



The Clinical Significance of MicroRNAs in Colorectal Cancer Signaling Pathways: A Review

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Glob Med Genet 2023;10:315–323.

Abstract

Colorectal carcinoma (colon and rectum) is currently considered among the most prevalent malignancies of Western societies. The pathogenesis and etiological mechanisms underlying colorectal cancer (CRC) development remain complex and heterogeneous. The homeostasis and function of normal human intestinal cells is highly regulated by microRNAs. Therefore, it is not surprising that mutations and inactivation of these molecules appear to be linked with progression of colorectal tumors. Recent studies have reported significant alterations of microRNA expression in adenomas and CRCs compared with adjacent normal tissues. This observed deviation has been proposed to correlate with the progression and survival of disease as well as with choice of optimal treatment and drug resistance. MicroRNAs can adopt either oncogenic or tumor-suppressive roles during regulation of pathways that drive carcinogenesis. Typically, oncogenic microRNAs termed oncomirs, target and silence endogenous tumor-suppressor genes. On the other hand, tumor-suppressive microRNAs are critical in downregulating genes associated with cell growth and malignant capabilities. By extensively evaluating robust studies, we have emphasized and distinguished a discrete set of microRNAs that can modulate tumor progression by silencing specific driver genes crucial in signaling pathways including Wnt/b-catenin, epidermal growth factor receptor, P53, mismatch repair DNA repair, and transforming-growth factor beta.

Keywords

- microRNAs
- genomics
- colorectal cancer
- molecular pathways

Introduction

Colorectal carcinoma (colon and rectum) is one of the most prevalent malignancies of Western societies. More specifically, the proportion of colorectal cancer (CRC) accounts for approximately 8 to 9% of newly diagnosed cancer cases in the United States, comprising the second leading cause of cancer-related deaths annually. Recently, the development of effective treatment and screening strategies have significantly

contributed to a steady decline in incidence and disease-specific mortality. Nevertheless, increasing rates of disease in younger adults and the exponential increases of cases in economically emerging countries such as Eastern Europe and Asia remain a significant concern.¹

The pathogenesis and etiologic mechanisms underlying the development of CRC remain complex and heterogeneous. A variety of environmental factors appear to contribute to the development of these tumors. Nevertheless, although CRC

DOI <https://doi.org/10.1055/s-0043-1777094>.
ISSN 2699-9404.

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Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

appears to be largely influenced by lifestyle factors, it is evident that interindividual genetic variation significantly affects the prevalence and severity of disease.² Eventually, over the last decades scientists have finally succeeded in linking CRC to genetic predisposition in certain family trees. Accordingly, they identified specific critical mutations that influence the tumorigenesis of CRC.

Actually, only 15 to 20% of CRCs show clear hereditary predisposition. The vast majority of colorectal tumors occurs sporadically and usually affects individuals without a family history or genetic predisposition. Although familial cases represent only a small proportion, they have provided key insights into the molecular pathways of disease. As several studies have shown, sporadic CRCs share the same genetic abnormalities as their inherited counterparts. In general, sporadic colorectal tumors exploit similar genetic mechanisms of proliferation as hereditary cancers but arise from driver mutations occurred in somatic cells. For example, APC gene, which has prominent role in the biology of FAP syndrome, also found to have somatic mutations in approximately 50 to 55% of sporadic CRC cases.³ These mutations influence the progressive acquisition of genetic and epigenetic alterations that eventually lead to malignancy (►Table 1).⁴

The most extensively studied sequences of the human genome are those of protein-coding genes.⁵ The Central Dogma of Biology, established by Francis Crick in 1970, dictates that DNA is transcribed into RNA, which is subsequently translated, and that proteins constitute the major players of biological mechanisms.⁶ However, after the publication of the Human Genome Project in 2003, the perspective of genomic research has significantly amended. During the last two decades the development and widespread implementation of innovative methods in the field of genomics led to the discovery that only 1.5 to 2% of human genome encodes for functional peptides.⁷ Eventually, it was revealed that our genome encodes approximately 20,000 to 25,000 protein genes, similarly with much simpler organisms such as the fruit fly.⁸ Therefore, it is not surprising that the majority of single-nucleotide polymorphisms, identified to correlate with human malignancies including colorectal tumors through large genome-wide association studies, are located in large introns or distal to coding regions, an area previously been considered as unexplored territory.⁹ More recently, the advent of next-generation sequencing and multiomics analyses have led to projects like ENCODE providing unequivocal evidence that nearly 90%

of the human genome is dynamically and pervasively transcribed in noncoding RNAs.^{10,11}

Since then, a substantial amount of research has been devoted to the identification and interpretation of these noncoding RNAs. The emerging landscape indicates that noncoding transcripts represent a broad and heterogeneous group of molecules with diverse functions and key regulatory roles in various biological processes.¹² Several studies show that noncoding RNAs utilize a variety of mechanisms and participate in fundamental cellular activities such as proliferation, differentiation, migration, and apoptosis, through transcriptional and posttranscriptional regulation of gene expression.¹³

High-throughput sequencing techniques and microarray analyses have identified thousands noncoding RNAs in the human genome. Clearly, the presence of a highly sophisticated regulatory network, interacting with the protein-coding genome (Exome), has a fundamental impact in human pathophysiology with significant biological and biomedical consequences. Currently, it is evident that noncoding RNAs influence almost all genomic processes and biological pathways of eukaryotic organisms. Therefore, obviously as CRC constitutes a genomic disease, mutations, and aberrant expression of noncoding RNAs have come to surface as hallmark of tumorigenesis. Accordingly, a plethora of important studies have revealed significant association between noncoding genomic regions and pathogenesis of intestinal malignancies.

