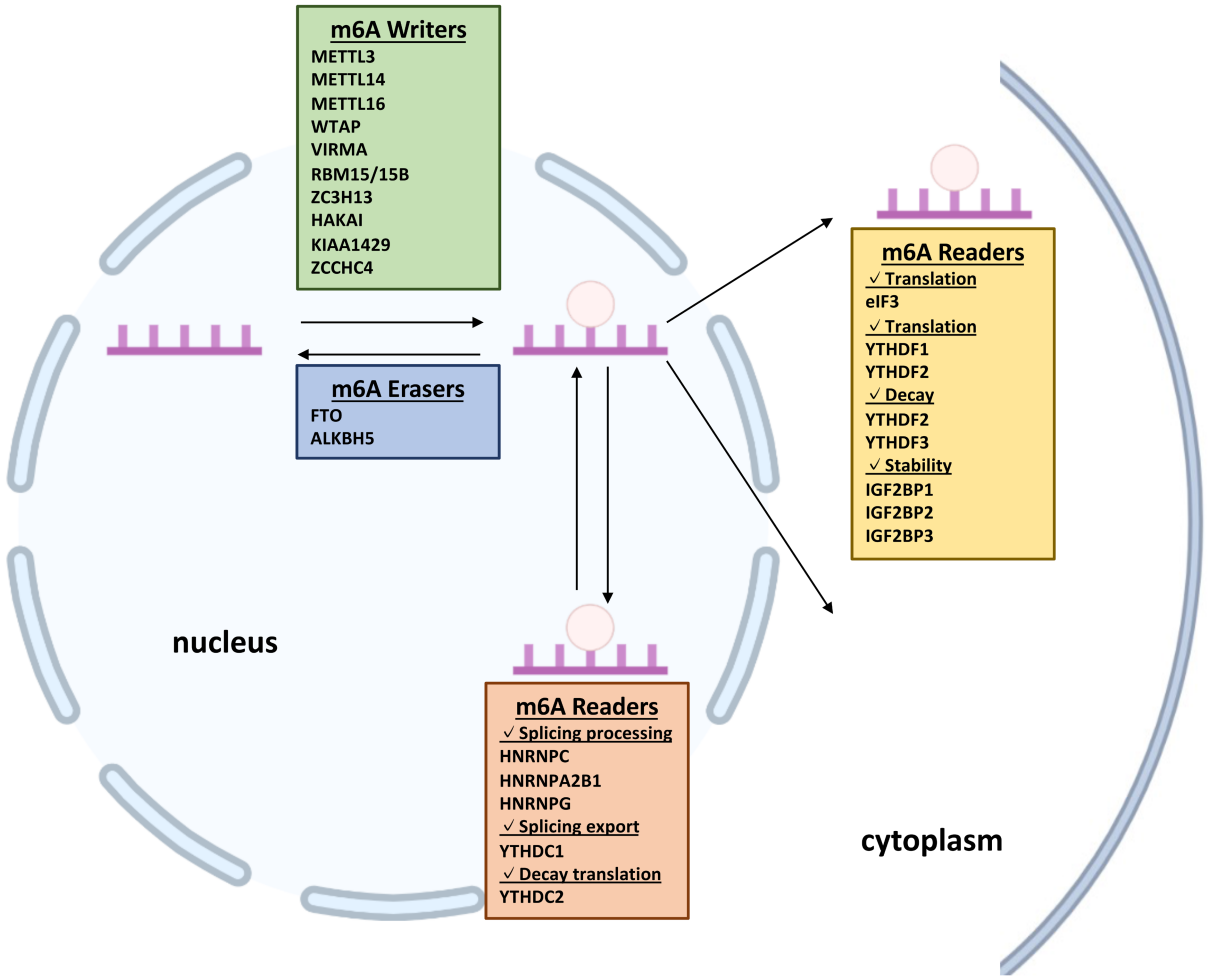

FIGURE 2 Genes involved in RNA methylation. The figure provides an overview of genes involved in RNA methylation, specifically focusing on the key players in the m6A RNA modification pathway. The m6A Writers, including METTL3, METTL14, METTL16, WTAP, VIRMA, RBM15/15B, ZC3H13, HAKAI, KIAA1429, and ZCCHC4, add m6A methyl groups to RNA. Conversely, the m6A Erasers, FTO and ALKBH5, remove m6A marks. The m6A Readers, such as eIF3, YTHDF2, YTHDF3, IGF2BP1–3, HNRNPC, HNRNPA2B1, HNRNPG, YTHDC1, and YTHDC2, recognize and interact with m6A-modified RNA, influencing various cellular processes including translation, decay, stability, splicing processing, splicing export, and decay translation. These processes and proteins are located in both the cytoplasm and nucleus, highlighting the complex regulation of RNA methylation within the cell.

