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Epigenetic Approach on Colorectal Cancer Systemic Therapy: Promising yet a Long Way to Go

Aproximación epigenética sobre la terapia sistémica del cáncer colorrectal: prometedor pero un largo camino por recorrer

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SUMMARY

Colorectal cancer (CRC) is the fourth leading cause of cancer-related deaths worldwide. Various genetic and epigenetic changes in colonic epithelial cells, including DNA methylation, histone modifications, and noncoding RNAs, were involved in the initiation and progression of CRC. Moreover, epigenetic changes played an important role in CRC drug resistance. The dynamic and reversibility of epigenetic changes provide new possible therapeutic targets for colorectal cancer. Recent studies have reached the field of development of drugs targeting DNA methyltransferase and histone deacetylases, followed by bromodomain and extra terminal inhibitors alongside RNA-based therapeutics as an emerging cancer therapy. Through this review, we discussed epigenetic changes in CRC alongside potential agents targeting epigenetics and the possibility of implementing these drugs in the clinical setting. As epigenetic regulation is dynamic and dependent, its process warrants further investigation. It is visible that future research needs a combination of completing the ongoing studies, initiating new clinical trials, and developing new agents capable of treating CRC. A combination of expertise needs to be formed to overcome the obstacle in several inhibitors and

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RNA-based therapeutics to bring forth novel epigenetic agents that are both effective and safe.

Keywords: *Colorectal cancer, epigenetics, DNA methylation, histone modification, RNA therapeutics, cancer.*

RESUMEN

El cáncer colorrectal (CCR) es la cuarta causa principal de muertes relacionadas con el cáncer en todo el mundo. Varios cambios genéticos y epigenéticos en las células epiteliales del colon, incluida la metilación del ADN, las modificaciones de histonas y los ARN no codificantes, se encuentran involucrados en el inicio y la progresión de CCR. Además, los cambios epigenéticos juegan un papel importante en la resistencia a los medicamentos de CCR. La dinámica y la reversibilidad de los cambios epigenéticos proporcionan nuevos posibles blancos terapéuticos para el cáncer colorrectal. Estudios recientes indican el desarrollo en el campo de medicamentos dirigidos a la ADN metiltransferasa e histona desacetilasas, seguido de los dominios del bromo y de los inhibidores de los extras terminales, junto con la terapéutica basada en ARN, todas ellas como terapias emergentes del cáncer. A través de esta revisión, discutimos los cambios epigenéticos en el CCR junto con los agentes potenciales dirigidos a la epigenética, así como la posibilidad de implementar estos medicamentos en el entorno clínico. Como la regulación epigenética es dinámica y dependiente, su proceso garantiza una mayor investigación. Es previsible que en investigaciones futuras se necesite una combinación que implica el completar los estudios en curso, iniciar nuevos ensayos clínicos y desarrollar nuevos agentes capaces de tratar el CCR. Se debe formar una combinación de experiencias para superar el obstáculo en varios inhibidores y terapias basadas en ARN para presentar nuevos agentes epigenéticos que sean efectivos y seguros.

Palabras clave: *Cáncer colorrectal, epigenética, metilación del ADN, modificación de histonas, terapéutica de ARN, cáncer.*

INTRODUCTION

Colorectal cancer (CRC) has been known to be caused by both genetic alteration and epigenetic changes (1). It accounts for almost 10 million cancer-caused death worldwide, with 1.93 million new cases in 2020 (2). This disease emerged from a polyp (1) and is generally asymptomatic (2). CRC had several subtypes characterized by genetic and epigenetic alterations (1). As epigenetic changes happen early in cancer pathogenesis, they can act as molecular hallmarks alongside biomarkers for diagnosis and prognosis (3). With modification in genetic and epigenetic profiles, CRC grew and had invasion capability supporting its metastases (4).

In cancer, epigenetic alterations commonly found were abnormal DNA methylation and histone modifications or altered expression of non-coding RNAs (5). It is somatically heritable yet reversible, making them a good candidate for potential drug treatments. Recently, epigenetics has been shown as an essential tool to understand further disease pathogenesis and alternative ways of therapy. Targeting epigenetic modifications may dictate the future of personalized medicine. Resistance to conventional anticancer drugs also involves epigenetic mechanisms (6). With the advancement of epigenome analysis technologies, a new area is emerging: pharmaco-epigenomics, where epigenetic profiles could be used to classify molecular pathways of cancer drug sensitivity, hence, deciding the best therapeutic strategy. In this review, we described the epigenetic mechanism of CRC alongside the emerging epigenetic modification targeting drugs for CRC treatment, which offers a solid reason to explore new therapeutic strategies with sufficient efficacy and safety for cancer treatment.