Two decades ago both the existence and the importance of these molecules remained unknown. Their detection was achieved by the collaboration of Ambros's and Ruvkun's laboratories, through experiments in the nematode *Caenorhabditis elegans*, with the discovery of the lin-4 microRNA.¹⁴ Since then, scientific research has elucidated their mechanisms of biogenesis, modes of action, and biological roles. MicroRNAs are small single-stranded RNA transcripts with 18 to 25 nucleotides length that comprise vital part of the genome in the majority of eukaryote organisms.¹⁵ Generally, they are well-characterized regulatory noncoding RNAs, which account approximately 1 to 2% of human genome in total. As plethora of scientific studies support, they represent a conserved family of small RNA molecules with extremely important role in the posttranscriptional regulation of gene expression. MicroRNAs are transcribed as long precursor molecules that undergo sequential multistep processing to produce mature single-stranded RNAs that manipulate the expression of other genes.¹⁶ Regarding location, microRNAs are found through the whole range of human genome. They often reside not only in intergenic (desert) regions, but also in introns of coding transcripts, although some may even overlap with exonic sequences.¹⁷ In addition, a large amount of microRNAs are organized in clusters and transcribed as polycistronic units.¹⁸ CRC progression involves several mechanisms, including most notably overproliferation, defects in apoptotic regulation, increased angiogenesis, and acquisition of invasive and metastatic phenotype.¹⁹ Since the first association between microRNA expression and CRC, made by Michael et al in 2003 who found decreased

Table 1 Percentage of sporadic, familial, and inherited cases of colorectal cancer⁴

Sporadic cases	65%	
Hereditary cases	High-penetrance genes (APC, BRCA, MLH1, MSH2, MSH6, TP53, etc.)	5%
	Moderate-penetrance genes (MUTYH, CHEK2, ATM)	5%
Familial cases	25%	

levels of miR-143 and miR-145 in tumor tissues compared with healthy adjacent, huge amount of research has been dedicated to unravel the molecular effects of these tiny molecules.^{20,21}

Methods

To elucidate the influential role of microRNAs on the major molecular signaling pathways that drive CRC tumorigenesis, we performed in-depth review and analysis of the robust and valid literature. The terms used were “microRNAs or mirs,” “colorectal cancer,” and “molecular signaling pathways.” Up to date (October 2023), totally 1,994 studies were retrieved in PubMed, Medline, and Queen Mary University of London official library, in which approximately 40 concerned clinical trials investigating microRNA differential expression patterns in CRC tissue specimen and plasma samples. By extensively evaluating these studies, we have emphasized and distinguished a discrete set of microRNAs that can modulate tumor progression by silencing specific driver genes crucial in signaling pathways like Wnt/b-catenin, epidermal growth factor receptor (EGFR), P53, mismatch repair DNA repair, and transforming-growth factor Beta (TGF- β).

Discussion

In general, CRC was thought correlated with a comparative decline in the total microRNA expression.²² Studies have shown that in the context of global microRNA depletion, the defects of tumor-suppressor microRNAs have greater effects on driving tumorigenesis than depletion of oncogenetic microRNAs (oncomirs). This phenomenon was further supported by animal-based models that revealed Dicer1, which is key enzyme of physiological microRNA biogenesis, as a major tumor-suppressor gene in CRC cell lines.²³ Nevertheless, that issue still remains ambiguous and highly controversial.²⁴ Interestingly, a work by Wang et al found the majority of microRNA expression to be globally elevated in CRC.²⁵ Converging with these findings, more recent studies of microRNA profiles in CRC indicated that approximately two-third of altered microRNAs in CRC have shown enhanced expression. Authors concluded that microRNA processing machinery probably is not compromised in colorectal malignancies.²⁶ These conflicting results suggest that microRNAs should be independently evaluated between diverse tumor types and patients.

Wnt/b-Catenin Signaling Pathway

Aberrant and hyperactivated Wnt/b-catenin signaling is a major mechanism of colorectal tumorigenesis and early-cancer progression. Therefore, microRNAs that regulate this pathway play important role in maintaining the physiology of normal colonic cells.²⁷ An increasing amount of studies have identified specific microRNA mutations that influence Wnt/b-catenin cascade, either by direct suppression of APC gene, or indirectly by targeting alternative pathway components.²⁸ Mir-135 is a well-studied molecule that directly targets the 3' UTR of APC gene in human

colorectal cells. Therefore, enhanced Mir-135 family expression implies upregulation of Wnt pathway and is associated with advanced tumor grade and poor clinical outcome.^{29,30} Colonic cancer progression via immediate inhibition of APC gene is also clear and extensively validated for miR-494 and miR-19.^{31,32} In addition, mir-21, which is among the most famous oncomirs, shows positive expressional correlation with key components of Wnt pathway including b-catenin and cyclin D1. This observation is modulated through repression of multiple mir-21 targets, most of which are genes that control cell-cycle like PTEN.³³ Moreover, animal model studies have shown that enhanced expression of mir-574-5p promotes colon cancer progression. This molecule activates Wnt stimulation, via downregulating Quaking 6/7 RNA-binding protein that controls several intracellular processes including proliferation, differentiation, and angiogenesis.³⁴ To further support the critical role of stimulatory Wnt/b-catenin oncogenic microRNAs, Li et al demonstrated that mir-224 targets and inhibits the GSK3 β and secreted Frizzled Related Protein 2, significant suppressors of this precise signaling pathway. Consequently, results in increased nuclear translocation of b-catenin thereby enhanced expression of target genes c-Myc and cyclin D1.³⁵ Furthermore, mir-145 is a tumor-suppressor agent that normally inspects and moderates the intracellular translocation of b-catenin into the nucleus, crucial step for signaling activation. Therefore, truncating mutations and reduced expression are typical events in colorectal tumors.³⁶ Moreover, mir-28-5b mechanistically attenuates Wnt signaling, by suppressing a calmodulin-binding transcription activator termed CAMTA2. As revealed by a contemporary research, CAMTA2 is significantly upregulated in many colorectal tumors and associates with poor survival. Authors concluded that mir-28-5b/CAMTA2 axis is critical in CRC development and might be a promising diagnostic and therapeutic marker too.³⁷ Similarly, mir-93 significantly decreases Wnt pathway activation, through targeting Smad-7, which is essential for nuclear accumulation of b-catenin.³⁸ Consistent with these findings, Zhang et al also recognized mir-7 as a strong tumor-suppressor gene for colorectal tumorigenesis, through downregulation of YY1 oncogene in normal colonic cells.³⁹ By applying in silico searches, luciferase reporter assays, and western blot analyses, researchers identified an evolutionary-conserved mir-7 binding site in the 3'UTR of YY1. This gene exerts broadly oncogenic functions through Wnt signaling pathway by activating b-catenin, antiapoptotic survivin, and fibroblast growth factor 4. Furthermore, a very recent report identified the significant implication of mir-103/107 in colon tumorigenesis. This molecule stimulates stem-like features in CRC cells including self-renewal, angiogenesis, and chemoresistance by directly repressing Axin 2, which is a strong inhibitor of Wnt/b-catenin signaling cascade.⁴⁰