effective interferon production via cyclic GMP–AMP synthase (cGAS)-STING32, a crucial system in the viral immune signaling pathway implicated in gut microbiota disruption.[31–33] ZCCHC4 is well known for its role in regulating the production of core cytokines associated with IBD.

3.2 | m6A in IBD immune cells

M1 macrophages, neutrophils, and Th cells are the predominant immune cells contributing to the progression of IBD.[34] During the pathological development of IBD, m6A methylation modifications may upregulate inflammation-associated innate immune cells while suppressing the activation of acquired immune B cells.[35]

Defects in METTL3 and METTL14 in T cells result in Treg dysfunction and spontaneous IBD.[36,37] Although the specific contributing genes and methylated cells remain unknown, methylation affects mRNAs encoding cytokines that play a role in IBD pathogenesis.[26] In UC,[38] IL2, IL4, GATA3, IL9, IL13, TGB, and TNF are involved, while in CD,[26] IL4, IL6, IL12, IL17, IL21, IL22, IL23, TNF, and IFN are implicated.[39]

Based on these findings and others, m6A modification may serve as a potential therapeutic target for IBD. However, further research and evidence are necessary. Current studies on m6A in IBD have primarily focused on its role in mRNA but neglect non-coding RNA. Non-coding RNAs play pivotal roles in IBD, as exemplified by the following discoveries: (1) TOB1 suppresses intestinal mucosa inflammation by inhibiting the differentiation of $CD4^+$ T cells into Th1/Th17 cells[40]; (2) miR-223 activates the NF-κB pathway by targeting SNIP1, promoting cellular pyroptosis, and ultimately contributing to IBD pathogenesis[41]; (3) miR-155 and miR-223 are known to regulate the NF-κB pathway, a key factor in IBD development[41]; (4) the miR-155/HBP1 axis promotes intestinal fibrosis by activating the Wnt/β-catenin signaling pathway.[42]

