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Review Article

Role and mechanisms of m6A demethylases in digestive system tumors

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Abstract: Digestive system tumors are common malignancies in humans, often accompanied by high mortality and poor prognosis. Therefore, intensive research on the pathogenesis of digestive system tumors is imperative. N6-methyladenosine (m6A) is the most common RNA modification in eukaryotes and exerts regulatory effects on RNA expression and metabolism, including splicing, translation, stability, decay, and transport. m6A demethylases belong to the AlkB family of dioxygenases that can catalyze m6A demethylation. Accumulating evidence in recent years has shown that abnormal m6A levels caused by m6A demethylases play crucial roles in different aspects of human cancer development. In this review, we comprehensively summarize the recent findings on the functions and underlying molecular mechanisms of m6A demethylases in cell proliferation, apoptosis, migration, invasion, metastasis, angiogenesis, resistance to chemo- and radiotherapy, and the tumor immune microenvironment (TIME) of digestive system tumors. Furthermore, we discuss the therapeutic potential of targeting these m6A demethylases for treatment.

Keywords: m6A, fat mass and obesity associated protein (FTO), AlkB homolog 5 (ALKBH5), digestive system tumors, cancer therapeutics

Introduction

Digestive system tumors, including colorectal, gastric, hepatic, pancreatic, and esophageal malignancies, have emerged as a growing concern for public health. Despite significant advancements in their diagnosis and treatment over the past decade, these tumors remain the leading cause of mortality worldwide [1, 2]. Therefore, it is crucial to elucidate the mechanisms underlying their initiation and progression of digestive system tumors, and to identify novel therapeutic targets that could potentially disrupt these processes.

Since the first report of RNA modification in 1951, over 170 types have been identified, encompassing modifications such as 5-methylcytosine (m5C), 1-methylguanosine (m1G), 7-methylguanosine (m7G), N1-methyladenosine (m1A), and m6A. Among these diverse modifications, m6A stands out as the most abundant modification in eukaryotes [3]. High-throughput sequencing technologies reveal that m6A consistently occurs at the consensus sequence RRm6ACH (where R=G or A; H=A, C, or U) [4, 5]. m6A modification is a dynamic process, which is primarily governed by m6A methyltransferases ("writers"), demethylases ("erasers"), and identified by some specific RNA-binding proteins ("readers") [6-14]. These proteins identify specific sites, playing crucial roles in mRNA processes such as degradation, translation, splicing, stability, and nuclear export [15-17] (**Figure 1**). Importantly, m6A demethylases, predominantly fat mass and obesity associated protein (FTO) and AlkB homolog 5 (ALKBH5), both of which belong to the AlkB family of dioxygenases, are responsible for catalyzing the reverse process of m6A methylation to regulate m6A level in the transcriptome [18, 19]. While recent studies have identified ALKBH3 as another AlkB family member with potential m6A demethylation capabilities [20], current research predominantly focuses on FTO and ALKBH5 as the core mediator of this epigenetic process.

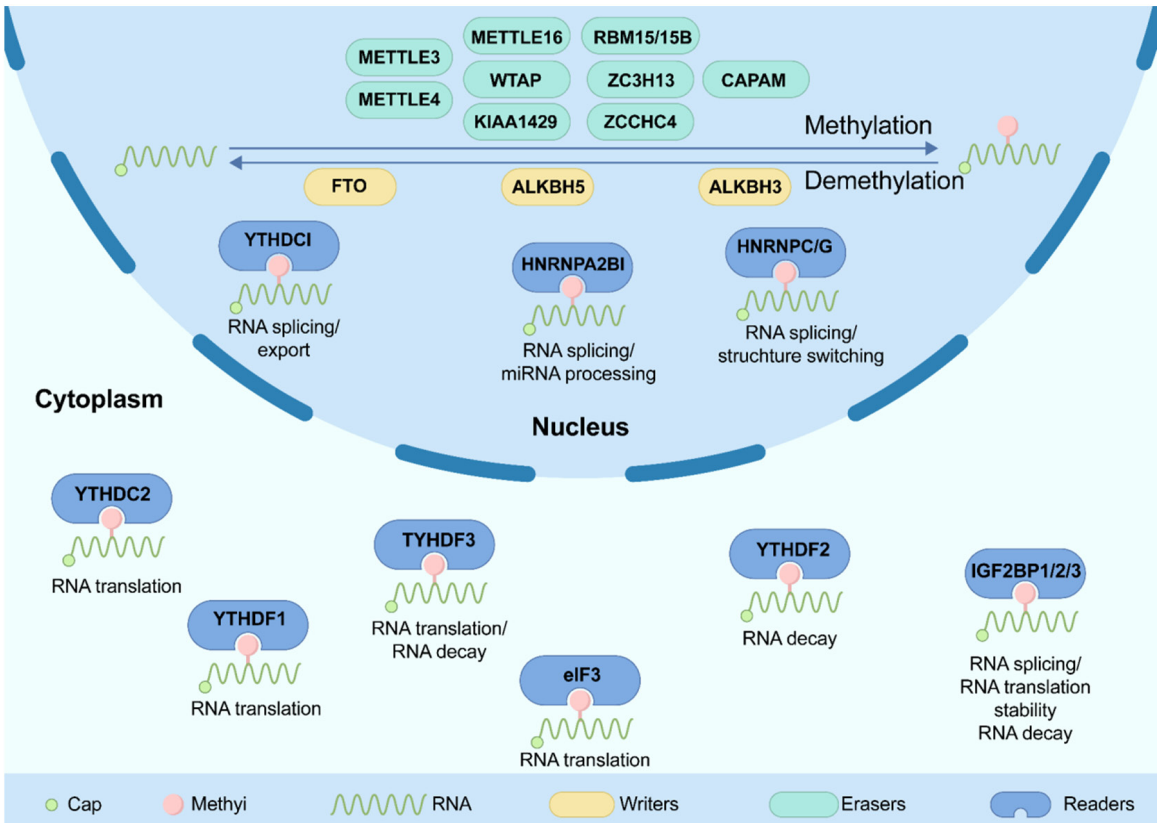

Figure 1. Mechanism of m6A modification mediated by writers, erasers and readers. Writers including methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), methyltransferase-like 16 (METTL16), wilms tumor 1-associated protein (WTAP), vir like m6A methyltransferase associated (VIRMA/KIAA1429), RNA binding motif protein 15 (RBM15), RNA binding motif protein 15B (RBM15B), zinc finger CCCH-type containing 13 (ZC3H13), zinc finger CCHC-type containing 4 (ZCCHC4), and cap-specific adenosine methyltransferase (CAPAM) are responsible for m6A methylation to target RNAs. Erasers, such as FTO, ALKBH5, and AlkB homolog 3 (ALKBH3) are responsible for removing the m6A modification. Various readers such as YTH domain containing 1 (YTHDC1), YTH domain containing 2 (YTHDC2), YTH domain family 1 (YTHDF1), YTH domain family 2 (YTHDF2), YTH domain family 3 (YTHDF3), insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1), insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2), insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3), heterogeneous nuclear ribonucleoprotein C (HNRNPC), heterogeneous nuclear ribonucleoprotein G (HNRNPG), heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1), and eukaryotic translation initiation factor 3 (eIF3) recognize m6A and modulate mRNA export, splicing, stability, decay, translation, structure switching, and microRNA (miRNA) processing (Figure was created with figdraw.com).

Notably, ALKBH3 was initially identified for its demethylation activity towards m1A and 3-methylcytosine (m3C) in methylated polynucleotides and subsequently has been found to predominantly function as an m6A demethylase for transfer RNA (tRNA) [20, 21]. Given the well-characterized mechanisms and broader regulatory implications of FTO and ALKBH5 in m6A modification, this discussion will concentrate on these two primary m6A demethylases that currently form the research foundation in this field.

m6A demethylases, acting as crucial regulators of m6A methylation homeostasis, have been implicated in various cancers, including digestive system tumors [22-25]. This review focuses on the functions of m6A demethylases in digestive system tumors, including cell proliferation, apoptosis, migration, invasion, metastasis, angiogenesis, resistance to chemo- and radiotherapy, and the TIME. Furthermore, it summarizes the therapeutic potential of small-molecule inhibitors targeting these m6A demethylases for the treatment of such conditions. This work will help to enhance our comprehension of the relationship between the potential role of m6A demethylases and digestive system tumors, and further identify novel targets for cancer treatment.

The structure of m6A demethylases

FTO

FTO, initially linked to fatty acid biogenesis, was later identified as the first m6A demethylase that removes RNA methylation through an Fe(II)/α-KG-dependent mechanism [26]. Structurally, its catalytic function is mediated by a 505-amino-acid architecture featuring two distinct domains: an N-terminal domain (NTD, residues 32-326) containing a jelly-roll motif formed by a distorted double-stranded β-helix (DSBH), and a C-terminal domain (CTD, residues 327-498) that stabilizes the NTD [27, 28]. The NTD harbors conserved residues (His231/Asp233/His307) coordinating Fe^{2+} and critical α-KG-binding sites (Arg316/Arg322), enabling oxidative catalysis. FTO can demethylate m6A within RRACH motifs of mRNAs and long noncoding RNAs (lncRNAs) in cellulo [29], while exhibiting broader substrate versatility including N6,2'-O-dimethyladenosine (m6Am) in mRNA transcripts and small nuclear RNAs (snRNAs), m1A in tRNAs, 3-methythymine (m3T) in single-stranded DNAs (ssDNAs), as well as 3-methyluracil (m3U) in single-stranded RNAs (ssRNAs) [30, 31]. Its unique b1-b2 loop structure facilitates recognition of both double-stranded and single-stranded nucleic acids [27], though m6A remains its predominant tumor-related substrate due to RNA transcript abundance [15].

