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RNA-based therapeutics for colorectal cancer: updates and future directions

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Abstract

Colorectal cancer (CRC) is one of the most common causes of cancer death worldwide. While standard chemotherapy and new targeted therapy have been improved recently, problems such as multidrug resistance (MDR) and severe side effects remain unresolved. RNAs are essential to all biological processes including cell proliferation and differentiation, cell cycle, apoptosis, activation of tumor suppressor genes, suppression of oncogenes. Therefore, there are various potential approaches to address genetic disease like CRC at the RNA level. In contrast to conventional treatments, RNA-based therapeutics such as RNA interference, antisense oligonucleotides, RNA aptamer, ribozymes, have the advantages of high specificity, high potency and low toxicity. It has gained more and more attention due to the flexibility in modulating a wide range of targets. Here, we highlight recent advances and clinical studies involving RNA-based therapeutics and CRC. We also discuss their advantages and limitations that remain to be overcome for the treatment of human CRC.

Graphical abstract

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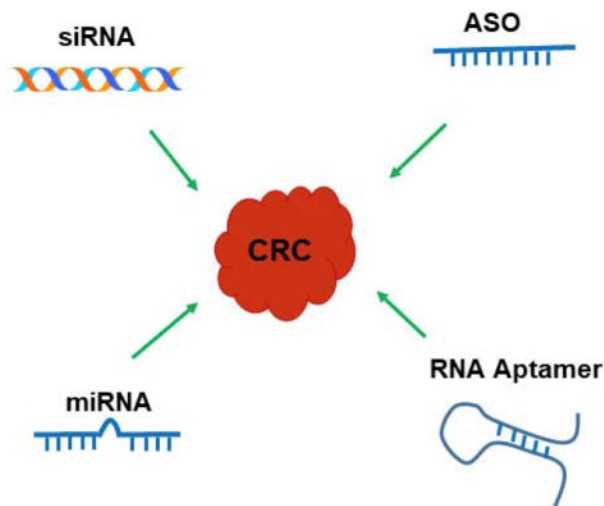
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Keywords

RNA-based therapeutics; Colorectal Cancer; Chemical modifications; RNA interference; Gene Delivery; Clinical trials

1. Introduction

Colorectal cancer (CRC) is the third commonly diagnosed and second leading cause of cancer death worldwide in 2018 [1]. Currently, the most common treatment for patients with stage I and II colorectal cancer is surgical resection. The patients with stage III disease may receive surgery and adjuvant chemotherapy to lower the risk of recurrence. The main treatment for stage IV metastatic colorectal cancer (mCRC) is chemotherapy. The use of targeted therapies is another option to treat stage IV colorectal cancer.

The treatment for early stage disease has the best effect, however, about half of colorectal cancer patients are diagnosed with metastatic disease or will develop advanced-stage disease subsequently or have cancer recurrence in the following months [2]. For patients with inoperable metastatic disease, the prognosis is poor, with a 5-year survival rate around 14% [3].

Chemotherapy drugs for CRC patients include fluorouracil (5-FU) plus leucovorin (folinic acid), capecitabine, irinotecan, oxaliplatin, and trifluridine plus tipiracil [4]. In most cases, two or more of these chemotherapy drugs are combined during treatment to achieve a better clinical outcome. For advanced colorectal cancer, chemotherapy improves life quality and prolongs survival rate [5]. However, chemotherapy has its limitations. The patients who receive chemotherapy may suffer from hair loss, bone marrow suppression, nausea, vomiting, diarrhea, increased risk of infections, and neuropathy [6]. Another limitation of chemotherapy is multidrug resistance (MDR), which may cause cancer relapse and metastasis [7]. Drug resistance is responsible for treatment failure in 90% of patients with metastatic cancers [8]. Currently, 5-FU remains the most effective chemotherapeutic agent

for the treatment of colorectal cancer, however, nearly half of the mCRC patients do not respond to it [9].

“One fits all” has been the principle of cancer therapeutics for a long time and chemotherapy is based on this principle. However, when CRC patients at the same disease stage receive the same treatment, the clinical outcomes for different individuals are quite different [10]. Advances in research and technologies have shown that cancer is a disease with high heterogeneity [11–13]. The CRC progression involves a variety of molecular biological changes such as genetic mutations or epigenetic abnormalities [14][15], the key property for cancer cells to gain drug resistance and evade the immune system of hosts. Studies have shown that correcting the abnormalities in gene expression can be detrimental to the cancer cells [16][17]. Therefore, new therapeutic strategies tailoring to the genetic changes and aiming at several cancer hallmarks simultaneously are in urgent need.

Ribonucleic acid (RNA)-based therapeutics with high specificity, high potency and low toxicity hold enormous potential as a new therapeutic approach [18]. The options for RNA therapeutics include RNA interference (RNAi), microRNA mimics and inhibitors, antisense oligonucleotides (ASO), aptamers, ribozymes, long non-coding RNA (lncRNA), and a variety of other RNAs. This wide range of choices offers flexible approaches to target disease-causing proteins at the RNA level. In contrast to conventional treatments, RNA-based therapeutics offer competitive advantages [19]. The “undruggable” proteins for conventional medicines can be selectively modulated by RNAs. They can even target proteins that have multiple homologous family members. In addition, RNAs can be designed for the targeted genes with a known sequence and easily be synthesized.

