Targeting key RNA methylation enzymes to improve the outcome of colorectal cancer chemotherapy (Review)

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Abstract. RNA methylation modifications are closely linked to tumor development, migration, invasion and responses to various therapies. Recent studies have shown notable advancements regarding the roles of RNA methylation in tumor immunotherapy, the tumor microenvironment and metabolic reprogramming. However, research on the association between tumor chemoresistance and N6-methyladenosine (m6A) methyltransferases in specific cancer types is still scarce. Colorectal cancer (CRC) is among the most common gastrointestinal cancers worldwide. Conventional chemotherapy remains the predominant treatment modality for CRC and chemotherapy resistance is the primary cause of treatment failure. The expression levels of m6A methyltransferases, including methyltransferase-like 3 (METTL3), METTL14 and METTL16, in CRC tissue samples are associated with patients' clinical outcomes and chemotherapy efficacy. Natural pharmaceutical ingredients, such as quercetin, have the potential to act as METTL3 inhibitors to combat chemotherapy resistance in patients with CRC. The present review discussed the various roles of different types of key RNA methylation enzymes in the development of CRC, focusing on the mechanisms associated with chemotherapy resistance. The progress in the development of certain inhibitors is also listed. The potential of using natural remedies to develop antitumor medications that target m6A methylation is also outlined.

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Abbreviations: M6A, N6-methyladenosine; METTL3, methyltransferase-like 3; MTC, m6A methyltransferase complex; TCM, Traditional Chinese Medicine; CRC, colorectal cancer; DES, deficiency and excessive syndrome; YAP, Yes-associated protein; GAS5, growth arrest-specific transcript 5; FTO, fat mass and obesity-associated protein; PDX, patient-derived tumor xenograft; CSC, colorectal cancer stem cell; FOLFOX, 5-FU + oxaliplatin; XELOX, oxaliplatin + capecitabine; DTP, drug-tolerant persister; EMT, epithelial-mesenchymal transition; H4K3, histone third subunit IV lysine; DDR, DNA damage repair; HR, homologous recombination repair

Key words: m6A methylation transferase, METTL3, colorectal cancer, chemotherapy, tumor microenvironment, epigenetic modifications

Contents

1. Introduction

Since 1960, when chemical modifications of RNA were first documented in detail as forms of epistatic modifications, >170 forms of RNA modifications, which have the role of maintaining mRNA stability and are involved in precursor shearing, transport and translation initiation of mRNA (1), have been identified (2). Of these modifications, the methylation modification of the nitrogen atom at position 6 of the RNA molecule adenine, i.e., N6-methyladenosine (m6A) modification, is the most widespread in eukaryotes (3). Although RNA methylation was discovered >60 years ago, owing to technical limitations, previous studies on epigenetic modifications in tumors have mostly focused on DNA methylation and histone modifications (4-7). It was not until 2012 when Meyer *et al* (8)

first applied m6A-sequencing (seq) technology to determine the overall m6A levels of human and mouse genes at the transcriptional level on a large scale, and until 2015, when Linder *et al* (9) first used m6A individual-nucleotide-resolution cross-linking and immunoprecipitation-sequencing technology to achieve single-base m6A level detection that m6A research was gradually developed.

An increasing number of studies have found that abnormal m6A methylation is closely associated with the development, metastatic recurrence and treatment failure of various tumors (10,11).

Due to the relevance of epigenetic modifications in the extracellular environment, the complex heterogeneity of tumors and the potential of immunotherapy, research on m6A and tumors has predominantly focused on the immune microenvironment and immunotherapy (12,13). Indeed, only a few studies have really focused on the relationship between chemoresistance and m6A methylation, and even less on a specific single cancer entity.

Colorectal cancer (CRC) is the third most common cancer type worldwide (14), and significant amounts of research have been devoted to the development of novel antitumor agents in the form of immune agents, lysosomal viruses and vaccines; however, chemotherapy is still the dominant treatment strategy (15). By contrast, primary or secondary chemoresistance is the main cause of treatment failure in patients with advanced CRC, and another limitation of conventional chemotherapy is the lack of specific targets (16). Primary drug resistance refers to poor response of the tumor tissue to the conventional dose of drugs at the initial treatment, while secondary drug resistance refers to the expected state that the tumor tissue can shrink or necrosis under the action of the drug at the initial treatment, but the efficacy decreases or metastatic recurrence may even occur after long-term use of the drug. The difference lies in the response to the initial tumor treatment.

Epigenetic modifications of RNA are associated with sensitivity to multiple chemotherapeutic agents (17). Therefore, the present study focused on the relationship between aberrant m6A methylation modifications and chemotherapeutic response mechanisms in CRC (18-20) and various potential therapeutic measures targeting the m6A methylation process to seek novel strategies to improve the therapeutic effect pertaining to CRC.

Numerous scholars posit that the reversible and protein-regulatory properties of m6A methylation offer a promising approach to overcoming the current shortcomings in multiple cancer therapies (8). However, the real application of m6A methylation and clinical transformation must also solve a myriad of issues, including the following problems: i) M6A methylation as the most extensive RNA modification-how to focus and target the key molecules; ii) how to screen out the most accurately targeted drug candidates in each cancer species; and iii) how to specifically target the regulatory axis involved in m6A methylation to reverse drug resistance in tumors (21).

2. The m6A key enzyme in the development of CRC

Methyltransferases. The key m6A methylation enzymes can be classified into methyltransferases, methyl recognition enzymes and demethylases.

M6A methyltransferases mostly function as complexes [the m6A methyltransferase complex (MTC). The MTC mainly comprises methyltransferase-like 3 (METTL3), METTL14, vir like m6A methyltransferase associated (VIRMA), WT1 associated protein (WTAP), zinc finger CCCH-type containing 13 (ZC3H13), RNA binding motif protein 15 and Cbl proto-oncogene like 1 (HAKAI) (22,23). METTL3 was the first m6A methyltransferase identified and the only catalytic subunit of MTC, indicating that the presence and activation of METTL3 are the basis for m6A methylation. Although METTL3 can act independently of other m6A methyltransferases, its catalytic activity is much weaker than that of the MTC formed by wrapping it with other transferases (24). METTL14, as the primary RNA binding platform, forms a complex with METTL3 through 10 positively charged binding sites (Fig. 1), activating and enhancing METTL3 activity and promoting the recognition of RNA substrates, and thus enhancing MTC methylation efficiency (25,26). By contrast, WTAP is essential for promoting the enrichment of METTL3, METTL14 and other methyltransferases, transporting MTC into the nucleus and stabilizing MTC activity in the organism (27,28). VIRMA and ZC3H13 are relatively newly discovered m6A methyltransferases. VIRMA, also known as KIAA1429, which are the largest molecular weight proteins of MTC components known to date. VIRMA may be a scaffold for the MTC structure, connecting the immobilized WTAP, HAKAI and ZC3H13 to form an envelope structure capable of accommodating the METTL3-METTL14 complex (29). ZC3H13 is also a linking protein that bridges WTAP to the METTL3-METTL14 complex and facilitates the recognition of RNA substrates.

It was discovered that m6A methyltransferases are extensively involved in various stages of CRC, including tumor stemness, microenvironmental remodeling, drug resistance, metastasis and recurrence. Some of the roles and related pathway molecules are shown in Table I.

For a long time, the methyltransferase METTL3 has been regarded as a pro-oncogene (43,44). However, in recent years, a limited number of studies have found that under specific conditions, such as tumor starvation, METTL3 may also inhibit tumor development and development through the activation of the p38 signaling pathway and interference with the cell cycle to bring tumor cells into a dormant state (38,45). More interestingly, it has been suggested that METTL3 can function not only as a methyltransferase, but also as a methyl recognition enzyme independent of YTH N6-methyladenosine RNA binding protein F (YTHDF)1, capturing the recognition of mRNAs undergoing m6A methylation modification in the cytoplasm, promoting the recruitment of E74 like ETS transcription factor 3 (elF3) (46), drive β-linked protein trans-activation and upregulate c-Myc, VEGF, cyclin D7, MMP-3, c-Jun and other key genes of intestinal cancer malignant phenotypes (47).

Of note, m6A methylation is not only related to the modern medical concept, but also similar to certain Traditional Chinese Medicine (TCM) concepts (48,49).

In the concept of TCM, the function of various parts of the body is associated and influenced by the external environment. This is similar to epigenetics. If the external evil is more severe than the physical weakness, it is classified as excessive syndrome (ES) according to TCM concepts; if