ALKBH5

ALKBH5, another highly conserved member of the AlkB family of Fe(II)/α-KG-dependent dioxygenases with distinct structural and functional characteristics. Its 395-amino-acid structure features a conserved DSBH core (β6-β13 sheets) and a CTD containing a catalytic triad (His204/Asp206/His266) for metal ion coordination, with α-KG stabilized by Asn193/Tyr195/Arg277/Arg283 [32, 33]. Unlike FTO, which recognizes both double-stranded and single-stranded nucleic acids, ALKBH5 possesses a unique Cys230-Cys267 disulfide bond that restricts substrate specificity to ssRNAs [32]. Notably, ALKBH5 binds substrates in a 5'-3' orientation opposite, which is reversed compared to other AlkB enzymes [34]. In addition, ALKBH5 exhibits a preference for substrates with an (A/G) m6AC motif, whereas FTO lacks discernible motif specificity. This enzyme differs from FTO in its subcellular localization, with FTO present in both the nucleus and cytosol, whereas ALKBH5 is predominantly found in nuclear speckles [35-37]. ALKBH5 preferentially targets coding sequences 5' region to regulate mRNA export, metabolism, and processing complex assembly [35]. FTO catalyzes the oxidation of m6A, resulting in a blend of adenosine, N6-formyladenosine (F6A), and primarily N6-hydroxymethyladenosine (hm6A) [26, 38-40]. Conversely, ALKBH5 functions to demethylate m6A through oxidation of the N6-methyl group, forming a transient hm6A intermediate that subsequently breaks down into adenosine and formaldehyde [41, 42].

Roles of m6A demethylases in the progression of digestive system tumors

Recent studies have shown that m6A demethylases, particularly FTO and ALKBH5, are closely associated with the processes involved in the progression of digestive system tumors, including cell proliferation, apoptosis, migration, invasion, metastasis, angiogenesis, resistance to chemo- and radiotherapy, and modulation of the TIME (**Figure 2**). This section mainly summarizes how m6A demethylases FTO and ALKBH5 influence the pathological progression of digestive system tumors (**Figure 3** and **Table 1**).

The role of m6A demethylases in the proliferation and apoptosis of digestive system tumors

The fundamental characteristics of cancer include unlimited proliferation and resistance to cell death [43]. Most colorectal cancers (CRCs) are adenocarcinomas, which are malignant tumors originating from glandular cells in the rectum and colon [44]. One study showed that Zinc finger protein 687 (ZNF687) protein levels were significantly elevated in colorectal adenocarcinoma tissues, predicting the poor prognosis of CRC [22]. Studies have shown that knockdown of ZNF687 suppressed CRC cell proliferation, while activation of the Wnt pathway by BML-284 has the reverse effects. Furthermore, FTO enhanced ZNF687 expression in an m6A-dependent manner. Additionally, other studies have indicated that FTO enhances CRC cell proliferative capacities and decreases apoptotic ability by mediating m6A demethylation of MYC through the miR-96/AMPKα2 axis

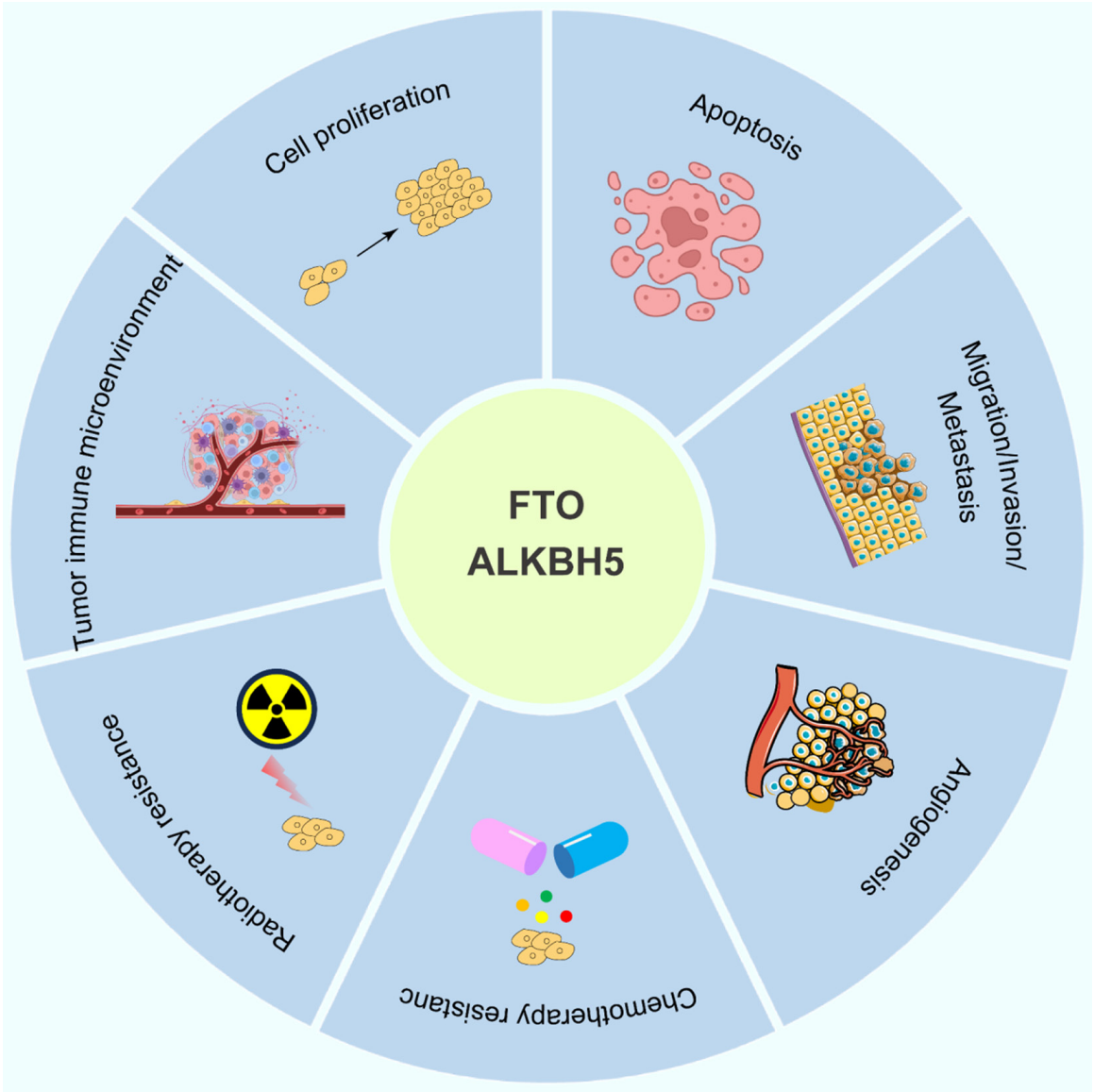

Figure 2. The functions of m6A demethylases in digestive system tumors. m6A demethylases are involved in various processes in the development of digestive system tumors, including cell proliferation, apoptosis, migration, invasion, metastasis, angiogenesis, resistance to chemo- and radiotherapy, and the TIME.

to enhance MYC expression and by stabilizing MZF1/c-Myc via GSK3β-regulated ubiquitination [45, 46]. Conversely, Ye et al. [47] reported a significantly reduced level of FTO in CRC patients. They observed that overexpression of FTO/ALKBH5 impedes CRC cell proliferation, while their downregulation promotes malignant behaviors in CRC cells. Mechanistically, FTO and ALKBH5 inhibit CRC cell proliferation by erasing m6A modifications on HK2 mRNA, thereby attenuating IGF2BP2-mediated stabilization and upregulation of HK2, which is critical for FOXO pathway activation. ALKBH5 is aberrantly elevated in CRC and is associated with poor patient survival [48]. Knockdown of ALKBH5 impairs CRC cell proliferation, whereas its overexpression increases tumor weight and volume [48]. Mechanistically, ALKBH5 binds to RAB5A and posttranscriptionally activates its expression through m6A demethylation, which inhibits YTHDF2-mediated mRNA degradation. However, emerging evidence has revealed the role of ALKBH5 in CRC is complex and controversial. Zhang et al. [49] demonstrated that the downregulation of ALKBH5 can promote CRC progression. Mechanistically, methylated RNA