In this review, we summarize the recent researches on the biological actions of different RNAs and their role in regulating genes that are involved in CRC progression, which include effects on CRC cell growth, apoptosis, invasion, and metastasis. We also summarize clinical studies of RNA-based therapeutic strategies and discuss their advantages and limitations in CRC treatment.

2. RNA-based Therapeutics in Colorectal Cancer

RNA therapeutics refer to the use of RNAs as therapeutic agents. These consist different classes of RNAs, each of them regulates gene expression or translation through different mechanisms of action. Small interfering RNAs (or short silencing RNAs, siRNA), microRNAs (miRNA), and antisense oligonucleotides (ASO) are the most commonly used RNA therapeutics for silencing gene expression. Examples of RNA-based therapeutics in CRC treatment are summarized in Table 1.

2.1 RNA interference (RNAi)

RNAi is a naturally biological process used by cells to inhibit gene expression through targeting the mRNA molecules. Fire and Mello revealed the mechanism of action in the nematode worm *Caenorhabditis* in 1998 and won the 2006 Nobel Prize for their work [20]. In many types of cancer, including colorectal cancer, RNAi opens up a new avenue for the treatment. It has been used for silencing oncogenes or proto-oncogenes that are up-regulated

due to gene mutations or overexpression. SiRNA and miRNA are the most well-known RNA interference.

2.1.1 siRNA—SiRNAs are a class of double-stranded RNAs (dsRNAs), usually 21~25 nucleotides in length with 3' overhanging nucleotides at each end [21]. SiRNAs can be either produced from long dsRNAs and hairpin looped RNA catalyzed by a RNase III enzyme Dicer, or artificially synthesized and introduced into the cells by transfection. They are incorporated into other proteins to form RNA-induced silencing complex (RISC), and unwound to single strand siRNAs subsequently. The single strand siRNAs which remain part of RISC find and bind to complementary mRNAs, and induce the mRNAs cleavage.

SiRNA can be used for a variety of purposes, including the suppression of CRC cell proliferation and induction of cell apoptosis, overcoming multi-drug resistance, and prevention of CRC metastasis. Peng et al. have reported that B7-H4 siRNA is able to inhibit proliferation, invasion, and migration of colorectal cancer cell line LOVO effectively through targeting CXCL12/CXCR4 and JAK2/STAT3 signaling [22]. In another study, the siRNA against *KRAS* has been applied to inhibit the proliferation of *KRAS* mutated CRC cells and to slow the tumor growth in a xenograft mouse model [23]. Ding et al. knocked down a histone demethylase called lysine-specific demethylase 1 (LSD1) with siRNA, resulting in suppressing of proliferation, migration and invasion of CRC *in vitro* [24]. Due to the complexity of cancer, combination therapy has gained more attention to overcome drug resistance or to active cancer cell apoptosis. Many studies have reported combination approaches for siRNA with small molecule anticancer drugs to achieve synergistic anticancer effects by combining different mechanisms of action. Lee et al. have shown that the synergistic effect to suppress tumor growth by using VEGF siRNA and SN-38 (7-ethyl-10- hydroxycamptothecin) in a LS174T tumor-bearing mouse xenograft model [25]. A study conducted by Sadreddini et al. used the combination of doxorubicin and snail siRNA. Snail is an important mediator of epithelial mesenchymal transition (EMT), a process by which epithelial cells gain migratory and invasive properties. The combination regimen inhibited proliferation, induced apoptosis, and reduced migration in human CRC cell line HCT-116 [26].

Combined siRNA treatments have also been studied. Valentino et al. applied a co-targeting strategy that targets mutated PI3K/AKT/mTOR and RAS pathways by siRNAs in CRC cell lines with PIK3CA and KRAS mutations. The combined therapy of siRNA (PIK3CA + KRAS or Akt2 + KRAS) provided a synergistic inhibition of CRC cell proliferation and an increase in apoptosis [27].

For siRNA to be effective to treat CRC, there are mainly three steps. Firstly, the targeted gene that plays an important role in the CRC progression need to be identified; secondly, the siRNAs for targeted gene silencing will be designed and synthesized; thirdly, the siRNAs need to be delivered into the CRC cells. Each step comes along with different challenges, which include unintended off-target effects [28], length of effect [29], short half-life [30], requirement of a delivery approach [31], and risk of immune system activation [32]. There are hypothesized theories for off-target effects. 1) siRNAs are able to bind to non-targeted mRNAs via imperfect complementarity and silence the non-targeted gene expression [33].

2) siRNAs may enter endogenous miRNA systems that could imperfectly bind to non-targeted mRNAs and silence those targets [34]. To be clinically applicable, siRNAs should exert effects over a certain period time since siRNA will be degraded quickly after introduction. This may require repeated siRNA delivery or chemical modification of the siRNA to increase stability [35]. In order for siRNA entered the target cells, certain delivery strategies need to be taken due to the clearance by nucleases in the bloodstream. In addition, when the cells are introduced with siRNAs, they may consider it as a by-product of viral and activate innate immune responses [32].