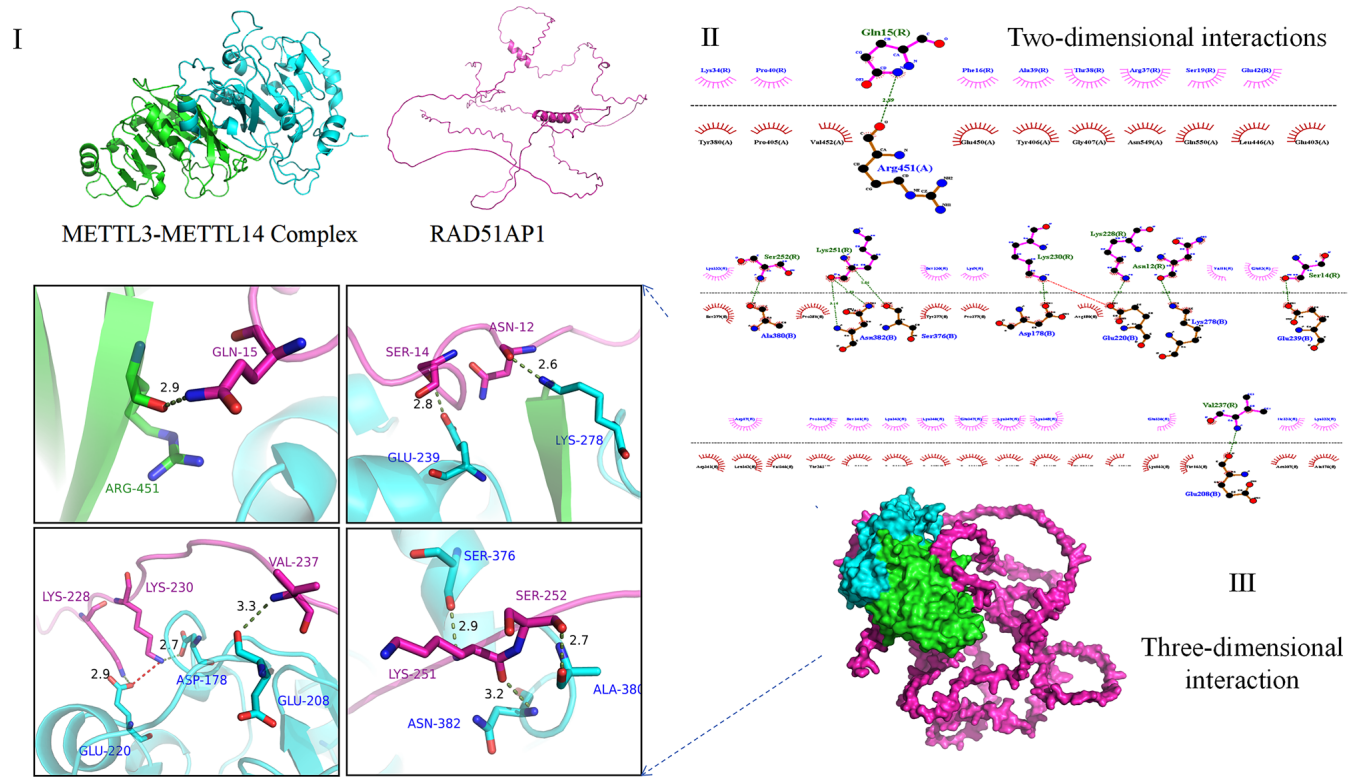

Figure 1. Schematic diagram of the downstream protein interactions of the human METTL3-METTL14 complex and related resistance mechanisms. (I) Left: Structure of the human METTL3:METTL14 complex. Right: Protein structure of the human RAD51AP1 (one of the downstream proteins of METTL3-mediated 5-FU resistance in colorectal cancer). (II) Two-dimensional interaction between the METTL3-METTL14 complex and the RAD51AP1: The red dotted line indicates a salt bridge, the green dotted line hydrogen bonds, (A) indicates METTL3, (B) METTL14 and (R) RAD51AP1, where (A) chain ARG451 and GLN15 of the R chain, (hydrogen bond chain A) mainly have a hydrophobic interaction and R chain, including R chain positive LYS230 with (B) protein negatively charged GLU220 have a salt bridge interaction. (III) Three-dimensional interaction between the METTL3-METTL14 complex and RAD51AP1: Sky blue represents METTL14, green stands for METTL3 and purple indicates METTL14. The dark green dashed line shows the hydrogen-bonding interactions, e.g., GLU239, LYS278 and GLU220 of METTL14 form hydrogen bond interactions with ASN12, SER14 and ASP178 of METTL3 proteins; its hydrogen bonds are 2.8, 2.6 and 2.7 Angstroms. METTL3, methyltransferase-like 3; RAD51AP1, RAD51-associated protein 1.

the two are of the same magnitude, it is classified as deficiency and excessive syndrome (DES); and if the physical weakness is more pronounced, it is defined as deficiency syndrome (DS). The early and early-middle stages of CRC often appear as ES or DES, while the late stages are DS (50,51). Under the guidance of these theories, TCM treatments need to be selected according to the different manifestations of different syndrome types in patients, so as to be used correctly (52).

It has been found that patients with CRC with different syndrome types have differences in gene expression, and elF3 and the downstream factors it regulates are typical representatives. High expression of the keratin 19, keratin 18, keratin 8, ELF3 and serpin family E member 1 genes is a potential marker to identify the TCM evidence type in CRC and, high expression of the ELF3 gene in CRC with DES or ES. High expression of mucin 2 and regenerating family member 4 in DES was mainly related to cell growth, as well as the MAPK and cyclic adenosine 3',5'-monophosphate signaling pathways, while high expression of collagen type I alpha 2 chain and periostin genes in ES was mainly related to angiogenesis and the PI3K/AKT pathway and the caveolae-associated protein 2 and glutathione peroxidase 1 genes were highly expressed in DS and are mainly related to vomiting, platelet catabolism and endocytosis (50,53).

Whether m6A methyltransferases can function as methylation recognition enzymes or other epigenetic factors remains to be elucidated. The combination of classical medical theories, including TCM and epigenetic modifications, which are emerging molecular biology concepts, provides new perspectives for antitumor therapy and warrants further exploration (54,55).

Methyl recognition enzymes. The main function of methyl recognition enzymes is to recognize bases that undergo modification, thus activating downstream pathways and participating in biological processes, including mRNA translation, transcription, fission, and degradation. The core members include YTHDF1-3 and YTHDC1-3 (56-58). YTHDF2 and YTHDF3 accelerate the degradation and fission of modified mRNAs by recruiting the C-C chemokine receptor 4-negative regulator of transcription deadenosylation complex (59) and upregulating forkhead box O3 (FOXO3) expression (60), respectively.

Wang *et al* (61) found that YTHDF1 was significantly amplified and upregulated in CRC tissues, a phenomenon closely associated with the inflammatory cancer transformation of intestinal lesions and liver and lung metastases in patients with the aforementioned disease.

Although the exploration of RNA methylases is not widely performed in clinical practice, it is not only limited

Table I. M6A methylation transferase and related pathway molecules in colorectal cancer development.

Methyltransferases	Role	Functions	Institutional approach	Author	Publication year	(Refs.)
METTL3	Cancer-promoting genes	Promotes the maturation and secretion of exosomes, leading to tumor metastasis	miR-1246/SPRED2/MAPK signaling pathway	Peng	2019	(20)
			pri-miR-196b	Huang	2023	(30)
			miR-107/PER3 axis	Zhang	2022	(31)
			miR-106b-5p/PTEN axis	Shi	2022	(32)
		Accelerates the transformation of inflammatory cancer	Upregulated by Fusobacterium nucleatum	Xu; Chen	2022; 2022	(33,34)
		Interferes with energy metabolism in bowel cancer	Targets on m6A-GLUT1-mTORC1 axis, promoting Warburg effect	Shen; Chen; Lu	2020; 2021; 2021	(19,35,36)
			Mitochondrial metabolism remodeling	Sun	2021	(37)
	Cancer-suppressor genes	Inhibits colorectal cancer cell proliferation and migration	p38/ERK signaling pathway	Deng	2019	(38)
METTL14	Cancer-suppressor genes	Accelerates mRNA degradation	Downregulates SOX4; delays the EMT process	Chen	2020	(39)
		Inhibits aerobic glycolysis	Downregulates SLC53A2, PGAM3	Hou	2023	(40)
WTAP	Cancer-suppressor genes	Suppresses the Wnt signaling pathway and degrades β-catenin	Targets on the WTAP-WT1-TBL1 axis	Zhang	2016	(41)
VIRMA	Cancer-promoting genes	Increases mRNA stability	Upregulates HK2 levels to promote the Warburg effect	Li	2022	(42)

WTAP, WT1 associated protein; VIRMA, vir like m6A methyltransferase associated; m6A, N6-methyladenosine; SPRED2, Sprouty-related, EVH1 domain-containing protein 2; MAPK, mitogen-activated protein kinase; METTL3, methyltransferase-like 3; PER3, period circadian regulator 3; PTEN, phosphatase and tensin homolog; GLUT-1, glucose transporter type 1; mTORC1, mechanistic target of rapamycin complex 1; SOX4, SRY-box transcription factor 4; WT1, Wilm's tumor gene; TBL1, transducin β-like protein 1; HK2, hexokinase 2.

to discovery. There are also some small molecule drugs for methylation recognition enzymes such as YTHDF1, which is helpful for subsequent research.

The group of the above-mentioned study also developed a lipid nanoparticle-encapsulated Rho guanine nucleotide exchange factor 1 small interfering RNA drug for *in vivo* tumor therapy. YTHDF1 also up-regulates the transcription factor glucocorticoid modulatory element binding protein 2 of the adhesion-regulating molecule-1/nuclear factor kappa pathway, thereby activating the pathway to resist apoptosis and drive CRC progression (62). Ni *et al* (63) demonstrated that YTHDF3 is a novel target for the Yes-associated protein 1 (YAP) signaling pathway. A long noncoding RNA called growth arrest-specific transcript 5 (GAS5) binds directly to YAP, promoting YAP phosphorylation and attenuating YAP-mediated YTHDF3 transcription and allowing YTHDF3 to reversibly and selectively bind to GAS5, which undergoes m6A-methylation to trigger its decay and form a negative feedback loop. Although methylation recognition enzymes have been relatively poorly studied, they are indispensable for the proper binding of methyltransferases to the modified site.

Insulin-like growth factor 2 mRNA binding proteins (IGF2BPs) are also significant methyl recognition enzymes. They can specifically recognize and then bind directly to the m6A modification site, subsequently upregulating SOX2 to activate CRC stem cells (CSCs) (64). IGF2BP2 also can induce chemoresistance in CRC cells by activating the PI3K/AKT signaling pathway and enhancing aerobic glycolysis (65). However, these functions are still inseparable from the upstream regulation of METTL3.

Demethylases. Thus far, the knowledge of demethylases is limited and the only known demethylases are fat mass and obesity associated (FTO) and alkylation repair homolog 5 (ALKBH5). Demethylases are key to the reversibility of m6A methylation (66,67) and are able to complete the demethylation process by oxidizing m6A to N6-hydroxymethyladenosine and N6-formyladenosine (68-70). Conventionally, the reversibility of methylation modifications creates a window for reversing chemoresistance and increasing demethylase activity to tilt the reaction rate toward demethylation, thus facilitating the re-sensitization of drug-resistant cells to chemotherapy.

Relier *et al* (71) found that, although the overall FTO expression did not change significantly at different CRC stages, the subcellular localization of FTO shifted from strictly in the nucleus to the cytoplasm in the mucosa during metastatic CRC infiltration, which may be related to the tumor metastatic process. Meanwhile, FTO knockdown as performed in several different colon cancer cell lines, patient-derived cells and Patient-derived tumor xenograft animal models enhanced aldehyde dehydrogenase (ALDH) activity and promoted the CSC phenotype. Mice subjected to FTO silencing exhibited significant resistance to FOXFOL [50 μM 5-fluorouracil (5-FU) + 1 μM oxaliplatin (OXA)] as a first-line treatment, suggesting FTO is an important marker for predicting CRC metastasis and chemoresistance. Furthermore, Ruan *et al* (72) illustrated that, under hypoxic conditions in the tumor microenvironment, FTO leads to increased degradation of serine/threonine kinase receptor-associated protein as an E3 ligase-mediated ubiquitinated protein that promotes CRC metastasis. Further studies to elucidate the specific mechanisms of interaction between how FTO achieves metastasis from the nucleus to the cytoplasm according to the progression of CRC staging, how it identifies and specifically binds to its downstream targets and tumor microenvironment features, including those of hypoxia, FTO and CRC metastasis, and the response to therapeutic measures such as chemotherapy, are warranted. High expression and the methylation degeneration of ALKBH5 are strongly associated with Lynch syndrome (73) and silencing ALKBH5 facilitates an enhanced immune response, reduces lactate accumulation in the tumor microenvironment and increases infiltration of T-regulatory cells and myeloid-derived suppressor cell production (74). These findings are of great significance for slowing down the trend of CRC rejuvenation (75) and inducing the conversion of microsatellite stable-type CRC to 'hot tumors' to benefit from immunotherapy.