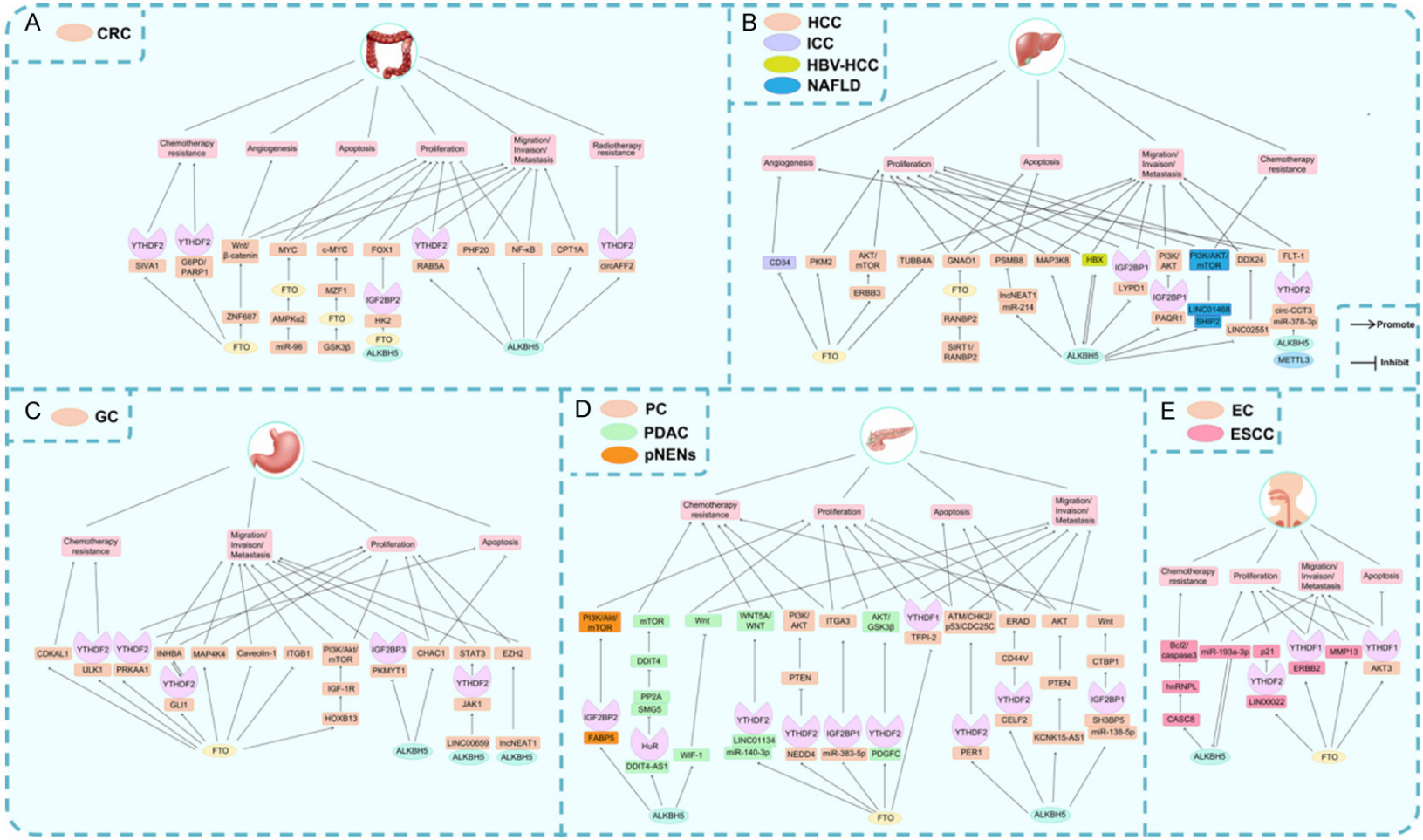

Figure 3. m6A demethylases promote or inhibit the progression of digestive system tumors by coordinating with reader proteins to target related molecules. A. m6A demethylases regulate the molecular mechanism of CRC. B. m6A demethylases regulate the molecular mechanism of HCC, ICC, HBV-HCC, and NAFLD. C. m6A demethylases regulate the molecular mechanism of GC. D. m6A demethylases regulate the molecular mechanism of PC, PDAC, and pNENs. E. m6A demethylases regulate the molecular mechanism of EC and ESCC.

Table 1. m6A demethylation under diverse factors in the TIME

Factor in the TIME	m6A demethylase	Cancer type	Molecular mechanism	Refs
Hypoxia	FTO	CRC	Destabilizing MTA1 mRNA and inhibiting MTA1 expression	[121]
Glycolysis reprogramming	FTO	GC	Enhancing RPPKAA1 mRNA stability	[63]
		HCC	FTO-IT1/FTO axis, repressing GLUT1 and PKM2 mRNA stability through YTHDF2	[124]
	ALKBH5	HCC	FTO-UBR7-Keap1-Nrf2-Bach1-HK2 axis, repressing UBR7 expression	[125]
Lipid metabolism reprogramming	FTO	NAFLD	Promoting the mRNA processing, translation and nuclear translocation of SREBP1C	[126]
	ALKBH5	NAFLD	Enhancing LIN01468 expression through m6A modification	[60]
	FTO	EC	Enhancing HSD17B11 expression by YTHDF1	[127]
	ALKBH5	pNENs	Enhancing FABP5 expression through IGF2BP2	[76]
	ALKBH5	CRC	Enhancing CPT1A expression	[129]
Amino acid metabolism reprogramming	FTO	CRC	FTO/YTHDF2/ATF4 axis, disrupting ATF4 RNA decay through YTHDF2	[128]
Immune suppression	FTO	CRC	Enhancing PD-L1 expression	[130]
	ALKBH5	ICC	ALKBH5/PD-L1, preventing YTHDF2-mediated PD-L1 RNA decay	[131]
		HCC	Enhancing MAP3K8 expression	[89]
	FTO and ALKBH5	EC	METTL3/FTO/ALKBH5 mediating m6A modification and stability of c-myc by IGF2BP2	[132]

FTO, fat mass and obesity associated protein; CRC, colorectal cancers; GC, Gastric cancer; HCC, Hepatocellular carcinoma; ICC, Intrahepatic cholangiocarcinoma; EC, esophageal cancer; NAFLD, non-alcoholic fatty liver disease.

immunoprecipitation sequencing (MeRIP-seq) and RNA sequencing (RNA-seq) data suggested that ALKBH5-mediated m6A modification downregulates PHF20 by reducing the stability of its mRNA in 3'UTR region. In line with the above findings, the inhibitory roles of ALKBH5 in proliferation have also been documented by other groups [50]. They showed that overexpression of ALKBH5 inhibits CRC cell proliferation by inhibiting the NF-κB signaling axis, thereby reducing CCL5 expression and promoting CD8+ T cell infiltration in the tumor microenvironment. Further research is needed to explore these contradictory conclusions.

Liver cancer ranks as the third most common cause of cancer-related deaths globally, exhibiting rising incidence rates [51]. Hepatocellular carcinoma (HCC) constitutes the most prevalent type of liver cancer, accounting for approximately 90% of all diagnosed cases [52]. More recently, a significant number of studies have focused on studying the functional roles of m6A demethylases, particularly in cell proliferation within HCC. For example, FTO is highly expressed in HCC and associated with poor prognosis [53]. It promotes HCC cell proliferation and tumor growth through triggering the demethylation of pyruvate kinase M2 (PKM2) and accelerating the translated production. Additionally, Jiang et al. [54] revealed that pharmacological inhibition of FTO increases m6A levels on erb-b2 receptor tyrosine kinase 3 (ERBB3) mRNA, leading to decreased ERBB3 expression, thereby inhibiting the AKT-mTOR signaling pathway and impairing HCC cell proliferation. However, there is some controversial evidence suggesting that reduced expression of FTO in HCC patient samples and mouse models may function as a tumor suppressor [55, 56]. Liu et al. [55] revealed that FTO promotes HCC proliferation and inhibits apoptosis by demethylating m6A modifications on tumor suppressor guanine nucleotide-binding protein G(o) subunit alpha (GNAO1) mRNA. However, silent information regulator 1 (SIRT1)/RANBP2-mediated SUMOylation of FTO at K216 promotes its degradation, leading to m6A hypermethylation of GNAO1 mRNA, reduced GNAO1 levels, and accelerated HCC progression. These findings provide a close correlation between aberrant FTO expression and the pathogenesis of HCC. Consistently, m6A demethylase ALKBH5 has been demonstrated to be involved in HCC cell proliferation and apoptosis. In addition to promoting HCC cell migration, Yeermaike et al. [24] found that ALKBH5 promotes HCC cell proliferation and suppresses apoptosis by demethylating m6A modification on long noncoding RNA nuclear paraspeckle assembly transcript 1 (lncNEAT1), leading to its upregulation. Elevated lncNEAT1 sponges miR-214, resulting in derepression of its target PSMB8. Hepatitis B virus (HBV) infection is regarded as

a significant factor that increases the risk of HCC [57]. Similarly, ALKBH5 has been uncovered as an oncogenic factor in hepatitis B virus (HBV)-related HCC (HBV-HCC), where it is upregulated in HBV-HCC tissues and has been shown to promote cell growth both *in vitro* and *in vivo* [58]. Notably, this upregulation is induced by HBV X protein (HBx)-mediated histone H3 lysine 4 (H3K4me3) modification, which occurs following HBV infection. In turn, ALKBH5 demethylates m6A modification on HBx mRNA and stabilizes its expression. As type 2 diabetes mellitus and obesity rates climb worldwide, non-alcoholic fatty liver disease (NAFLD) is emerging as a notably important contributor to the risk of HCC [59]. Wang et al. [60] reported that ALKBH5 upregulates long intergenic noncoding RNA 01468 (LINC01468) through m6A demethylation in NAFLD. Elevated LINC01468 binds SHIP2, promoting its cullin 4A (CUL4A)-linked ubiquitination and degradation. This activates the PI3K/AKT/mTOR pathway, driving de novo lipid biosynthesis and NAFLD cell proliferation. In contrast, another study by Chen et al. [61] revealed that ALKBH5 was downregulated in HCC, associated with worse clinical outcomes. They functionally confirmed that ALKBH5 suppresses cell proliferation. Mechanistically, ALKBH5 inhibits HCC cell proliferation by erasing m6A modifications on LY6/PLAUR Domain Containing 1 (LYPD1) mRNA, thereby attenuating IGF2BP1-mediated stabilization of LYPD1 and promoting its degradation.