DNA methylation in colorectal cancer

DNA methylation could alter the activity of a DNA fragment without changing the sequence (7). Methylation is an enzymatic addition of a methyl group to cytosine in a 5-position with a donor from S-adenosyl-L-methionine (SAM), catalyzed by DNA methyltransferases (DNMTs) (8,9). In CRC, aberrant DNA methylation occurs in CpG dinucleotides, giving the name of CpG island methylation phenotype (CIMP) (10). CpG islands are DNA segments with approximately 1 000 base pairs long complemented with at least 50 % cytosines and guanines (9,11). They are commonly unmethylated (12). There are three DNMTs; named DNMT1, DNMT3a,

and DNMT3b. These three enzymes had specific roles. However, they shared a large regulatory N-terminal domain and a catalytic C-terminal domain. DNMT1 usually methylates hemimethylated DNA (13). During replication, DNMT1 helps the addition of methyl group to CpG dinucleotides on DNA strands (14). Moreover, DNMT1 could also repair DNA methylation, as it is called DNMT maintenance, because it preserves DNA methylation's original pattern in a cell lineage (15). The structure and function of DNMT3a and DNMT3b are very similar. Compared to DNMT1, both DNMT3a and DNMT3b are called de novo DNMT as they can methylate native and synthetic DNA without hemimethylated DNA preference (16).

The first identified aberrant DNA methylation in CRC is hypermethylation of CpG in tumor suppressor genes. Hence, disturbing its transcription leads to gene silencing and loss of function. Several CIMP markers have been identified, e.g. CKDN2A, MINT1, MINT2, MINT3, and MLH1 (17,18). Different epigenotype classifications were suggested. According to the proportion of methylated marker loci, CRC was grouped into three different epigenotypes: high CIMP, low CIMP, and negative CIMP (18). Another study divides CRC into five molecular subtypes depending on CIN and CIMP status. Group 1 had high MSI status and MLH1 methylation alongside BRAF mutation (14). Currently, CRC classification showed insights into CRC origin but not a distinct impact on therapy.

DNMT inhibitors (DNMTIs)

DNMTIs work by reversing the DNA hypermethylation status of tumor suppressor genes (TSGs). According to the nucleotide regulatory mechanisms, DNMTIs can be divided into cytosine-analog inhibitors and non-nucleotide analogue inhibitors (14). Azacytidine, an analog of cytidine, is a demethylation agent used in cancer. Combining azacytidine and entinostat with anti-PD-1 and anti-CTLA-4 inhibitors reduced tumor growth and metastasis in CRC (5). Azacytidine act as ribonucleoside, preferentially incorporated into RNA as it phosphorylated three times into the active form, azacytidine triphosphate (14), while decitabine only integrates to DNA (19). Azacytidine could also integrate into DNA through the ribonucleotide reductase pathway (8). Azacytidine administration causes hypomethylation of NDN (Necdin, MAGE Family Member) promoter. Azacytidine has been tested in clinical trials for CRC in combination with capecitabine and oxaliplatin (NCT01193517), pembrolizumab (NCT02260440), and entinostat (NCT01105377). On the other hand, decitabine has been tested alone (NCT00879385) and combined with capecitabine and oxaliplatin (NCT01193517). Decitabine was found to influence PD-L1 expression *in vivo* and affect the tumor microenvironment *in vitro* and *in vivo* (20). Methylation inhibition by decitabine could increase Fas expression, restoring the sensitivity of Fas-mediated apoptosis (21). Despite their effectiveness, both drugs lacked chemical and metabolic stability and had high toxicity (22). However, decitabine is less toxic than azacytidine (14). Even so, a study of colorectal cancer cell lines reported that decitabine deteriorates cell condition over time (19). Azacytidine and decitabine have been used in combination with gefitinib and exhibit synergistic activity through inducing BAX (Bcl-2 associated X protein), PARP (poly-ADP protease polymerase) cleavage, and reducing Bcl-2 (21). Combining decitabine with HDACi also promotes BAC, p53, and reduces Bcl-2 (12).

Guadecitabine (SGI-110, S110), a second-generation hypomethylating prodrug, was developed as a replacement for 5-AZA and DAC, as it had a longer plasma half-life and lower peak plasma concentration compared to decitabine. It has DAC attached to deoxyguanosine through a phosphodiester bond (14). In the Phase I/II clinical trial, a combination of SGI-110 and irinotecan resulted in serious adverse events for 24 % of participants (NCT01896856). Zebularine (ZEB) is another DNMTi having a more stable with longer half-life and can be administered orally (23). As it has a competitive mechanism with cytidine deaminase, zebularine alone is not as efficient as azacytidine or decitabine. However, it prevents the re-methylation of genes after treatment with other DNMTIs; hence, it may lower the dose (24). Zebularine can induce apoptosis and regulate genes in extrinsic apoptotic pathways in colon cancer cell lines.

