Review

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Role of microRNA in colorectal carcinoma (CRC): a narrative review

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Abstract

MicroRNAs (miRNAs) are short non-coding RNAs that play a critical role in regulating gene expression by binding to target messenger RNAs (mRNAs). They were first discovered around 8 years after the identification of the first miRNA in 1993, and since then, there has been a significant increase in miRNA-related research and discoveries. MiRNAs have been implicated in various biological processes, including cancer, particularly in colorectal cancer (CRC). In CRC, miRNAs act as either oncogenes or tumor suppressors, influencing essential cellular functions such as cell proliferation, apoptosis, angiogenesis, and metastasis. The dysregulation of miRNAs in CRC can arise from different factors, leading to abnormal expression levels of their target mRNAs and subsequently affecting protein production. Consequently, miRNAs may directly target oncogenes or tumor suppressor genes, thereby contributing to cancer initiation and progression. Notably, tumors often exhibit reduced expression of mature miRNAs. In CRC research, miRNAs offer potential as diagnostic biomarkers and therapeutic targets. Specific miRNA profiles could serve as non-invasive tools for early CRC detection and risk assessment. Additionally, miRNA-based therapies present a promising approach for targeted cancer treatment by modulating miRNA expression. However, challenges related to delivery systems and long-term safety must be addressed to fully harness their therapeutic potential.

Keywords: colorectal carcinoma, microRNA, miRNA

Introduction

MicroRNAs (miRNAs) are short, non-coding regulatory RNAs ranging in size from 17 to 25 nucleotides. Their classification as miRNAs stems from their production through the activity of Dicer, an enzyme responsible for converting hairpin-shaped precursor molecules (known as pre-miRNAs) into fully mature miRNAs[1]. They exert their regulatory role by suppressing gene expression at the post-transcriptional level through the recognition of complementary target sites within the 3′ untranslated region (UTR) of target messenger RNAs (mRNAs). The era of miRNAs officially began around 8 years after the discovery of the first miRNA by Ambros and Ruvkun in 1993[2]. At that time, three separate research groups identified numerous small RNAs in *Caenorhabditis elegans*, Drosophila, and humans, marking a significant advancement in understanding their functions[3,4]. Over the past 7 years, there has been a tremendous expansion in both the number of miRNAs discovered and the volume of related scientific publications[5].

In the context of cancer, particularly colorectal carcinoma (CRC), miRNAs play a pivotal role in regulating various biological processes. These tiny RNA molecules, which do not encode proteins themselves, instead regulate gene expression by attaching to mRNA and preventing its conversion into proteins. In CRC, miRNAs can function as either oncogenes, promoting cancer development, or tumor suppressors, inhibiting it. They exert their influence by modulating the expression of genes involved in essential cellular functions like cell proliferation, apoptosis, angiogenesis, and metastasis[5]. MiRNAs are similar to protein-coding genes in that they can act as tumor suppressors, and any dysfunction in their activity may contribute to the

HIGHLIGHTS

- MicroRNAs' (miRNA) pivotal role in regulating gene expression, especially in colorectal cancer (CRC), impacting crucial cellular functions.
- Dysregulation of miRNAs in CRC, with reduced mature miRNA expression in tumors, contributing to cancer initiation and progression.
- Potential of miRNAs as diagnostic biomarkers and therapeutic targets in CRC, with noted challenges in delivery systems and long-term safety for clinical use.

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initiation or progression of cancer in normal cells. Such impairments can result from various factors, including genomic deletions, mutations, epigenetic modifications, or alterations in miRNA processing. Dysregulation of miRNAs can lead to changes in the expression of their target mRNAs, resulting in either downregulation or upregulation of the corresponding protein products. Their involvement in cancer becomes evident when they directly target oncogenes or tumor suppressor genes. Notably, tumor tissues and cells often exhibit reduced expression of mature miRNAs[6]. This study aims to evaluate the role of miRNA in CRC.

Histology and pathophysiology

Most CRCs predominantly exhibit adenocarcinoma with intestinal differentiation, while mucinous, serrated, and signet ring cell differentiation are less prevalent. Intestinal-type adenocarcinomas are characterized by large angulated or fused glands with ovoid nuclei, coarse chromatin, and numerous mitotic figures. Approximately 10% of CRCs are mucinous adenocarcinomas[7,8] which are defined by the presence of extracellular mucin accounting for at least 50% of the tumor volume. However, recent data suggest that the extent of mucinous differentiation does not reliably predict the outcome[9].