Gastric cancer (GC) ranks as the fifth most prevalent cancer globally and similarly holds the fifth position in terms of cancer-related mortality [51]. It accounts for approximately 4.9% of all cancers and 6.8% of all cancer-related deaths worldwide. A recent study demonstrated that FTO is overexpressed in GC, and this elevated expression correlates with a poor prognosis [62]. Furthermore, it indicates that FTO may function in promoting cell proliferation. Recent findings showed that FTO-mediated m6A demethylation at the 3'UTR of AMPK catalytic subunit α1 (PRKAA1) mRNA reduces its m6A modification, thereby diminishing YTHDF2 binding and preventing YTHDF2-mediated mRNA degradation [63]. This stabilization upregulates PRKAA1 expression, promoting GC cell proliferation. In addition, a recent study identified that FTO enhances the expression of HOXB13 to promote GC cell proliferation through upregulating insulin-like growth factor 1 receptor (IGF-1R) and subsequent activation of the PI3K/AKT/mTOR signaling pathway [64]. Interestingly, Sun et al. [65] found that the GLI1/INHB1 positive feedback loop drives GC cell proliferation, and further revealed that FTO erases m6A modifications on GLI1 mRNA, thereby blocking YTHDF2-mediated degradation and upregulating GLI1 expression. Additionally, silencing ALKBH5 hampers GC cell proliferation and induces apoptosis by upregulating CHAC1 expression [66]. On the other hand, ALKBH5 also functioned on oncogenic non-coding RNAs (ncRNAs). A mechanistic study demonstrated that ALKBH5, in cooperation with long intergenic noncoding RNA 00659 (LINC00659), promotes GC cell proliferation by erasing m6A modifications on JAK1 mRNA, thereby blocking YTHDF2-mediated RNA decay and stabilizing JAK1 transcripts to upregulate its expression, which activates the JAK1/STAT3 oncogenic signaling pathway [67]. Further research is necessary to determine whether other ncRNAs are involved in ALKBH5-mediated regulation of these processes in GC.

Pancreatic cancer (PC) is a highly aggressive malignancy of the digestive system, known for its insidious onset, challenges in early diagnosis, and low surgical resection rates [68, 69]. Of all PC cases, approximately 90% are represented by pancreatic ductal adenocarcinoma (PDAC), making it the most common histological subtype [70]. It has been reported that FTO exerts an oncogenic role in PC/PDAC [71, 72]. Further studies have shown that the expression of FTO is more abundant in PC tissues compared to normal tissues. Knockdown of FTO stabilizes tissue factor pathway inhibitor 2 (TFPI-2) mRNA through YTHDF1, leading to increased TFPI-2 expression and inhibiting the proliferation of PC cells [71]. Moreover, another study demonstrated that elevated FTO levels in PDAC decrease m6A modifications in the 3'UTR of platelet-derived growth factor C (PDGFC), attenuating YTHDF2-mediated RNA degradation and stabilizing PDGFC expression, which reactivates the AKT/GSK3β signaling pathway to drive PDAC cell proliferation [72]. However, recent studies showed that another m6A demethylase ALKBH5 acts as a tumor suppressor in PC [25, 73, 74]. Guo et al. [25] revealed that decreased expression of ALKBH5 in PC is

associated with poor prognostic indicators. Overexpression of ALKBH5 inhibits PC cell proliferation both *in vitro* and *in vivo*, while its knockdown produces opposite effects. Mechanistically, ALKBH5 reduces the m6A modification of PER1 mRNA, thereby attenuating YTHDF2-mediated RNA degradation and stabilizing PER1 expression, which reactivates the ATM-CHK2-P53/CDC25C signaling cascade to inhibit tumor cell proliferation. Another study has shown that CUGBP Elav-like family member 2 (CELF2) is downregulated in PC tissues and functions as a tumor suppressor [73]. Furthermore, they discovered that ALKBH5 mediates m6A-dependent degradation of CELF2 via YTHDF2, which disrupts CD44 alternative splicing (shifting CD44s to CD44v), inhibits PC cell proliferation, and promotes apoptosis by activating the endoplasmic reticulum-associated degradation (ERAD) signaling. In addition to regulating mRNA, ALKBH5 also influences the metabolism of ncRNAs and related biological functions in PC. For instance, He et al. [74] revealed that ALKBH5 can stabilize K member 15 and WISP2 antisense RNA 1 (KCNK15-AS1) mRNA to mediate its upregulation. Overexpressing KCNK15-AS1 impedes cell proliferation and promotes cell apoptosis in PC by increasing levels of phosphatase and tensin homolog (PTEN) mRNA and inactivating the AKT pathway. While pancreatic neuroendocrine tumors (pNENs) rank as the second most frequent malignancy of PC and their incidence is growing [75], the function of ALKBH5 in these tumors remains largely unknown.

Studies have shown that ALKBH5 upregulates FABP5 expression by erasing m6A modifications on FABP5 mRNA, which enhances IGF2BP2-mediated RNA stabilization and increases FABP5 expression, thereby disrupting lipid metabolism and synergizing with PI3K/AKT/mTOR signaling to promote tumor cell proliferation in pNENs [76].

The m6A demethylases not only play a crucial role in the aforementioned common digestive system tumors, but they are also involved in the pathogenesis of esophageal cancer (EC), a highly lethal cancer that ranks as the seventh leading cause of cancer-related mortality globally [51]. Researchers have demonstrated that FTO is significantly upregulated in EC and functions as an oncogene by influencing cell proliferation and apoptosis in EC cells [77]. Mechanistically, FTO enhances AKT3 expression through YTHDF1-dependent stabilization of its 3'UTR, while METT14 antagonizes FTO's oncogenic role by counteracting its m6A demethylase activity. Esophageal squamous cell carcinoma (ESCC), which is the primary subtype of EC, accounts for over 80% of all cases [78]. Studies have shown that elevated FTO protein levels in ESCC patients are associated with a poor prognosis [23]. FTO can enhance ESCC cell proliferation by enhancing matrix metalloproteinase 13 (MMP13) expression. Zhao et al. [79] showed that FTO removes m6A modification on human epidermal growth factor receptor 2 (ERBB2) mRNA in ESCC cells, facilitating YTHDF1-mediated stabilization of ERBB2 transcripts and ultimately promoting tumor cell proliferation, migration, and invasion. Additionally, FTO upregulates long intergenic noncoding RNA 00022 (LIN00022) expression by erasing m6A modifications to evade YTHDF2-mediated mRNA degradation, thereby driving ESCC cell proliferation through LIN00022-dependent destabilization of p21 via ubiquitination [80]. Studies have also indicated a strongly positive correlation between Cancer Susceptibility Candidate 8 (CASC8) and ALKBH5 in promoting the proliferation of ESCC cells [81]. Further investigation showed that the upregulation of CASC8 induced by ALKBH5 enhances the expression of heterogeneous nuclear ribonucleoprotein L (hnRNPL) and activates the Bcl2/caspase3 signaling pathway. However, the role of ALKBH5 in the proliferation of ESCC cells remains controversial [82, 83]. Xue et al. [82] showed that depletion of ALKBH5 enhances ESCC cell proliferation, whereas knockdown of miR-193a-3p has the opposite effects. Furthermore, they uncovered a reciprocal positive feedback loop between miR-193a-3p and ALKBH5, wherein miR-193a-3p directly targets and suppresses ALKBH5 expression, while ALKBH5 in turn suppresses miR-193a-3p maturation by reducing its m6A modification, thereby driving ESCC growth.

Taken together, substantial evidence demonstrates that FTO and ALKBH5 are generally highly expressed in many digestive system tumors, acting as oncogenes by promoting cell proliferation and inhibiting apoptosis through targeting relevant targets and activating path-

ways. This provides a novel opportunity to explore the potential of RNA regulation-based therapy and pathway-based therapy in digestive system tumors.

The role of m6A demethylases in the migration, invasion, and metastasis of digestive system tumors

The initiation of invasion and metastasis, which is responsible for over 90% of solid tumor-related deaths, represents a key hallmark of cancer [43, 84]. Aggressive CRC is commonly accompanied by elevated expression of FTO, suggesting its oncogenic role in CRC. Studies have shown that increased levels of FTO in CRC promote migration, invasion, and metastasis through multiple mechanisms [22, 45]. For instance, FTO enhances ZNF687 expression through m6A demethylation, which activates the Wnt/β-catenin pathway to drive tumor cell migration and invasion [22]. On the other hand, FTO can suppress CRC cell migration and invasion under certain conditions [47]. Mechanistically, FTO and ALKBH5 suppress CRC progression by erasing m6A modifications on HK2 mRNA, thereby blocking IGF2BP2-mediated stabilization and translation of HK2 - a key glycolytic enzyme - which subsequently attenuates FOXO signaling and malignant behaviors such as migration and invasion. As for ALKBH5, some studies have found that ALKBH5 can function as both an oncogene and a tumor suppressor in CRC cell proliferation and metastasis by modulating crucial factors such as RAB5A and plant homeodomain finger-containing protein 20 (PHF20) in an m6A-dependent manner [48, 49]. Besides, ALKBH5 was involved in suppressing the NF-κB pathway, thereby inhibiting CRC cell motility [50]. Further research is required to clarify the potential oncogenic or tumor- suppressive effects of FTO and ALKBH5 on migration, invasion, and metastasis of CRC.

