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MicroRNAs in colorectal cancer: potential biomarkers and therapeutic targets

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1. ABSTRACT

Colorectal Cancer (CRC) is one of the most common malignant tumors of gastrointestinal tract. The occurrence, development, diagnosis and prognosis have become a hot topic in current clinical research. However, current diagnosis and treatment still depend on the results of tumor imaging or pathology test. Therefore, to reveal the biological pathogenesis of CRC and to discover specific biomarkers for early diagnosis and better prognosis assessment of CRC have become key roles in current studies. Recent literature has revealed that aberrant expression of microRNAs (miRNAs) could contribute to the development and progression of CRC through the regulation of several critical pathways, including apoptosis, epithelial-mesenchymal transition, angiogenesis and signal transduction. These findings have also indicated that miRNAs could be a novel biomarker for early clinical diagnosis and prognosis evaluation of CRC. In this review, we have summarized the recent studies of miRNAs on the diagnosis, treatment and prognosis clinical biomarker of CRC. We also discussed the regulatory mechanisms of miRNAs in CRC, which may guide the future treatment for CRC.

2. INTRODUCTION

Colorectal Cancer (CRC) is one of the most common malignant tumors of grastrointestinal tract. The number of patients is approximately 28 million worldwide (1). In 2012, there are 103170 new cases of colon cancer, and 40290 new cases of rectal cancer (2). Statistically, in 2009, the incidence of colorectal cancer ranked the third most common malignant tumor in China. With a percentage of 29.4.4/100,000, the mortality ranked the fifth among malignant tumors (3). Despite many studies trying to reveal the effective monitoring measures of diagnosing and forecasting colorectal cancer, resolution of clinical diagnosis and treatment mainly depends on the tumor imaging or pathology test results. Limited clinical diagnosis may lead to difficulty in distinguishing between high-risk CRC patients and low-risk CRC patients. Therefore, patients may accept inadequate or unnecessary treatment (4). Currently, to reveal the biological pathogenesis of CRC and to discover specific biomarkers for early diagnosis and prognosis assessment of CRC has become essential in the clinical treatment of colorectal cancer. MicroRNAs (miRNAs) is a class of endogenous, small (19-25nucleotides), noncoding, conserved RNA molecules. MiRNAs are critical in activities of human life through the mechanism of complementary pairing the 3'untranslated region (3'-UTR) of target gene mRNA molecules to cause inhibition of translation of target mRNA molecules. MiRNAs participates in the occurrence, development and metastasis of tumors, which also play a role as oncogene or tumor suppressor gene (5-6). Recently, some studies showed that abnormal expression of miRNAs is partially associated with the occurrence and development of CRC. It is suggested that miRNA can be

used as a specific novel biomarkers of CRC in the early clinical diagnosis and prognostic evaluation (7). In this paper, we reviewed the studies on miRNAs as specific novel biomarkers of CRC in the early clinical diagnosis and prognostic evaluation. We also discussed about the molecular mechanism of the regulation miRNAs in colorectal cancer and a wide clinical application of miRNAs as a novel diagnostic markers and therapeutic targets in CRC.

3. BIOGENESIS OF MIRNAS

MiRNAs are a group of endogenous non-protein-coding RNA molecules that are highly evolutionary and conservative. They can mingle with specific mRNAs to negatively-regulate gene expression at a post-transcriptional level. This process starts in the nucleus, where miRNAs are encoded by genomic DNA and transcribed by RNA polymerase II or III into the primary miRNA (pri-miRNA). Pri-miRNA is cut by the nucleic acid enzyme, Drosha to form a precursor miRNA (premiRNA). Pre-miRNA is exported into the cytoplasm through a shuttle protein, exportin 5, and is then cut by another nucleic acid enzyme, Dicer into approximately 22 nucleotides of double-stranded miRNAs. When two strands in duplex miRNAs are divided into two miRNAs, one strand is called the mature miRNA, and the other is degraded. Single stranded mature miRNAs associate with the RNA-induced silencing complex (RISC) and becomes the active miRNAs. The active complex interacts with target mRNA at its 3'UTR region through the complete or incomplete pairing machinery to induce the degradation or inhibition of translation of the target mRNA. Active miRNAs function through silencing the expression of genes at their post transcriptional level (8). MiRNAs play an important part in a variety of biological processes, such as cell development, differentiation, multiplication, apoptosis and metabolism.