In addition, zebularine and decitabine ignited T-cells' mitochondrial apoptotic pathway (25). Zebularine can inhibit invasion through AKT signaling silencing and increase let-7b expression levels in SW620 cell line (26). As few drugs had gone through clinical trials for CRC, clinical responses were not clearly reported, and the search for effective demethylating drugs for CRC is ongoing.

Histone Modification in Colorectal cancer

Nucleosomes were formed by two histones copies of H2A, H2B, H3, and H4, wrapped by 147 base pairs of DNA (27). Histone has a long tail protruding from the nucleosome and is prone to covalent modification in many areas, including acetylation, phosphorylation, methylation, ubiquitylation, deamination, ribosylation, and glycosylation (28). Histone modification can change nucleosome structure to alter chromatin structure and gene expression (29).

Histone acetylation is catalyzed by histone acetyltransferase (HAT) (30). The enzyme transfers the acetyl group of acetyl coenzyme A to the end of histone amino acid, thereby relaxing chromatin structure under electric charge and increasing DNA accessibility (31). On the contrary, histone deacetylases (HDACs) remove the terminal acetyl group of histone lysine and compressed chromatin structure, inhibiting transcription (30,32). HDACs are in charge of tumor suppressor gene silencing (32). HDAC had 18 members, divided into four classes : I (HDAC1-3 and HDAC 8), II (HDAC4-1, HDAC 9, and HDAC 10), III (sirtuins 1-7) and IV (HDAC 11) (32). In CRC, increased expression of HDAC2 was found alongside HDAC 1-3, HDAC5, and HDAC7. Moreover, abnormal expression of HDAC is often related to poor CRC survival (32), as the expression of HDAC1, HDAC2, and HDAC3 were higher in distant metastasis tumors (31).

Histone methylation, on the other hand, is regulated by histone methyltransferases (HMTs) and histone dimethyl transferases (HDMTs). HMTs could be classified into histone arginine methyltransferase (PRMTs) and histone lysine methyltransferase (HKMTs) (12). Lysine methylation occurred mainly on histone H3 or H4 (23). Depending on modified-residues locations (27), histone lysine methylation could activate or repress gene expression (27,33). In CRC, methylation by PMRT on H4R3 recruits SMARCA4, promoting CRC migration through activation of TNS4 and EGFR (31). Lysine-specific demethylase (LSD1) was one of the earliest histone demethylases, catalyzing the demethylation of histone H3K4 at the CDH1 promoter, downregulating its expression, hence, hastening the EMT process of CRC (31).

Histone phosphorylation attached phosphoryl group at serine (S), threonine (T), and tyrosine (Y) residues of histone tails. It is a vital step in chromosome condensation during cell division, transcriptional regulation, and DNA damage repair (34). Phosphorylation disturbs the DNA and histone interaction, affecting chromatin structure stability required in mitosis. Specific phosphorylated sites within histone are associated with different chromatin functions (27). Nowadays, studies have been directed to evaluate the effect of dysregulated phosphorylation on disease development (24). Moreover, there is only a few research addressing the association between histone phosphorylation and CRC.

Histone Methyltransferase Inhibitors for Colorectal Cancer Treatment

As determining a cell's fate is necessary to prevent uncontrolled cell growth, a polycomb group (PcG) system is used to regulate transcription (15). Enhancer of zeste homolog 2 (EZH2), a subunit of the poly-comb repressive complex 2 (PRC2), had been observed as a regulator of cell proliferation, migration, and stemness in cancer cells, hence making it a potential drug target. Increased EZH2 has been noted in various cancers, e.g. bladder, lung, endometrial, gastric, breast, and colorectal cancer (35,36). EZH2 could alter downstream gene expression by trimethylation of H3K27 and by regulating gene expression beside H3K27 (35). 3-deazaneplanocin A (DZNep) is the first EZH2 inhibitor that could directly inhibit EZH2 through hydrolase inhibition of S-adenosyl-L-homocysteine (SAH) (35). It inhibits CRC proliferation and induces cell cycle arrest through upregulation of p27 and

downregulation of p-CDC2 expression (12). Afterward, several S-adenosyl-methionine (SAM) competitive inhibitors were developed. Few of it being GSK346, which can suppress migration, invasion, and proliferation of CRC cells, and GSK 343, which elicit autophagy via upregulation of LC3 gene expression resulting in CRC cell death (27). Tazemetostat, which was approved for epithelioid sarcoma treatment, was tested in combination with 5-FU, showing synergistic antitumor activity both *in vitro* and *in vivo* (37). This drug is currently in clinical trials for CRC alongside other cancers (NCT04705818). Other SAM-competitive inhibitors, UNC-1999, could induce autophagy by stimulating reticulum endoplasmic stress, resulting in death in CRC (12). A more recent study introduced two orally bioavailable EZH1/2 inhibitors, (R)-OR-S1 and (R)-OR-S2 could suppress methylation of H3K27 in colorectal cancer cells (35). In addition, chaetocin, a fungal mycotoxin, could inhibit HMT on methylating H3K9 and is observed to reduce CRC cell migration. It suppresses cancer cell growth through induction of apoptosis (32). Overall, efforts have been made to develop HMT inhibitors to tackle cancer progression.