Mucinous adenocarcinomas demonstrate an expansile, pushing growth pattern with strips, clusters, or singly arranged tumor cells floating in mucin pools. Serrated adenocarcinomas account for about 8% of all CRCs[10] and are largely composed of neoplastic epithelial cells with clear or eosinophilic cytoplasm, vesicular nuclei, prominent nucleoli, and preserved nuclear polarity[11]. While areas of mucinous differentiation and tumor budding are often found at the advancing tumor edge, serrated carcinomas generally lack the abundant luminal necrotic debris typically seen in intestinal-type carcinomas[12]. Signet ring cell carcinomas are defined by dyshesive, medium to large cells with abundant mucinous cytoplasm and eccentric, hyperchromatic nuclei, accounting for at least 50% of the tumor volume[13]. Three patterns of signet ring cell carcinoma have been described, including single infiltrating signet ring cells reminiscent of diffuse-type gastric carcinoma, signet ring cells floating in mucin pools, and sheet-like growth of tumor cell nests without a desmoplastic stromal response. The latter feature nests of mucin-containing cells with peripherally compressed nuclei resembling goblet cell carcinoma of the appendix. Signet ring cell carcinomas with sheet-like growth and those accompanied by extracellular mucin are often associated with mucinous carcinomas[14]. Cribriform comedo-type carcinomas are composed of expansile sheets and aggregates of tightly packed glands with round lumina and minimal intervening stroma[15]. They often display a peripheral rim of tumor cells with cribriform growth surrounding necrotic material similar to ductal carcinoma in situ of the breast. Despite the presence of extensive glandular differentiation, this distinctive histologic variant is associated with a worse prognosis than low-grade intestinal-type adenocarcinoma[16]. Micropapillary carcinomas are characterized by lacunar spaces containing tumor cells without supportive stroma. Tumor cells demonstrate reverse polarity, with their apical surfaces oriented toward the periphery of the cluster. They exhibit eosinophilic cytoplasm and high-grade nuclear features with frequent lymphovascular invasion, along with characteristic apical MUC1 (Mucin1) immunopositivity. This glycoprotein, which normally inhibits stromal interactions, likely contributes to the morphologic appearance of this tumor type[17]. Medullary carcinomas are solid tumors showing minimal gland formation. They comprise syncytial nests of polygonal tumor cells with eosinophilic cytoplasm and large vesicular nuclei with prominent nucleoli. Frequent intratumoral and/or peritumoral lymphocytes may be associated with Crohn-type lymphoid aggregates at the advancing tumor edge[18].

The pathology of CRC involves several factors that play a crucial role in its classification and management. One of these factors is tumor grade, which is usually divided into four grades. However, due to inconsistencies in interpretation and limited clinical relevance, it is often simplified into two categories: poor (encompassing poorly differentiated and undifferentiated tumors) and other (including well and moderately differentiated tumors). Poorly differentiated tumors lack tubular formation and are associated with a worse prognosis and a higher risk of lymphatic spread. Various other factors have been proposed as prognostic indicators, such as lymphovascular invasion and infiltrative tumor margin (considered poor prognostic factors), as well as tumor-infiltrating lymphocytes and pushing margin (considered good prognostic factors). Nevertheless, their independent significance is often challenging to determine due to limitations in available studies, which are usually small and retrospective[19]. Accurate staging of CRC is essential for proper management. The primary factor in T staging is the depth of tumor invasion through the bowel wall, and it remains the most reliable predictor of prognosis in CRC patients. In the UK, there are currently two staging systems in use[19] (Fig. 1).

Hereditary component of CRC

In the past decade or so, molecular genetics has had a significant impact on our understanding of CRC development by identifying both germline and somatic mutations associated with the disease. Approximately 6–7% of all CRC cases are attributed to single-gene germline mutations that confer a hereditary susceptibility to the disease. These mutations are linked to specific hereditary

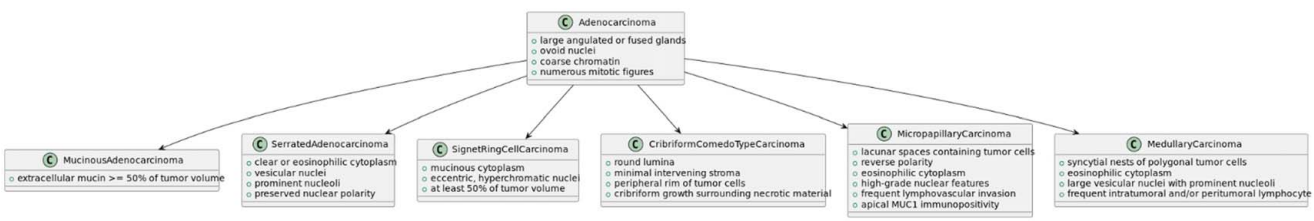

Figure 1. Various aspects of histology and pathophysiology of colorectal cancer (CRC).

syndromes and affect genes involved in DNA repair (such as MutL Homolog 1, MutS Homolog 2, MutS Homolog 6, Postmeiotic Segregation Increased 2, MutY Homolog) and signal transduction (like Adenomatous Polyposis Coli and SMAD family member 4). For the remaining familial cases of CRC, it is believed that much of the inherited risk is likely due to polygenic factors, involving multiple genes, but many of the specific genetic changes in these cases are yet to be identified[20].

The inherited syndromes associated with CRC are a group of distinct diseases caused by specific mutations that increase the risk of developing CRC. These syndromes are generally more aggressive and have a poorer prognosis due to their association with other tumors, and some may not respond well to chemotherapy. Hereditary colorectal cancer (HCRC) is not a single disease but a collection of different diseases or syndromes, each with its unique mutational genetic basis, leading to specific clinical characteristics. HCRC can be broadly divided into two main groups, namely the hereditary nonpolyposis colorectal cancer (HNPCC) and the hereditary polyposis colorectal cancer (HPCC).