2.1.2 microRNA (miRNA)—MiRNAs are short non-coding RNAs of 20~25 nucleotides in length that regulate gene expression post-transcriptionally by imperfect base pairing to the complementary sequences in the 3' untranslated region (UTR) of mRNAs. MiRNAs are able to regulate cellular functions including cell development, proliferation, invasion, and apoptosis [36]. It has been found aberrant expressions of miRNAs are associated with the initiation, progression and metastasis of CRC [37–39]. MiRNA biogenesis is controlled by various enzymes such as Drosha, Dicer, DGCR8, thus abnormal expression of these proteins due to gene mutations or dysregulated epigenetic changes such as DNA hypomethylation [40], DNA hypermethylation [41], and histone deacetylation [42]. In CRC, some miRNAs whose expression are increased have been identified as oncogene miRNAs (or oncomiRs). OncomiRs regulate cancer development by inhibiting tumor suppressor genes, genes involved in cell differentiation or apoptosis. For example, Asangani et al. have reported that miR-21 induces invasion, intravasation and metastasis in colorectal cancer cell lines by negatively regulating tumor suppressor Pcd4 at the post-transcriptional level [43]. Fang et al. have found that miR-17–5p induces drug resistance by targeting PTEN, a tumor suppressor that dominates the PTEN/AKT/PI3K pathway [44].

Other miRNAs whose expression is decreased in CRC cells act like tumor suppressors. Yin et al. have demonstrated that miR-204–5p is a tumor suppressor in CRC by inhibiting *RAB22A*, a member of the RAS oncogene family [45]. Tang et al. have shown that miR-93 suppresses CRC development by down regulating Wnt/ β -catenin [46]. Zhang et al. have reported that miR-217 inhibits tumor growth and enhances cell apoptosis in CRC via down regulating MAPK signaling [47].

A single miRNA can regulate a variety of disease-associated targets simultaneously. It is a major advantage for the treatment of a disease like cancer where multiple genes are mutated or dysregulated. It has been reported that miR-143 suppresses CRC cell growth by regulating the oncogene *KRAS* expression [48]. Another study has demonstrated that miR-143 exerts its tumor suppressor role in CRC by repressing *DNMT3A* [49]. Furthermore, miRNA-143 has been found to inhibit CRC cell invasion and migration by targeting metastasis-associated in colon cancer-1 (*MACC1*), a CRC tumorigenesis and metastasis related gene [50].

Although miRNA therapy possesses great potential for the treatment of CRC, several obstacles remain to be overcome including an efficient miRNA delivery system, and the safety issues related to side effects.

2.2 Antisense Oligonucleotides (ASOs)

ASOs are synthesized nucleic acids and their base sequences are complementary to the targeted RNAs in the nucleus (e.g., pre-mRNA) or cytoplasm (e.g., miRNA, mRNA) via Watson-Crick hybridization [51]. Among all the RNA therapeutics, ASOs have a relatively longer track record, first being introduced by Paterson et al. to inhibit translation in 1977 [52]. Since then different modifications in the structure (e.g., backbone, sugar moiety) have been applied to optimize their therapeutic potential. ASOs have been tested for the treatment of cancer in more than 50 clinical trials (<https://clinicaltrials.gov>).

Targets of ASOs for treatment of CRC include genes that play an essential role in cell proliferation, cell differentiation, cell cycle, and cell death. Growth factors such as epidermal growth factor receptor (EGFR) play an important role in CRC cellular proliferation [53]. Najar et al. established EGFR ASO encapsulated within polyamidoamine (PAMAM) nanoparticles. The nanoparticles reduced the proliferation of human colon cancer cell line HT29 [54].

In addition, genes that are important for proliferation-independent processes in CRC progression such as adhesion, invasion, and migration are also potential targets. For example, Tao et al. constructed a eukaryotic expression vector encoding ASOs against an oncomiR miR-21. The down-regulation of miR-21 by ASOs significantly reduced the invasion and migration of CRC cells [55].

Regulation of proto-oncogenes such as *c-MYC* by ASOs has been explored. Abaza et al. have reported that *c-MYC* ASOs inhibited the growth of CRC cells and increase the sensitivity of CRC cells to several chemotherapeutic drugs including paclitaxel, 5-FU, doxorubicin, and vinblastine [56]. Oncogene *MDM2* is a negative regulator of the tumor suppressor p53. Wang et al. inhibited *MDM2* expression with an ASO and found *MDM2* ASO had both *in vitro* and *in vivo* antitumor activity, indicating its potential as a CRC cancer therapeutic [57]. It has been reported that mutations of the *RAS* oncogenes result in persistent activation of the downstream pathways, leading to carcinogenesis in several human cancers including CRC. Ross et al. evaluated a high-affinity constrained ethyl-containing therapeutic ASO AZD4785 targeting *KRAS* mRNA and found AZD4785 effectively inhibited proliferation in *KRAS* mutant cells [58].

2.3 RNA Aptamer

RNA aptamers are single-strand RNA oligonucleotides with various shapes that can bind to targets such as proteins, peptides, and small molecules. RNA aptamers are identified through several rounds of selection called systematic evolution of ligands by exponential enrichment (SELEX) [59]. Due to their stable three-dimensional shape, the binding to targets has high affinity and specificity [60].