Epidermal Growth Factor Receptor Signaling Pathway

EGFR is a transmembrane glycoprotein, member of the human EGFR family. When activated, it promotes intracellular signaling via stimulation of two major subnetworks,

KRAS/RAF/MEK and PI3K/AKT, respectively. It constitutes a well-characterized pathway with critical role in the survival, proliferation, migration, angiogenesis, and apoptosis of human cells, involved in several types of epithelial cancers including CRC.⁴¹ A series of investigations in the field revealed the extensive implication of microRNAs in regulation of EGFR signaling that significantly enhanced our detailed understanding of intestinal carcinogenesis.⁴² Two of the most important EGFR-related microRNA tumor-suppressor genes are mir-143 and mir-145. Expression analyses have shown that these molecules are significantly diminished in cells of colorectal tumor origin compared with normal epithelium. Among their gene targets are several members of the EGFR pathway, notably KRAS and BRAF.⁴³ As identified, mir-143 and mir-145 orchestrate a well-coordinated program of gene repression, where either they share a target transcript, or both their target transcripts converge in a common signaling cascade.⁴⁴ In addition, Let-7 microRNA is a well-known tumor-suppressor molecule that regulates EGFR. Let-7 directly targets KRAS mRNA in a variety of tumors including lung and breast cancer.⁴⁵ However, surprisingly, an analysis showed that Let-7 provided increased expression in metastatic CRC tissues compared with normal mucosa, but only in KRAS-mutated tumors.⁴⁶ Researchers concluded that this microRNA could be applied as a distinctive biomarker regarding treatment with anti-EGFR targeted therapy like cetuximab. Moreover, recent studies have acknowledged several novel suppressor microRNAs associated with EGFR cataract. For example, mir-19, mir-217, and mir-181d can inhibit angiogenesis, proliferation, and invasion of colorectal tumors by interacting with downstream regulators like PEAK1.^{47–49} On the other hand, mir-31 appears to be a potent stimulator of KRAS in colorectal tumors, through negative regulation of RASA1, which is inhibitor of KRAS function.⁵⁰ Furthermore, mir-210 and mir-181a constitute closely related with KRAS activation molecules, as significant oncomirs that promote CRC progression and invasiveness.⁵¹

At the same time, AKT-PI3K-mTOR/PTEN represents the second signaling hub of the EGFR pathway, being amplified in almost 20% of CRCs.⁵² Multiple oncomirs and tumor-suppressor microRNAs interact and control functioning of this cascade under both physiological circumstances and malignancy. For instance, miR-17–92 cluster, also known as oncomir-1, directly targets 3'UTR and inhibits PTEN gene, driving aberrant cell proliferation and atypical angiogenesis.⁵³ Furthermore, mir-21, mir-19, and mir-96 are important oncogenes also found to be involved in CRC carcinogenesis through stimulation of AKT/PI3K pathway.⁵⁴ In addition, mir-126 is a significant tumor-suppressor gene with crucial implications in the control of EGFR signaling. This molecule specifically silences p85b protein-coding gene, which is necessary substrate regarding the stability and propagation of PIK3 network.⁵⁵ Interestingly, mir-126 also activates VEGF-induced angiogenesis by modulating expression of PIK3R2 (PI3K regulatory subunit 2).⁵⁶ Consequently, abrogation of mir-126 potentially provides a selective growth advantage during tumorigenesis. Moreover, through animal

model experiments, mir-497 was also identified to exhibit antiproliferative and antiapoptotic action by targeting VEGF receptor 2 in colonic tissues.⁵⁷ Furthermore, a very recently published study revealed the significant involvement of mir-27b in the control of PIK3CA signaling cascade.⁵⁸ Overexpression of this molecule inhibits colorectal tumor cells proliferation and migration, via suppressing PI3K p110a subunit. Researchers concluded that mir-27b could be tested as potential therapeutic antitumor agent for colorectal malignancies.

P53 Signaling Pathway

Tumor-suppressor gene P53 is one of the most frequently mutated genomic regions in human malignancies. It is known as 'the guardian of the genome' gene, owing to the numerous fundamental roles in maintaining the cellular physiology under diverse stress conditions.⁵⁹ Specifically, more than 50% of CRCs display of some degree inactivation in p53 function.⁶⁰ Several microRNAs have been recognized recently as significant components of p53 signaling cascade across these tumors.⁶¹ Contemporary scientific knowledge suggests that albeit P53 regulates transcription and maturation of multiple downstream tiny RNAs by activation or repression of distinct molecules, vice versa, TP53 expression is also under the tight control of particular microRNAs.⁶² For instance, Shi et al reported that P53 can induce miR-15a/16–1 to form a double-negative feedback loop with transcription factor AP4.⁶³ Then, activated AP4 performs critical function regarding invasiveness and metastasis of colorectal malignancies. In addition, *in silico* searches identified miR-504 as a novel microRNA that can negatively regulate p53 expression via two binding sites in the human p53–3'UTR.⁶⁴ Ectopic expression of this molecule impaired p53 protein levels and function, especially in mediating apoptosis and G1 cell cycle arrest. As experimentally revealed, mir-504 overexpression promotes colon cancer tumorigenicity *in vivo*.⁶⁵ Actually, translational repression of TP53 is controlled by a wide variety of microRNAs including mir-125b, mir-25, mir122, mir-30d, and mir-518c.⁶⁴ MicroRNA-125b is a brain-enriched tiny RNA that acts as negative regulator of p53 in both zebrafish and humans.⁶⁶ *In vitro* overexpression of Mir-125b reduces endogenous levels of p53 protein and impairs physiological apoptosis during development and stress response. Moreover, mir-25 and mir-30 compromise tumor-suppressive functions, via negative regulation of both gene expression and protein level of p53.⁶⁷ Interestingly, mir-518c can simultaneously target and inactivate both p53 and PTEN genes.⁶⁸ The huge intricacy of p53 signaling pathway was further illustrated through studies around mir-34a. This molecule is a downstream transcription target of p53 protein during apoptosis but concomitantly was found to enhance p53 expression through direct negative regulation of SIRT1 that physically interacts and disrupts the protein.⁶⁹ Additionally, p53 induces and manipulates the expression of several supplementary microRNA molecules, to enhance tumor suppression. These include mir-107, mir215, mir-143, mir-145, and mir-192.^{64,70–72} Such findings demonstrate the huge importance of microRNAs expression and

interactions, to mediate the proper function of p53 tumor-suppressive signaling cascade.