HNPCC is an autosomal dominant cancer syndrome, accounting for 1.7–4.2% of all CRC cases (representing 3–8 cases per million inhabitants worldwide, according to Globocan 2018)[21–23]. The commonest of these hereditary cancers is Lynch syndrome (LS), which is associated with an increased risk of colorectal, endometrial, ovarian, stomach, and small bowel cancer. LS is linked to mutations in DNA mismatch repair (MMR) genes, including MLH1 (76%), MSH2 (40%), MSH6, PMS2, and EPCAM, with an incidence of 3–5 cases per million inhabitants[24]. Other forms of HNPCC include sporadic CRCs with MLH1-/PMS2-deficient tumors[25], Muir–Torre syndrome (involving the genes MLH1, MSH2, MSH6, and PMS2) with an incidence of 2 cases per 10 million inhabitants[26], and Turcot syndrome type I, which occurs in ~20–25% of all Turcot syndrome cases and is related to mutations in the MMR gene[27]. HPCC accounts for ~3–5% of all CRC cases[27,28] (representing 5–9 cases per million inhabitants globally, according to Globocan 2018). It includes various syndromes such as familial adenomatous polyposis (FAP) linked to germline mutations in the APC gene, with an incidence of 2–3 cases per million inhabitants[29]. Adenomatous polyposis syndromes (APC and MUTYH), juvenile polyposis coli (associated with BMPR1A and SMAD4 genes) with an estimated incidence of 1 per one million inhabitants[30,31]. Peutz–Jeghers syndrome (involving STK11/LKB1 gene) with an incidence of 1.2 per one million inhabitants[32,33], PTEN hamartoma tumor syndrome (PHTS) related to the PTEN gene, Cowden syndrome affecting the PTEN gene with an incidence of 6 cases per 10 million inhabitants[34–36], Turcot syndrome type II (associated with the APC gene mutation) with a frequency of ~75–80% among all Turcot syndromes, and Gardner syndrome (involving the APC gene) with an incidence of 1 per one million people within the United States[37,38].

Colorectal-specific miRNAs

Multiple miRNAs play diverse roles in human CRC proliferation by targeting various genes involved in cancer cell regulation. Some miRNAs, such as miR-106a, miR-106a/b, miR-20a/b, miR-17, and miR-10b, are upregulated and target genes like PTEN, GABBR1, and KLF4. Conversely, miR-125, miR-1258, miR-1271, miR-141-3p, miR-143, miR-143/miR-145, miR-143-3p, miR-16-5p, miR-185, miR-193a-3p, miR-200b-3p, and others are either upregulated or downregulated, and they target various genes. Collectively, these miRNAs and their target genes contribute to unrestricted CRC proliferation, emphasizing their significant role in cancer development[39]. A recent review of 23 miRNA expression studies revealed that among the 164 significantly altered miRNAs in CRC, approximately 2/3 were upregulated and 1/3 were downregulated in tumors[40]. Functional studies have shown that specific miRNAs play significant oncogenic or tumor suppressor roles, and their functions need to be assessed individually within the context of the specific tissue and tumor type. Michael and colleagues were pioneers in demonstrating altered miRNA expression patterns in CRC[41]. They reported reduced levels of miR-143 and miR-145 in CRC, suggesting their role as tumor suppressors. Subsequent studies have validated these findings and confirmed the tumor-suppressive functions of miR-143 and miR-145 in CRC[41]. Another relevant miRNA in CRC is miR-21, known for its oncogenic properties. At least seven studies reported elevated levels of miR-21 in CRC[42], and it has been implicated in cancer initiation, progression, and metastasis in various solid tumor types[43]. Several other miRNAs have been consistently found to be altered in CRC across multiple studies, including the miR-17-92 cluster, miR-106a, miR-31, miR-181b, miR-183, miR-135a/b, the miR-200a/b/c family, miR-203, and miR-224[42]. Genome-wide profiling of chromatin signatures has demonstrated that DNA methylation plays a role in regulating their expression in CRC cell lines *in vitro*[41]. Various miRNAs, such as let-7, miR-34, miR-342, miR-345, miR-9, miR-129, and miR-137, are frequently hypermethylated in colon tumors, leading to their reduced expression[44–47]. In addition to their roles in gene expression regulation, miRNAs also contribute to global epigenetic regulation in CRC[48].

Cellular proliferation and survival play crucial roles in the process of carcinogenesis. The abnormal expression of miRNAs regulates the development of CRC by targeting various cell cycle regulators. These regulators include survivin and cyclins. Notably, miR-16 directly targets survivin[49]. MiR-16 exerts control over the growth of CRC cells, promoting apoptosis by modulating the p53/survivin signaling pathway. Another miRNA involved in cell cycle regulation is miR-218, which induces cell cycle arrest in colon cancer cells during the G2 phase. It achieves this by inhibiting cyclin-dependent kinase 4 (CDK4) and elevating p53 levels[50]. The p53 protein serves as a transcription factor, activated in response to cellular stress, to inhibit cell proliferation and trigger cell death. Disruption of this p53 pathway can facilitate the development of tumors[51]. MiR-34a is a specific miRNA with a pivotal role in gene expression regulation, well-known for its tumor-suppressive functions. It is often linked to the control of genes involved in cell cycle regulation, apoptosis (cell death), and other processes related to cancer development. MiR-34a's action involves inhibiting the expression of SIRT1, an enzyme engaged in various cellular processes and the regulation of protein acetylation, including that of p53. Consequently, this inhibition leads to apoptosis due to an increase in acetylated p53 levels, forming a positive feedback loop involving miR-34a and p53[52]. Furthermore, a brief introduction of miR-34a into SW480 cells results in a significant reduction in migration and invasion, attributed to the upregulation of acetylated p53 and p21[53]. Additionally, it suggests that an overexpression of miR-34a