Varieties of RNA aptamers have been identified to bind different targets and have shown great potential as diagnostic, prognostic and therapeutic tools in CRC. Mi et al. have screened a large number of nuclease-resistant RNA oligonucleotides in a tumor-bearing mouse model to identify RNA aptamers that can localize to hepatic colon cancer metastases. They have found an RNA aptamer that binds to p68, an RNA helicase that is upregulated in

CRC [61]. RNA aptamers are isolated against not only cell surface markers, but also intracellular key components. Mi et al. have identified another aptamer that binds to the protein DHX9, another RNA helicase that is upregulated in CRC. The aptamer is selectively localized to the nucleus of cancer cells *in vivo*, indicating that it may facilitate targeted delivery to the nucleus [62].

In addition to their targeting capability, RNA aptamers can act like antagonists to inhibit the interactions between extracellular targets and their ligands. The overexpression of carcinoembryonic antigen (CEA) in CRC cells induces cell adhesion, enhances the resistant to anoikis, and promotes hepatic metastasis [63]. Lee et al. have identified an RNA aptamer against the domain of CEA required for metastasis with high affinity and specificity using SELEX. They have demonstrated that the RNA aptamer could inhibit CEA interactions with heterogeneous nuclear ribonucleoprotein M4 and death receptor 5, resulting a suppression of hepatic metastasis of colon cancer cells in mice [64].

Aptamers are called “chemical antibodies”, which have several competitive advantages comparing to their protein counterpart. Cell-free assembly allows aptamer production to be cost effective, rapid, and reproducible in large scale [65]. Aptamers induce less immunogenicity after chemical modifications [66], and they have an enhanced tissue penetration due to their smaller size [67]. Another significant advantage of aptamers is being able to introduce other functional groups to the backbone. For example, aptamers can form a chimeric structure with siRNA to deliver siRNA to targeted cell population [68], conjugated to chemo drugs such as doxorubicin [69], or combined with three-way junction RNA to construct multifunctional RNA nanoparticle to target metastatic CRC cells [70].

2.4 Ribozymes

Ribozymes are RNA molecules that function as enzymes to catalyze biochemical reactions, thus they have great potential for gene therapy. The most common activities of natural ribozymes are to catalyze RNA cleavage and ligation reactions [71]. Gene-tailored ribozymes have been designed, synthesized and delivered to specific cells to regulate the expression of targeted genes [72].

Ribozymes have been reported to target a variety of oncogenes and the drug resistance genes in CRC. A hammerhead ribozyme has been designed to preferentially cleave oncogene *KRAS* mRNA in human colon cancer cell lines with *KRAS* mutations, resulting in tumor growth suppression and alteration of angiogenic gene expression [73]. Jeong et al. have shown that systemic delivery of adenovirus harboring trans-splicing ribozyme can recognize cancer-specific transcripts and significantly reduce tumor burden in colon cancer mouse model [74]. Glutamylcysteine synthetase (γ -GCS) plays an important role in both cisplatin and multidrug resistance. Nagata et al. have designed a hammerhead ribozyme against γ -*GCS* mRNA to specifically down-regulated γ -*GCS* gene expression in the HCT-8DDP human colon cancer cell line. The downregulation of γ -*GCS* expression leads to reversal of resistance to cisplatin, doxorubicin and etoposide [75]. RPI.4610 (Angiozyme) is a ribozyme that targets vascular endothelial growth factor receptor 1 (VEGF-1). It has been reported that Angiozyme can inhibit metastases in a human KM12 colorectal cancer xenograft model and increased survival in a murine 4T1 tumor model [76].

2.5 Long Noncoding RNAs (lncRNAs)

lncRNAs are a class of RNAs with a length over 200 nucleotides that are not translated to proteins. The role of lncRNA in cancer development has attracted more and more attention [77]. Although the research on lncRNAs are still developing and at a preliminary stage, it is believed that lncRNAs are important regulators for gene expression.

lncRNAs have complex secondary and tertiary structures due to their large sizes. The structure complexity enables their regulatory abilities via binding to protein, DNA, or RNA (see Table 2 for details). Accumulating evidence has indicated that lncRNAs are a powerful factor in the CRC progression. lncRNAs can epigenetic and transcriptional regulate gene expressions. For example, a lncRNA called CCAT1-L is transcribed specifically in human CRC from a locus 515 kb upstream of *MYC*. CCAT1-L is important for *MYC* transcriptional regulation and promotes long-range chromatin looping. CCAT1-L knockdown have reduced long-range interactions between the *MYC* promoter and its enhancers [78]. CRC patients have higher HOTAIR expression levels in CRC tissue than adjacent normal tissues [79]. It has been demonstrated that lncRNA HOTAIR acts as a scaffold to assemble polycomb repressive complex 2 (PRC2) and lysine specific demethylase 1 (LSD1) complexes, leading to epigenetic silencing of cancer-related genes [80].