Consistent with CRC, overexpression of FTO is associated with tumorigenesis of HCC [54, 85]. Our findings show that FTO is highly expressed in HCC and is essential for in vitro migration as it positively influences the stability of human tubulin beta class Iva (TUBB4A) mRNA [54]. Apart from the tumor-promoting effect, Zhao et al. [86] identified a decrease in FTO expression in HCC tissues, implying the tumor suppressor role of FTO in regulating cell migration, invasion, and metastasis in HCC. Liu et al. [55] also supported this suppressor role of FTO in the invasive capabilities of HCC cells. Mechanistically, SIRT1 promotes HCC progression by destabilizing FTO via SUMOylation-mediated degradation, which increases m6A modification of tumor suppressor GNAO1 and suppresses its mRNA expression, thereby driving malignant phenotypes including metastasis. Another m6A demethylase ALKBH5 also plays a crucial role in promoting or inhibiting cell migration and invasion. For instance, ALKBH5 and METTL3 bind to circ-CCT3 and regulate its m6A modification, which stabilizes circ-CCT3 in a YTHDF2-dependent manner and enhances its function as a sponge for miR-378-3p [87]. By sequestering miR-378-3p, circ-CCT3 relieves its suppression of FLT1, leading to FLT1 upregulation and subsequent promotion of HCC cell migration and invasion. Zhang et al. [88] revealed that ALKBH5 promotes HCC metastasis by downregulating long intergenic noncoding RNA 02551 (LINC02551) through m6A demethylation, which disrupts its role as a molecular adaptor, enabling TRIM27-mediated ubiquitination and degradation of DDX24, thereby facilitating HCC growth and metastasis. Similarly, You et al. [89] demonstrated in their studies that ALKBH5 upregulates MAPK kinase kinase 8 (MAP3K8) expression by erasing m6A modifications to antagonize YTHDF2-mediated mRNA destabilization, thereby promoting HCC cell proliferation and metastasis. Conversely, poor prognosis of HCC was associated with lower expression of ALKBH5, suggesting the tumor suppressing role of ALKBH5 [61]. Mechanistically, ALKBH5-mediated m6A demethylation of LYPD1 mRNA disrupts IGF2BP1-mediated stabilization of LYPD1, reducing its expression, and thereby inhibiting the motility of HCC cells. Additionally, Wang et al. [90] reported that ALKBH5 suppresses HCC progression by downregulating Progestin and AdipoQ Receptor 4 (PAQR4) in an m6A-dependent manner. ALKBH5 demethylates m6A on PAQR4 mRNA, reducing its stability via the m6A reader IGF2BP1. Decreased PAQR4 expression inhibits the interaction between PAQR4 and AKT, thereby suppressing PI3K/AKT pathway activation, which restrains HCC cell proliferation and metastasis.

Previous studies reported that FTO was able to promote GC cell migration, invasion, and

metastasis by upregulating the expression of integrin beta1 (ITGB1) and MAP4K4 [91, 92]. Zhou et al. [93] also found that FTO enhances the degradation of caveolin-1 mRNA via its demethylation, which regulates the mitochondrial fission/fusion and metabolism, thereby promoting GC cell metastasis. Besides, FTO-induced activation of the PI3K/AKT signaling pathway also potentiates the migratory and invasive capabilities of GC cells [64, 94]. The epithelial-mesenchymal transition (EMT), a fundamental process for the progression of metastasis, involves the loss of epithelial cell polarity, tight junctions, and cell-cell adhesion properties, accompanied by the acquisition of interstitial cell morphology and migratory capabilities [95]. Recent research indicates that FTO functions as an oncogene through the EMT pathway, where knockdown of FTO results in increased expression of E-cadherin and decreased expression of vimentin [96]. Consistently, ALKBH5 has been identified as highly expressed in GC, the knockdown of ALKBH5 inhibits metastasis *in vitro*, while its overexpression has the opposite effect [66]. *In vivo* experiments also show a reduction in lymph node metastasis with ALKBH5 knockdown. Mechanistically, ALKBH5 interferes the stabilization of CHAC1 to promote GC metastasis. The binding of lncNEAT1 with ALKBH5 was recently found to support the invasion and metastasis of GC cells by enhancing the posttranscriptional expression of enhancer of zeste homolog 2 (EZH2) [97]. Consistent with this observation, another study demonstrated that ALKBH5 binds to JAK1 mRNA in the nucleus and removes m6A modifications at the GGAC motif within its coding sequence, thereby reducing YTHDF2-mediated mRNA degradation and stabilizing JAK1 expression in an m6A-dependent manner [67]. This stabilization, facilitated by the ALKBH5-LINC00659 complex, activates the JAK1/STAT3 signaling pathway, which drives gastric cancer cell metastasis. However, a controversial study showed that decreased expression of ALKBH5 is associated with clinical tumor distal metastasis and lymph node metastasis [98]. More evidence demonstrated that ALKBH5 inhibits GC metastasis by removing m6A modifications on PKMYT1 mRNA, which diminishes IGF2BP3 binding to its m6A sites and destabilizes PKMYT1 mRNA. This destabilization leads to the downregulation of PKMYT1 expression.

Recent studies demonstrate that m6A demethylases participate in the migration, invasion, and metastasis of PC as well. Knockdown of FTO in PC cells promoted the expression of TFPI-2 transcripts, thereby inhibiting cell migration and invasion [71]. Additionally, some studies have shown that FTO can play a crucial role in miRNA regulation by modulating m6A modification, which promotes cancer metastasis in PC. For instance, the silence of FTO can promote the maturation of miR-383-5p in an m6A-IGF2BP1-dependent manner, leading to the upregulation of its target gene integrin alpha-3 (ITGA3) and inhibiting the metastasis of PC cells [99]. Another m6A demethylase ALKBH5 also plays a key role in metastasis of PC. He et al. [74] have shown that the expression of the KCNK15-AS1 is markedly reduced in PC, highlighting its role in inhibiting cell migration and invasion. Furthermore, they demonstrated that ALKBH5 binds directly to KCNK15-AS1 and removes m6A modifications at 11 conserved RRACH motifs. This demethylation stabilizes KCNK15-AS1. The upregulated KCNK15-AS1 recruits MDM2 proto-oncogene (MDM2) to promote RE1 silencing transcription factor (REST) ubiquitination and degradation, which transcriptionally activates PTEN. This PTEN activation inhibits the AKT pathway, ultimately suppressing PC cell motility. Besides, PER1 and CELF2 were identified as critical targets of ALKBH5, and ALKBH5 inhibited the migration and invasion of tumor cells by regulating the expression of PER1 and CELF2 in PC [25, 73]. Similarly, in PDAC, ALKBH5 acts as a tumor suppressor by demethylating m6A modifications at the 3'UTR region of Wnt inhibitory factor-1 (WIF-1) mRNA, thereby stabilizing its expression and inhibiting the Wnt signaling pathway to inhibit cell proliferation and metastasis [100].

Research into the association between m6A demethylases and the migration, invasion, and metastasis of EC/ESCC, as well as the underlying mechanisms, is still in its early stages. Liu et al. [23] conducted a study revealing that overexpression of FTO could promote ESCC cell migration. Conversely, the loss of FTO function resulted in a reduction in the migration of cancer cells. Mechanistically, FTO upregulates MMP13 at both mRNA and protein levels, thereby promoting ESCC cell migration. Wei et al. [77] revealed that FTO is highly expressed in

EC, where it drives m6A demethylation at specific 3'UTR clusters of AKT3, thereby stabilizing AKT3 mRNA through a YTHDF1-mediated mechanism to promote EC metastasis. Interestingly, m6A methyltransferase METTL14 negatively regulates FTO function in the progression of EC. Additionally, another study revealed that pharmacological inhibition of FTO can hinder the EMT pathway in EC by decreasing the expression of N-cadherin and Vimentin, while simultaneously increasing the expression of E-cadherin [101]. Consequently, these results suggest that FTO holds potential as a novel predictive biomarker for EC/ESCC. As for ALKBH5, a negative correlation between ALKBH5 and metastasis of ESCC cells was recently illustrated [82]. Further investigation is required to fully elucidate the role of ALKBH5 in the migration, invasion, and metastasis of EC.

As a critical component of the digestive system, the biliary tract is frequently involved in malignancies, among which gallbladder cancer (GBC) represents the most common biliary tract tumor [102]. Within the spectrum of digestive system tumors, GBC shares common pathogenic drivers such as chronic inflammation, but is uniquely characterized by its strong association with cholelithiasis [103]. Few studies have explored the role of ALKBH5 in GBC, with only one study indicating that transforming growth factor beta 1 (TGFβ1) suppresses the translation efficiency of forkhead box A1 (FOXA1) by hindering ALKBH5's binding to the FOXA1 coding sequence [104]. This, in turn, facilitates metastasis in GBC associated with EMT.