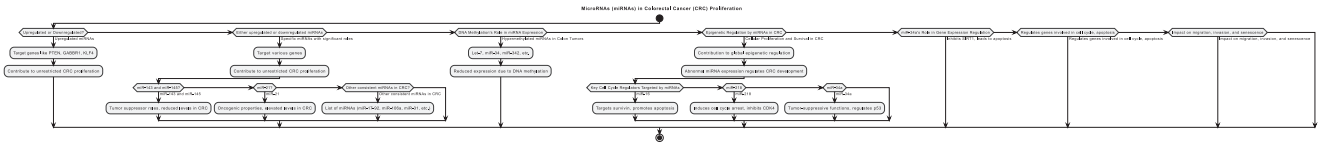

Figure 2. An overview of the role of miRNA in CRC.

induces cell growth arrest and behaviors resembling senescence through the upregulation of the p53 pathway[54,55] (Fig. 2).

Current diagnostic modalities of CRC

Various diagnostic tests are available for CRC screening and diagnosis. The fecal occult blood test (FOBT) detects occult blood leaked by CRC into the gut lumen, with two types being guaiac-based (gFOBTs) and immunochemical (iFOBTs) tests[56]. The immunochemical FOBT (FIT) utilizes human hemoglobin detection and has higher sensitivity than gFOBT for detecting advanced neoplasia[57,58]. The multitarget stool DNA test (mt-sDNA) includes immunochemical and molecular assays for specific markers, providing an accurate test result[59]. Computed Tomography Colonography (CTC) uses a colon CT scan for imaging[60]. Colon Capsule Endoscopy (CCE) captures video footage through a disposable capsule passing through the colon[61]. Flexible Sigmoidoscopy (FS) allows visualization and sampling on the left side of the colon, reducing CRC incidence and death[62]. Colonoscopy is the gold standard, enabling detection, sampling, and therapeutic interventions[63,64]. The Methylated Septin 9 (SEPT9) test is a potential blood-based biomarker for CRC diagnosis[65,66].

Use of miRNA as a biomarker and diagnostic modality

Recent studies have unveiled the potential of various miRNA classes as biomarkers for diagnosing CRC. Their clinical benefits as biomarkers lie in their accurate diagnostic value, stable presence in human fluids, and non-invasive detection[67]. Specific miRNAs like miR-21*, miR-92a, miR-18a, miR-144*, and miR-29a have been extensively investigated and identified as promising non-invasive CRC biomarkers[68–80]. For instance, miR-21-5p regulates various target genes and pathways associated with tumor growth, invasion, metastasis, and even 5-FU resistance and CRC cell radiosensitivity[81–83]. Moreover, miR-425-5p has been shown to influence chemoresistance in CRC cells through its role in programmed cell death[84]. While the exact reasons for miRNA dysregulation in CRC are not always clear, consistently downregulated or upregulated miRNAs in the presence of the disease may also serve as reliable biomarkers. A panel comprising miR-15b, miR-21, and miR-31, proposed by Han *et al.*, showed the highest sensitivity and specificity in distinguishing the healthy control group from the CRC group[85].

In CRC, miR-21 has consistently shown upregulation both in tissue samples and in circulation (plasma and serum) when compared to healthy controls[86–88]. A recent meta-analysis, which included 18 studies comprising 1129 blood specimens from CRC patients and 951 control specimens, also confirmed the diagnostic potential of circulating miR-21 with a moderate sensitivity of 77% and a good specificity of 83% for CRC[89]. Another miRNA, miR-210, known for its hypoxia-regulated nature, exhibits oncogenic properties in various cancers, mediating cell proliferation, migration, invasion, and clonogenicity[90]. Along with eight other miRNAs, miR-210 has been identified as a potentially useful diagnostic biomarker in CRC tissues[91]. Several studies have reported its diagnostic potential, as it is upregulated in the serum of CRC patients, consistent with our findings in plasma[92].

Circulating miRNAs show immense potential as cancer biomarkers. However, their translation into clinical practice necessitates validation and standardized procedures to mitigate technical biases, as discussed in this review. The development and implementation of standard operating procedures (SOPs) governing various aspects of circulating miRNA analysis are crucial for transforming miRNA signatures into clinically meaningful tests. Standardization must cover processes from whole blood collection to plasma/serum preparation, handling, banking, RNA extraction, and miRNA quantification, reducing interlaboratory variations and ensuring consistency among different users[93]. Consensus procedures will enable accurate interpretation and comparison of study results and the identification of miRNAs as specific and sensitive cancer biomarkers. Nevertheless, it is important to acknowledge that while methodological parameters can be addressed, certain individual and environmental factors may still influence circulating miRNA applications in clinical practice without full evaluation[93]. The process of obtaining sufficient RNA for analyzing and profiling numerous miRNAs still lacks consistency and standardization in the field. Consequently, there is a need for concerted efforts to establish common practices. Additionally, there are several crucial considerations to address when designing studies focused on analyzing circulating miRNAs. As this field progresses, there is significant potential for technological advancements in profiling circulating miRNA signatures from plasma or serum[94]. Furthermore, the possibility of extracting extracellular miRNAs from non-invasive samples, such as saliva or urine, based on the knowledge gained from isolating them in plasma or serum, opens up new avenues. Ultimately, this information has the potential to pave the way for developing diagnostic tests using easily accessible biofluids. As future research endeavors bridge existing knowledge gaps, the utilization of not only extracellular miRNAs but also other RNA species has the potential to greatly enhance our understanding of diseases and may even lead to exciting new therapeutic approaches[94] (Table 1).