In brief, the m6A methylation key enzymes are widely involved in all aspects of CRC development and have considerable interventional value. Accordingly, it is reasonable to ask what the role of these enzymes is in the current mainstream chemotherapeutic benefits pertaining to CRC, and what the potential opportunities for intervention are.

3. Mechanisms of chemoresistance in CRC involving key m6A methylation enzymes

The mechanisms by which chemoresistance occurs in CRC are complex and there is no shortage of links involving m6A methylation enzymes. In particular, the mechanism of chemotherapy resistance due to an altered tumor microenvironment regulated by m6A methylation key enzymes has gained attention. The known molecular mechanisms underlying the m6A methylation of key enzymes that regulate common chemotherapeutic drug resistance in pan-cancer are presented in Fig. 2.

Li *et al* (64) found that METTL3 expression was elevated in both primary and metastatic CRC foci compared to normal tissue using a The Cancer Genome Atlas database analysis, and that patients with high METTL3 expression benefited less from chemotherapy when XELOX (OXA + capecitabine) and FOLFOX (5-FU + OXA) were used as first-line regimens. Chen *et al* (76) found that overexpression of IGF2BP1 promoted the colony-forming ability and resistance to 5-FU and etoposide in CRC cells.

As the study of m6A methyltransferase is the most extensive and because 5-FU and OXA are the most commonly used chemotherapeutic agents in the treatment of CRC, the resistance mechanisms associated with m6A methylation were found to be highly dependent on the regulation of METTL3 (54) (as shown in Fig. 2). Therefore, this study will further focus on the 5-FU and OXA resistance mechanisms of m6A methyltransferases, particularly METTL3, in CRC. Due to the high complexity of the many genes and proteins involved and the associated mechanisms, the present review categorizes these mechanistic processes into drug transporter protein-related, stem cell activity, EMT process and cellular autophagy, as illustrated in Fig. 3 and below.

Mechanisms associated with the microenvironment surrounding CRC cells. Tumor microenvironment refers to the special survival environment of tumor cells with their

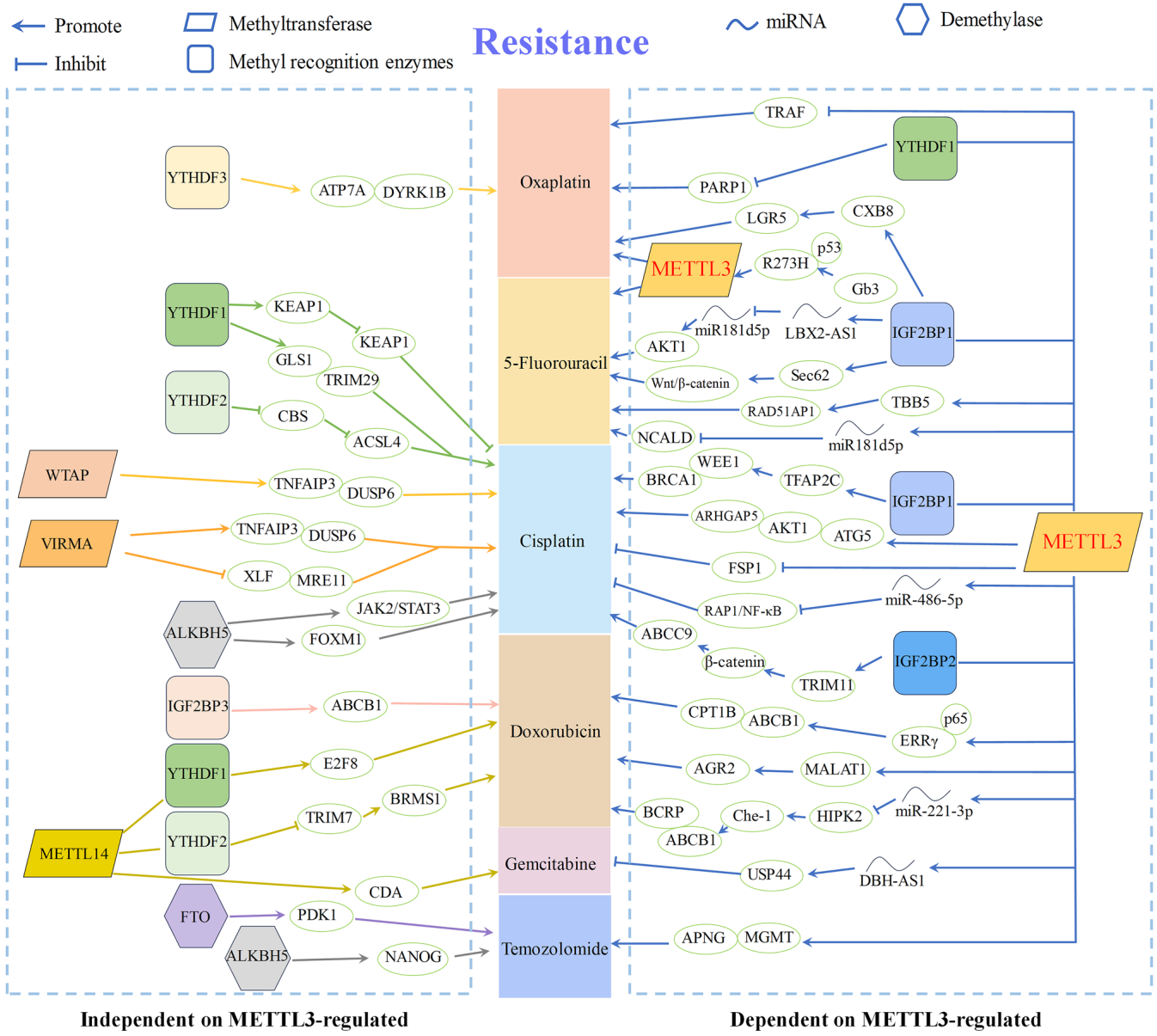

Figure 2. Role and molecular mechanisms of m6A regulators in chemotherapy resistance. Adapted from Liu *et al* (54). The data and figures of the article can be freely quoted and edited, in accordance with the CC0 protocol. Copyright link: Rightslink® by Copyright Clearance Center. Reasons for the present design: i) METTL3 is the catalytic center in the m6A methylation process and it was identified as the most widely available species of m6A methylation key enzyme. Accordingly, the m6A-associated mechanisms of common chemotherapeutic drugs were categorized into combinations based on whether they are dependent on METTL3 regulation. ii) The mechanisms were further classified and some of the latest findings were updated. Specifically, overexpression of TBB5 upregulated RAD51AP1 expression and induced an increase in m6A methylation modification of this gene, resulting in improved resistance to 5-FU in CRC cells. Knocking down METTL3 decreased the expression of RAD51AP1 and TBB5, while reducing the level of m6A methylation modification of RAD51AP1. As a result, resistant cells were once again sensitized to 5-FU. Upregulated by METTL3 and recognized by IGF2BP1, expression of the preprotein translocator known as Sec62 is upregulated, activating the Wnt/β-linker pathway and leading to enhanced 5-FU resistance in CRC cells. Due to the dysregulated glycolipid metabolism in CRC, there is an abundance of glycolipid complexes within CRC cells. These complexes containing Gb3 can upregulate R273H, leading to p53 mutation and induction of METTL3 for m6A methylation, ultimately resulting in resistance to 5-FU and oxaliplatin in CRC cells. iii) Methylases, recognition enzymes and demethylases were distinguished by different shapes. Enzymes of the same family are filled using the same color system (e.g., IGF2BP1-3 belong to the IGF2BP family and YTHDF1-3 belong to the YTHDF family). Through 3 or 4 steps of classification, it was found that the mechanism of 5-FU resistance is closely related to the regulation of METTL3 and the recognition function of the IGF2BP family. Cisplatin resistance is more closely related to the function of recognition proteins, and also involves the YTHDF family. METTL3, methyltransferase-like 3; m6A, N6-methyladenosine; 5-FU, 5-fluorouracil; RAD51AP1, RAD51-associated protein 1; YTHDF, YTH m6A RNA binding protein F; IGF2BP, insulin-like growth factor 2 mRNA binding protein; CRC, colorectal cancer; TBB5, tubulin beta class I; Sec62, SEC62 homolog, preprotein translocation factor.

surrounding chemokines, apoptotic factors, immune cells, adhesion proteins, joint composition of metabolic disorders, defective apoptosis, lack of oxygen and acidification. Such a special environment is conducive to the evasion of tumor cells in response to therapy and epigenetic alterations such as m6A methylation induce the inevitable adaptation of tumor cells to this special environment.

Metabolic remodeling in CRC. Metabolic remodeling is one of the 14 features of the tumor environment (77) and CRC is characterized by abnormal active glycolipid