Collectively, m6A demethylases FTO and ALKBH5 are critical factors in the cell migration, invasion, and metastasis of digestive system tumors, providing valuable insights into the understanding and treatment of these diseases. The biological function and underlying mechanism of m6A demethylation in facilitating cancer metastasis in digestive system tumors remain incompletely understood, and further research is needed for validation.

The role of m6A demethylases in the angiogenesis of digestive system tumors

Angiogenesis, the generation of new blood vessels, serves as a crucial driver of tumor growth and metastasis [105]. Emerging evidence underscores that m6A demethylases participate in the angiogenesis of digestive system tumors. In CRC, Li et al. [22] revealed that FTO-mediated upregulation of ZNF687 levels in CRC contributes to angiogenesis via activation of the Wnt/β-catenin pathway. Intrahepatic cholangiocarcinoma (ICC), the second most common primary malignant liver cancer, arises from epithelial cells of the bile duct or hepatic ducts [106]. Rong et al. [107] reported a significant association between a decrease in FTO protein levels in ICC and angiogenesis, which was demonstrated by the expression of CD34, a crucial marker for microvessel density related to angiogenesis [107, 108]. Recent findings also revealed the critical role of ALKBH5 in HCC angiogenesis. Circ-CCT3, a highly expressed circular RNA in HCC, is closely associated with angiogenesis in human umbilical vein endothelial cells (HUVECs) [87]. Mechanistically, ALKBH5 and METTL3 bind to circ-CCT3 and reduce its m6A methylation levels, thereby stabilizing circ-CCT3 expression in a YTHDF2-dependent manner. The stabilized circ-CCT3 acts as a competing endogenous RNA to sponge miR-378a-3p, which relieves miR-378a-3p-mediated suppression of FLT1. Consequently, FLT1 upregulation drives angiogenesis and promotes HCC progression. However, further research is required to determine whether m6A demethylases play a role in the angiogenesis of other digestive system tumors and consequently impact the malignant progression of these tumors.

The role of m6A demethylases in the resistance to chemo- and radiotherapy of digestive system tumors

Chemotherapy and radiotherapy are commonly utilized in the treatment of solid tumors [109]. However, the effectiveness of these therapeutic approaches is limited due to a series of genetic and epigenetic alterations that lead to resistance. A study demonstrated that FTO was significantly upregulated in both primary and 5-fluorouracil (5-FU)-resistant CRC tissues, where it functionally promoted chemoresistance through epigenetic regulation of apoptosis-related genes [110]. Mechanistically, FTO selectively erased m6A modifications within the coding sequence (CDS) region of SIVA1 mRNA, a critical pro-apoptotic factor. This demethylation event triggered YTHDF2-mediated recognition and subsequent degradation of

SIVA1 transcripts, effectively suppressing its protein expression. Additionally, Wang et al. [111] systematically elucidated that FTO enhances chemoresistance in CRC by stabilizing G6PD/PARP1 mRNA through m6A-YTHDF2 axis. Mechanistically, FTO catalyzes removal of m6A modifications at specific sites within the 3'UTRs of their transcripts. This demethylation process disrupts YTHDF2's recognition and binding to m6A-marked mRNAs, thereby blocking YTHDF2-mediated mRNA decay. Another m6A demethylase ALKBH5 has been shown to be involved in the radiosensitivity of CRC. Research by Shao et al. [112] indicated that circAFF2 is highly expressed in patients with radiosensitive CRC and correlates with better prognosis. Mechanistically, ALKBH5 demethylates circAFF2, stabilizing its expression by preventing YTHDF2-mediated degradation. The upregulated circAFF2 enhances the radiosensitivity in CRC cells both *in vitro* and *in vivo* by binding to Cullin-associated and neddylation dissociated 1 (CAND1), promoting its interaction with Cullin1 and inhibiting its neddylation process.

Drug resistance is a major factor in treatment failure in GC. The role of FTO in mediating drug resistance in GC cells has been established [113, 114]. FTO expression is highly upregulated in cisplatin-resistant GC cells [113]. Functionally, FTO knockdown enhances cisplatin sensitivity in GC cells both in vitro and in vivo, whereas FTO overexpression promotes cisplatin resistance. Mechanistically, FTO reduces m6A methylation on Unc-51-like kinase 1 (ULK1) mRNA, thereby diminishing YTHDF2 binding and preventing YTHDF2-mediated mRNA degradation. This stabilization upregulates ULK1 expression, which drives autophagy and contributes to cisplatin resistance in GC. Another study demonstrated that FTO up-regulates CDK5 Regulatory Subunit Associated Protein 1 Like 1 (CDKAL1) through m6A demethylation, thereby promoting GC cell proliferation and inducing mitochondrial fusion [114]. This mechanism ultimately enhances resistance to 5-FU treatment.

Recently, studies also revealed a crucial role of m6A demethylase-mediated m6A modification in PC/PDAC chemoresistance. Lin et al. [115] demonstrated that FTO is upregulated in gemcitabine-resistance PDAC cells and tissues, where it drives chemoresistance by reducing m6A methylation on Neuronal precursor cell-expressed developmentally downregulated 4 (NEDD4) mRNA. This demethylation diminishes YTHDF2 binding to NEDD4, thereby stabilizing NEDD4 mRNA and increasing its expression. The elevated NEDD4 promotes ubiquitination-mediated degradation of PTEN, leading to sustained activation of the PI3K/AKT signaling pathway, which enhances cell survival and confers gemcitabine resistance in PDAC. The expression of long intergenic noncoding RNA 01134 (LINC01134) is significantly elevated in primary tumors from PDAC patients [116]. FTO upregulates LINC01134 by demethylating its m6A modification, thereby diminishing YTHDF2 binding and preventing YTHDF2-mediated mRNA degradation. This stabilization of LINC-01134 promotes chemoresistance by acting as a molecular sponge for miR-140-3p, which relieves miR-140-3p-mediated suppression of WNT5A. The subsequent upregulation of WNT5A activates the WNT signaling pathway, driving gemcitabine resistance in PDAC. As for ALKBH5, researchers have found that gemcitabine treatment downregulates ALKBH5 expression in a PDAC patient-derived xenograft model, highlighting its role in reducing m6A modification at the 3'UTR of WIF-1 mRNA, thereby upregulating WIF-1 expression and suppressing Wnt signaling, which collectively enhances the chemosensitivity of PDAC cells [100]. In addition to its chemo-sensitizing effects, ALKBH5 promotes chemoresistance in PDAC by reducing m6A modification at the GGACU motif of DDIT4-AS1, which enhances HuR binding and stabilizes DDIT4-AS1 expression [117]. This upregulation of DDIT4-AS1 promotes UPF1 phosphorylation by preventing the binding of SMG5 and PP2A to UPF1, leading to DDIT4 mRNA destabilization and mTOR pathway activation. In PC, ALKBH5 positively regulates the expression of lncRNA SH3BP5-AS1 by demethylating its m6A modification, thereby stabilizing SH3BP5-AS1 mRNA in an IGF2BP1-dependent manner [118]. The upregulated SH3BP5-AS1 acts as a competing endogenous RNA to sponge miR-139-5p, which relieves miR-139-5p-mediated suppression of C-terminal binding protein 1 (CTBP1). Consequently, increased CTBP1 expression activates the Wnt signaling pathway, promoting chemoresistance in PC cells.

Recent studies demonstrate that ALKBH5 participates in the chemoresistance of ESCC cells. The high expression of CASC8 in ESCC is associated with poor prognosis [86]. ALKBH5-mediated upregulation of CASC8 promotes chemoresistance in ESCC cells by increasing the levels of hnRNPL and activating the Bcl2/caspase 3 pathway. Given the limited understanding of the mechanisms involved so far, it is imperative to conduct functional studies aimed at elucidating the mechanisms of m6A demethylases in EC/ESCC.

To conclude, these results suggest that the interaction among m6A demethylases, mRNA, and ncRNAs contributes to chemo- and radioresistance in digestive system tumors. Disrupting this interplay may offer promising novel therapeutic targets for cancer treatment strategies. Notably, there is a scarcity of studies exploring the role of m6A demethylases in chemotherapy and radiotherapy resistance during the progression of digestive system tumors. More extensive research is required to improve the clinical outcome for patients.

The role of m6A demethylases in regulating the TIME of digestive system tumors

m6A demethylases play a crucial role in determining the complexity and diversity of the TIME, thereby exerting significant regulatory influence on the initiation and progression of various cancers, including digestive system tumors [119]. The TIME is characterized by three key features: hypoxia, metabolic reprogramming, and immune suppression, which together contribute to the formation of an immunosuppressive microenvironment and regulate tumor immune evasion through diverse mechanisms.