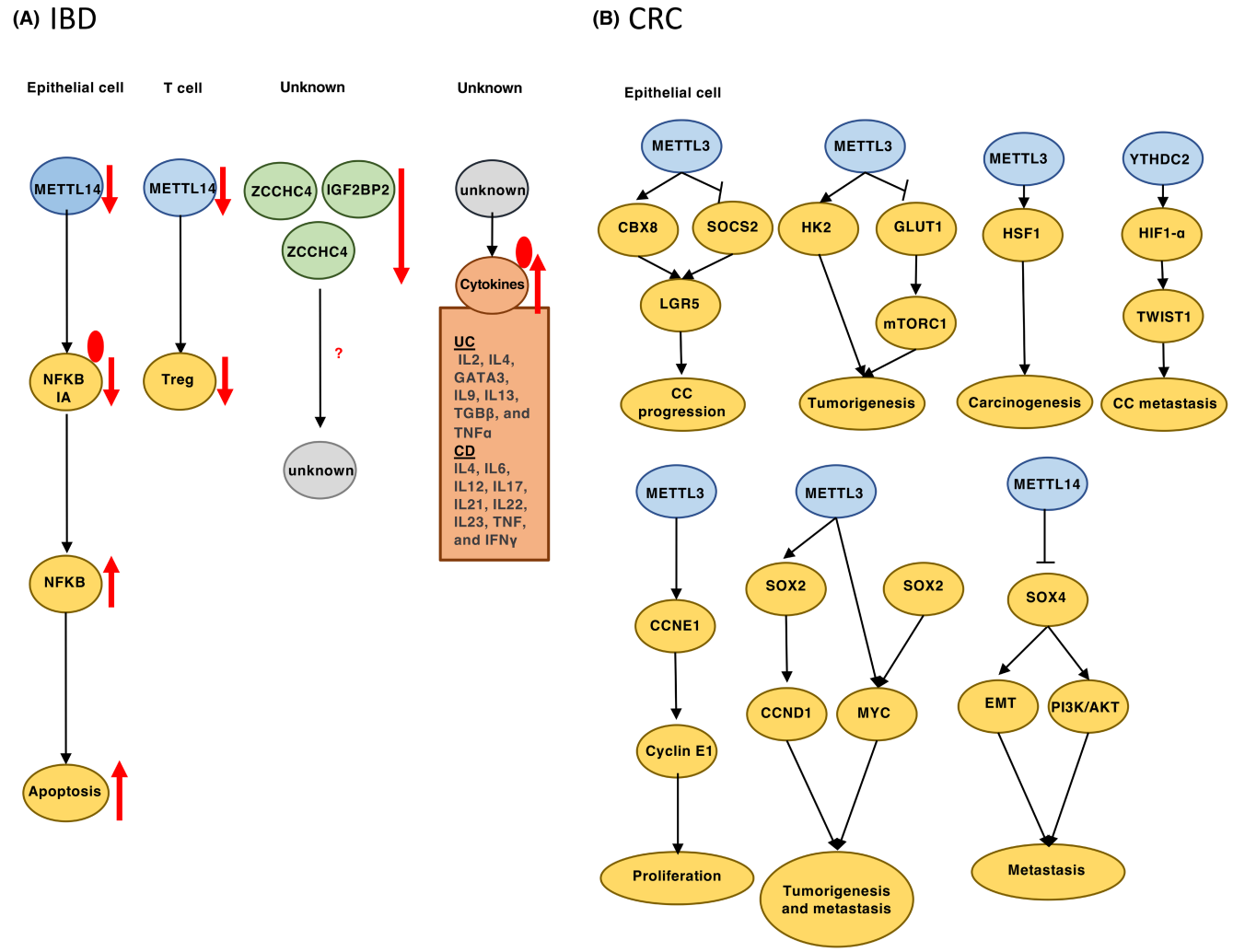

FIGURE 3 Role of m6A in IBD and cancer. (A) Role of m6A in IBD. This figure illustrates the role of m6A RNA methylation in IBD and CRC. In IBD, m6A modifications play a pivotal role in regulating apoptosis in epithelial cells through METTL4, NFKB1A, and NFKB. In T cells, m6A modifications mediated by METTL14 promote the development of regulatory T cells (Tregs). The network extends to ZCCHC4 and IFG2BP2, forming a functional axis in IBD progression. These m6A modifications influence cytokine production, affecting the disease phenotype in UC and CD. Cytokines such as IL2, IL4, GATA3, IL9, IL13, TGBβ, TNFα are influenced in UC, while IL4, IL6, IL12, IL17, IL21, IL22, IL23, TNF, and IFNγ are impacted in CD. This highlights how m6A RNA methylation modulates crucial cellular processes and cytokine profiles, contributing to the distinct pathogenesis of UC and CD in IBD. (B) Role of m6A in CRC. This figure showcases the multifaceted role of m6A RNA methylation in CRC. METTL3 plays a central role in CRC progression, influencing pathways such as CBX8, SOCS2, HK2, GLUT2, mTORC1, HSF1, YTHDC2, HIF1a, TWIST1, CCNE1, CyclinE1, and MYC1. METTL14 regulates EMT with SOX4 and the PI3K-AKT pathway. These precise modifications of RNA molecules and their interactions with various genes and pathways significantly influence disease progression, tumorigenesis, metastasis, and proliferation in CRC.

Therefore, further research into m6A non-coding RNA in IBD is imperative.

3.3 | Roles of m6A-associated genes in mRNA

While numerous reports have discussed m6A in colorectal cancer (Figure 3B), we focus on genes implicated in m6A within the context of IBD, an emerging disease with links to cancer origins.[43] Our discussion is divided into two parts: protein-coding mRNAs and non-protein-coding RNAs.