Use of mRNA as therapeutics

MiR-9 has been identified as a tumor suppressor gene in CRC, as its upregulation correlates with decreased expression of UHRF1, involved in DNA methylation and cell proliferation in CRC tissue samples. It counteracts UHRF1 function and promotes apoptosis of CRC cells *in vitro*[95]. An example of a miRNA with dual action is miRNA-873, which acts as an oncogene, stimulating lung

Table 1

Role of specific miRNAs in terms of their diagnostic modality. (This table elaborates on the use of miRNA as a biomarker for cancer as well as explains its diagnostic feasibility.)

miRNA name	Role as a biomarker	Diagnostic potential	References
miRNA-21	Upregulated	Yes	[61–73][68–80]
miR-92a	Investigated	Yes	[61–73][68–80]
miR-18a	Investigated	Yes	[61–73][68–80]
miRNA-144	Investigated	Yes	[61–73][68–80]
miR-29a	Investigated	Yes	[61–73][68–80]
miR-21-5p	Upregulated	Yes	[74–76][81–83]
miR-425-5p	Influences	Yes	[77][84]
miR-15b	Part of a panel	Yes	[78][85]
miR-31	Part of a panel	Yes	[78][85]
miR-210	Upregulated	Yes	[83][90]
Consensus miRNAs	Potential panel	Yes	[84][91]
miRNA analysis	Technical biases	SOPs needed	[93]
Standardization	SOPs implementation	Critical for accuracy	[93]
Future potential	Non-invasive samples	Promising development	[94]

adenocarcinoma cell migration and proliferation by targeting SRC kinase signaling inhibitor 1. However, its expression is decreased in mouse CRC samples, human CRC clinical specimens, and highly metastatic CRC cell lines. MiRNA-873 suppresses CRC metastasis by targeting the ELK1-Cyclin D1 pathway and STRN4, while promoting the epithelial–mesenchymal transition (EMT) process *in vitro* and *in vivo*[96,97]. MiR-17 is another significant CRC-related miRNA that enhances cell growth, proliferation, and cell cycle transitions from G0 to G1 and from G1 to S by suppressing the RND3 tumor suppressor gene. RND3 is involved in the adenoma to carcinoma transition in CRC patients. Additionally, miR-17-5p is associated with metastasis and the clinical stage of CRC[98,99]. Mir-21 is known to reduce apoptosis, increase invasion depth, and promote lymphatic metastasis by inhibiting PTEN. It is also linked to enhanced cell proliferation and advanced stages of TNM clinical classification[100,101]. In the context of chemotherapy, miRNAs play a crucial role in regulating pathways for chemical resistance and sensitivity[102]. MiR-7 has been found to suppress EGFR *in vitro*, potentially regaining sensitivity to EGFR inhibitors like cetuximab for CRC patients who have developed resistance to these drugs[103].

Radiation therapy resistance in CRC involves complex processes, including increased expression of DNA repair proteins, dysregulation in signaling pathways, angiogenesis, cancer stem cells (CSCs), and autophagy. MiRNAs have the potential to predict and modify cancer treatments like radiotherapy, and recent evidence highlights their significant role in cellular responses against ionizing radiation[104–106].

In the context of CRC, miRNAs hold a pivotal position as they can function as either oncogenes, promoting tumor growth, or as tumor suppressors, influencing vital cellular processes. The disruption of miRNA regulation in CRC, stemming from a variety of factors, leads to irregular mRNA expression, which in turn disrupts the production of proteins. The reduced levels of miRNAs in CRC tumors underscore their potential utility as non-invasive biomarkers for early detection and as targets for innovative miRNA-based therapeutic approaches. However, several challenges must be addressed, including the necessity for rigorous quality control, ethical considerations regarding the use of genetic data, the need to account for epigenetic variations, and ensuring the long-term safety of miRNA-based applications. In summary, miRNAs play a central role in governing gene activity in CRC, offering promising prospects for both diagnosis and treatment, but these prospects must be approached with careful attention to these complex factors.

Certain miRNAs have contrasting roles in CRC, either promoting its progression, metastasis, and drug resistance or exerting tumor-suppressing effects. They can be broadly classified into two types: tumor suppressor miRNAs and oncogenic miRNAs. To leverage them for treatment, strategies include upregulating tumor suppressor miRNAs using miRNA mimics, such as synthetic miRNAs or miRNA expression vectors, when they are downregulated. Conversely, oncomiRs can be targeted with miRNA antagonists like antisense oligonucleotides, antagomirs, or miRNA sponges when they are overexpressed. They play pivotal roles in CRC by regulating signaling pathways, EMT, angiogenesis, and more, impacting cell proliferation, metastasis, and chemoresistance. They offer potential directions for future CRC treatments. However, developing miRNA-based therapies faces challenges in creating a delivery system that ensures durability, tissue-specific targeting, and avoids potential toxicities and off-target effects[106,107].

Altered miRNA expression significantly impacts the intricate gene regulatory mechanisms involved in cancer development. Notably, miRNAs possess the remarkable ability to function as both oncogenes, referred to as oncomiRs, and tumor suppressors[108]. It is essential to recognize that a single miRNA can regulate multiple genes and may also be targeted by several other miRNAs. Emerging evidence suggests that miRNA expression is subject to epigenetic regulation, akin to DNA methylation (both hypermethylation and hypomethylation), RNA modifications, and post-translational modifications of histones[109].