Besides the transcriptional and epigenetic regulations, it has been found that lncRNAs play important roles in posttranscriptional regulation. Damas et al. have demonstrated that lncRNA SNHG5 promotes tumor cell survival in CRC by binding to target mRNAs to block double-stranded RNA-binding protein staufen homolog 1 (STAU1) mediated degradation [81]. Bian et al. have demonstrated that lncRNA UCA1 exerts its regulatory function by sponging and inhibiting a tumor suppressive miRNA miR-204-5p [82]. Another study has shown that lncRNA UCA1 can also control cancer-related pathways by interacting with mRNA 3'-UTRs, preventing them from miRNA-mediated degradation [83].

Recent breakthroughs have provided many examples of lncRNAs' regulatory capacities. lncRNAs associated with CRC development include AFAP1-AS1 [84], CCAT1 [78], CRNDE [85], DUSP [86], DANCR [87], GHET1 [88], HOTAIR-G [79], HNF1A-AS1 [89], MALAT1 [90], NEAT1 [91], SNHG12 [92], UCA1 [83] and others.

2.6 Small Activating RNAs

Small activating RNAs (saRNAs) are small double-stranded RNAs, which work through a distinct mechanism called RNA activation [93][94]. Several studies have demonstrated that saRNAs are able to up-regulate protein that are important for tumor progression by targeting gene promoters to induce transcriptional gene activation [95] [96]. Wang et al. have demonstrated that saRNA p21-saRNA-322 can inhibit CRC growth by stimulating the expression of p21, a downstream protein of P53, *in vivo* [97].

3. Improving the Effectiveness of RNA therapeutics for the treatment of Colorectal Cancer

Although RNA-based therapeutics have great potential for the treatment of CRC, problems such as degradation by nuclease, delivery to the CRC cells, and potential immunogenicity remain to be addressed [98]. Many studies have been reported that these issues can be overcome with the chemical modification of the RNAs [99]. In addition, advances in nanotechnology have improved systemic delivery of RNA therapeutics.

3.1 Chemical Structure Modifications

RNAs are unstable and rapidly cleaved *in vivo* due to a large amount of ribonucleases in serum and cells. RNAs, coding and non-coding, can be modified to affect RNA structure and function. In order to improve the stability of RNAs without reducing their activities in biological fluids, various chemical modifications to the base, sugar or phosphate moieties of nucleotides have been performed.

Chemical modifications to RNAs may also increase specificity and reduce off-target effects [100], enhance activity [101], improve pharmacokinetic (PK) and pharmacodynamic (PD) properties [102], and decrease immunological activation [103].

The chemical modifications for short synthesized RNA oligonucleotides include phosphorothioate (PS) backbone modification; 2'-fluoro (2'-F), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), locked nucleic acid (LNA) sugar substitutions; and conjugation with cholesterol or polyethylene glycol (PEG). The PS backbone modification replaces the non-bridging phosphate oxygen by a sulfur atom. This simple substitution increases resistance to nuclease degradation and improved affinity to plasma proteins with less renal clearance [104]. Substitutions at the 2' position of the sugar ring with 2'-F, 2'-OMe, 2'-MOE or LNS group improve the potency, stability, and overall PK and PD properties [105].

Non-nucleotide chemical modifiers can also be applied. Kitade et al. added aromatic benzene-pyridine (BP-type) analogs to the 3'-overhang region of the miR-143, resulting in greater activity and increased resistance to nuclease. The modified miR-143 showed a significant tumor-suppressive effect on xenografted tumor of CRC DLD-1 cells [106].

3.2 Targeted Nanoparticles for RNA Delivery to Colorectal Cancer Cells

RNAs are negatively charged and have large molecular weight, they cannot pass into the CRC cells. This obstacle can be overcome with delivery systems. There are various delivery systems currently being applied to improve the efficacy of RNA therapeutics. The two major types are viral and non-viral delivery vector.

Viral vectors such as retroviral, lentiviral, adenovirus, adeno-associated virus have high transfection efficiency, however the shortcomings of potential immunogenicity, cytotoxicity and insertional mutagenesis limit their applications [107]. Nanoparticle-based non-viral vectors such as liposomes, polymersomes, dendrimers, and inorganic nanoparticles have the advantages of low cost, ease of production in large amount, and lower pathogenicity, which offer a promising alternative for RNA therapeutics delivery [108]. Nanoparticles can reach

the tumor tissue passively due to their sizes. In addition, the enhanced permeation and retention (EPR) effect allows the accumulation within the cancer tissue longer to improve the therapeutic efficacy [109].

Tanggudu et al. [110] have reported that oral delivery of c-Myc conjugated polyethyleneimine (PEI)-macromolecule polyglycidyl methacrylate (PGMA) nanoparticles suppressed tumor growth efficiently and increased animal survival in a colon cancer model. Sureban et al. [111] encapsulated siDCAMKL-1 in Poly(lactide-co-glycolide)-based nanoparticles (NP-siDCAMKL-1) and treated mice carrying CRC tumor with NP-siDCAMKL-1, resulting in CRC tumor xenograft growth arrest.