3.3.1 | Genes related to m6A and their impact on DNA

Many of the genes associated with m6A have been found to harbor genetic mutations.[43] However, it remains unclear whether these mutations enhance or impair their functionality. Further research is warranted. Additionally, the analysis of DNA copy numbers related to gene expression has revealed that 60% of genes are diploid, while 30% exhibit deletions. Notably, three genes (*VIRMA*, *ZC3H13*, and *YTHDF1*) deviate from this trend.[43] Future studies should explore whether these genes are linked to mRNA expression levels.

TABLE 2 Molecules modifying mRNA on m6A in IBD.

Type	Enzyme	Function
Writers	METTL3	Promoting CRC tumorigenesis and metastasis (SOX)
Writers	METTL3	Promoting CC cells proliferation (MYC)
Writers	METTL3	Promoting CRC cells proliferation (CCNE1)
Writers	METTL3	Promoting CRC tumorigenesis (HK2/GLUT1)
Writers	METTL3	Promoting CRC development (GLUT1)
Writers	METTL3	Promoting CC stemness and chemoresistance (CBX8)
Writers	METTL3	Promoting CC cells proliferation (SOCS2)
Writers	METTL3	Promoting CRC development (HSF1)
Writers	METTL3	Promote vasculogenic mimicry (VM) formation (EphA2 and VEGFA)
Writers	METTL3	Promote angiogenesis and metastasis (PLAU)
Writers	METTL3 WTAP	Promoting CC cells proliferation (UCA1)
Writers	METTL3	Promoted the proliferation, migration, and invasion of CRC cells (CRB3)
Writers	KIAA1429	Promote CRC carcinogenesis (HK2)
Writers	KIAA1429	Promotes the growth and motility of colorectal cancer (SIRT1)
Writers	METTL14	Inhibiting CRC metastasis (SOX4)
Writers	METTL14	Inhibiting CRC metastasis (ARRDC4)
Writers	WTAP	Promoting CC development (–)
Writers	RBM15	Promote the CRC growth and metastasis(MyD88)
Erasers	FTO	Promoting CRC occurrence and progression (MYC)
Erasers	ALKBH5	Inhibit cell proliferation and the metastasis (FOXO3)
Readers	YTHDC2	Promoting CC cells proliferation (HIF-1α)
Readers	YTHDC1	Promoted the proliferation, migration, and invasion of CRC cells (ARHGEF2)

3.3.2 | Writers

Numerous studies have focused on RNA m6A writers in colorectal cancer, with the majority concentrating on METTL3. However, a few have examined WTAP, KIAA1429, METTL14, WTAP, and RBM15. Writers are implicated in a wide array of mRNAs associated with colorectal cancer, including *SOX2* (*METTL3* oncogene: metastatic potential), *MYC* (*METTL3* oncogene: proliferative potential, tumorigenic), *YPEL5* (*METTL3* oncogene: tumorigenic), *Sec62* (*METTL3* oncogene: stem cell-like, chemotherapy resistance), *HSF1* (*METTL3* oncogene: tumorigenic), *HK2/GLUT1* (*METTL3* oncogene: tumorigenic), *GLUT1* (*METTL3* oncogene: tumorigenic), *SOCS2* (*METTL3* oncogene: tumorigenic), *CCNE1* (*METTL3* oncogene: proliferative), and *CBX8* (*METTL3* oncogene: stemness). Interestingly, *METTL3* and *METTL14* may exert opposing effects on tumor grade, with *METTL3* suggesting an oncogenic function and *METTL14* exhibiting an anti-oncogenic role. Notably, each gene targets different methyltransferases.[43]

3.3.3 | Erasers

Published studies on erasers in colorectal cancer are scarce. However, ALKBH5 stands out as a notable eraser, as it has been demonstrated to suppress the proliferative and metastatic potential of colorectal cancer via FOXO3.[15,16,43] This suggests that different eraser types may exert varying effects on distinct genes.

3.3.4 | Readers

There is a limited body of research on readers in the context of colorectal cancer. Notably, YTHDC1 and YTHDC2 have been shown to enhance the proliferative and invasive potential of colorectal cancer through interactions with ARHGEF2 and HIF-1α.[15,16,43] These findings indicate that these two genes may have distinctive roles.