Histone Deacetylase Inhibitors for Colorectal Cancer Treatment

HDACi were divided into five groups based on their compounds: benzamides, cyclic tripeptides, short-chain fatty acids, hydroxamic acids, and sirtuin inhibitors. Vorinostat or suberoylanilide hydroxamic acid (SAHA), an oral HDACis which mainly inhibits HDAC class I and II, was first approved by the United States Food and Drug Administration (FDA) as cutaneous T-cell lymphoma (CTCL) treatment. Vorinostat induces ROS-dependent apoptosis and blocks angiogenesis (32). Moreover, it had antiproliferative activity in KRAS-mutant CRC lines *in vitro* (12). Afterward, vorinostat entered clinical trials for CRC. It was used alone (NCT00126451) and in combination with 5-FU (NCT00336141), 5-FU, leucovorin (NCT00942266), hydroxychloroquine or regorafenib (NCT02316340), and 5-FU-leucovorin-oxaliplatin (NCT00138177). However, no clear result has been determined.

Sulforaphane (SFN), found in vegetables like broccoli, cauliflower, and cabbage, has been found as a possible chemopreventive agent (32,38). It targets HDAC1, HDAC2, HDAC3, and HDAC8 in CRC cells. SFN and HDAC inhibition alter apoptosis and cell cycle, blocking tumor growth *in vitro* and *in vivo*. Moreover, it intercedes cancer invasion and angiogenesis by reducing matrix metalloproteinase (MMP) (32). Domatinostat, a selective HDAC class I inhibitor, and Resminostat, HDAC class I and II inhibitor, both could be administered orally. Domatinostat could inhibit proliferation, survival, and cell cycle in CRC cells. However, it is particular to cells with high expression of certain HDACs, and no clinical trial has been done. On the other hand, resminostat has been used in several cancers but is in clinical trials for CRC (NCT01277406). Belinostat was another drug tested on colon cancer as it can induce TSG expression gene TGFβRII with the restoration of the downstream cascade, resulting in cancer cell death (32). No results were available for clinical trials on belinostat in CRC treatment (NCT00413075, NCT00413322). Panobinostat could also activate tumor suppressor gene death-associated protein kinases and alter genes involved in angiogenesis, mitosis, and DNA replication (12,32). In addition, it could prolong histone hyperacetylation, providing an intermittent dosing schedule (32). However, clinical trials using panobinostat alone (NCT00690677) had no published results, while combination therapy with 5-FU (NCT01238965) was terminated due to adverse events. Entinostat, an HDAC class I inhibitor, could trigger the expression of various genes and increase overall acetylation (39). Clinical trials for CRC have been done, combining other drugs with entinostat; hydroxychloroquine and entinostat (NCT03215264), pembrolizumab (NCT02437136), and azacytidine.

A new class of histone deacetylase inhibitor, romidepsin, showed its effect in CRC cell lines through alteration in protein modification (27). A clinical trial of romidepsin on metastatic CRC has been done (NCT00077337) alongside a combination with MK-3475 in advanced CRC (NCT02512172). Trichostatin A (TSA) suppresses CRC cell growth *in vivo* by generating cell cycle arrest and apoptosis via JAK2/STAT3 signaling (27). TSA with 5-fluorouracil (5-FU)

could suppress CRC viability by downregulation of TS mRNA and TS protein (33). In addition, ACY-1215, an HDAC6 inhibitor, could reinforce the apoptosis effect of oxaliplatin in CRC (12). HDACis could perform its anti-tumor effects as an emerging anticancer drug by inducing cell cycle arrest, inhibiting tumor cell growth, differentiation, and apoptosis. However, despite few efforts to initiate clinical trials on HDACis-, few HDACi-s lacked specificity; hence, developing specific targets for CRC is necessary for more optimal treatments.

Bromodomain and extra terminal inhibitors (BETIs)

The bromodomain and extra-terminal domain (BET) is a protein family having two N-terminal acetyl-lysine binding bromodomains in tandem (BD1 and BD2), as well as BRD2, BRD3, BRD4, and BRDT (40). BRD4 had three isoforms named BRD4L, BRD4Sa, and BRD4Sb, characterized by a unique C-terminal motif (CTM). BET proteins serve as an epigenetic readers in lysine-acetylated histones or transcription factors. BET could be modulated through expression regulation, post-translational modification, and RNA-DNA mediated mechanism (41).