Targeting is essential for the treatment of CRC. The most commonly used biomarkers in CRC include carcinoembryonic antigen (CEA) [112], death receptor 5 (DR5, also known as TRAIL receptor 2) [113], epithelial growth factor receptor (EGFR) [114], tumor-associated glycoprotein (TAG)-72 [115], and folate receptor- α (FR α). The conjugation of ligands on the surface of nanocarriers for the targeted delivery of RNA therapeutics to CRC cells has been widely used. Pi et al. have demonstrated that folate-displaying extracellular vesicles (EVs) can specifically deliver survivin siRNA to CRC cells and efficiently inhibit colorectal cancer growth in patient-derived colorectal cancer xenograft mouse model [117]. Kim et al. have developed anti-TAG-72 PEG-immunoliposomes (PILs) to target TAG-72 overexpressing CRC cells. Intravenous administration of anti-TAG-72 PILs efficiently accumulated in the tumor tissues, indicating that the immunoliposomes have great potential as gene delivery to human CRC cells.

However, nanocarriers still have several limitations such as immunogenicity and toxicity, which limit the therapeutic applications. Thus, more studies on the nanocarrier delivery in CRC therapy are needed.

4. Clinical Application of RNA-based Therapy in Colon Cancer

To date, the U.S. Food and Drug Administration (FDA) has approved only a limited number of RNA-based therapeutics: five ASO drugs (fomivirsen, mipomersen, eteplirsen, nusinersen, inotersen), one aptamer drug (pegaptanib), and one siRNA drug (patisiran). These successful clinical translations show great potential of utilizing of RNA-based therapy for tackling various diseases through gene regulation. In August 2018, the FDA approved the first targeted RNA-based therapy siRNA for treat a rare disease polyneuropathy, which fully demonstrates siRNAs as a new drug class with great potential. Although currently no RNA therapeutics is approved by the FDA for CRC treatment, many clinical investigations are ongoing.

An example of this is Angiozyme, the first synthetic ribozyme tested in clinical trial. Angiozyme targets the mRNA of vascular endothelial growth factor receptor-1 (VEGFR-1) to inhibit angiogenesis and tumor growth. A Phase I clinical trial has demonstrated that Angiozyme have minimal toxicities and good bioavailability for 31 patients with refractory solid tumors, including colorectal cancer [118].

Several ASOs have reached clinical trials for the treatment of CRC. ASO LY2275796 targets an oncogene eukaryotic translation initiation factor 4E (eIF-4E) in solid tumors, including colon cancer. In a phase 1 dose escalation, pharmacokinetic and pharmacodynamics study of LY2275796, tumor eIF-4E expression was decreased but no tumor responses observed, suggesting LY2275796 should combine with other treatment modalities [119]. ISIS 183750 is another ASO that inhibits the production of eIF4E. In preclinical study, the EIF4e ASO demonstrated its abilities to reduce EIF4e expression and inhibit CRC cell proliferation. In addition, it had enhanced activity in combination with irinotecan (Iri). The researchers conducted a phase I/II clinical trial to test the combination therapy activity. The study has shown that the ASO can penetrate tumor cells, however its activity has been compromised by extensive stromal binding. The combination of ISIS183750 and Iri did not show objective responses. Future studies may need combination with other chemotherapies or treatment for patient with less advanced disease [120].

RNAi is a highly targeted therapeutics for gene silencing, however, only a few of them have progressed into clinical trials. Atu027 is a RNA interference therapeutic that consists of liposomal particles and chemically stabilized siRNA that silences the expression of protein kinase N3 (PKN3) in the vascular endothelium. A phase 1 study of Atu027 was tested in patients with advanced solid tumors including colon cancer [121]. The study demonstrated that Atu027 was well tolerated in the patients. Based on the mode of action, future studies that examine efficiency of combination with traditional cytotoxic drugs are recommended. CALAA-01 is a cyclodextrin-based polymeric nanoparticle including a siRNA that reduces expression of the M2 subunit of ribonucleotide reductase (R2) [122]. A phase I study reported that the delivery system can provide a targeted delivery of siRNA and CALAA-01 was well tolerated during the phase Ia study [123].

5. Advantages and Limitations in developing RNA-based therapeutics

RNA-based therapeutics modulate the internal machinery of cells, changing the expression of targeted genes. It offers a broad range of potential applications and provides a high degree of flexibility, making it possible to manipulate previously “undruggable” targets. A variety of different approaches are available for researchers to either up-regulate or down-regulate certain genes within a cell. In addition, depending on the approach being used, a specific protein or a broader range of proteins can be targeted at the same time. In contrast, DNA-based therapies or gene editing technologies such as CRISPR aims to repair a dysfunctional gene or introduce a correct version into the cells. They have the high risk to permanently alter the genome, which limits a wide application in human.

Although RNA-based therapeutics offer advantages as mentioned above, there are challenges remain to be overcome. Besides stability and delivery issues, one challenge for RNAi is the “off-target” effect [124]. Due to the tolerance for mismatches with targets, those small RNAs may have a large number of potential targets in the genome and could affect many mRNAs that are not their intended targets [125]. In addition, the off-target effects may affect the phenotype of cells much more extensively than we originally anticipated [28].

Another challenge is tissue distribution. The majority of the systematically administered siRNAs and miRNAs tend to accumulate in organs that are parts of monoclear phagocyte system such as spleen, liver and lung [126]. ASOs have been shown to have a high distribution in liver and kidney, while the distribution to tumors is less extensive [127]. The identification of an appropriate target is another issue. To apply RNA-based therapeutics, we need to understand the importance of target gene for the progress of CRC, the degree of gene suppression needed, and the mechanisms that other genes may compensate to the loss of function of targeted gene.