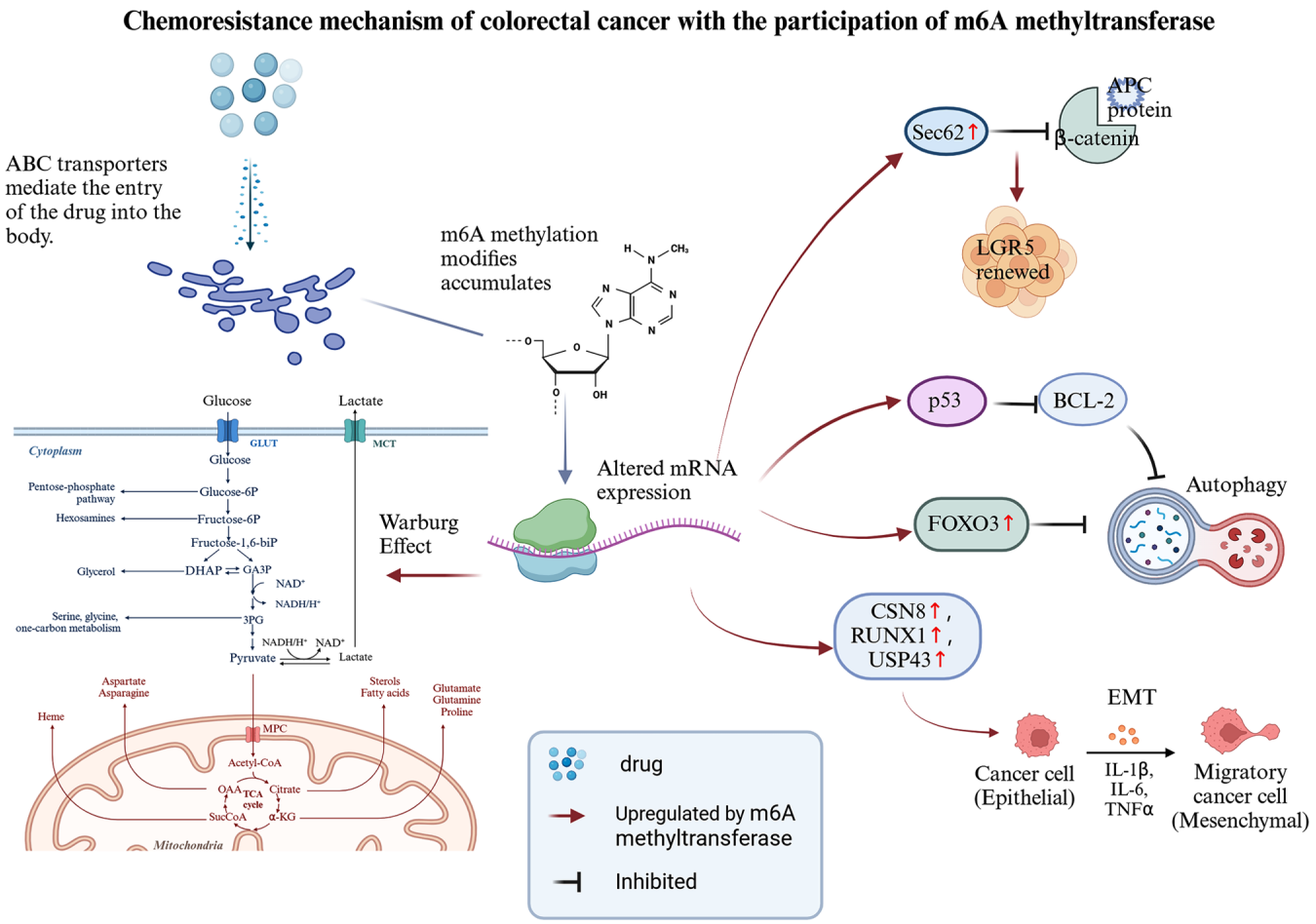

Figure 3. M6A methylation key enzyme may mediate the chemotherapy resistance process of colorectal cancer (using methyltransferase as an example). Created with BioRender.com. Several studies have indicated that METTL3 can impact the effectiveness of chemotherapy drugs such as cisplatin, oxaliplatin and 5-fluorouracil. These drugs are commonly used to treat various types of cancer by influencing different enzymes involved in m6A methylation. Understanding this mechanism is crucial, as it sheds light on the role of METTL3. However, the specific impact and mechanism of METTL3 on chemotherapy treatment for individual cancers have not received sufficient attention or been adequately summarized. This article aims to address and enlighten fellow researchers on this matter. METTL3, methyltransferase-like 3; m6A, N6-methyladenosine; miRNA, microRNA, EMT, epithelial to mesenchymal transition; ABC, ATP-binding cassette transporter; APC, adenomatosis polyposis coli; LGR5, leucine rich repeat containing G protein-coupled receptor 5; BCL-2, B-cell CLL/lymphoma 2, apoptosis regulator; FOXO3, forkhead box O3; CSN8, COP9 signalosome subunit 8; RUNX1, runt-related transcription factor 1; USP43, ubiquitin specific peptidase 43; TCA, tricarboxylic acid.

metabolic rearrangement. Interference with metabolic pathways by inhibiting the activity of key enzymes of glycolysis and blocking oxidative phosphorylation pathways may improve the sensitivity of CRC-resistant cells to cisplatin and vincristine (78-80). Overexpression of METTL3 recruits YTHDF1 to trigger the translation of LDHA mRNA, catalyze glycolysis and increase the resistance of CRC cells to 5-FU (81). By contrast, knockdown of METTL3 reduces the protein translation efficiency of hypoxia-inducible factor-1α, inhibits the activity of lactate dehydrogenase A, hexokinase 2, GADPH and other key enzymes of glycolysis, and blocks the occurrence of the Warburg effect in intestinal cancer cells (82), thus re-sensitizing drug-resistant cells to chemotherapy. In addition, YTHDF1 and 2 are involved in the activation of transcription factor 4-mediated glutamine metabolism, and deletion of YTHDF1 and 2 enhances cisplatin tolerance in colorectal cancer (83,84).

Autophagy in CRC cells. Autophagy refers to the formation of autophagic lysosomes stimulated by various adhesion and apoptotic factors that transfer intracellular material into lysosomes for degradation. When the drug-tolerant persister (DTP) state is activated, key autophagy genes such as unc-51 like autophagy activating kinase 1 and autophagy related 2A are upregulated and when autophagy inhibitors are administered, cells exit the DTP state and cannot survive chemotherapy (85,86).

Hao *et al* (60) found that the m6A methylation recognition protein YTHDF3 is required for the maintenance of autophagy and that YTHDF3 depletion interrupts the formation of autophagosomes. Although YTHDF3 promotes and recognizes the translation of the autophagy gene FOXO3, it does not maintain its stability. Silencing METTL3 without interfering with YTHDF3 can still impair autophagy-lysosome synthesis and destabilize FOXO3 mRNA. Thus, the complete regulation of autophagy in CRC cells is influenced by METTL3 and the METTL3/YTHDF3 complex. Lin *et al* (87) demonstrated that the expression of light chain (LC)3B, a marker of autophagy activation, was positively correlated with the expression of FTO in normal and 5-FU-resistant CRC tissues and that FTO knockdown resulted in a significant increase in apoptosis,

inhibition of autophagy in CRC cells and re-sensitization of drug-resistant cell lines to 5-FU.

Epithelial-mesenchymal transition (EMT). A study from China published in 2020 found that METTL3 regulates the TGF-β and Snail pathways to affect EMT in intestinal cancer cells (88). EMT is also an important mechanism for the development of chemoresistance in CRC, indicating a decrease in adhesion factors such as epithelial surface calmodulin, detachment from basement membrane connections, and cytoskeletal and morphological convergence to mesenchymal features. Various molecules such as COP9 signalosome subunit 8, runt-related transcription factor 1, ubiquitin specific peptidase 43, histone deacetylase 2 and tumor microenvironment-associated fibroblasts can regulate EMT in CRC cells (89). When EMT occurs, the malignant phenotype of intestinal cancer becomes more prominent and resistant to OXA and 5-FU (90).

Mechanisms associated with the function of CRC cells themselves

Stem cell activity in CRC. CSCs are a special class of cells with self-healing and multi-differentiation potential. The most clinically relevant feature of CSCs is their ability to metastasize and evade standard chemotherapy (91). The differentiation homeostasis of this class of cells is usually regulated by the Wnt/β-catenin signaling pathway. Sec62 is a key protein in this pathway, and its increased expression enhances the sphere-forming ability of CSCs. Liu *et al* (92) found that Sec62 expression was positively correlated with METTL3 expression and that the METTL3-mediated accumulation of m6A methylation, which upregulates Sec62 levels, competitively disrupted the binding of β-linked protein and oncogene adenomatosis polyposis coli, leading to 5-FU resistance. METTL3-mediated m6A methylation accumulation also drives the methyltransferase recruitment of histone third subunit IV lysine (H4K3) to the promoter of leucine rich repeat containing G protein-coupled receptor 5 (LGR5), a colon cancer stem cell marker, leading to irinotecan and OXA resistance (93). Bai *et al* (94) found that silencing YTHDF1 downregulated CSC markers, including CD133, CD44, ALDH1, octamer-binding transcription factor 4 and LGR5, and inhibited Wnt/β-linked protein pathway activity in *ex vivo* experiments. Accordingly, it is proposed that YTHDF1 recognizes and promotes the translation of m6A-modified frizzled class receptor 9 and Wnt6 mRNAs, leading to aberrant activation of Wnt/β-linked protein signaling and ultimately affecting tumorigenicity, stem cell-like activity and response to chemical agents in CRC as a scientific hypothesis.

In other words, some researchers have found that m6A methylation key enzymes can regulate the expression of various CSC markers and, consequently, affect CSC activity and sensitivity to chemotherapeutic agents. However, the specific mechanisms involved remain to be further elucidated.

DNA damage repair (DDR). Defects in the DDR pathway are a hallmark of genomically unstable tumors, and up to 15-20% of CRCs have DDR pathway defects (95,96). In the past, it was often thought that DDR pathway defects were mainly associated with peroxisome proliferators-activated receptors (PPAR) inhibitor efficacy. Furthermore, defects in the DDR pathway were primarily associated with the efficacy of PPAR inhibitors. Mechanism of action of common chemotherapeutic agents, including 5-FU and topoisomerase inhibitors, is related to the DDR pathway. METTL3 in the physiological state can promote cell repair after physical damage, including that caused by UV light. By contrast, METTL3 is pathologically upregulated in tumors, leading to an excessive rate of homologous recombination repair (HR), non-homologous end recombination and failure of chemotherapeutic drugs (97). Li *et al* (98) found that after METTL3 silencing treatment of HCT-8/5-FU resistant colon cancer cells, the resistant cells were able to be re-sensitized to 5-FU, while RAD51-associated protein 1, a key factor on the HR pathway, was downregulated. Zhang *et al* (99) discovered that METTL3 knockdown in OXA-resistant colon cancer cells also improved the chemosensitivity of resistant cells, while METTL3 overexpression restored the drug-resistant phenotype. Further sequencing suggested that the differentially expressed genes were mainly enriched in classical drug resistance pathways, including the Hippo and DDR pathways.

4. Therapeutic exploration of targeting key m6A methylation enzymes

Currently, the exploration of METTL3 inhibitors is being conducted mainly from the following three perspectives: Application of natural drug ingredients, small molecule drug synthesis development and clinical trials, and the combination of huge data with computer model predictions and screening of drug targets and pathways (as shown in Fig. 4).

Although m6A methylesterase inhibitors are currently less used in the treatment of CRC, the METTL3 inhibitor STM2457 has been reported to exhibit significant therapeutic effects in acute myeloid leukemia (100) and was able to reverse chemoresistance in small cell lung cancer (101) However, its derivative STC-15, the first clinical candidate for an oral agent targeting METTL3, is in phase I clinical trials for patients with advanced solid tumors (NCT05584111). Therefore, researchers have begun to explore the application of key m6A methylation enzyme modulators in solid tumors from multiple perspectives, including natural drug components, small molecule targeted drugs and programmed analysis of potential drug components using computerized big data. CRC has also received attention as a highly prevalent solid tumor, and an urgent clinical need to improve the efficacy of its treatments has emerged.