Furthermore, a correlation between miRNA biogenesis and chromatin characteristics in the genomic regions containing pre-miRNAs has been documented. Specifically, genes situated within active chromatin regions are more likely to be targeted by miRNAs[110]. Additionally, it has been observed that the promoters of miRNA target genes tend to be preferentially located within specific chromatin domains[111]. In addition to chromatin influence on miRNA target gene regulation, there are the effects of post-translational modifications of histones. These modifications include events such as phosphorylation of serine or threonine residues, acetylation and deacetylation of lysine residues, and methylation of lysine or arginine residues. Histone modifications that impact epigenetics encompass methylation (catalyzed by histone demethylases – HDM) and acetylation (regulated by histone acetyltransferases – HAT, and histone deacetylases – HDAC). In cancer, gene demethylation by HDMs often leads to the upregulation of genes associated with cellular proliferation, migration, and invasion. This creates an opportunity for miRNA-based agents that can modulate proteins in these pathways, ultimately inhibiting malignant cellular proliferation[112,113].

Developing miRNA-based therapeutics presents substantial obstacles. The negative charge of miRNAs renders them unable to penetrate cells, posing a significant challenge in terms of delivery. Furthermore, miRNAs exhibit a short circulation duration, tend to aggregate with serum proteins, and undergo rapid degradation. As a result, nanostructures like polymeric micelles and liposomes have gained extensive use as nanocarriers for the precise delivery of miRNAs to particular cells or tissues[114]. Research also found that

miR-21 contributes to resistance against the chemotherapeutic drug 5-FU by reducing the levels of human DNA MutS homolog 2 (hMSH2) in CRC[115]. Moreover, studies have demonstrated that reinstating the dysregulated miRNAs can successfully surmount drug resistance[116]. Consequently, it is our conjecture that simultaneously delivering miRNAs that reverse multidrug resistance (MDR) alongside chemotherapeutic agents holds great promise as an approach to combat MDR in cancer chemotherapy[117].

Synthetic miRNA mimics or antimiR oligonucleotides have a short half-life and are rapidly degraded by nucleases in biological fluids[118]. To address this challenge, several strategies have been developed. These include chemical modifications like phosphodiester and phosphorothioate internucleotide linkages, the addition of a 2′-O-methyl group, or the synthesis of locked nucleic acids that constrain the ribose ring with a methylene linkage between the 2-oxygen and the 4-carbon. Beyond chemical modifications, therapeutic miRNAs have been encapsulated within functionalized nanoparticles to enhance their protection from degradation, reduce immune responses, and prolong their circulation time. Furthermore, the conjugation of nanoparticles with targeting ligands, such as proteins, peptides, and antibodies, has improved cellular uptake and enabled specific targeting of tumor sites[119] (Table 2).

Animal and human trials

A series of studies have been conducted which observe and evaluate the role of miRNA in CRC. In this study by Ibrahim *et al*., miR-33a was identified as a tumor suppressor by repressing the proto-oncogene Pim-1. The researchers used a polyethyleneimine (PEI) complexation method to deliver miR-33a *in vivo* in mice, which demonstrated antitumor effects and target downregulation. The method was found to be biocompatible and allowed systemic delivery. However, the study also acknowledges the challenges of efficient delivery and potential off-target effects. Despite these challenges, miRNA-based therapies, using unmodified miRNAs and PEI-based delivery, hold promise as a treatment option for cancer.

The study conducted on mice suggests that miRNA replacement therapy may have potential as a therapeutic strategy for cancer treatment, but further research and preclinical studies are necessary to fully explore its safety and efficacy in human patients[120]. Geng *et al*. utilized female athymic nude mice aged 4–6 weeks, acquired from the Transgenic Animal Research Center. Human colon adenocarcinoma cell lines HT29 and HCT116, as well as human hepatocellular carcinoma cell lines HepG2 and Huh7, were obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). Human embryo kidney epithelial cell line HEK293 was also used. These cell lines were maintained in specific culture media with fetal bovine serum (FBS) and L-glutamine. The research aimed to investigate the role of miRNAs in the regulation of Fas expression in colon carcinoma and their involvement in the intrinsic and extrinsic apoptotic pathways. The study investigated the role of miRNAs in regulating Fas expression in colon carcinoma. It found that the let-7 family of miRNAs is involved in downregulating Fas in colon cancer cells. Fas activation suppressed Dicer processing, leading to an accumulation of pre-let-7a-1, indicating post-transcriptional control of let-7a by Fas. Interestingly, there was a double-negative feedback mechanism between Fas and let-7, where Fas suppressed let-7, and let-7 downregulated Fas expression. This feedback loop may play a crucial role in Fas-related apoptosis and antitumor immune responses. The study also demonstrated that inhibiting let-7 increased sensitivity to Fas-related apoptosis in colon cancer cells, suggesting potential therapeutic implications. Combining let-7 inhibitors with IFN-c could potentially enhance treatment efficacy while reducing adverse effects, making it a promising focus for future clinical investigations in colon carcinoma therapy[121].