Mismatch Repair Machinery

Microsatellite instability (MSI) is a hallmark feature of defective mismatch repair (MMR) system machinery. Lynch syndrome is a well-known syndrome related to germline defective mutations of MMR system. This hypermutagenic phenomenon is present in approximately 15% of sporadic CRC cases. Although, the microRNA profiles of CRC have been studied extensively, comparatively few analyses have specifically investigated microRNA signatures in the presence of MSI.⁷³ Nevertheless, certain microRNAs have recently emerged as significant regulators of genes associated with MMR, including MLH1, MSH2, MSH6, and PMS2.⁷⁴ Valeri et al identified that mir-155 compromises MMR mission by targeting the 3'UTR of MLH1, MSH2, and MSH6.⁷⁵ Overexpression of this molecule correlated with a hypermutated phenotype and MSI in CRC cell lines. In addition, mir-21 downregulates the MMR recognition complex hMutSa, by interacting with the two core gene subunits MSH2 and MSH6.⁷⁶ Colorectal tumors overexpressing mir-21 showed MSI and also displayed reduced response to 5-fluorouracil-based chemotherapy.⁷⁷ Therefore, mir-21 could be further evaluated as treatment response biomarker. Moreover, Sarver et al identified a small panel of six microRNAs that could successfully distinguish MSI from microsatellite stable colon tumors.⁷⁸ Among them, mir-31 and mir-625 were overexpressed in MSI malignancies, whereas mir-552, mir-

592, mir-181c, and mir-196b performed decreased expression in stable cancers. More interestingly, microRNA patterns could be applied to distinguish hereditary and sporadic colorectal tumors with MSI. Although these two conditions share the same molecular mechanism of tumor development, the underlying cause is quite different. Balaguer et al applied microarray analysis data, to reveal a group of 31 microRNA molecules that could be used as classifiers with high accuracy (area under the receiver operating characteristic curve: 0, 94).⁷⁹ Consequently, microRNAs prove to have fundamental roles for the diligent function of DNA damage/repair machinery, especially in the terms of colorectal carcinogenesis.

TGF- β Signaling Pathway

TGF- β /Smad is an important molecular pathway involved in cell proliferation and associated with cancer invasiveness and metastatic potential. Regarding CRC, this pathway performs dual functions as it is a tumor-suppressor at the early stages but simultaneously acts as a powerful cancer promoter in advanced neoplasms.⁸⁰ Several microRNAs have been identified to interact and regulate the TGFBR2 receptor (TGFBR2), which is the key component of initiating signaling.⁸¹ For instance, mir-135 is a well-studied molecule, confirmed by many researchers to be significantly elevated in CRC cells.⁸² As recently revealed mir-135 directly disturbs TGFBR2 translation and consequently promotes cell proliferation and inhibits apoptosis.⁸³ Similarly, mir-301a also targets TGFBR2 and is correlated with increased tumor

Table 2 microRNA molecules and their respective gene targets and biological effect

MicroRNAs with respective mRNA targets associated with colorectal cancer				
Key signaling pathway	microRNA	Targets	Target effects	References
Wnt/b-catenin pathway	Mir-135	APC	Proliferation	90
	Mir-21	PTEN	Progression/invasion/metastasis	91
	Mir-145	b-catenin	Proliferation/migration	92
	Mir 103/107	Axin 2	Angiogenesis/chemoresistance	93
	MIR-494	b-catenin	Progression/metastasis	94
	TrkC-miR2	TrkC	Proliferation	95
EGFR pathway	MIR-143	BRAF	Proliferation/angiogenesis	96
	LET-7	KRAS	Progression/invasion/metastasis	97
	Mir-210	KRAS	Progression/invasion/metastasis	98
	Mir-217	KRAS/MAPK1	Proliferation/angiogenesis	98
	Mir-19	KRAS, VEGF	Proliferation/angiogenesis	99
TP53 pathway	Mir-339-5p	MDM2 gene	Proliferation/invasion	100
	Mir-34a	c-Kit gene	Proliferation	101
	Mir-600	TP53 gene	Proliferation/invasion	102
MMR	Mir-155	MLH1 gene	Microsatellite instability	103
	Mir-625	MMR	Microsatellite instability	104
TGF- β	Mir-20a	TGF- β gene	Proliferation/invasion	105
	miR-20a/b	Bcl-2	Apoptosis	106

Abbreviations: EGFR, epidermal growth factor receptor; MMR, mismatch repair.