It has been suggested that most of the natural drug components that can regulate the action of m6A methylation key enzymes are polyphenols, alkaloids, flavonoids, anthraquinones and terpenoids (102). For instance, curcumin is a phenolic compound extracted from turmeric root that can reduce the expression of ALKBH5 and enhance the translation of tumor necrosis factor receptor associated factor 4 (TRAF4), prompting TRAF4 to bind to YTHDF6, a methyl recognition enzyme with m6A, and improve the efficiency of m6A methylation modification (103). Curcumin was also able to drive the conversion of microtubule-associated protein LC3-I to LC3-II or upregulate Beclin-1 to induce autophagy in CRC cells, reduce CSC generation and re-sensitize drug-resistant cells to 5-FU and OXA (104,105). The combination of curcumin with another polyphenol, resveratrol, could alter the distribution of key m6A methylation enzymes such as METTL3 and

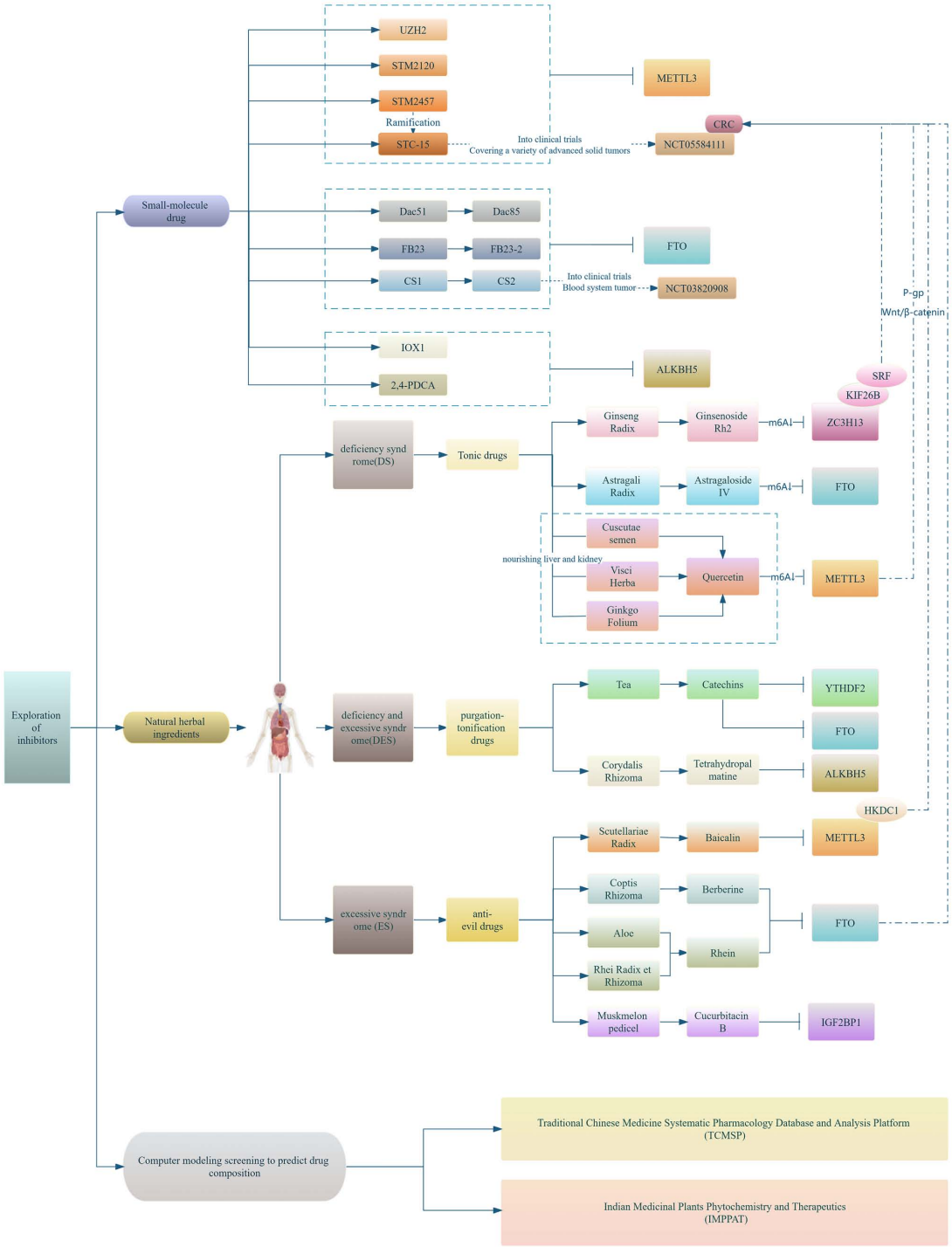

Figure 4. Exploration of inhibitors. The names and definitions of Chinese medicinal herbs in the figure are based on the Chinese Pharmacopoeia 2020 Edition. The drawings of the human body and drugs in the figure are created with BioRender.com. Ginseng Radix is the dried root and rhizome of the plant *Panax ginseng* (C. A. Mey.) of the Acanthopanax family. Astragalus is the dried root of the legume *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *Astragalus membranaceus* (Fisch.) Bge. Cuscutae semen is the dried mature seed of the species *Cuscuta australis* R.Br. or *Cuscuta chinensis* Lam. of the composite family. Visci Herba is the dry leafy stem branch of the plant *Viscum coloratum* (Komar.) Nakai of the family Viscaceae. Ginkgo Folium is the dried leaves of the *Ginkgo biloba* L. plant of the Ginkgo family. Corydalis Rhizoma is the dried tuber of *Corydalis yanhusuo* W.T. Wang, a member of the Papaveraceae family. Scutellariae Radix is the dried root of *Scutellaria baicalensis* Georgi, a plant in the family Lablabaceae. Coptis Radix is the dried rhizome of *Coptis chinensis* Franch., *Coptis deltoidea* C.Y. Cheng et Hsiao, or *Coptis teeta* Wall., all of the buttercup family. Aloe is a concentrated dried juice of the leaves of the lily plant *Aloe barbadensis* Miller, *Aloe ferox* Miller or other related plants of the same genus. Rhei Radix et Rhizoma refers to the dried roots and rhizomes of *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf. or *Rheum officinale* Baill. Muskmelon pedicel is the fruiting stalk of cucumber in Cucurbitaceae. UZH2 is a 1,4,9-triazaspiro[5.5]undecan-2-one derivative that can act as a potent inhibitor of METTL3. However, UZH2 does not singularly target METTL3; it also partially inhibits the activity of METTL1 and METTL16. STM2120 is one of the very few METTL3 inhibitors that belong to the non-S-adenosylmethionine-dependent class. STM2457 is a METTL3 inhibitor with higher activity and proven efficacy in cells and in vitro and in vivo, discovered on the basis of the studies of STM2120. STC-15 is the first STM2457 derivative to enter clinical trials. Test number: NCT05584111. Dac51 is a rationally designed FTO inhibitor based on structural similarity screening. Dac85 is a derivative of Dac51. FB23 is an FTO competitive inhibitor that selectively inhibits the N6-methyladenosine demethylase activity of FTO. FB23-2 is a more efficient derivative of FB23. CS1, Bisantrene, an anthracene derivative with antitumor activity. CS2 is a derivative of CS1 and is in clinical trials, trial no: NCT03820908. IOX1, a potent broad-spectrum inhibitor of 2OG oxygenases. 2,4-PDCA, lutidinic acid, 2,4-dicarboxypyridine, a potent broad-spectrum inhibitor of 2OG oxygenases.

YTHDF2, reduce the overall m6A methylation level in the intestine and improve intestinal mucosal integrity (106).

An increasing number of studies support the anti-tumor effects of herbal medicines as epigenetic modification modulators, including turmeric, tannin, yam and *Kalanchoe pinnata* (107), which can target DNA (cytosine-5-)-methyltransferase 1 to inhibit P65 gene methylation and interfere with CRC cell infiltration and migration (108). Although the relationship between Chinese medicine and m6A methylation has not yet been fully elucidated, some studies have found that herbal extracts can regulate DNA methylation, including chaihu saponin (109), quercetin (110) and catechins (111). Flavonoid components such as chaihu saponin, quercetin and catechin can also increase the expression of METTL3 and METTL14 and decrease the methyl recognition proteins such as FTO and ALKBH5 (109,112).

As for small-molecule drug development, besides STM2457 and its derivative STC-15, modulators targeting methylation recognition enzymes are also under active development. An inhibitor of FTO called CS1 inhibited the proliferation of six CRC cell lines, including HT-29, COLO, HCT-116 and 5-FU-resistant cell lines (HCT-116/5FU). It also induced G25/M phase cell cycle arrest and promoted apoptosis of HCT-116 cells by downregulating doublecortin domain containing 2C (113).

Virtual screening and *in vitro* assays of 1,042 commercially available natural products by Du *et al* (114) identified quercetin as a natural inhibitor of METTL3, which binds to METTL3 to form stable protein-ligand complexes. Manna *et al* (115) identified hesperidin as a potent inhibitor of METTL3 by computer screening and molecular dynamics simulation. Deng *et al* (102) are also exploring the development of traditional drugs as novel and effective therapeutic agents to inhibit m6A modification-mediated tumor progression using the TCM Systematic Pharmacology Database and Analysis Platform, Indian Medicinal Plants Phytochemistry and Therapeutics database combined with artificial intelligence to build a framework for traditional or natural drug-based targeting of m6A drugs.

5. Discussion

M6A methylation modifications are a bridge between the tumor microenvironment and phenotypic alterations, including chemoresistance, the mechanisms of which are complex, and the knowledge of epigenetics is yet to be refined. Of note, it has been found that various epigenetic modifications are not completely isolated from each other and there is a strong correlation between m6A methylation and DNA methylation, which can specifically lead to increased DNA methylation at proximal sites when METTL3 is depleted, resulting in downregulated chromatin binding levels of fragile X mental retardation, autosomal homolog 1 and tet methylcytosine dioxygenase 1 (116). Although current studies on m6A methylation have started from a combination of various high-throughput screens, molecular deconstruction techniques and metabolic alteration assays, the specific mechanisms of the interactions between the various aspects of m6A methylation, how to accurately achieve a homeostatic balance between methylation and demethylation, and the specific mechanisms of migration, invasion resistance and other malignant phenotypes of tumors induced by key m6A methylation enzymes, as well as the regulation of the tumor microenvironment remain unresolved. More importantly, some of the currently developed m6A modification inhibitors and activators have poor target specificity, therapeutic efficacy, and safety and pharmacokinetic limitations. The large-scale development and application of artificial intelligence provide new opportunities to assist in the preclinical screening of more efficient drug components. More m6A methylation key enzyme modulators can enter clinical trials in the near future, providing an effective way to improve the treatment of CRC.