3.4 | Roles of m6A-related genes in non-coding RNA

3.4.1 | Writers

ncRNAs, such as *circ1662* (*METTL3* oncogene: invasiveness, metastatic potential), *lncRP11* (*METTL3* oncogene: tumorigenicity), *miR-1246* (*METTL3* oncogene: metastatic potential), and *circNSUN2* (*METTL3* oncogene: metastatic potential), are implicated as writers. Conversely, *METTL14* (anti-oncogene: tumor growth, metastatic potential), lncRNA *XIST* (*METTL14* anti-oncogene: tumor growth, invasion, metastatic potential), miR-125b (*Nsun2* anti-oncogene: invasion potential), and lncRP11 (*ALKBH5* oncogene: tumorigenicity) regulate the proliferative, invasive potential, and drug resistance of colorectal cancer cells.[44–46] The expression of methylation genes as oncogenes or anti-oncogenes lacks a consistent pattern. This variance could be attributed to differences in the genes targeted by methylation.

3.4.2 | Erasers

This gene, through rare non-coding RNA (lncRNA *RP11*), regulates CRC cell formation (*ALKBH5* anti-oncogene: tumorigenesis).[44] Further research is imperative.

3.4.3 | Readers

There is a paucity of reports on readers in colorectal cancer. They play a role in regulating CRC cell formation through ncRNAs, such as circNSUN2 (*YTHDC1* oncogene: tumorigenesis), lncRNA *XIST* (*YTHDC2* anti-oncogene: proliferative potential, invasive potential), and lncRNA *GAS5* (*YTHDC3* oncogene: tumorigenesis). The expression pattern of methyl organelle genes as oncogenes or anti-oncogenes remains inconsistent.[44–46] This inconsistency may arise from variations in the genes subjected to methylation.

The significance of m6A RNA modification in colorectal cancer is substantial, suggesting its potential as a therapeutic target for the disease. Notably, *METTL14* is a common gene associated with both IBD and colorectal cancer. We speculate that METTL14 might mitigate the onset of IBD or IBD-associated colorectal cancer.[34,35,43] However, the suppression of METTL14 expression is not observed, necessitating further research using single-cell samples.

Three key points warrant emphasis: First, METTL14 plays a role in the pathogenesis of both IBD and colorectal cancer, suggesting its involvement in the development of IBD-associated colorectal cancer, although this hypothesis lacks supporting studies. Second, erasers and readers remain unexplored and require investigation in future studies. Third, research should delve into the roles of writers, erasers, and readers in stromal cells.

TABLE 3 Therapeutic agents targeting m6A mRNA.

Drug	Target
STM2457	METTL3
Rhein	FTO
MA	FTO
FL1-11	FTO
FB23	FTO
FB23-2	FTO

3.5 | Therapeutics targeting RNA methylation

Limited drugs targeting genes regulating or reading RNA methylation have been identified and are discussed below (Table 3).

3.5.1 | METTL3 inhibitors

STM2457 has emerged as a competitive inhibitor of METTL3.[47] It achieves inhibition of intracellular methylation by binding to METTL3 *S*-adenosyl-L-methionine (SAM) binding site.

3.5.2 | FTO inhibitors

Rhein has been identified as a competitive inhibitor of FTO, capable of increasing intracellular m6A on mRNA. It binds reversibly to FTO and AlkB, forming a complex that inhibits m6A substrates within the cell.[48] Additionally, a new inhibitor, the non-steroidal MA, was found to inhibit FTO demethylation of ssDNA and ssRNA containing m6A by targeting FTO instead of ALKBH5. Among these FTO inhibitors, MA stands out as a highly selective inhibitor. Inspired by the discovery of MA, Wang et al. introduced a new FTO inhibitor, named FL1-11.[49] Toh et al. synthesized a series of selective compounds, including tethered nucleotide mimics with 2OG-binding components. These compounds demonstrated high potential in inhibiting FTO activity.[50] Huang et al. utilized structure-based rational design to screen two FTO inhibitors, FB23 and FB23-2, which directly bind to the FTO protein and inhibit its m6A demethylase activity both in vitro and in vivo.[51]

3.6 | Methods for testing RNA methylation

Methods such as MeRIP-seq,[52] m6A-seq,[53] methylated RNA immunoprecipitation sequencing, DART seq,[54] and single-cell DART seq are used for testing RNA methylation.[55] Previously, the detection of m6A RNA modification was limited to bulk samples, with single-cell-level data remaining elusive. However, recent advancements, including single-cell DART seq, now enable the identification of RNA modifications at the single-cell level. This

breakthrough holds promise for a deeper understanding of the disease.