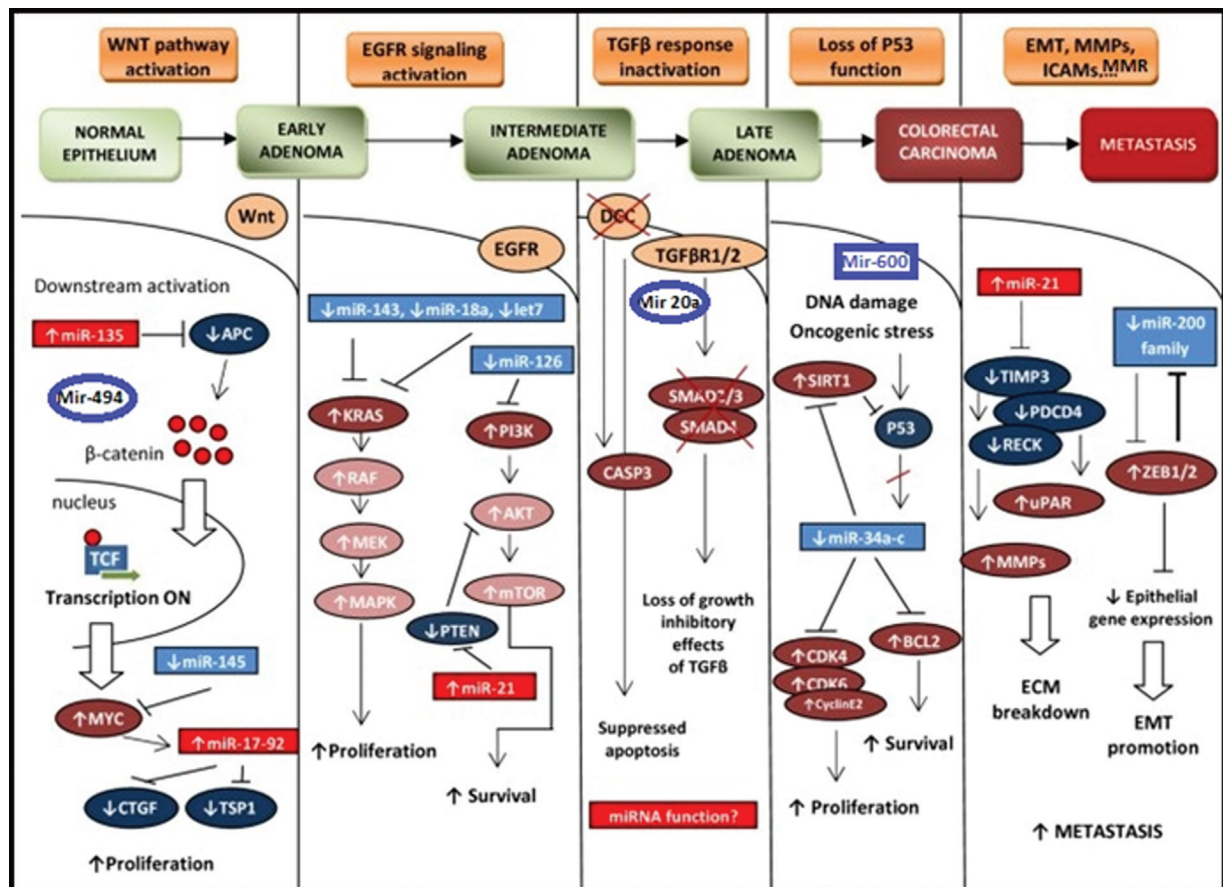


Fig. 1 A proposed graphic scheme of miRNAs impact on development of colorectal cancer. EGFR, epidermal growth factor receptor; MMR, mismatch repair.

aggressiveness and metastatic dissemination.^{84,85} To further support the critical role of TGFBR2, studies have shown that the prominent miR-21 oncogene also regulates that critical receptor via direct binding to the 3'UTR.⁸⁶ Moreover, Smad-4 is an important mediator of TGF- β signaling cascade, whose abrogation results in distal metastases and generally poor prognosis. MicroRNA-20-5p and miR-224 significantly silence Smad gene and induce proliferation and cancer invasiveness.^{87,88} Furthermore, miR-1269 is a recently well-studied molecule, usually found increased in advanced stage cancer tissues. TGF- β activates miR-1269, whereas vice versa miR-1269 enhances TGF- β signaling by targeting Smad-4, hence forming a positive feedback loop.⁸⁹ This molecule is associated with treatment relapse and metastasis, suggesting a potential useful biomarker regarding choice of adjuvant chemotherapy.

A summary of distinct microRNA molecules and their respective gene targets and biological effect is illustrated in **Table 2** (**Fig. 1**).

Conclusion

CRC is one of the most commonly diagnosed malignancies worldwide, with considerable morbidity and mortality. The recent and revolutionary advancements in comprehension of disease biology have significantly elucidated the diverse nature and pathogenesis of these tumors, promising a more

favorable future for patients and doctors. Noncoding genomic regions and most notably noncoding RNAs appear to have crucial functional implication in both physiological and pathological processes of colorectal cells. Somatic genetic variations of loci without protein coding potential have been detected to contribute to the progressive transformation of normal colonic mucosa to adenocarcinoma, through regulation of several fundamental signaling pathways involved in chromosome instability, MSI, and serrated neoplasia cascades. As demonstrated through in-depth investigation of numerous robust studies, colorectal malignant cells possess a variety of mutational alterations and express abnormally several noncoding genomic regions that influence important signaling cascades toward carcinogenesis. Thus, identification and detailed interpretation of noncoding driver mutations may enable effective screening and risk assessment as well as individualized therapeutic approaches targeting specific proteins. The huge scientific interest around regulatory genome and especially noncoding RNAs promises that the upcoming years will be marked by the development of noncoding RNAs-based therapy and establishment of a contextual role next to current diagnostic, preventive, and treatment strategies of colorectal tumors.

Ethical Approval and Consent to Participate

This is a literature-based review. This study does not contain any studies or trials performed by any of the authors.

Consent for Publication

Not applicable.

Authors' Contributions

A.M. made the literature research and composed the text. V.M. and E.A. analyzed and edited the supplementary material (image, table). M.G., M.T., and N.T. interpreted the analysis. All authors have read and approved the final manuscript. All authors have made significant work toward completion of the manuscript.

Funding

None.

Conflict of Interest

None declared.

Acknowledgments

I would like to offer special thanks to our patients, who are our constant source of inspiration and happiness.