Emerging evidence suggests that excessive hypoxia within tissues disrupts microenvironmental homeostasis, leading to the development of a hypoxic, hypoglycemic, and acidic TIME that promotes tumor initiation and growth [120]. Recent evidence has shown that m6A demethylases are regulated in hypoxic cancer cells, highlighting their critical role in hypoxia-mediated progression of digestive system tumors. For instance, in CRC, studies indicate that hypoxia triggers ubiquitination-mediated degradation of FTO protein via the E3 ligase STRAP at the K216 site, leading to its downregulation [121]. Reduced FTO levels are associated with higher recurrence rates and poor prognosis in CRC patients. Mechanistically, FTO loss enhances m6A methylation on metastasis-associated protein 1 (MTA1) mRNA, which is recognized and stabilized by IGF2BP2. This stabilization increases MTA1 mRNA and protein expression, thereby accelerating the metastasis and progression of CRC. Notably, a recent study links ALKBH5 to a hypoxia-driven tumor-associated macrophages in glioblastoma and suggests that m6A demethylases are involved in immune evasion [122]. Despite the absence of direct evidence regarding m6A demethylase-mediated immunoregulation in digestive system tumors, these studies have prompted us to further explore the regulatory function of m6A demethylases in the hypoxic tumor immunity microenvironment and their implications for immunotherapy.

Metabolic reprogramming is a key hallmark of malignancies, with dysregulated glucose and lipid metabolism not only closely linked to the initiation and progression of tumors, but also emerges as a significant mechanism for tumor immune evasion [43, 119, 123]. Emerging evidence highlights the critical role of m6A demethylases in metabolic reprogramming of digestive system tumors. In GC, FTO up-regulates the level of PRKAA1 expression by reducing m6A modification at its 3'-UTR, which diminishes YTHDF2-mediated mRNA degradation and enhances PRKAA1 stability [63]. This stabilization of PRKAA1 could promote glycolytic metabolism and redox balance. In HCC, Wang et al. [124] revealed that lncRNA FTO Intronic Transcript 1 (FTO-IT1), an intronic region transcript of FTO gene, stabilizes FTO mRNA and promotes its protein expression by enhancing the interaction between interleukin enhancer binding factor 2/3 (ILF2/ILF3) complex and FTO mRNA. Furthermore, FTO-IT1/FTO axis mediates the m6A modification of GLUT1 and PKM2 to promote glycolysis and progression of HCC. However, it is reported that ALKBH5 expression is downregulated in HCC, where it positively regulates Ubiquitin protein ligase E3 component N-recognin 7 (UBR7) expression through m6A-dependent mechanisms [125]. The upregulated UBR7 inhibits glycolysis and tumorigenesis by modulating the Keap1/Nrf2/Bach1/HK2 signaling axis. Recent findings also suggested that elevated FTO levels in the liver can enhance lipogenesis and

enlarge lipid droplets through the sterol regulatory element binding protein-1c (SREBP1c) pathway [126]. Consequently, this results in excessive accumulation of lipids and contributes to the development of NAFLD. Additionally, ALKBH5 also promotes lipid biosynthesis in NAFLD by stabilizing LINC01468 RNA through m6A demethylation, which reduces its m6A modification and enhances RNA stability [60]. This stabilization facilitates LINC01468-mediated degradation of SHIP2 via CUL4A-dependent ubiquitination, leading to sustained activation of the PI3K/AKT/mTOR pathway. Consequently, this signaling cascade drives lipogenesis and tumorigenesis. In line with these findings, other findings have also demonstrated the oncogenic role for FTO in EC progression [127]. Mechanistically, FTO enhances estradiol 17beta-dehydrogenase 11 (HSD17B11) expression by removing m6A modification from its mRNA, which stabilizes the transcript and promotes its translation via the m6A reader YTHDF1. This upregulation of HSD17B11 ultimately drives lipid droplet formation and tumorigenesis in EC cells. In pNENs, ALKBH5 drives aberrant lipid metabolism by reducing m6A methylation on FABP5 mRNA, which enhances its binding to IGF2BP2 and stabilizes FABP5 expression. This stabilization of FABP5, in turn, activates the PI3K/AKT/mTOR pathway [76]. m6A demethylation has emerged as a pivotal process in regulating amino acid metabolism [128]. Glutaminolysis inhibition enhances the activity of FTO, resulting in decreased m6A modification of activating transcription factor 4 (ATF4) mRNA and prolonging its half-life by interfering with YTHDF2-facilitated RNA degradation [128]. Consequently, the increased expression of ATF4 triggers the transcription of DDIT4, which inactivates mTOR signaling and promotes pro-survival autophagy. As such, how does the regulation of metabolic reprogramming by m6A demethylases specifically influence anti-tumor immunity and immunotherapy of digestive system tumors? Recently, in co-culture model of CRC cells and macrophages, aberrant upregulation of ALKBH5 mediates the upregulation of CPT1A by removing m6A modification, followed by the enhancement of fatty acid metabolism and M2 polarization of macrophages [129]. This discovery is of great significance for understanding metabolic reprogramming-mediated by m6A demethylation and immune regulation in the TIME of digestive system tumors. However, further compelling evidence is required to establish a direct connection between metabolic reprogramming-mediated by m6A demethylases in reshaping the TIME and regulating tumor immune evasion of digestive system tumors.

The TIME of almost all tumors is characterized by a highly immunosuppressive state [133]. Apart from the immune evasion induced by m6A demethylases-mediated hypoxia and metabolic reprogramming of digestive system tumors, many tumor cells further enhance this process by manipulating intrinsic regulators. PD-L1 is the primary immune suppressive molecule in tumor cells, and studies by Nobuhiro et al. [130] found that both FTO and PD-L1 are highly expressed in CRC cells. Furthermore, FTO knockdown inhibits the expression of PD-L1 in an IFN-γ signaling-independent manner, leading to immune escape of CRC cells. In ICC, ALKBH5 interacts with PD-L1, leading to the removal of m6A methylation from the 3'UTR region of PD-L1 mRNA [131]. This process hinders YTHDF2-induced degradation, thereby maintaining PD-L1 expression and inhibiting the proliferation of cytotoxic T cells. Gaining insight into the mechanism by which m6A demethylases modulate PD-L1 expression could offer perspectives for addressing the clinical hurdles faced in immunotherapy. In the context of EC, an abnormal upregulation of serine hydroxymethyltransferase 2 (SHMT2) is found to correlate with prognosis [132]. Furthermore, they found that an elevated expression of SHMT2 promoted PD-L1 expression. Conversely, a reduction in SHMT2 expression led to a suppression of PD-L1 expression. Mechanistically, SHMT2 maintains the stability of c-myc mRNA in a METTL3/FTO/ALKBH5/IGF2BP2-dependent manner to mediate immune evasion. The increase in immune-suppressing cells and the heightened secretion of inhibitory cytokines by these cells in the TIME impair the function of tumor-killing immune cells, maintaining an immune-hostile environment and facilitating tumorigenesis [134]. You et al. [89] demonstrated that ALKBH5-mediated the upregulation of MAP3K8 is essential for promoting the enrichment of PD-L1^{+} macrophage in HCC, thereby accelerating tumor progression and fostering an immunosuppressive TIME. As a mechanism controlling the above processes in regulating the TIME of digestive system tumors,

Table 2. The identified m6A demethylase-targeted compounds

Target gene	Inhibitor	Function	Refs
FTO	Rhein	Competes with FTO binding for the m6A-containing nucleic acid.	[135]
	MO-I-500	Inhibits the 2-OG-dependent hydroxylase FTO.	[136]
	MA/MA2	Competes with FTO binding for the m6A-containing nucleic acid.	[137]
	FB23/FB23-2	Competes with FTO binding for the m6A-containing nucleic acid.	[138]
	Dac51	Competes with FTO binding for the m6A-containing nucleic acid.	[140]
	CHTB	Competes with FTO binding for the m6A-containing nucleic acid.	[141]
	FL6/FL8	Competes with FTO binding for the m6A-containing nucleic acid.	[142]
	FTO-04	Competes with FTO binding for the m6A-containing nucleic acid.	[143]
	FTO-43	Competes with FTO binding for the m6A-containing nucleic acid.	[144]
	18077/18097	Binds to the active site of FTO.	[145]
	C1-C8	Binds to the active site of FTO.	[146]
	CS1/2	Binds to FTO and blocks its catalytic pocket.	[147]
	N-CDPCB,1a	Binds to the non-conserved amino acids of FTO.	[150]
	R-2HG	Competitively binds to FTO.	[151]
ALKBH5	Ena15/21	Uncompetitively and competitively inhibits 2OG.	[152]
	MV1035	Binds to the active site of ALKBH5.	[153]
	2-[(1-hydroxy-2-oxo-2-phenylethyl) sulfanyl] acetic acid	Binds to the active site of ALKBH5.	[154]
	4-[(furan-2-yl) methyl] amino-1,2-diazinane-3,6-dione	Binds to the active site of ALKBH5.	[154]
	Curcumin	Unknown.	[155]
	ALK-04	Unknown.	[156]

FTO, fat mass and obesity associated protein.

m6A demethylases are emerging as promising targets and prognostic indicators for improving immunotherapy.

In summary, the significance of m6A demethylases in reshaping the TIME through hypoxic, metabolic reprogramming, and immune suppression, subsequently influencing tumor immune evasion of digestive system tumors is gaining attention. The deeper understanding of the molecular mechanisms by which m6A demethylases regulate the TIME in these tumors offers promising targets for improving immunotherapy.

Targeting m6A demethylases for digestive system tumors treatment

As mentioned above, FTO and ALKBH5 play crucial roles in the pathogenesis and progression of digestive system tumors, offering novel therapeutic targets. Currently, several inhibitors targeting FTO and ALKBH5 have been identified, with some showing promising potential for clinical application. In this section, we present an overview of existing FTO and ALKBH5 inhibitors (**Table 2**) and their prospects in the treatment of digestive system tumors.