BRD4 is the most studied BETs due to its capability to assemble both "super-enhancers" and hyper-acetylated gene promoters to promote RNA polymerase (Pol) II-mediated transcriptional elongation through its kinase activity (41). Super-enhancers are a group of acetylated histones controlling oncogenes transcription (33). Specific oncogenes have been found to target BRD4, including cMyc, FOSL1, RUNX2, BCL-2, KRAS, BRAF, ARAF, and c-KIT (42). In 70 % of CRC cases, overexpression of c-Myc is observed alongside an activated c-Myc-dependent transcription program (43). *In vitro*, the mouse model of CRC with BRD4 knockdown showed decreased cell proliferation and growth, mainly through c-Myc downregulation (33). Hence, BET inhibitors' mechanism of action could be an option in hematopoietic and solid tumors through downregulating c-Myc expression. Several promising BET inhibitors have been found alongside their admissible toxicity and potent efficacy, including thienodiazepine JQ1, I-BET762 (GSK525762), I-BET151 (GSK1210151A), GS5829, TEN-010, OTX-015, and ZEN003694 (39,44). JQ1, a well-known BET inhibitor, replaces BRD4 from acetylated histones, leading to BRD4 loss in super-enhancers and transcriptional elongation of these genes, Myc included (40). JQ1efficacy *in vivo* had been shown in mouse models of multiple cancers (41). A combination of JQ1 and bortezomib in CRC could inhibit angiogenesis due to c-Myc expression inhibition (43) and induce cell cycle arrest (45). A novel orally administered JQ1, OTX015, has been formed as an inhibitor of BRD2/3/4 (39). It is the first clinically tested BET inhibitor with antitumor activity towards hematologic malignancy alongside breast cancer, prostate cancer, neuroblastoma, and glioblastoma. In CRC, OTX-015 targets Myc and induces G1 cell cycle arrest (45). BMS-986158 is another BET inhibitor with an inhibitory potential toward CRC in mouse xenograft models through downregulating c-Myc expression (8). Multiple BET inhibitors have been in clinical trials for solid tumors, including CRC (NCT02431260, NCT02711137). However, none goes past phases I and II, and searching for suitable BET inhibitors remains.

Alteration of non-coding RNA (ncRNA) in colorectal cancer

Even though not translated, non-coding RNA (ncRNA) was markedly participating in regulating gene expression. It can both have anti and pro-tumorigenic effects (3). ncRNA is classified into housekeeping ncRNAs and regulatory ncRNAs (46,47). Regulatory RNA could be divided into two groups depending on nucleotides length, small ncRNAs and long ncRNAs (48). miRNA belongs to the first group, having approximately 20 nucleotides in length and serving as post-transcriptional repressors through binding in the 3'-untranslated region (UTRs) of target mRNA (3,49). As miRNA controls most protein-coding genes, it regulates pro-tumorigenic processes from cell differentiation, proliferation, and apoptosis (49,50). However, the expression of miRNA could be disrupted through various genetic alterations and methylation processes (3). In tumor tissues, miRNA can be upregulated or downregulated, as it can be both an oncogenic miRNA (onco-miRs) through

inhibition of tumor suppressor genes (TSGs) or a tumor-suppressive mRNAs (ts-miRs) through inhibition of oncogene expression (3,49,51). Moreover, miRNA affects CRC resistance and sensitivity to therapeutic agents (12). Studies reported that miRNA administration could sensitize tumors to drugs (49).

One of the miR-34 family, miR-34a, is one of the tumor-suppressive mRNAs targeting NAD-dependent protein deacetylase sirtuin-1 (SIRT1), inducing positive feedback, enhancing its activity and holding the cell cycle at G1-S checkpoint. Moreover, it is involved in regulating TGF-β by directly targeting decapentaplegic homolog 4 (SMAD4), whereas downregulation of miR-34a results in increased epithelial-mesenchymal transition (EMT) and invasion (3). Let-7 family miRNA were well known as tumor-suppressing miRNAs targeting various oncogenes, including Ras and Myc. miR-200 family also performs as tumor suppressor miRNAs as all its members inhibit EMT, invasion, adhesion, and metastasis (49). Conversely, miR-34b and miR-34c were correlated to worse prognosis and metastasis in CRC. miR-31 is another oncogenic miRNA through activation of the RAS signaling pathway. Suppression of miR-31 might be able to inhibit migration and invasion alongside increasing sensitivity to 5-FU (12). *In vitro* and *in vivo* experiments of miR-21, which is commonly overexpressed in CRC, showed downregulated phosphatase and tensin homolog (PTEN) expression, resulting in migration, invasion, and metastasis (3). Sprouty1 (SPRY1) and 2, programmed cell death protein 4 (PDCD4), and reversion-inducing-cysteine rich protein with Kazal motifs (RECK) were also targets for miR-21, contributing to the tumorigenesis process (49). Few miRNAs were found to be involved in drug resistance. Upregulated miR-10b contributed to 5-FU resistance by inhibiting BIM (Bcl-2-like protein 11) (21).