References

- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020;70(01):7–30
- Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodríguez Yoldi MJ. Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. *Int J Mol Sci* 2017;18(01):197
- Polakis P. The many ways of Wnt in cancer. *Curr Opin Genet Dev* 2007;17(01):45–51
- Kastrinos F, Samadder NJ, Burt RW. Use of family history and genetic testing to determine risk of colorectal cancer. *Gastroenterology* 2020;158(02):389–403
- Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet* 2011;12(12):861–874
- Crick F. Central dogma of molecular biology. *Nature* 1970;227(5258):561–563
- Alexander RP, Fang G, Rozowsky J, Snyder M, Gerstein MB. Annotating non-coding regions of the genome. *Nat Rev Genet* 2010;11(08):559–571
- Hood L, Rowen L. The Human Genome Project: big science transforms biology and medicine. *Genome Med* 2013;5(09):79
- Yao L, Tak YG, Berman BP, Farnham PJ. Functional annotation of colon cancer risk SNPs. *Nat Commun* 2014;5:5114
- Venkatesh T, Suresh PS, Tsutsumi R. Non-coding RNAs: functions and applications in endocrine-related cancer. *Mol Cell Endocrinol* 2015;416:88–96
- Yang Y, Du Y, Liu X, Cho WC. Involvement of non-coding RNAs in the signaling pathways of colorectal cancer. *Adv Exp Med Biol* 2016;937:19–51
- Leonardi T. Insights into the function of non-coding RNAs [Dissertation]. Sidney Sussex College, University of Cambridge; 2016:255
- Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 2009;10(03):155–159
- Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993;75(05):843–854
- Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. *Nat Rev Cancer* 2015;15(06):321–333
- Towler BP, Jones CI, Newbury SF. Mechanisms of regulation of mature miRNAs. *Biochem Soc Trans* 2015;43(06):1208–1214
- Olena AF, Patton JC. Genomic organization of microRNAs. *J Cell Physiol* 2010;222(03):540–545
- Yuan X, Liu C, Yang P, et al. Clustered microRNAs' coordination in regulating protein-protein interaction network. *BMC Syst Biol* 2009;3:65
- Thomas J, Ohtsuka M, Pichler M, Ling H. MicroRNAs: clinical relevance in colorectal cancer. *Int J Mol Sci* 2015;16(12):28063–28076
- Michael MZ, O' Connor SM, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol Cancer Res* 2003;1(12):882–891
- Calin GA, Croce CM. MicroRNA-cancer connection: the beginning of a new tale. *Cancer Res* 2006;66(15):7390–7394
- Schee K, Fodstad Ø, Flatmark K. MicroRNAs as biomarkers in colorectal cancer. *Am J Pathol* 2010;177(04):1592–1599
- Lambertz I, Nittner D, Mestdagh P, et al. Monoallelic but not biallelic loss of *Dicer1* promotes tumorigenesis in vivo. *Cell Death Differ* 2010;17(04):633–641
- Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer* 2006;6(04):259–269
- Wang J, Du Y, Liu X, Cho WC, Yang Y. MicroRNAs as regulator of signaling networks in metastatic colon cancer. *BioMed Res Int* 2015;2015:823620
- Rokkas T, Kothonas F, Rokka A, Koukoulis G, Symvoulakis E. The role of circulating microRNAs as novel biomarkers in diagnosing colorectal cancer: a meta-analysis. *Eur J Gastroenterol Hepatol* 2015;27(07):819–825
- Beni FA, Kazemi M, Dianat-Moghadam H, Behjati M. MicroRNAs regulating Wnt signaling pathway in colorectal cancer: biological implications and clinical potentials. *Funct Integr Genomics* 2022;22(06):1073–1088
- Balacescu O, Sur D, Cainap C, et al. The impact of miRNA in colorectal cancer progression and its liver metastases. *Int J Mol Sci* 2018;19(12):3711
- Nagel R, le Sage C, Diosdado B, et al. Regulation of the adenomatous polyposis coli gene by the miR-135 family in colorectal cancer. *Cancer Res* 2008;68(14):5795–5802
- Valeri N, Braconi C, Gasparini P, et al. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. *Cancer Cell* 2014;25(04):469–483
- Zhang Y, Guo L, Li Y, et al. MicroRNA-494 promotes cancer progression and targets adenomatous polyposis coli in colorectal cancer. *Mol Cancer* 2018;17(01):1
- Liu Y, Liu R, Yang F, et al. miR-19a promotes colorectal cancer proliferation and migration by targeting TIA1. *Mol Cancer* 2017;16(01):53
- Wu D, Shi M, Fan XD. Mechanism of miR-21 via Wnt/ β -catenin signaling pathway in human A549 lung cancer cells and Lewis lung carcinoma in mice. *Asian Pac J Trop Med* 2015;8(06):479–484
- Ji S, Ye G, Zhang J, et al. miR-574-5p negatively regulates *Qki67* to impact β -catenin/Wnt signalling and the development of colorectal cancer. *Gut* 2013;62(05):716–726
- Li T, Lai Q, Wang S, et al. MicroRNA-224 sustains Wnt/ β -catenin signaling and promotes aggressive phenotype of colorectal cancer. *J Exp Clin Cancer Res* 2016;35:21
- Yu Y, Nangia-Makker P, Farhana L, G Rajendra S, Levi E, Majumdar AP. miR-21 and miR-145 cooperation in regulation of colon cancer stem cells. *Mol Cancer* 2015;14:98
- Luan X-F, Wang L, Gai X-F. The miR-28-5p-CAMTA2 axis regulates colon cancer progression via Wnt/ β -catenin signaling. *J Cell Biochem* 2021;22(09):945–957
- Tang Q, Zou Z, Zou C, et al. MicroRNA-93 suppress colorectal cancer development via Wnt/ β -catenin pathway downregulating. *Tumour Biol* 2015;36(03):1701–1710
- Zhang N, Li X, Wu CW, et al. microRNA-7 is a novel inhibitor of YY1 contributing to colorectal tumorigenesis. *Oncogene* 2013;32(42):5078–5088
- Chen HY, Lang YD, Lin HN, et al. miR-103/107 prolong Wnt/ β -catenin signaling and colorectal cancer stemness by targeting Axin2. *Sci Rep* 2019;9(01):9687