The critical oncogenic role of FTO across various cancer types has prompted an increasing focus on the study of developing FTO inhibitors. Rhein, discovered in 2012 as the first FTO inhibitor, is a natural product that hinders FTO's m6A demethylation activity by competitively inhibiting its nucleic acid substrate [135]. Another inhibitor, MO-I-500, a synthetic compound analogous to ascorbic acid, shows broad-spectrum inhibitory activity against 2-oxoglutarate (2-OG) oxygenase similar to Rhein, though its applications are limited [136]. Additionally, meclofenamic acid (MA)/MA2, a non-steroidal anti-inflammatory-drug approved by the USA Food and Drug Administration (FDA), also competes with FTO for binding to m6A-containing nucleic acid [137]. Researchers showed that FTO inhibition by MA2 effectively suppresses the tumor growth in GC [96]. FB23, derived from MA, is significantly more effective than MA, showing approximately 140 times greater efficacy in inhibiting FTO [138]. Its derivative, FB23-2, can inhibit PC cell proliferation [72]. Additionally, Xiao et al. [139] found that nanomedicine can be effectively utilized to specifically deliver bioactive candidate FB23-2 to immune cells in the TIME of HCC. Based on these findings, Liu et al. [140] developed Dac51, a potent inhibitor based on FB23 and FB23-2, targeting FTO for immunotherapy applications [138, 140]. Apart from the aforementioned compounds, several inhibitors including

4-chloro-6-(6'-chloro-7'-hydroxy-2',4',4'-trimethyl-chroman-2'-yl) benzene-1,3-diol (CHTB), FL6/8, FTO-04, and FTO-43 have been identified as FTO inhibitors. They competitively bind to m6A-containig nucleic acid and their therapeutic effects have been evaluated across various tumor cell models in vitro [141-144]. Notably, FTO-04 shows potent antitumor effects in GC by effectively suppressing GC cell proliferation through attenuating the Wnt/PI3K/AKT signaling pathway [144]. Through structure-based rational design, compounds 18077, 18097, and 1,2,3-triazole-pyridine hybrids (C1-C8) were developed to inhibit FTO by binding to its active site [145, 146]. Notably, C8 displays the most potent inhibition, achieving an IC50 value of 780 nM in EC cells [101]. Su et al. [147] developed two small-molecule FTO inhibitors, CS1 and CS2, which bind tightly to FTO, blocking its catalytic pocket and obstructing the entry of m6A-modified oligos. The findings revealed that pharmacological intervention mediated by CS1 inhibits the viability, migration, and invasiveness of CRC and PC cells [148, 149]. Studies also revealed that CS1 and CS2 can effectively block the self-renewal of leukemia-initiating cells and reduce immune infiltration by destabilizing LILRB4 mRNA [147]. Unlike CS1/CS2, N-(5-Chloro-2,4-dihydroxyphenyl)-1-phenylcyclobutanecarboxamide (N-CDPCB,1a) inhibits FTO by binding to the non-conserved amino acids of FTO [150], requiring further investigation to understand its effect on digestive system tumors. R-2HG, structurally similar to α-KG, competitively inhibits a range of Fe(II)/α-KG-dependent dioxygenases [151]. Continued research is necessary to elucidate the role of these FTO inhibitors in the progression of digestive system tumors.

In contrast to extensive research on FTO inhibitors, the progress made towards developing small molecule drugs targeting ALKBH5 has been comparatively sluggish. Ena15 and Ena21 uncompetitively and competitively inhibit 2OG to show inhibitory on the enzymatic activity of ALKBH5 [152]. Notably, ALKBH5-specific inhibitors such as MV1035, 2-[(1-hydroxy-2-oxo-2-phenylethyl) sulfanyl] acetic acid, and 4-[(furan-2-yl) methyl] amino-1,2-diazinane-3,6-dione have shown promise by binding to the active site of ALKBH5 in glioblastoma [153, 154]. Additionally, ALK-04 has been demonstrated to improve responses to immunotherapy in the TIME [155]. The effects of curcumin on ALKBH5 activity warrant further study [156]. Given ALKBH5's role in demethylation, its potential as a therapeutic target for digestive system tumors is significant. More research is required to explore the impact of ALKBH5 inhibitors on these tumors.

In conclusion, therapeutic targeting of m6A demethylases remains in its early stages. With an enhanced comprehension of the structural aspects and mechanisms underlying m6A demethylase-mediated carcinogenesis and drug response, inhibitors targeting m6A demethylases hold promising potential for future clinical applications.

Conclusion and perspectives

In recent years, emerging evidence has shown that m6A modification and its regulators play a significant role in the development of various malignances [14]. Among these regulators, m6A demethylases, particularly FTO and ALKBH5, have attracted extensive research attention [157]. Studies have shed light on their effects on various cellular processes including proliferation, apoptosis, migration, invasion, metastasis, angiogenesis, as well as resistance to chemo- and radiotherapy (**Figure 3**). These findings indicate that FTO and ALKBH5 hold potential as biomarkers for clinical diagnosis and as therapeutic targets in digestive system tumors. Despite advancements achieved in multiple areas, several contradictions and uncertainties have emerged, encompassing the following situations: (1) The dual regulatory roles of FTO and ALKBH5 in various cancer types exhibit distinct patterns. Specifically, FTO functions as an oncogene in CRC, GC, PC/PDAC, and EC/ESCC (**Figure 3A**, **3C-E**), whereas it exhibits tumor-suppressive properties in ICC (**Figure 3B**). And ALKBH5 serves as an oncogene in HBV-HCC/NAFLD, GC, pNENs, and ESCC (**Figure 3B-E**), but demonstrates tumor-suppressive effects in GBC [104]. This complexity underscores the importance of cautiously considering the application of FTO/ALKBH5 activators or inhibitors to avoid potentially inducing other tumorigenic processes. (2) In the context of the same cancer, researchers have reached divergent conclusions regarding the roles of FTO and ALKBH5. In HCC, Li et al. [53] reported that FTO knockdown inhibits can-

cer cell proliferation and tumor growth in vivo. In contrast, Mittenbühler and colleagues [56] provided evidence that FTO plays a protective role in the initiation of HCC by dynamically regulating m6A-dependent demethylation of Cul4a mRNA, thereby suppressing hepatocarcinogenesis. Similarly, there are conflicting findings concerning ALKBH5. Hu et al. [98] revealed that interfering with ALKBH5 promotes the metastasis of GC cells. Conversely, Chen et al. [66] demonstrated that silencing ALKBH5 impairs the metastatic capacity of GC cells. This functional paradox may originate from intrinsic tumor sample heterogeneity (including discrepancies between cell line models and primary tumor specimens), statistical limitations from restricted cohort sizes, technical variability across detection platforms, and undetermined biological biases in epigenetic regulatory networks. Collectively, these complexities necessitate large-scale multicenter studies integrating multi-omics approaches to elucidate the context-dependent roles of FTO and ALKBH5 in digestive system tumors, which will establish a mechanistic framework for developing precision oncology strategies targeting m6A-mediated epigenetic dysregulation.

In addition, m6A demethylases are also involved in modulating the TIME and the effects on immune evasion in digestive system tumors, which are characterized by their primary features: hypoxia, metabolic reprogramming, and immune suppression (**Table 1**). As previously discussed, the restriction of FTO by hypoxia affects MTA1 mRNA m6A methylation and stability, subsequently triggering CRC metastasis [121]. These findings indicated a novel m6A-mediated mechanism of gene regulation in the epigenetic landscape of cancer metastasis induced by hypoxia. In addition, since m6A demethylases influence metabolic reprogramming within the TIME of digestive system tumors, future research should clarify how this m6A-dependent metabolic remodeling drives immune escape in those tumors. Furthermore, it is critical to explore whether m6A demethylases regulate additional immunosuppressive molecules in digestive system tumors via m6A-dependent mechanisms, as this represents a promising direction.

In modern oncology, chemotherapy and targeted therapy remain cornerstone strategies for tumor treatment. Emerging evidence reveals that pharmacological inhibition of m6A demethylases, particularly FTO and ALKBH5, effectively suppresses tumorigenesis across malignancies, with recent advancements in demethylase inhibitor development (**Table 2**) demonstrating significant therapeutic efficacy. Notably, m6A demethylase inhibitors emerge as promising innovative targeted therapies for digestive system tumors. While current research on m6A demethylase inhibitors in digestive system cancers predominantly focuses on FTO and ALKBH5, the clinical translation of these targets faces dual challenges: (1) Biomarker validation - large-scale multicenter studies and prospective trials are imperative to confirm m6A demethylase's diagnostic precision and their utility in guiding personalized therapeutic regimens. (2) Drug development - despite progress in combinatorial strategies (e.g., with nilotinib or bortezomib) [158, 159], the field urgently requires tumor-specific inhibitors optimized for digestive system tumors.

Overall, the current findings highlight the pivotal roles of m6A demethylases in digestive system tumors, with emerging therapeutic potential through targeted inhibition. However, contradictions in their oncogenic versus tumor-suppressive roles and challenges in biomarker validation and drug development necessitate future studies to clarify context-dependent mechanisms, validate reliable biomarkers, and develop tumor-specific inhibitors for precision oncology.

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Disclosure of conflict of interest

None.

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