Despite the limited scope of m6A studies in IBD, the focus has predominantly been on mRNA. scRNAseq, whether 10× or smart, captures and analyzes only 2.9%–9.6% of ncRNAs. Consequently, single-cell DART seq for IBD is indispensable for unraveling the roles of m6A in mRNA in both stromal cells and epithelial cells, as well as its impact on ncRNAs.

4 | DISCUSSION

The role of RNA methylation, particularly m6A modification, in IBD and CRC is a topic of growing interest in the field of molecular biology and disease research. In this discussion, we will delve deeper into the implications of the findings presented in the previous sections and highlight key points regarding the role of m6A modifications in these diseases.

Regarding the significance of RNA methylation in IBD and CRC, the review emphasizes the significance of RNA methylation, particularly m6A, in the pathogenesis and progression of IBD and CRC. It is evident that RNA methylation plays a crucial role in regulating various cellular processes, including mRNA stability, translation, and splicing. Dysregulation of these processes can lead to the development and exacerbation of IBD and CRC.[14–16,56]

Regarding potential therapeutic targets, we demonstrated that one of the key takeaways from this review is the potential of RNA methylation as a therapeutic target in both IBD and CRC. The identification of specific methyltransferases (writers) and demethylases (erasers) involved in these diseases opens up opportunities for developing targeted therapies. For instance, METTL3 inhibitors and FTO inhibitors have been discussed as potential therapeutic agents that could help modulate the dysregulated RNA methylation patterns in these diseases. These inhibitors may offer a new avenue for the development of precision medicine approaches tailored to individual patients.[43]

As indicated in the dual role of METTL14, the review highlights the intriguing dual role of METTL14 in both IBD and CRC. While METTL3 and METTL14 are both involved in m6A methylation, they seem to have opposite effects in terms of tumor progression. METTL3 is described as an oncogene, whereas METTL14 is referred to as an anti-oncogene. This distinction may have important implications for future therapeutic strategies. Further research is needed to elucidate the precise mechanisms through which these methyltransferases exert their effects and how they can be harnessed for therapeutic purposes.[34–36]

Recent studies indicate the dual role of METTL14 in IBD and CRC. In IBD, METTL14 may contribute to the regulation of genes associated with inflammation, potentially influencing disease progression.[27] However, in the context of CRC, METTL14 exhibits a more complex role. It has been identified both as a potential tumor suppressor, regulating mRNA modifications such as glycolysis genes related to cancer pathways, and as a factor that might promote CRC progression.[57] The dual functionality of METTL14 underscores the intricacies of its involvement in these distinct gastrointestinal conditions,[27,57,58] and further research is essential to unravel the precise mechanisms underlying its actions in IBD and CRC. Understanding the dual role of METTL14 provides valuable insights that can guide future investigations and therapeutic approaches for these complex diseases.

Here we noted the importance of non-coding RNAs. Although the review primarily focuses on m6A modifications in mRNA, it highlights the potential significance of non-coding RNAs (ncRNAs) in IBD and CRC. ncRNAs, such as microRNAs and long non-coding RNAs, have been implicated in these diseases and are also subject to RNA methylation.[44–46] The interplay between m6A modifications and ncRNAs in disease pathogenesis warrants further investigation. Understanding how m6A modifications influence the stability and function of ncRNAs could provide novel insights into disease mechanisms.

As challenges and future directions, the review also acknowledges several challenges and gaps in our current understanding of RNA methylation in IBD and CRC. These challenges include the need for comprehensive studies on different types of RNA modifications (e.g., m^3A, m5C) and their roles in these diseases. Additionally, the review underscores the importance of exploring the roles of erasers and readers in disease pathogenesis, which has received limited attention in present medicine.

We noted as single-cell analysis and precision medicine, that advancements in single-cell RNA sequencing techniques, such as single-cell DARTseq, are highlighted as promising tools for elucidating the complexity of m6A modifications in epithelial and stromal cells.[54,55] This technology has the potential to uncover disease-specific m6A modifications that could serve as biomarkers for early detection and targets for precision medicine approaches.