4. ROLE OF MIRNAS IN COLON CARCINOGENESIS

4.1. miRNAs and apoptosis of tumor cells in CRC

Apoptosis of tumor cells is completed by both exogenous and endogenous pathway. The exogenous pathway depends on the combination of cell membrane apoptotic receptor (FASL, TRAIL, TNFR) and the ligand. The pathway activates the downstream caspase8 to trigger the "waterfall reaction" of apoptosis. The endogenous pathway that is based on the mitochondria releases mitochondrial proteins mediated by the upstream BCL-2 family proteins, including the cytochrome C (cyt C) with involvement of caspase9, to activate the "waterfall reaction" of downstream caspase pathway.

As an important protein promoting apoptosis of tumor cells, p53 is involved in the regulation of a variety of miRNAs. The regulation of transcriptional and post transcriptional level affects the translation of different mRNAs and then the targeting of miRNAs mRNAs (9). On the contrary, p53 is also regulated by some miRNAs, such as the miR29 family, including miR29a, miR29b, and miR29c. The act of miR29 on CDC42 and p85a leads to a positive regulation on p53 by elevating the levels of p53 to promote apoptosis-inducing effect on malignant cells (10).

The miR34 family targets directly the downstream gene of tumor protein p53 (TP53) (11). Study reported that transfection of miR34a in CRC cells induced cell apoptosis, increased the intracellular p53 and reduced transcription factor E2F (12)., The effects were absent in p53-depleted tumor cells, indicating that TP53 was a possible target of suppressor effect of miR34a. It could be speculated that the positive regulation loop existed between miR34a and p53. Both miR34a and p53 mutually promoted apoptosis effect on cancer cells (13). Another study showed that Bcl-2 protein family in the apoptosis pathway was directly negatively regulated by miR34a, through translating miR34a-depleted mouse embryonic stem cells by oligonucleotide transfection (14). Loss of miR34 gene caused resistance to apoptosis in tumor cells. Clinically, it appeared in decreasing sensitivity on pro-apoptotic chemotherapeutic drugs (15). MiR195 also had the same effect on the pro-apoptotic effect of BcL-2 (16).

Macrophage migration inhibitory factor (MIF) is an important factor in cell immune response and inflammation, which has great effect in the occurrence and development of CRC and apoptosis induced by hypoxia. MIF is suggested to have inhibitory effect of p53 activity (17-18). MIF is also a potential target for miR451. Bandres, *et al*. observed that overexpression of miR451 in gastrointestinal tract tumors inhibited mRNA and protein expression of MIF in biopsy specimens, which inhibited tumor growth (19).

4.2. miRNAs and epithelial-mesenchymal transition

Epithelial-mesenchymal transition (EMT) is a cell program that turns epithelial cells with polarity and weak activity into interstitial cells with non-polarity and strong activity *in vivo*. Tumor cells adapt to the change of microenvironment, separate from tumors, shift and spread into the vascular system through EMT. Transforming growth factorβ (TGFβ) that is released during tumor cell growth is one of the most important promoting factors in EMT, which can significantly upregulate the expression level of zinc finger binding protein (ZEB), reduces E-cadherin and increases vimentin, and induces tumor cells to lose polarity, EMT also decreases adhesion and improves activity ability and promotes cells to separate from tumors into circulation (20-21).

EMT is closely related to the activity of miRNAs, such as the family of miR200. Reduced level of miR200 was detected in CRC cells with EMT. Further results

confirmed that with low level of miR200 decreased the effect of E-cadherin on promoting intercellular tight junction, increased the effect of vimentin on promoting the EMT and increased EMT in tumor cells. In contrast, tumor cells transfected with high levels of miR200 had increasing EMT and E-cadherin level. ZEB is considered to inhibit the effect of miR200 on inhibiting EMT. ZEB forms mutual restrain loop and thus regulates the activity of miR200 and its function in EMT (22-23).

In addition to miR200 family, miR21 is also involved in the process of EMT in tumor cells. The increasing gene copy of MiR21 in CRC cells is more common, especially in the CRC metastasis. Histological study confirmed that miR21 level in CRC hepatic metastases was significantly higher than that in *in situ* CRC tissue (24). TGFβcould be a upstream factor of miR21. It was observed that MiR21 expression level increased in CRC treated with high concentration of TGFβ (25). The mechanism of MiR21 in CRC promoting EMT could relate to the inhibition of programmed cell death factor 4 (PDCD4). Selcuklu *et al*. observed that miR21-deplted tumor cells had increased PDCD4 and declined invasive ability (26), which was also confirmed in CRC tissues (27).