- 41 Gomez GG, Wykosky J, Zanca C, Furnari FB, Cavenee WK. Therapeutic resistance in cancer: microRNA regulation of EGFR signaling networks. *Cancer Biol Med* 2013;10(04):192–205
- 42 Wei S, Hu W, Feng J, Geng Y. Promotion or remission: a role of noncoding RNAs in colorectal cancer resistance to anti-EGFR therapy. *Cell Commun Signal* 2022;20(01):150
- 43 Pagliuca A, Valvo C, Fabrizi E, et al. Analysis of the combined action of miR-143 and miR-145 on oncogenic pathways in colorectal cancer cells reveals a coordinate program of gene repression. *Oncogene* 2013;32(40):4806–4813
- 44 Yan X, Chen X, Liang H, et al. miR-143 and miR-145 synergistically regulate ERBB3 to suppress cell proliferation and invasion in breast cancer. *Mol Cancer* 2014;13:220
- 45 Esquela-Kerscher A, Trang P, Wiggins JF, et al. The Let-7 microRNA reduces tumor growth in mouse models of lung cancer. *Cell Cycle* 2008;7(06):759–764
- 46 Vickers MM, Bar J, Gorn-Hondermann I, et al. Stage-dependent differential expression of microRNAs in colorectal cancer: potential role as markers of metastatic disease. *Clin Exp Metastasis* 2012;29(02):123–132
- 47 Zhang N, Lu C, Chen L. miR-217 regulates tumor growth and apoptosis by targeting the MAPK signaling pathway in colorectal cancer. *Oncol Lett* 2016;12(06):4589–4597
- 48 Huang L, Wen C, Yang X, et al. PEA3, acting as a tumor promoter in colorectal cancer, is regulated by the EGFR/KRAS signaling axis and miR-181d. *Cell Death Dis* 2018;9(03):271
- 49 Chen M, Lin M, Wang X. Overexpression of miR-19a inhibits colorectal cancer angiogenesis by suppressing KRAS expression. *Oncol Rep* 2018;39(02):619–626
- 50 Kent OA, Mendell JT, Rottapel R. Transcriptional regulation of miR-31 by oncogenic KRAS mediates metastatic phenotypes by repressing RASA1. *Mol Cancer Res* 2016;14(03):267–277
- 51 Ota T, Doi K, Fujimoto T, et al. KRAS up-regulates the expression of miR-181a, miR-200c and miR-210 in a three-dimensional-specific manner in DLD-1 colorectal cancer cells. *Anticancer Res* 2012;32(06):2271–2275
- 52 Velho S, Oliveira C, Ferreira A, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. *Eur J Cancer* 2005;41(11):1649–1654
- 53 Fang L, Li H, Wang L, et al. MicroRNA-17-5p promotes chemotherapeutic drug resistance and tumour metastasis of colorectal cancer by repressing PTEN expression. *Oncotarget* 2014;5(10):2974–2987
- 54 Danielsen SA, Eide PW, Nesbakken A, et al. Portrait of the PI3K/AKT pathway in colorectal cancer. *Biochim Biophys Acta* 2015;1885(01):104–121
- 55 Guo C, Sah JF, Beard L, Willson JKV, Markowitz SD, Guda K. The noncoding RNA, miR-126, suppresses the growth of neoplastic cells by targeting phosphatidylinositol 3-kinase signaling and is frequently lost in colon cancers. *Genes Chromosomes Cancer* 2008;47(11):939–946
- 56 Fish JE, Santoro MM, Morton SU, et al. miR-126 regulates angiogenic signaling and vascular integrity. *Dev Cell* 2008;15(02):272–284
- 57 Tu Y, Liu L, Zhao D, et al. Overexpression of miRNA-497 inhibits tumor angiogenesis by targeting VEGFR2. *Sci Rep* 2015;5:13827
- 58 Chen Y, Zhang B, Jin Y, Wu Q, Cao L. MiR-27b targets PI3K p110 α to inhibit proliferation and migration in colorectal cancer stem cell. *Am J Transl Res* 2019;11(09):5988–5997
- 59 Ozaki T, Nakagawara A. Role of p53 in cell death and human cancers. *Cancers (Basel)* 2011;3(01):994–1013
- 60 Kandoth C, McLellan MD, Vandin F, et al. Mutational landscape and significance across 12 major cancer types. *Nature* 2013;502(7471):333–339
- 61 Mozammel N, Amini M, Baradaran B, Mahdavi SZB, Hosseini SS, Mokhtarzadeh A. The function of miR-145 in colorectal cancer progression; an updated review on related signaling pathways. *Pathol Res Pract* 2023;242:154290
- 62 Rokavec M, Li H, Jiang L, Hermeking H. The p53/microRNA connection in gastrointestinal cancer. *Clin Exp Gastroenterol* 2014;7:395–413
- 63 Shi L, Jackstadt R, Siemens H, Li H, Kirchner T, Hermeking H. p53-induced miR-15a/16-1 and AP4 form a double-negative feedback loop to regulate epithelial-mesenchymal transition and metastasis in colorectal cancer. *Cancer Res* 2014;74(02):532–542
- 64 Feng Z, Zhang C, Wu R, Hu W. Tumor suppressor p53 meets microRNAs. *J Mol Cell Biol* 2011;3(01):44–50
- 65 Hu W, Chan CS, Wu R, et al. Negative regulation of tumor suppressor p53 by microRNA miR-504. *Mol Cell* 2010;38(05):689–699
- 66 Le MTN, Teh C, Shyh-Chang N, et al. MicroRNA-125b is a novel negative regulator of p53. *Genes Dev* 2009;23(07):862–876
- 67 Kumar M, Lu Z, Takwi AA, et al. Negative regulation of the tumor suppressor p53 gene by microRNAs. *Oncogene* 2011;30(07):843–853
- 68 Tay Y, Tan SM, Karreth FA, Lieberman J, Pandolfi PP. Characterization of dual PTEN and p53-targeting microRNAs identifies microRNA-638/Dnm2 as a two-hit oncogenic locus. *Cell Rep* 2014;8(03):714–722
- 69 Yamakuchi M, Ferlito M, Lowenstein CJ. miR-34a repression of SIRT1 regulates apoptosis. *Proc Natl Acad Sci U S A* 2008;105(36):13421–13426
- 70 Sachdeva M, Zhu S, Wu F, et al. p53 represses c-Myc through induction of the tumor suppressor miR-145. *Proc Natl Acad Sci U S A* 2009;106(09):3207–3212
- 71 Yamakuchi M, Lotterman CD, Bao C, et al. P53-induced microRNA-107 inhibits HIF-1 and tumor angiogenesis. *Proc Natl Acad Sci U S A* 2010;107(14):6334–6339
- 72 Georges SA, Biery MC, Soo-yeon K, et al. Coordinated regulation of cell-cycle transcripts by p-53 inducible mir-192 and mir-215. *Cancer Res* 2008;68:24
- 73 Earle JS, Luthra R, Romans A, et al. Association of microRNA expression with microsatellite instability status in colorectal adenocarcinoma. *J Mol Diagn* 2010;12(04):433–440
- 74 Sievänen T, Korhonen TM, Jokela T, et al. Systemic circulating microRNA landscape in Lynch syndrome. *Int J Cancer* 2023;152(05):932–944
- 75 Valeri N, Gasparini P, Fabbri M, et al. Modulation of mismatch repair and genomic stability by miR-155. *Proc Natl Acad Sci U S A* 2010;107(15):6982–6987
- 76 Yu Y, Wang Y, Ren X, et al. Context-dependent bidirectional regulation of the MutS homolog 2 by transforming growth factor β contributes to chemoresistance in breast cancer cells. *Mol Cancer Res* 2010;8(12):1633–1642
- 77 Valeri N, Gasparini P, Braconi C, et al. MicroRNA-21 induces resistance to 5-fluorouracil by down-regulating human DNA MutS homolog 2 (hMSH2). *Proc Natl Acad Sci U S A* 2010;107(49):21098–21103
- 78 Sarver AL, French AJ, Borralho PM, et al. Human colon cancer profiles show differential microRNA expression depending on mismatch repair status and are characteristic of undifferentiated proliferative states. *BMC Cancer* 2009;9:401
- 79 Balaguer F, Moreira L, Lozano JJ, et al. Colorectal cancers with microsatellite instability display unique miRNA profiles. *Clin Cancer Res* 2011;17(19):6239–6249
- 80 Principe DR, Doll JA, Bauer J, et al. TGF- β : duality of function between tumor prevention and carcinogenesis. *J Natl Cancer Inst* 2014;106(02):djt369
- 81 Lin E, Kuo PH, Liu YL, Yang AC, Tsai SJ. Transforming growth factor- β signaling pathway-associated genes SMAD2 and TGFBR2 are implicated in metabolic syndrome in a Taiwanese population. *Sci Rep* 2017;7(01):13589
- 82 Valeri N, Braconi C, Gasparini P, et al. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. *Cancer Cell* 2014;25(04):469–483