Despite initial attempts at treatment with 5-ASA, corticosteroids, and AZA/6-MP, challenges have been performed in achieving a complete cure and maintaining remission in IBD patients.[59] The reasons for the lack of success with these treatments and the challenges faced by patients can be multifaceted, as follows:

(1) Disease complexity[59]: IBD encompasses a spectrum of diseases with its own unique and heterogeneous pathophysiology, which makes it challenging to develop universal treatment approaches.

(2) Individual variability[59,60]: patients with IBD exhibit significant variability in their response to treatments, which is relevant to several factors such as genetic predisposition, environmental influences, and the specific manifestations of the disease.

(3) Immune system dysregulation[61]: IBD is characterized by dysregulation of the immune system, leading to chronic inflammation in the gastrointestinal tract, the complexity of which may render the treatments insufficient to achieve complete and sustained remission.

(4) Treatment resistance and dependence[59]: some patients may develop resistance to certain medications over time, leading to diminished efficacy.

(5) Incomplete understanding of disease mechanisms[59,62]: most importantly, the exact etiology of IBD remains incompletely understood, and the lack of comprehensive understanding specific molecular targets in the pathogenesis, insufficient understanding of the targeted molecules or the potential impact of treatment stages and individual variations among patients hampers the development of targeted therapies that address the root causes of IBD.

Given that we here update the novel findings in RNA methylation and suggested the possibility of discovering innovative therapeutic approaches, the diversity of patient responses emphasizes the need for personalized medicine in IBD treatment.[59] Tailoring therapies based on individual patient characteristics, disease severity, and responsiveness to specific medications may enhance the overall success of treatment strategies. In this regard, the concept of RNA modifications gives promising options for therapeutic approaches.

Taken together, RNA methylation, particularly m6A modification, holds great promise as a key player in the pathogenesis of IBD and CRC. This review has provided valuable insights into the current state of research in this field, highlighting potential therapeutic avenues and emphasizing the importance of further investigations to fully understand the molecular mechanisms underlying these diseases. As we continue to unravel the complexities of RNA methylation, it is hoped that novel diagnostic and therapeutic strategies will emerge, ultimately improving the prognosis and treatment of IBD and CRC patients.

5 | CONCLUSIONS

Posttranscriptional RNA modifications are pivotal players in biological processes. In this study, our focus has been on unraveling the intricate role of m6A in the genesis and progression of inflammatory diseases and cancer. By governing the expression of downstream target genes, m6A-regulated genes exert a profound impact on the pathogenesis of both IBD and colorectal cancer. The outcomes underscore the significant sway of m6A in shaping the trajectory of IBD and colorectal cancer through its masterful orchestration of downstream gene expression.

This comprehensive review has succinctly encapsulated recent breakthroughs, therapeutic avenues, and methodological strides in understanding m6A modifications in the context of IBD and colorectal cancer. The future endeavors in IBD and colorectal cancer research hold the promise of unveiling the intricacies of disease mechanisms by delving into the kaleidoscope of m6A modifications within epithelial and stromal cells. Leveraging advanced techniques such as single-cell DARTseq and others will be pivotal in this pursuit. Further strides are requisite to pinpoint disease-specific m6A modifications for early detection and to engineer more effective m6A-targeted therapeutic interventions.

AUTHOR CONTRIBUTIONS

Yuki Ozato: Writing – original draft; writing – review and editing. **Tomoaki Hara:** Formal analysis; methodology; writing – review and editing. **Sikun Meng:** Formal analysis; writing – review and editing. **Hiromichi Sato:** Formal analysis; writing – review and editing. **Shotaro Tatekawa:** Writing – review and editing. **Mamoru Uemura:** Conceptualization; supervision. **Takeshi Yabumoto:** Conceptualization. **Shizuka Uchida:** Conceptualization; software; supervision. **Kazuhiko Ogawa:** Supervision. **Yuichiro Doki:** Supervision. **Hidetoshi Eguchi:** Supervision. **Hideshi Ishii:** Conceptualization; funding acquisition; project administration.

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CONFLICT OF INTEREST STATEMENT

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ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board; N/A.

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