In a research by Bao *et al*., mouse colonic epithelial cells were collected from two groups: Muc2 +/+ and Muc2 −/− mice, with four mice in each group. Total RNAs were extracted for miRNA array analysis, which was performed using Affymetrix GeneChip miRNA Arrays version 3.0 at the Genomic Facility in Illinois. Additionally, 41 paired human CRC tissues and their adjacent normal colonic mucosa were collected from November 2012 to October 2013. Some samples were snap-frozen in liquid nitrogen and stored at − 80°C for RNA and protein extraction for quantitative RT-PCR (qRT-PCR) and western blotting analysis[122]. In summary, this study demonstrated that miR-27a is frequently downregulated in CRC, and its reduced expression is associated with cancer distant metastasis and histopathological stages, indicating its role as a tumor suppressor. Both in-vivo and in-vitro studies identified SGPP1 and Smad2 as novel targets of miR-27a, which are linked to STAT3 to regulate cancer cell proliferation, apoptosis, and migration. Thus, miR-27a holds potential as a biomarker for CRC development and progression and may serve as a therapeutic target for CRC therapy by affecting SGPP1, Smad2, and Stat3[122].

He *et al*.[50] did a study on CRC. They took samples from tumors and nearby normal tissue during surgery before any treatment. They discovered that miR-218, a tiny molecule, was less active in cancer. They also found that miR-218 can slow down cancer cell growth, stop cell division, and help cells die. One way it does this is by stopping a gene called BMI-1, which can make cancer worse. This means miR-218 might help fight CRC[50].

In a research by Vidic *et al*., the human colorectal adenocarcinoma cell line LoVo, obtained from the American Type Culture Collection in Rockville, MD, was cultured in F12 (HAM) medium supplemented with 10% FBS, 10 mM L-glutamine from

Table 2

Specific miRNAs that are found to be useful in the therapeutics of CRC. (This table summarizes their role, mechanism, and use in a specific strategy for cancer treatment).

miRNA name	Role in CRC	Mechanism of action	Therapeutic strategy	References
MiR-9	Tumor suppressor	Inhibits UHRF1	Promotes apoptosis in CRC cells *in vitro*	[86]
MiR-873	Dual action	Targets SRC kinase	Suppresses CRC metastasis, promotes EMT	[87,88]
MiR-17	Oncogene	Suppresses RND3	Enhances cell growth and proliferation	[89,90]
MiR-21	Oncogene	Inhibits PTEN	Reduces apoptosis, increases invasion	[91,92]
MiR-7	Sensitization	Suppresses EGFR	Regains sensitivity to EGFR inhibitors	[94]

Gibco BRL in Paisley, UK, 350 mg/l gentamicin from Krka in Novo mesto, Slovenia, and 1 ml/l crystacillin from Pliva d.d in Zagreb, Croatia. The cells were incubated in a 5% CO_2 humidified incubator at 37°C. LoVo cells were specifically chosen for this study based on previous literature indicating the presence of a K-ras point mutation at codon 13. The study's findings demonstrated the therapeutic potential of K-ras silencing in treating various cancers. Remarkably, this study is, to the best of our knowledge, the first investigation showcasing the potential of miRNA molecules for localized electrogene treatment of colorectal adenocarcinoma tumors[123].

Dong *et al*. included two cohorts comprising a total of 95 patients with histologically confirmed CRC who underwent surgery in Hong Kong, between 1999 and 2009. The first cohort consisted of four patients used to establish miRNA expression profiles, while the second cohort had 91 patients for miRNA validation[124]. The findings of the study revealed that miR-133a was frequently downregulated in both CRC tissues and colon cancer cell lines. When miR-133a was reintroduced into colon cancer cells, it led to cell cycle arrest in the G0–G1 phase, effectively suppressing cancer cell growth. The inhibition of tumor growth by miR-133a was attributed, in part, to its ability to directly reduce RFFL translation and activate the p53/p21 pathway. Moreover, miR-133a also enhanced the sensitivity of cancer cells to chemotherapeutic agents, indicating its potential as a promising clinical therapeutic intervention[124].

The main objective of the research by Hiraki *et al*. was to identify a potent therapeutic miRNA capable of targeting mutant KRAS in CRC. To achieve this, the study conducted a microarray analysis on KRAS-transfected human embryonic kidney 293 (HEK293) cells and human lung fibroblasts (MRC5). The findings revealed that miR-4689 effectively suppressed the oncogenic KRAS-driven EGFR signaling pathways by directly inhibiting KRAS and AKT1[125].

The research by Sun *et al*. collected patient samples comprising primary tumor (II and III stages) and corresponding adjacent normal tissue (paracancerous tissues) from Xiangya, between 2009 and 2011. Human CRC cell lines SW-480, SW-620, and HT-29 were obtained from the Cell Bank of Shanghai (China). The results revealed that miR-429 inhibits the initiation of EMT by targeting Onecut2, along with Onecut2-mediated regulation of EMT-related genes and transcript activators in CRC cells[126].

Ye *et al*. conducted a study in which the human CRC cell lines, SW620, SW480, RKO, HT29, and 293T, were obtained from the cell bank at the China Academy of Medical Science (China). The findings of this study demonstrate that miR-27b is derived from CSCs in CRC and plays a crucial role as a tumor suppressor and angiogenic factor by targeting VEGFC. Further research focused on CSCs and angiogenesis holds promise for the development of innovative anticancer therapeutic approaches. Additionally, miRNA-based therapeutic strategies show potential for enhancing tumor management in the near future. These results not only advance our understanding of the mechanisms governing CRC cells but also pave the way for the progressive development of more effective cancer treatments[127] (Table 3).