6. Conclusion

RNA-based therapeutics have gained increased attention, mainly due to the ability to target disease genes that are previously unmanipulatable and the flexibility in modulating a large range of targets. However, because of challenges in stability and delivery, it may take time for clinical practice becoming a reality. Advances in medicine chemistry and nanotechnology will help to solve those issues and RNA based therapies will become more widely adopted. To be a new class of treatment modality alongside medicines such as small molecules or antibodies, RNA modalities need to have superior outcomes for patients. Currently pharmaceutical scientists equipped with new biotechnology are working on different RNA-based mechanisms to reveal the fullest potential for RNA-based therapeutics.

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Chemical compounds studied in this article

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Abbreviations

CRC	colorectal cancer
lncRNA	long non-coding RNA
ASO	antisense oligonucleotides
RNAi	RNA interference
RISC	RNA-induced silencing complex
miRNA	microRNA
saRNA	Small activating RNA
PK	pharmacokinetic

EPR	enhanced permeation and retention
EMT	epithelial-mesenchymal transition
PD	pharmacodynamics
MDR	multidrug resistance
CXCL12	C-X-C motif chemokine ligand 12
JAK2	janus kinase 2
STAT3	signal transducer and activator of transcription 3
LSD1	lysine-specific histone demethylase 1
PEG	polyethylene glycol
VEGF	vascular endothelial growth factor
MAPK1	mitogen-activated protein kinase 1
DHX9	DEXH-box helicase 9
CEA	carcinoembryonic antigen
EpCAM	epithelial cell adhesion molecule
hTERT	human telomerase reverse transcriptase
γ-GCS	gamma-glutamylcysteine synthetase
VEGF-1	vascular endothelial growth factor-1
UTR	3' untranslated region
PAMAM	polyamidoamine
EGFR	epidermal growth factor receptor
CEA	carcinoembryonic antigen
SELEX	systematic evolution of ligands by exponential enrichment
CCAT	colon cancer associated transcript 1
HOTAIR	HOX transcript antisense RNA
PRC2	polycomb repressive complex 2
CRNDE	colorectal neoplasia differentially expressed
UCA1	urothelial cancer associated 1
HNF1A-AS1	HNF1A antisense RNA 1
SIRT1	sirtuin 1

SNHG5	small nucleolar RNA host gene 5
PS	phosphorothioate
2'-F	2'-fluoro
2'-OMe	2'-O-methyl
2'-MOE	2'-O-methoxyethyl
LNA	locked nucleic acid
PEI	polyethyleneimine
PGMA	polyglycidal methacrylate
DR5	death receptor 5
FRα	folate receptor-a
EVs	extracellular vesicles
eIF-4E	eukaryotic translation initiation factor 4E
PKN3	protein kinase N3

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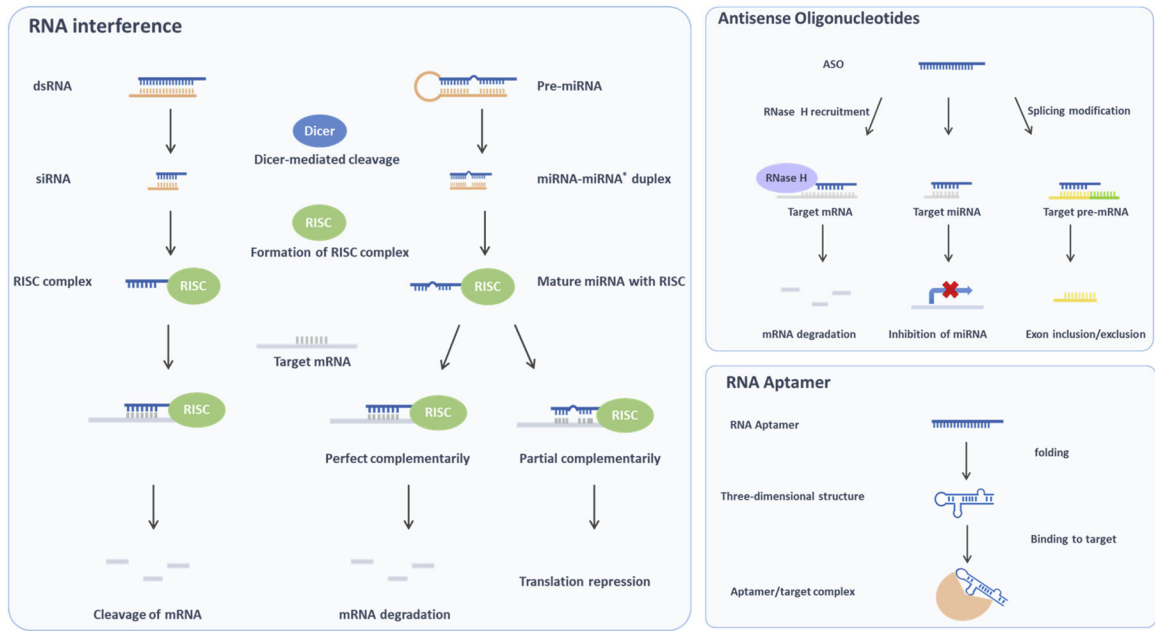


Figure 1. Common RNA-based therapeutics (RNA interference, ASO, and RNA aptamer) mechanism of action.