Of note, the present study had certain limitations. First, the specific expression of each key m6A methylase was not summarized and discussed. The expression of these methylation key enzymes in different cancer species and their close relation to clinical characteristics and prognosis was not focused on, and may be discussed in the future. The present review focused on summarizing some directions of the basic findings of each methylation key enzyme in CRC chemoresistance and the subsequent clinical transformation. Furthermore, the relationship between the complex immune microenvironment of CRC and key m6A methylases was not summarized and discussed. The present review focused on the relationship of the immune microenvironment with m6A modification, and indeed, considerable studies have focused on this aspect. This is another topic that is not very closely related to the focus of this paper, but the knowledge system is huge, so it was not discussed. In the future, a focus will be placed on the role of m6A in the immune microenvironment.

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Availability of data and materials

Not applicable.

Authors' contributions

SCY and HYJ designed the study. SCY and TXK consulted the literature on the composition and role of key enzymes for m6A methylation. SCY, HYY and ZZ collected information on the relationship between key m6A enzymes and CRC and tracked the development of m6A-targeted drugs. ZYJ and FMZ categorized the retrieved materials according to year and differences in the types of key enzymes involved, and were responsible for the organization and design of the tables. SCY wrote this manuscript. HX, GT and LM critically reviewed

the manuscript. HX directed and participated in information gathering, image conception and design, drawing figures and subsequent revision of the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Gilbert WV, Bell TA and Schaening C: Messenger RNA modifications: Form, distribution, and function. Science 352: 1408-1412, 2016.
2. Cohn WE: Pseudouridine, a carbon-carbon linked ribonucleoside in ribonucleic acids: Isolation, structure, and chemical characteristics. J Biol Chem 235: 1488-1498, 1960.
3. Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, Pestova TV, Qian SB and Jaffrey SR: 5' UTR m(6) A promotes cap-independent translation. Cell 163: 999-1010, 2015.
4. Dawson MA and Kouzarides T: Cancer epigenetics: from mechanism to therapy. Cell 150: 12-27, 2012.
5. Nishiyama A and Nakanishi M: Navigating the DNA methylation landscape of cancer. Trends Genet 37: 1012-1027, 2021.
6. Huang W, Li H, Yu Q, Xiao W and Wang DO: LncRNA-mediated DNA methylation: An emerging mechanism in cancer and beyond. J Exp Clin Cancer Res 41: 100, 2022.
7. Esteller M: Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet 8: 286-298, 2007.
8. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE and Jaffrey SR: Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell 149: 1635-1646, 2012.
9. Linder B, Grozhik AV, Olarerin-George AO, Meydan C, Mason CE and Jaffrey SR: Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nat Methods 12: 767-772, 2015.
10. Sun T, Wu R and Ming L: The role of m6A RNA methylation in cancer. Biomed Pharmacother 112: 108613, 2019.
11. Song N, Cui K, Zhang K, Yang J, Liu J, Miao Z, Zhao F, Meng H, Chen L, Chen C, *et al*: The role of m6A RNA methylation in cancer: Implication for nature products anti-cancer research. Front Pharmacol 13: 933332, 2022.
12. Li X, Ma S, Deng Y, Yi P and Yu J: Targeting the RNA m^6A modification for cancer immunotherapy. Mol Cancer 21: 76, 2022.
13. An Y and Duan H: The role of m6A RNA methylation in cancer metabolism. Mol Cancer 21: 14, 2022.
14. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
15. Shi JF, Wang L, Ran JC, Wang H, Liu CC, Zhang HZ, Yang L, Shi SS, Jiang LM, Fan JH, *et al*: Clinical characteristics, medical service utilization, and expenditure for colorectal cancer in China, 2005 to 2014: Overall design and results from a multicenter retrospective epidemiologic survey. Cancer 127: 1880-1893, 2021.
16. Alfarouk KO, Stock CM, Taylor S, Walsh M, Muddathir AK, Verduzco D, Bashir AH, Mohammed OY, Elhassan GO, Harguindey S, *et al*: Resistance to cancer chemotherapy: Failure in drug response from ADME to P-gp. Cancer Cell Int 15: 71, 2015.
17. Liu K, Ouyang QY, Zhan Y, Yin H, Liu BX, Tan LM, Liu R, Wu W and Yin JY: Pharmacoepitranscriptomic landscape revealing m6A modification could be a drug-effect biomarker for cancer treatment. Mol Ther Nucleic Acids 28: 464-476, 2022.
18. Pan J, Liu F, Xiao X, Xu R, Dai L, Zhu M, Xu H, Xu Y, Zhao A, Zhou W, *et al*: METTL3 promotes colorectal carcinoma progression by regulating the m6A-CRB3-Hippo axis. J Exp Clin Cancer Res 41: 19, 2022.
19. Shen C, Xuan B, Yan T, Ma Y, Xu P, Tian X, Zhang X, Cao Y, Ma D, Zhu X, *et al*: m^6A-dependent glycolysis enhances colorectal cancer progression. Mol Cancer 19: 72, 2020.
20. Peng W, Li J, Chen R, Gu Q, Yang P, Qian W, Ji D, Wang Q, Zhang Z, Tang J and Sun Y: Upregulated METTL3 promotes metastasis of colorectal cancer via miR-1246/SPRED2/MAPK signaling pathway. J Exp Clin Cancer Res 38: 393, 2019.
21. Liu Z, Zou H, Dang Q, Xu H, Liu L, Zhang Y, Lv J, Li H, Zhou Z and Han X: Biological and pharmacological roles of m^6A modifications in cancer drug resistance. Mol Cancer 21: 220, 2022.
22. Yan J, Liu F, Guan Z, Yan X, Jin X, Wang Q, Wang Z, Yan J, Zhang D, Liu Z, *et al*: Structural insights into DNA N^6-adenine methylation by the MTA1 complex. Cell Discov 9: 8, 2023.
23. Yan X, Pei K, Guan Z, Liu F, Yan J, Jin X, Wang Q, Hou M, Tang C and Yin P: AI-empowered integrative structural characterization of m^6A methyltransferase complex. Cell Res 32: 1124-1127, 2022.
24. Zhou Z, Lv J, Yu H, Han J, Yang X, Feng D, Wu Q, Yuan B, Lu Q and Yang H: Mechanism of RNA modification N6-methyladenosine in human cancer. Mol Cancer 19: 104, 2020.
25. Wang Z, Pan Z, Adhikari S, Harada BT, Shen L, Yuan W, Abeywardana T, Al-Hadid Q, Stark JM, He C, *et al*: m^6 A deposition is regulated by PRMT1-mediated arginine methylation of METTL14 in its disordered C-terminal region. EMBO J 40: e106309, 2021.
26. Liu X, Du Y, Huang Z, Qin H, Chen J and Zhao Y: Insights into roles of METTL14 in tumors. Cell Prolif 55: e13168, 2022.
27. Ping XL, Sun BF, Wang L, Xiao W, Yang X, Wang WJ, Adhikari S, Shi Y, Lv Y, Chen YS, *et al*: Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Res 24: 177-189, 2014.
28. Fan Y, Li X, Sun H, Gao Z, Zhu Z and Yuan K: Role of WTAP in cancer: From mechanisms to the therapeutic potential. Biomolecules 12: 1224, 2022.
29. Yue Y, Liu J, Cui X, Cao J, Luo G, Zhang Z, Cheng T, Gao M, Shu X, Ma H, *et al*: VIRMA mediates preferential m^6A mRNA methylation in 3'UTR and near stop codon and associates with alternative polyadenylation. Cell Discov 4: 10, 2018.
30. Huang L, Liang D, Zhang Y, Chen X, Chen J, Wen C, Liu H, Yang X, Yang X and Lin S: METTL3 promotes colorectal cancer metastasis by promoting the maturation of pri-microRNA-196b. J Cancer Res Clin Oncol 149: 5095-5108, 2023.
31. Zhang F, Su T and Xiao M: RUNX3-regulated circRNA METTL3 inhibits colorectal cancer proliferation and metastasis via miR-107/PER3 axis. Cell Death Dis 13: 550, 2022.
32. Shi K, Yang S, Chen C, Shao B, Guo Y, Wu X, Zhao L, Yang X, Zhang Q, Yuan W and Sun Z: RNA methylation-mediated LINC01559 suppresses colorectal cancer progression by regulating the miR-106b-5p/PTEN axis. Int J Biol Sci 18: 3048-3065, 2022.
33. Xu Q, Lu X, Li J, Feng Y, Tang J, Zhang T, Mao Y, Lan Y, Luo H, Zeng L, *et al*: Fusobacterium nucleatum induces excess methyltransferase-like 3-mediated microRNA-4717-3p maturation to promote colorectal cancer cell proliferation. Cancer Sci 113: 3787-3800, 2022.
34. Chen S, Zhang L, Li M, Zhang Y, Sun M, Wang L, Lin J, Cui Y, Chen Q, Jin C, *et al*: Fusobacterium nucleatum reduces METTL3-mediated m^6A modification and contributes to colorectal cancer metastasis. Nat Commun 13: 1248, 2022.
35. Chen H, Gao S, Liu W, Wong CC, Wu J, Wu J, Liu D, Gou H, Kang W, Zhai J, *et al*: RNA N^6-methyladenosine methyltransferase METTL3 facilitates colorectal cancer by activating the m^6A-GLUT1-mTORC1 axis and is a therapeutic target. Gastroenterology 160: 1284-1300.e16, 2021.
36. Lu S, Han L, Hu X, Sun T, Xu D, Li Y, Chen Q, Yao W, He M, Wang Z, *et al*: N6-methyladenosine reader IMP2 stabilizes the ZFAS1/OLA1 axis and activates the Warburg effect: Implication in colorectal cancer. J Hematol Oncol 14: 188, 2021.
37. Sun L, Wan A, Zhou Z, Chen D, Liang H, Liu C, Yan S, Niu Y, Lin Z, Zhan S, *et al*: RNA-binding protein RALY reprogrammes mitochondrial metabolism via mediating miRNA processing in colorectal cancer. Gut 70: 1698-1712, 2021.