On the contrary, lncRNAs are diverse depending on their structure, localization, and mode of action (46), making it possible for a tremendous amount of function as either positive or negative transcription regulators (3). Some ncRNA could perform as competing endogenous RNAs (ceRNAs), able to sponging miRNA, which commonly happens in tumorigenesis (46). 'Sponging' miRNA refers to the competitively occupying binding site of miRNAs, sequestering miRNAs and altering the downstream gene expression (52), resulting in restored translation, which otherwise, the translation would stop (3). Moreover, lncRNA can function as decoys that block other regulatory RNAs or proteins to DNA (46,48).

In CRC pathogenesis, lncRNAs took part in various pathways such as EGFR, WNT, TGF-β, and p53 signaling pathways. However, epigenetic modifications could happen in parallel; for example, the overexpression of HOX transcript antisense RNA (HOTAIR) recruits PRC2, hence silencing its target genes through H3K27 trimethylation (3,46). HOTAIR has been shown to increase metastatic capacity in vivo, alongside reduced survival in CRC (3). DUXAP10, a transcription silencer, formed a complex with LSD1, shutting down the expression of p53 (48). Oncogenic lncRNAs such as CASC11 (cancer susceptibility 11), PVT1 (plasmacytoma variant translocation 1), and CCAT (colon cancer-associated transcript) family were found to promote CRC progression through interaction with proteins stimulating Myc or other Wnt target gene expression, in post-translational level. Increased PVT1 is associated with cell proliferation, invasion, and metastasis as it could bind to miR-30d-5p, decreasing its expression and reversing the repression of RUNX2 (runt-related transcription factor-2). Moreover, it also sponged miR-455, which naturally functions as a tumor suppressor in cancer (52). lncRNA named CCAT1-L and small nucleolar RNA host gene-1 (SNHG1) were also found in CRC. CCATL-1 acts as Myc transcriptional regulator while SNHG1 promotes Myc expression (48). On the other hand, lncRNA could function as a tumor suppressor. Low levels of growth arrest-specific 5 (GAS5) were found to be positively related to advanced TNM stage and poor survival in CRC (3). Like miRNA, lncRNAs were also involved in drug resistance. CASC15 was overexpressed in oxaliplatin-resistance CRC cells through sponging miR-145 and promoting ABCC1 (ATP-binding cassette, sub-family C, member 1). ABCC1 notices and effluxes antitumor agents, making tumor cells resistant to chemotherapeutic drugs (21).

ncRNA Targeted Therapy

Due to ncRNA's inseparable involvement in CRC pathogenesis, it has been proposed as a potential therapeutic target. Two basic modes of action were suggested, replace the downregulated ts-miRs using miR-mimics or inhibit the upregulation of onco-miRs (3,49). Before implementing these strategies, it is necessary to differentiate between which miRNA is upregulated and downregulated in CRC. After pointing out the potential miRNA target, a pharmacological analysis should be performed through *in vivo* miRNA delivery studies. Furthermore, clinical trials were carried out to assess efficacy and safety. Various methods have been formed to hinder mature miRNAs, e.g., antisense oligonucleotides (ASOs, also known as anti-miRs, or antagomiRs), anti-miR peptides, miRNA masking, and miRNA sponges. ASOs were synthesized nucleic acids complementary to target mRNAs in the nucleus (e.g. pre-mRNA) and cytoplasm (53). Anti-miR binds to miRISC (RNA-induced silencing complex), blocking miRNA interaction with target mRNA. MiRNA masking uses a complement of miRNA binding sites in the 3'UTR region of target mRNAs to interfere with particular miRNA-mRNA interactions (54). On the other hand, miRNA mimics were used to recover the tumor suppressor activity. These miRNAs had one strain identical to the target miRNA, linked to molecules (e.g. cholesterol, peptides), and modified to avoid binding to RISC while still allowing degradation (54). To optimize the delivery of miRNAs or antagomiRs, tissue specificity is required alongside minimal potential off-targets. For a ts-miRNAs replacement to succeed, miRNA uptake by cells at a physiologically adequate level must be considered (49). Local or systemic delivery of miRNA should be considered according to the tumor. Only the liver and kidney had a high concentration of miRNA mimics or antagonists after intravenous administration. After that, miRNA levels decrease rapidly in another organ 24h after injection (49). Moreover, unmodified RNA oligonucleotides were prone to damage by serum nucleases and unable to penetrate cell membranes to enter cell cytoplasm. Modification at the 2' sugar position has been made, such as 2'-O-methoxyethyl (2'-MOE), 2'-fluoro (2'-F), 2'-O-methyl (2'-OMe), locked nucleic acid (LNA), and conjugation to cholesterol or polyethylene glycol (PEG) to increase miRNA stability (49,53,54).