Future prospects

miRNAs show promise as potential biomarkers and therapeutic targets in CRC. However, further validation and standardization are

Table 3

Summary of all the previous studies done on the role of miRNA in CRC.

References	Animal model	Human model	Key findings
Ibrahim *et al*.[100]	Mice	—	miR-33a identified as tumor suppressor, delivered using PEI complexation method, demonstrating antitumor effects and target downregulation. miRNA-based therapies hold promise for cancer treatment.
Geng *et al*.[101]	Mice and human cell lines	HT29, HCT116, HepG2, Huh7, HEK293	Investigated miRNA role in Fas expression regulation in colon carcinoma. Let-7 family involved in downregulating Fas in colon cancer cells. Inhibiting let-7 increased sensitivity to Fas-related apoptosis, potential therapeutic implications.
Bao *et al*.[102]	Mice and human tissues	Colonic epithelial cells from Muc2 + / + and Muc2 − / − mice	miR-27a frequently downregulated in colorectal cancer, associated with distant metastasis and histopathological stages. SGPP1 and Smad2 identified as novel targets of miR-27a, linked to STAT3 to regulate cancer cell proliferation, apoptosis, and migration. Potential therapeutic target for CRC.
He *et al*.[103]	—	Paired CRC tumor and adjacent normal tissue	miR-218 expression reduced in CRC. Functional analysis revealed suppression of colon cancer cell growth, induction of cell cycle arrest, and promotion of apoptosis. Potential tumor-suppressive miRNA in CRC.
Vidic *et al*.[104]	—	Human colorectal adenocarcinoma cell line LoVo	Therapeutic potential of K-ras silencing in treating various cancers using miRNA for localized electrogene treatment of colorectal adenocarcinoma tumors.
Dong *et al*.[105]	—	95 CRC patients' tumor tissues and colon cancer cell lines	miR-133a frequently downregulated in CRC tissues and cell lines. Reintroduction of miR-133a suppressed cancer cell growth, induced cell cycle arrest in G0–G1 phase, enhanced sensitivity to chemotherapeutic agents. Potential therapeutic intervention for CRC.
Hiraki *et al*.[106]	—	Human embryonic kidney 293 cells and human lung fibroblasts	miR-4689 effectively suppressed oncogenic KRAS-driven EGFR signaling pathways by directly inhibiting KRAS and AKT1. Potential therapeutic miRNA for targeting mutant KRAS in CRC.
Sun Y *et al*.[126]	Human CRC tissues and cell lines	SW-480, SW-620, HT-29	miR-429 inhibits initiation of EMT by targeting Onecut2, with Onecut2-mediated regulation of EMT-related genes and transcript activators in CRC cells.
Ye *et al*.[106]	—	Human colorectal cancer cell lines SW620, SW480, RKO, HT29, and 293T	miR-27b derived from CSCs in CRC, plays a crucial role as a tumor suppressor and angiogenic factor by targeting VEGFC. Potential for innovative anticancer therapeutic approaches.

crucial. Large-scale studies across diverse populations are needed to confirm the diagnostic and prognostic value of specific miRNAs.

The dysregulation of miRNAs in CRC presents an opportunity for miRNA-based therapeutics. Using miRNA mimics or antagonists to regulate specific miRNAs could be explored as targeted CRC treatment. Efficient delivery of miRNA mimics or antagonists to target tissues is a challenge in miRNA-based therapies. Research should focus on improving delivery systems to ensure effective and safe miRNA treatment. Advancements in miRNA research may lead to personalized treatment strategies for CRC patients. Incorporating miRNA profiles into individualized treatment plans could improve therapy efficacy.

Combining miRNA-based therapies with other treatment modalities like chemotherapy or radiation may enhance treatment outcomes and overcome drug resistance in CRC. Long-term follow-up studies are essential to assess the impact of miRNA-based therapies on CRC patient outcomes and understand potential side effects for successful clinical integration.

Conclusion

miRNAs have emerged as crucial regulators in the development and progression of CRC. They can function as oncogenes or tumor suppressors, influencing various biological processes involved in cancer growth, invasion, metastasis, and drug resistance. The dysregulation of miRNAs in CRC offers potential as diagnostic biomarkers and therapeutic targets. Specific miRNA profiles may serve as non-invasive diagnostic tools for early CRC detection and risk assessment. Additionally, miRNA-based therapies provide a promising avenue for targeted cancer treatment. By using miRNA mimics or antagonists, miRNA expression can be modulated to restore normal cellular functions, potentially inhibiting tumor growth and metastasis. However, challenges related to efficient delivery systems and long-term safety need to be addressed. The future of miRNA research in CRC holds promise for personalized medicine and the development of innovative treatment approaches. A better understanding of the roles of specific miRNAs in CRC biology and advancements in miRNA-based therapies could usher in a new era of precision medicine, leading to improved patient outcomes and transforming the management of CRC.

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Ethics approval was not required for this review.

Consent

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Author contribution

A.I.S. and W.B.: designed the study and conceived the idea; and A.A., S.Z., S.K.P., A.M., and U.Z.: wrote the first draft; A.M. and H.A.: wrote the second draft; U.T. and V.K.: worked on the revisions. All authors equally contributed to this work.

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No conflicts of interest were declared.

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