38. Deng R, Cheng Y, Ye S, Zhang J, Huang R, Li P, Liu H, Deng Q, Wu X, Lan P and Deng Y: m^{6}A methyltransferase METTL3 suppresses colorectal cancer proliferation and migration through p38/ERK pathways. Onco Targets Ther 12: 4391-4402, 2019.
39. Chen X, Xu M, Xu X, Zeng K, Liu X, Pan B, Li C, Sun L, Qin J, Xu T, *et al*: METTL14-mediated N6-methyladenosine modification of SOX4 mRNA inhibits tumor metastasis in colorectal cancer. Mol Cancer 19: 106, 2020.
40. Hou Y, Zhang X, Yao H, Hou L, Zhang Q, Tao E, Zhu X, Jiang S, Ren Y, Hong X, *et al*: METTL14 modulates glycolysis to inhibit colorectal tumorigenesis in p53-wild-type cells. EMBO Rep 24: e56325, 2023.
41. Zhang J, Tsoi H, Li X, Wang H, Gao J, Wang K, Go MY, Ng SC, Chan FK, Sung JJ and Yu J: Carbonic anhydrase IV inhibits colon cancer development by inhibiting the Wnt signalling pathway through targeting the WTAP-WT1-TBL1 axis. Gut 65: 1482-1493, 2016.
42. Li Y, He L, Wang Y, Tan Y and Zhang F: N^6-methyladenosine methyltransferase KIAA1429 elevates colorectal cancer aerobic glycolysis via HK2-dependent manner. Bioengineered 13: 11923-11932, 2022.
43. Wei X, Huo Y, Pi J, Gao Y, Rao S, He M, Wei Q, Song P, Chen Y, Lu D, *et al*: METTL3 preferentially enhances non-m^{6}A translation of epigenetic factors and promotes tumourigenesis. Nat Cell Biol 24: 1278-1290, 2022.
44. Zeng C, Huang W, Li Y and Weng H: Roles of METTL3 in cancer: Mechanisms and therapeutic targeting. J Hematol Oncol 13: 117, 2020.
45. Collignon E, Cho B, Furlan G, Fothergill-Robinson J, Martin SB, McClymont SA, Ross RL, Limbach PA and Ramalho-Santos M: m^{6}A RNA methylation orchestrates transcriptional dormancy during paused pluripotency. Nat Cell Biol 25: 1279-1289, 2023.
46. Lin S, Choe J, Du P, Triboulet R and Gregory RI: The m(6)A methyltransferase METTL3 promotes translation in human cancer cells. Mol Cell 62: 335-345, 2016.
47. Wang JL, Chen ZF, Chen HM, Wang MY, Kong X, Wang YC, Sun TT, Hong J, Zou W, Xu J and Fang JY: Elf3 drives β-catenin transactivation and associates with poor prognosis in colorectal cancer. Cell Death Dis 5: e1263, 2014.
48. Xiang Y, Guo Z, Zhu P, Chen J and Huang Y: Traditional Chinese medicine as a cancer treatment: Modern perspectives of ancient but advanced science. Cancer Med 8: 1958-1975, 2019.
49. Xin T, Zhang Y, Pu X, Gao R, Xu Z and Song J: Trends in herb-genomics. Sci China Life Sci 62: 288-308, 2019.
50. Wang YN, Zou M, Wang D, Zhang ZK, Qu LP, Xu J, Shi CD and Gao F: An exploratory study on TCM syndrome differentiation in preoperative patients with colorectal cancer assisted by laboratory indicators. Heliyon 8: e10207, 2022.
51. Wang CY, Ding HZ, Tang X and Li ZG: Comparative analysis of immune function, hemorheological alterations and prognosis in colorectal cancer patients with different traditional Chinese medicine syndromes. Cancer Biomark 21: 701-710, 2018.
52. Zhang X, Wang X, Shi R, Ran X, He X and Dou D: Effective substances and mechanism of red ginseng on rats with spleen-deficiency syndrome based on the substance and energy metabolism as well as the 'brain-gut' axis. J Ethnopharmacol 311: 116438, 2023.
53. Lu Y, Zhou C, Zhu M, Fu Z, Shi Y, Li M, Wang W, Zhu S, Jiang B, Luo Y and Su S: Traditional chinese medicine syndromes classification associates with tumor cell and microenvironment heterogeneity in colorectal cancer: A single cell RNA sequencing analysis. Chin Med 16: 133, 2021.
54. Liu WW, Zhang ZY, Wang F and Wang H: Emerging roles of m6A RNA modification in cancer therapeutic resistance. Exp Hematol Oncol 12: 21, 2023.
55. Ma SC, Zhang JQ, Yan TH, Miao MX, Cao YM, Cao YB, Zhang LC and Li L: Novel strategies to reverse chemoresistance in colorectal cancer. Cancer Med 12: 11073-11096, 2023.
56. Zou Z, Sepich-Poore C, Zhou X, Wei J and He C: The mechanism underlying redundant functions of the YTHDF proteins. Genome Biol 24: 17, 2023.
57. Chen L, Gao Y, Xu S, Yuan J, Wang M, Li T and Gong J: N6-methyladenosine reader YTHDF family in biological processes: Structures, roles, and mechanisms. Front Immunol 14: 1162607, 2023.
58. Sarraf G and Chhabra R: Emerging role of mRNA methylation in regulating the hallmarks of cancer. Biochimie 206: 61-72, 2023.
59. Chang G, Shi L, Ye Y, Shi H, Zeng L, Tiwary S, Huse JT, Huo L, Ma L, Ma Y, *et al*: YTHDF3 Induces the translation of m^{6}A-enriched gene transcripts to promote breast cancer brain metastasis. Cancer Cell 38: 857-871, 2020.
60. Hao W, Dian M, Zhou Y, Zhong Q, Pang W, Li Z, Zhao Y, Ma J, Lin X, Luo R, *et al*: Autophagy induction promoted by m^{6}A reader YTHDF3 through translation upregulation of FOXO3 mRNA. Nat Commun 13: 5845, 2022.
61. Wang S, Gao S, Zeng Y, Zhu L, Mo Y, Wong CC, Bao Y, Su P, Zhai J, Wang L, *et al*: N6-methyladenosine reader YTHDF1 promotes ARHGEF2 translation and RhoA signaling in colorectal cancer. Gastroenterology 162: 1183-1196, 2022.
62. Ning Z, Wu Z, Zhang F, Yang M, Lu Z, Yu B, Long F, Guo Y, Yang K, Hu G, *et al*: GMEB2 promotes the growth of colorectal cancer by activating ADRM1 transcription and NF-κB signalling and is positively regulated by the m^{6}A reader YTHDF1. Cancers (Basel) 14: 6046, 2022.
63. Ni W, Yao S, Zhou Y, Liu Y, Huang P, Zhou A, Liu J, Che L and Li J: Long noncoding RNA GAS5 inhibits progression of colorectal cancer by interacting with and triggering YAP phosphorylation and degradation and is negatively regulated by the m^{6}A reader YTHDF3. Mol Cancer 18: 143, 2019.
64. Li T, Hu PS, Zuo Z, Lin JF, Li X, Wu QN, Chen ZH, Zeng ZL, Wang F, Zheng J, *et al*: METTL3 facilitates tumor progression via an m^{6}A-IGF2BP2-dependent mechanism in colorectal carcinoma. Mol Cancer 18: 112, 2019.
65. Liu X, He H, Zhang F, Hu X, Bi F, Li K, Yu H, Zhao Y, Teng X, Li J, *et al*: m6A methylated EphA2 and VEGFA through IGF2BP2/3 regulation promotes vasculogenic mimicry in colorectal cancer via PI3K/AKT and ERK1/2 signaling. Cell Death Dis 13: 483, 2022.
66. Zhao Y and Peng H: The role of N^6-methyladenosine (m^{6}A) methylation modifications in hematological malignancies. Cancers (Basel) 14: 332, 2022.
67. Qu X and Shi Y: Bile reflux and bile acids in the progression of gastric intestinal metaplasia. Chin Med J (Engl) 135: 1664-1672, 2022.
68. Li H, Wu H, Wang Q, Ning S, Xu S and Pang D: Dual effects of N^6-methyladenosine on cancer progression and immunotherapy. Mol Ther Nucleic Acids 24: 25-39, 2021.
69. Liu C, Yang S, Zhang Y, Wang C, Du D, Wang X, Liu T and Liang G: Emerging roles of N6-methyladenosine demethylases and its interaction with environmental toxicants in digestive system cancers. Cancer Manag Res 13: 7101-7114, 2021.
70. Uddin MB, Wang Z and Yang C: Dysregulations of functional RNA modifications in cancer, cancer stemness and cancer therapeutics. Theranostics 10: 3164-3189, 2020.
71. Relier S, Ripoll J, Guillorit H, Amalric A, Achour C, Boissière F, Vialaret J, Attina A, Debart F, Choquet A, *et al*: FTO-mediated cytoplasmic m^{6}A$_m$ demethylation adjusts stem-like properties in colorectal cancer cell. Nat Commun 12: 1716, 2021.
72. Ruan DY, Li T, Wang YN, Meng Q, Li Y, Yu K, Wang M, Lin JF, Luo LZ, Wang DS, *et al*: FTO downregulation mediated by hypoxia facilitates colorectal cancer metastasis. Oncogene 40: 5168-5181, 2021.
73. Ballester V, Taylor WR, Slettedahl SW, Mahoney DW, Yab TC, Sinicrope FA, Boland CR, Lidgard GP, Cruz-Correa MR, Smyrk TC, *et al*: Novel methylated DNA markers accurately discriminate Lynch syndrome associated colorectal neoplasia. Epigenomics 12: 2173-2187, 2020.
74. Li N, Kang Y, Wang L, Huff S, Tang R, Hui H, Agrawal K, Gonzalez GM, Wang Y, Patel SP and Rana TM: ALKBH5 regulates anti-PD-1 therapy response by modulating lactate and suppressive immune cell accumulation in tumor microenvironment. Proc Natl Acad Sci USA 117: 20159-20170, 2020.
75. Wang YQ, Li HZ, Gong WW, Chen YY, Zhu C, Wang L, Zhong JM and Du LB: Cancer incidence and mortality in Zhejiang Province, Southeast China, 2016: A population-based study. Chin Med J (Engl) 134: 1959-1966, 2021.
76. Chen HM, Lin CC, Chen WS, Jiang JK, Yang SH, Chang SC, Ho CL, Yang CC, Huang SC, Chao Y, *et al*: Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is a prognostic biomarker and associated with chemotherapy responsiveness in colorectal cancer. Int J Mol Sci 22: 6940, 2021.
77. Hanahan D: Hallmarks of cancer: New dimensions. Cancer Discov 12: 31-46, 2022.
78. Yang W, Wang Y, Tao C, Li Y, Cao S and Yang X: CRNDE silencing promotes apoptosis and enhances cisplatin sensitivity of colorectal carcinoma cells by inhibiting the Akt/mTORC1-mediated Warburg effect. Oncol Lett 23: 70, 2022.
79. Wei TT, Lin YT, Tang SP, Luo CK, Tsai CT, Shun CT and Chen CC: Metabolic targeting of HIF-1α potentiates the therapeutic efficacy of oxaliplatin in colorectal cancer. Oncogene 39: 414-427, 2020.