Table 1

RNA-based therapeutics used in the targeted treatment of CRC

Agent	Targeted cells	Targets	Functions	References
B7-H4 siRNA	LOVO	CXCL12/CXCR4 and JAK2/STAT3 signaling	Inhibits proliferation, invasion, and migration	22
Antibody-siRNA complexes	HCT116, LoVo, SW480, HCT15, DLD1	<i>KRAS</i>	Deactivates ERK and the MAPK pathway; inhibits tumor growth	23
siRNA	SW620	<i>LSD1</i>	Suppresses proliferation, migration and invasion of CRC cells	24
siRNA-PEG and SN-38	LS174T	<i>VEGF</i>	Inhibits tumor growth, and enhances the antitumor effect of the chemotherapeutic drug	25
siRNA and Dox	HCT116	Snail	Changes EMT genes, induces cell apoptosis, inhibit migration	26
siRNAs	HCT116	<i>KRAS</i> and <i>PK3CA</i>	A synergistic decrease in proliferation and an increase in apoptosis	27
miR-204–5p	LoVo and HCT116	<i>RAB22A</i>	Inhibits migration and invasion, promotes the sensitivity to chemotherapy	45
miR 217	RKO and SW480	<i>MAPK1</i>	Inhibits tumor growth and enhances apoptosis in CRC	46
	SW480, Lovo	<i>KRAS</i>	Suppresses CRC cell growth	48
miR-143	SW480, 228	<i>DNMT3A</i>	Decreases tumor cell growth	49
	SW620	<i>MACC1</i>	Inhibits cell growth, migration and invasion	50
ASO	HT29	<i>EGFR</i>	Reduces cell proliferation	54
ASO	HCT116	miR-21	Inhibits cell proliferation, reduces invasion and migration	55
ASO	LS174T and DLD-1	<i>MDM2</i>	Anti-tumor activity <i>in vitro</i> and <i>in vivo</i>	57
ASO AZD4785	SW480	<i>KRAS</i> mRNA	Inhibits the proliferation of tumor cells	58
S-1 aptamer	119X	protein DHX9	Binds to DHX9 and localizes to tumors <i>in vivo</i>	62
Aptamer	LS174T, LoVo, SW480	CEA	Inhibits homotypic aggregation, migration, and invasion	64
Aptamer	HT29	EpCAM	Delivers siRNA to cancer stem cells <i>in vivo</i>	68
Hammerhead ribozyme	SW480	<i>KRAS</i>	Induces growth suppression, apoptosis and alters angiogenic factor expression	73
Ribozyme	HT-29	hTERT	Targets and treats the tumor	74
Hammerhead ribozyme	HCT-8DDPA	γ -GCS	Suppresses multidrug resistance-associated protein	75
Ribozyme (Angiozyme)	KM12	<i>VEGF-1</i>	Reduces the number of metastases	76
smRNA	HCT116, HT29	P21	Inhibits cell proliferation and induces apoptosis	97

CXCL12: C-X-C Motif Chemokine Ligand 12, JAK2: Janus Kinase 2, STAT3: Signal Transducer and Activator of Transcription 3, LSD1: Lysine-specific Histone Demethylase 1, PEG: Polyethylene Glycol, VEGF: Vascular Endothelial Growth Factor, MAPK1: Mitogen-Activated Protein Kinase 1, DHX9: DEXH-Box Helicase 9, CEA: Carcinoembryonic Antigen, EpCAM: Epithelial Cell Adhesion Molecule, hTERT: human Telomerase Reverse Transcriptase, γ -GCS: gamma-glutamylcysteine synthetase, VEGF-1: vascular endothelial growth factor-1.

Table 2

Examples of lncRNAs serve as important regulators in CRC progression

Category of lncRNA mechanisms	Examples	Functions	References
Chromatin interactions	CCAT1	Promotes long-range chromatin looping at the MYC locus	78
Protein interactions	HOTAIR	Acts as a scaffold to assemble PRC2 and LSD1 complexes	80
	CRNDE	Represses miR-181a-5p expression	85
miRNA interactions	UCA1	Inhibits a tumor suppressive miRNA miR-204-5p	82
	HNF1A-AS1	Regulates SIRT1 by competitively binding miR-34a	89
mRNA interactions	SNHG5	Binds target mRNAs to block STAU1 mediated degradation	81
	UCA1	Interacts with mRNA 3-UTRs	83

CCAT: Colon Cancer Associated Transcript 1, HOTAIR: HOX Transcript Antisense RNA, PRC2: Polycomb Repressive Complex 2, LSD1: Lysine-specific Histone Demethylase 1, CRNDE: Colorectal Neoplasia Differentially Expressed, UCA1: Urothelial Cancer Associated 1, HNF1A-AS1: HNF1A antisense RNA 1, SIRT1: Sirtuin 1, SNHG5: Small Nucleolar RNA Host Gene 5, STAU1: Staufen Double-Stranded RNA Binding Protein 1.