In a mouse model experiment, an intra-tumoral and intravenous injection of the miR-143-liposome complex, miR-143 being a ts-miRNA in CRC, showed growth inhibitory effects (3). In human colon cancer SW480 cells, researchers have been able to precisely silence miR-135b, inhibiting cell proliferation and inducing apoptosis (54). Recently, few miRNA-based drugs have been tested in clinical trials (39). MRX34, an RNA mimic for tumor suppressor miR-34, was the first miRNA mimic to enter clinical trials. Results were promising as it produces partial responses for renal cell and hepatocellular carcinoma. However, trials were halted due to severe adverse events (49). MRG-106, an LNA anti-miR-155, is in phase I and II clinical trials for lymphoma and leukemia, while RGLS5579, an anti-miR-10, remained in the preclinical phase for glioblastoma multiforme (49). Although miRNA therapy has been shown as a potential treatment for CRC, an efficient delivery system and safety issues must be addressed before using the drugs in the clinical setting.

In addition to miRNA, small interfering RNAs (siRNAs), the other group of small ncRNAs, have been studied to be used as an onco-miRs antagonist (3). siRNA is a double-stranded RNAs (dsRNAs), able to separate into single-strand and bind to mRNA. Its binding results in cleavage and mRNA degradation, hence, preventing translation. A complementary siRNA could be created by identifying a target mRNA sequence (55). siRNA could suppress CRC proliferation, induce apoptosis, manage drug resistance, and prevent metastasis. B7-H4 siRNA can inhibit proliferation, invasion, and migration of CRC cell line LOVO through specific signaling. Studies have also reported a combination of siRNA with anticancer drugs to have a synergistic effect. A combination of doxorubicin and snail siRNA; a mediator of EMT, inhibits proliferation and reduces migration alongside inducing apoptosis in human CRC cell line HCT-116 (53). In 2018, the first siRNA drug, patisiran, was approved by the FDA for rare polyneuropathy treatment via targeting mRNA transcription of transerythrin (39). In the

Table 1

Epigenetic therapy in colorectal cancer

Epigenetic drug categories	Agent Name	Proposed mechanism of action	Concern issue(s)
DNA Methylase inhibitor (DNMTi)	Azacytidine**	Induce viral mimicry via the MDA5/ MAVS/IRF7 pathway, inhibit Wnt signaling pathway	Lacked chemical and metabolic stability, had high toxicity, and had no significant effect in monotherapy. Side effects include fetal abnormalities and decrease male fertility.
	Guadecitabine**	Prolong cell exposure to decitabine, enhancing its uptake to DNA and division of cancer cells	More stable than decitabine. Clinical trial results not posted.
	Decitabine**	Inhibit MAPK pathway, increase NALP-1 expression	Lacked chemical and metabolic stability and had high toxicity, with no significant effect in monotherapy. Side effects include myelosuppression.
	Zebularine	Induce p53-dependent ER stress and autophagy, increase expression of Let-7b (a ts-miRNA)	High selectivity for cancer cells but no clinical trial for CRC yet
Histone Methyltransferase inhibitor (HMTi)	Chaetocin	Inhibit methylation of H3K9, affects NFκB, ERK-signaling, and caspase-dependent apoptosis.	No clinical trial information
	CBB1003	Suppressing cell growth via down-regulation of LGR5 levels inactivates Wnt/β-catenin pathway.	No clinical trial information
	DZNep	Inhibit CRC proliferation, induce cell cycle arrest through upregulation of p27 and downregulation of p-CDC2 expression, and directly inhibit EZH2.	No clinical trial information
	GSK346	Induce cell cycle arrest and inhibit EZH2.	No clinical trial information
	GSK343	Promoted autophagy through upregulation of LC3 gene expression resulting in CRC cell death.	No clinical trial information

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Epigenetic drug categories	Agent Name	Proposed mechanism of action	Concern issue(s)
	UNC-1999	Induce autophagy through stimulating endoplasmic reticulum stress.	No clinical trial information
	(R)-OR-S1 and (R)-OR-S2	Suppress methylation of H3K27 in colorectal cancer cells.	No clinical trial information
	Tazemetostat**	Increased proapoptotic proteins, promotes PUMA induction, and increased sensitivity to 5-FU.	No clinical trial results published
Histone Deacetylase inhibitor (HDACi)	Vorinostat (SAHA)**	Inhibit HDAC class I and II. Induces ROS-dependent apoptosis and blocks angiogenesis.	Clinical trial was completed with few adverse events.
	Sulforaphane	Inhibit HDAC1, HDAC2, HDAC3, HDAC8. Block tumor growth by altering apoptosis and cell cycle. Intercedes cancer invasion and angiogenesis by reducing matrix metalloproteinase (MMP).	No clinical trial information
	Domatinostat	Inhibit HDAC1, HDAC2, HDAC3. Targets Hedgehog (HH)/Gli signaling pathway.	No clinical trial information
	Resminostat**	Inhibit HDAC class I and II. Inhibit proliferation, induced G0/G1 cell cycle arrest, and upregulate p21.	No clinical trial results published
	Belinostat**	Induce tumor suppressor gene expression gene TGFβRII with the restoration of the downstream cascade.	No clinical trial information
	Panobinostat**	Non-selective HDACi. Induce cell cycle arrest in G1, G2/M. Affects EGFR/HER2 signaling, MAPK signaling, PI3K-Akt, and the NFκB pathway.	Allow intermittent doses to reduce thrombocytopenia. No clinical trial results were published. Clinical trials in combination with 5-FU were terminated due to adverse events.
	Romidepsin	Induce cell cycle arrest and proliferation.	No clinical trial results published