80. Peng L, Jiang J, Chen HN, Zhou L, Huang Z, Qin S, Jin P, Luo M, Li B, Shi J, *et al*: Redox-sensitive cyclophilin A elicits chemoresistance through realigning cellular oxidative status in colorectal cancer. Cell Rep 37: 110069, 2021.
81. Zhang K, Zhang T, Yang Y, Tu W, Huang H, Wang Y, Chen Y, Pan K and Chen Z: N^6-methyladenosine-mediated LDHA induction potentiates chemoresistance of colorectal cancer cells through metabolic reprogramming. Theranostics 12: 4802-4817, 2022.
82. Yang Z, Quan Y, Chen Y, Huang Y, Huang R, Yu W, Wu D, Ye M, Min Z and Yu B: Knockdown of RNA N6-methyladenosine methyltransferase METTL3 represses Warburg effect in colorectal cancer via regulating HIF-1α. Signal Transduct Target Ther 6: 89, 2021.
83. Han S, Zhu L, Zhu Y, Meng Y, Li J, Song P, Yousafzai NA, Feng L, Chen M, Wang Y, *et al*: Targeting ATF4-dependent pro-survival autophagy to synergize glutaminolysis inhibition. Theranostics 11: 8464-8479, 2021.
84. Chen P, Liu XQ, Lin X, Gao LY, Zhang S and Huang X: Targeting YTHDF1 effectively re-sensitizes cisplatin-resistant colon cancer cells by modulating GLS-mediated glutamine metabolism. Mol Ther Oncolytics 20: 228-239, 2021.
85. Rehman SK, Haynes J, Collignon E, Brown KR, Wang Y, Nixon AML, Bruce JP, Wintersinger JA, Singh Mer A, Lo EBL, *et al*: Colorectal Cancer cells enter a diapause-like DTP state to survive chemotherapy. Cell 184: 226-242.e21, 2021.
86. Wang Y, Yang L, Zhang J, Zhou M, Shen L, Deng W, Liang L, Hu R, Yang W, Yao Y, *et al*: Radiosensitization by irinotecan is attributed to G2/M phase arrest, followed by enhanced apoptosis, probably through the ATM/Chk/Cdc25C/Cdc2 pathway in p53-mutant colorectal cancer cells. Int J Oncol 53: 1667-1680, 2018.
87. Lin Z, Wan AH, Sun L, Liang H, Niu Y, Deng Y, Yan S, Wang QP, Bu X, Zhang X, *et al*: N6-methyladenosine demethylase FTO enhances chemo-resistance in colorectal cancer through SIVA1-mediated apoptosis. Mol Ther 31: 517-534, 2023.
88. Li J, Chen F, Peng Y, Lv Z, Lin X, Chen Z and Wang H: N6-methyladenosine regulates the expression and secretion of TGFbeta1 to affect the epithelial-mesenchymal transition of cancer cells. Cells 9: 296, 2020.
89. Yang M, Sun M and Zhang H: The interaction between epigenetic changes, EMT, and exosomes in predicting metastasis of colorectal cancers (CRC). Front Oncol 12: 879848, 2022.
90. Sabouni E, Nejad MM, Mojtabavi S, Khoshduz S, Mojtabavi M, Nadafzadeh N, Nikpanjeh N, Mirzaei S, Hashemi M, Aref AR, *et al*: Unraveling the function of epithelial-mesenchymal transition (EMT) in colorectal cancer: Metastasis, therapy response, and revisiting molecular pathways. Biomed Pharmacother 160: 114395, 2023.
91. Oskarsson T, Batlle E and Massagué J: Metastatic stem cells: Sources, niches, and vital pathways. Cell Stem Cell 14: 306-321, 2014.
92. Liu X, Su K, Sun X, Jiang Y, Wang L, Hu C, Zhang C, Lu M, Du X and Xing B: Sec62 promotes stemness and chemoresistance of human colorectal cancer through activating Wnt/β-catenin pathway. J Exp Clin Cancer Res 40: 132, 2021.
93. Zhang Y, Kang M, Zhang B, Meng F, Song J, Kaneko H, Shimamoto F and Tang B: m^6A modification-mediated CBX8 induction regulates stemness and chemosensitivity of colon cancer via upregulation of LGR5. Mol Cancer 18: 185, 2019.
94. Bai Y, Yang C, Wu R, Huang L, Song S, Li W, Yan P, Lin C, Li D and Zhang Y: YTHDF1 regulates tumorigenicity and cancer stem cell-like activity in human colorectal carcinoma. Front Oncol 9: 332, 2019.
95. Mauri G, Arena S, Siena S, Bardelli A and Sartore-Bianchi A: The DNA damage response pathway as a land of therapeutic opportunities for colorectal cancer. Ann Oncol 31: 1135-1147, 2020.
96. Catalano F, Borea R, Puglisi S, Boutros A, Gandini A, Cremante M, Martelli V, Sciallero S and Puccini A: Targeting the DNA damage response pathway as a novel therapeutic strategy in colorectal cancer. Cancers (Basel) 14: 1388, 2022.
97. Zhang C, Chen L, Peng D, Jiang A, He Y, Zeng Y, Xie C, Zhou H, Luo X, Liu H, *et al*: METTL3 and N6-methyladenosine promote homologous recombination-mediated repair of DSBs by modulating DNA-RNA hybrid accumulation. Mol Cell 79: 425-442.e7, 2020.
98. Li M, Xia M, Zhang Z, Tan Y, Li E, Guo Z, Fang M, Zhu Y and Hu Z: METTL3 antagonizes 5-FU chemotherapy and confers drug resistance in colorectal carcinoma. Int J Oncol 61: 106, 2022.
99. Zhang SH QQLP: METTL3-mediated m6A modification in oxaliplatin resistance in colorectal cancer. J Chongqing Med Univ 47: 941-947, 2022 (In Chinese). https://kns.cnki.net/kcms/detail/detail.aspx?FileName=ZQYK202208010&DbName=DKFX2022. DOI: 10.13406/j.cnki.cyxb.003077.
100. Yankova E, Blackaby W, Albertella M, Rak J, De Braekeleer E, Tsagkogeorga G, Pilka ES, Aspris D, Leggate D, Hendrick AG, *et al*: Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. Nature 593: 597-601, 2021.
101. Sun Y, Shen W, Hu S, Lyu Q, Wang Q, Wei T, Zhu W and Zhang J: METTL3 promotes chemoresistance in small cell lung cancer by inducing mitophagy. J Exp Clin Cancer Res 42: 65, 2023.
102. Deng LJ, Deng WQ, Fan SR, Chen MF, Qi M, Lyu WY, Qi Q, Tiwari AK, Chen JX, Zhang DM and Chen ZS: m6A modification: recent advances, anticancer targeted drug discovery and beyond. Mol Cancer 21: 52, 2022.
103. Chen Y, Wu R, Chen W, Liu Y, Liao X, Zeng B, Guo G, Lou F, Xiang Y, Wang Y and Wang X: Curcumin prevents obesity by targeting TRAF4-induced ubiquitylation in m^6 A-dependent manner. EMBO Rep 22: e52146, 2021.
104. Weng W and Goel A: Curcumin and colorectal cancer: An update and current perspective on this natural medicine. Semin Cancer Biol 80: 73-86, 2022.
105. Su P, Yang Y, Wang G, Chen X and Ju Y: Curcumin attenuates resistance to irinotecan via induction of apoptosis of cancer stem cells in chemoresistant colon cancer cells. Int J Oncol 53: 1343-1353, 2018.
106. Gan Z, Wei W, Wu J, Zhao Y, Zhang L, Wang T and Zhong X: Resveratrol and curcumin improve intestinal mucosal integrity and decrease m^6A RNA methylation in the intestine of weaning piglets. ACS Omega 4: 17438-17446, 2019.
107. Hernández-Caballero ME, Sierra-Ramírez JA, Villalobos-Valencia R and Seseña-Méndez E: Potential of *Kalanchoe pinnata* as a cancer treatment adjuvant and an epigenetic regulator. Molecules 27: 6425, 2022.
108. Zhu D, Li A, Lv Y and Fan Q: Traditional Chinese medicine: A class of potentially reliable epigenetic drugs. Front Pharmacol 13: 907031, 2022.
109. Sun K, Du Y, Hou Y, Zhao M, Li J, Du Y, Zhang L, Chen C, Yang H, Yan F and Su R: Saikosaponin D exhibits anti-leukemic activity by targeting FTO/m^6A signaling. Theranostics 11: 5831-5846, 2021.
110. Xu W, Xie S, Chen X, Pan S, Qian H and Zhu X: Effects of quercetin on the efficacy of various chemotherapeutic drugs in cervical cancer cells. Drug Des Devel Ther 15: 577-588, 2021.
111. Wu R, Yao Y, Jiang Q, Cai M, Liu Q, Wang Y and Wang X: Epigallocatechin gallate targets FTO and inhibits adipogenesis in an mRNA m^6A-YTHDF2-dependent manner. Int J Obes (Lond) 42: 1378-1388, 2018.
112. Jiao Y, Williams A and Wei N: Quercetin ameliorated insulin resistance via regulating METTL3-mediated N6-methyladenosine modification of PRKD2 mRNA in skeletal muscle and C2C12 myocyte cell line. Nutr Metab Cardiovasc Dis 32: 2655-2668, 2022.
113. Phan T, Nguyen VH, Su R, Li Y, Qing Y, Qin H, Cho H, Jiang L, Wu X, Chen J, *et al*: Targeting fat mass and obesity-associated protein mitigates human colorectal cancer growth in vitro and in a murine model. Front Oncol 13: 1087644, 2023.
114. Du Y, Yuan Y, Xu L, Zhao F, Wang W, Xu Y and Tian X: Discovery of METTL3 small molecule inhibitors by virtual screening of natural products. Front Pharmacol 13: 878135, 2022.
115. Manna S, Samal P, Basak R, Mitra A, Roy AK, Kundu R, Ahir A, Roychowdhury A and Hazra D: Amentoflavone and methyl hesperidin, novel lead molecules targeting epitranscriptomic modulator in acute myeloid leukemia: In silico drug screening and molecular dynamics simulation approach. J Mol Model 29: 9, 2022.
116. Deng S, Zhang J, Su J, Zuo Z, Zeng L, Liu K, Zheng Y, Huang X, Bai R, Zhuang L, *et al*: RNA m^6A regulates transcription via DNA demethylation and chromatin accessibility. Nat Genet 54: 1427-1437, 2022.