- 83 Li J, Liang H, Bai M, et al. miR-135b promotes cancer progression by targeting transforming growth factor beta receptor II (TGFB β 2) in colorectal cancer. *PLoS One* 2015;10(06):e0130194
- 84 Zhang W, Zhang T, Jin R, et al. MicroRNA-301a promotes migration and invasion by targeting TGFB β 2 in human colorectal cancer. *J Exp Clin Cancer Res* 2014;33(01):113
- 85 Wang M, Li C, Yu B, et al. Overexpressed miR-301a promotes cell proliferation and invasion by targeting RUNX3 in gastric cancer. *J Gastroenterol* 2013;48(09):1023–1033
- 86 Yu Y, Kanwar SS, Patel BB, et al. MicroRNA-21 induces stemness by downregulating transforming growth factor beta receptor 2 (TGFB β 2) in colon cancer cells. *Carcinogenesis* 2012;33(01):68–76
- 87 Cheng D, Zhao S, Tang H, et al. MicroRNA-20a-5p promotes colorectal cancer invasion and metastasis by downregulating Smad4. *Oncotarget* 2016;7(29):45199–45213
- 88 Ling H, Pickard K, Ivan C, et al. The clinical and biological significance of MIR-224 expression in colorectal cancer metastasis. *GUT* 2016;65:6
- 89 Bu P, Wang L, Chen KY, et al. miR-1269 promotes metastasis and forms a positive feedback loop with TGF- β . *Nat Commun* 2015;6:6879
- 90 Wu ZMM, You LMM, Zhang YMM, et al. Colorectal cancer screening methods and molecular markers for early detection. *Technol Cancer Res Treat* 2023;19:1533033820980426
- 91 Yazdani Y, Farazmandfar T, Azadeh H, Zekavatian Z. The prognostic effect of PTEN expression status in colorectal cancer development and evaluation of factors affecting it: miR-21 and promoter methylation. *J Biomed Sci* 2016;23:9
- 92 Mozammel N, Amini M, Baradaran B, Mahdavi SZB, Hosseini SS, Mokhtarzadeh A. The function of miR-145 in colorectal cancer progression; an updated review on related signaling pathways. *Pathol Res Pract* 2023;242:154290
- 93 Chen H-Y, Lang Y-D, Lin HN, et al. miR-103/107 prolong Wnt/ β -catenin signaling and colorectal cancer stemness by targeting Axin2. *Sci Rep* 2019;9(01):9687
- 94 Zhang Y, Guo L, Li Y, et al. MicroRNA-494 promotes cancer progression and targets adenomatous polyposis coli in colorectal cancer. *Mol Cancer* 2018;17(01):1
- 95 Dokanehiifard S, Yasari A, Najafi H, et al. A novel microRNA located in the *TrkC* gene regulates the Wnt signaling pathway and is differentially expressed in colorectal cancer specimens. *J Biol Chem* 2017;292(18):7566–7577
- 96 Pagliuca A, Valvo C, Fabrizio E, et al. Analysis of the combined action of miR-143 and miR-145 on oncogenic pathways in colorectal cancer cells reveals a coordinate program of gene repression. *Oncogene* 2013;32(40):4806–4813
- 97 Sha D, Lee AM, Shi Q, et al. Association study of the Let-7 miRNA-complementary site variant in the 3' untranslated region of the KRAS gene in stage III colon cancer (NCCTG N0147 clinical trial). *Clin Cancer Res* 2014;20(12):3319–3327
- 98 Wu X, Li Z, Huang N, Li X, Chen R. Study of KRAS-related miRNA expression in colorectal cancer. *Cancer Manag Res* 2022;14:2987–3008
- 99 Ibrahim H, Lim YC. KRAS-associated microRNAs in colorectal cancer. *Oncol Rev* 2020;14(02):454
- 100 Zhang C, Liu J, Wang X, et al. MicroRNA-339-5p inhibits colorectal tumorigenesis through regulation of the MDM2/p53 signaling. *Oncotarget* 2014;5(19):9106–9117
- 101 Fawzy MS, Ibrahim AT, AlSel BTA, Alghamdi SA, Toraih EA. Analysis of microRNA-34a expression profile and rs2666433 variant in colorectal cancer: a pilot study. *Sci Rep* 2020;10(01):16940
- 102 Liebl MC, Hofmann TG. The role of p53 signaling in colorectal cancer. *Cancers* 2021;13:9
- 103 Scarpa M, Ruffolo C, Kotsafti A, et al. MLH1 deficiency down-regulates TLR4 expression in sporadic colorectal cancer. *Front Mol Biosci* 2021;8:624873
- 104 Zhang Y, Yin C, Wei C, et al. Exosomal miR-625-3p secreted by cancer-associated fibroblasts in colorectal cancer promotes EMT and chemotherapeutic resistance by blocking the CELF2/WWOX pathway. *Pharmacol Res* 2022;186:106534
- 105 Sokolova V, Fiorino A, Zoni E, et al. The effects of miR-20a on p21: two mechanisms blocking growth arrest in TGF- β -responsive colon carcinoma. *J Cell Physiol* 2015;230(12):3105–3114
- 106 Xiao Z, Chen S, Feng S, et al. Function and mechanisms of microRNA-20a in colorectal cancer. *Exp Ther Med* 2020;19(03):1605–1616