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Epigenetic drug	Agent Name	Proposed mechanism of	Concern issue(s)
	Trichostatin A (TSA)	Induce cell cycle arrest and apoptosis via JAK2/STAT3 signaling.	No clinical trial information
	Entinostat	Enhances anti-PD-1 activity, used in combination with pembrolizumab	No clinical trial results in monotherapy were published. A clinical trial combination with azacytidine results in few adverse events in half of the participants.
Bromodomain and Extra Terminal inhibitor (BETi)	JQ1	Prevent BRD4-mediated recruitment of p53 to chromatin targets, and induce cell cycle arrest and apoptosis via a c-Myc-independent mechanism.	A preclinical study in mice showed toxicities include intestinal crypt disruption, impaired long-term memory, hematopoietic cell depletion, and neuronal defects.
	BMS-986158	Downregulates c-Myc expression, causing cell death	No clinical trial information
RNA-based therapy	TKM-080301**	Inhibit PLK-1	Clinical trial results are only available for CRC with hepatic metastasis.
	NBF-006**	Inhibit GST-P	No clinical trial results published

DNA : deoxyribonucleic acid; MDA5/ MAVS/IRF7 : melanoma differentiation protein-5/mitochondrial antiviral-signaling protein/interferon regulatory factor-7; MAPK : mitogen activating protein kinase; ER : endoplasmic reticulum; NFκB : nuclear factor kappa-light-chain-enhancer of activated B cells; ERK-signaling: extracellular regulated kinase-signaling; EZH2 : enhancer zeste homoloh-2; LC3 : light chain 3; 5-FU : 5-fluorouracil; ROS : reactive oxygen species; EGFR: epidermal growth factor regulator; HER-2 : human epidermal growth factor-2; PLK-1 : polo-like kinase-1; GST-P : glutathione S-transferase P.

following years, givosiran was approved in 2019 and lumasiran in 2020. Seven other siRNA drug is in phase 3 clinical trials, some close to being approved by FDA (55). However, no approved drugs were used to treat colorectal cancer.

Future insight and conclusion

Currently, several approaches using epigenetic modifying agents as cancer therapy have been developed. Epigenetic drugs had been used as a single therapy or in combination with conventional anti-cancer drugs with some promising outcomes. For colorectal cancer, various DNMT inhibitors, HMT inhibitors, HDAC inhibitors, BET inhibitors, and miRNA therapy had been proposed. For DNMTis, clinical trials for azacytidine, guadecitabine, and decitabine have been conducted for colorectal cancer. Furthermore, few HDACis were on Phase I and II clinical trials for CRC while Tazemetostat, an EZH2 inhibitor is currently in phase II clinical trial. Other drug categories demonstrate a promising potential in cancer but none were in clinical trials for CRC. RNA-based therapeutics

also provide an intriguing method to tackle cancer as it gives the possibility to target the previously undruggable proteins. In addition, epigenetic drugs could be used in combination with each other or with conventional therapies. Few had preliminary results of increasing sensitivity for conventional drug therapies, hence shedding a light in drug resistance problem. However, some obstacles appeared for each of epi-drug categories. While DNMT and HDAC enzymes lacked specificity, leading to reactivation of non-specific genes, upregulated or downregulated miRNA need to be identified specifically as it differs from one cancer to another, to be able to act as potential drugs. Moreover, providing effective delivery with minimal off-target effect was vital for these agents to be used clinically. The varieties of preclinical and clinical agents were numerous, nevertheless, the conclusion of whether the agent is safe and effective could only be confirmed after completing clinical trials.

In conclusion, as epigenetic regulation is always changing and dependent, the mechanism behind it warrants further investigation. It is visible that future research needed a combination of completing the ongoing studies, initiating new clinical trials, and developing new agents capable to treat CRC. A combination of expertise needs to be formed to overcome the obstacle in several inhibitors and RNA-based therapeutics to bring forth novel epigenetic agents that are both effective and safe.

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