4.3. miRNAs and angiogenesis

Angiogenesis is a committed step in the process of tumor metastasis. Hypoxia is one of the most important factors to induce angiogenesis. Vascular endothelial growth factor (VEGF) is another most critical cell factor. When hypoxia appears in tumor organization, hypoxia-inducible factor 1 (HIF-1) is upregulated subsequently. VEGF-A is an important member of the VEGF family. HIF-1 stimulates the upregulation of VEGF-A mRNA, the levels of VEGF-A organization and the expression of VEGF receptor. This induces angiogenesis to resist hypoxia (28).

A variety of miRNAs are involved in the regulation of above hypoxia-HIF-VEGF pathway. HIF-1 is a heterodimeric basic helix-loop-helix structure composed of HIF-1a and the aryl hydrocarbon receptor nuclear translocator. Yamakuchi *et al*. found the level of miR22 in colorectal cancer was lower than that in normal tissue, and confirmed that high levels of miR22 in CRC cells could inhibit HIF-1a and reduce the downstream VEGF expression (29). On the contrary, HIF-1a and VEGF levels increased after knocking down the endogenous miR22. Results confirmed that miR22 acted on HIF-1a and inhibited angiogenesis effect induced by hypoxia in CRC cells.

MiR619 acted on VEGF gene and promoted tumor angiogenesis by upregulating the VEGF protein synthesis. MiR619 itself was positively regulated by the VEGF protein to form a mutual positive feedback loop. MiR619 was upregulated in CRC cells treated by anti-VEGF peptide (30).

Tumor suppressor genes TP53 inhibited tumor angiogenesis and tumor cells into the apoptosis program. MiRl07 was located in the chromosome 10q23.3.1 region, which was absent in 11% of patients with CRC (31). TP53 promoted downstream miRl07 to express increasingly, which combined mRNA inhibiting translation of HIF-1 to inhibit tumor angiogenesis (32). In the CRC cell treated by hypoxia, VEGF in high miRl07 level was significantly lower than that in low miRl07 level. Animal experiments also confirmed high miRl07 caused the feature of low VEGF expression, low blood vessel density and low tumor size.

4.4. miRNA and CRC intracellular signal transduction

The tumor cells utilize multiple intracellular signal transduction pathways to obtain the ability of proliferation, invasion and migration, including the Kirsten rat sarcoma viral oncogene homolog (KRAS gene) pathway. Let-7 family is one of the earliest discovered miRNAs with its action site in KRAS. The inhibition of KRAS leads to suppression of tumors. Akao *et al*. reported that let-7 miRNA was mostly underexpressed in the CRC organization (33). Transfection of let-7 in CRC cells downregulated intercellular KRAS at translational level to resist angiogenesis and promoted tumor angiogenesis of c-myc expression.

MiR143 is located in chromosome 5q32, which is often lost in 5q24-32 region of CRC specimens. MiRl143 was underexpressed in tumor tissues (31). The precursor of miRNA143 was transfected into CRC cells, causing the decline of downstream factor ERK5 level in KRAS pathway. Although the specific target was still not clear, it was certain that miR143 inhibited KRAS pathway to block tumor development (34).

MiR17-92 was positively regulated by c-myc protein. Knockdown of c-myc or transfection of miR17-92 in CRC cells proved the direct downregulation of tumor suppressor factor.The synthesis of anti-angiogenic thrombospondin-1 (Tsp1) and connective tissue growth factor (CTGF) promoted the invasion and metastasis of CRC (35).

Activation of the tyrosine kinase receptor activated the anti-apoptotic response in tumor cells through PI3K-Akt pathway. Pi3K-Akt pathway is a critical cellular pathway for the survival and migration of tumor cells. Schimanski *et al*. observed that increasing phosphorylation of Akt after the transfection of miR196 in CRC cells, indicating that PI3K -Akt pathway was promoted by miR196. In addition, miR196 level in CRC metastasis tissue was higher than that in *in situ* tissue, which showed its importance in promoting CRC migration (Fig.1).

5. ROLE OF miRNAS AS BIOMARKERS

Literature indicated that the expression level of miRNAs was altered between the CRC organization

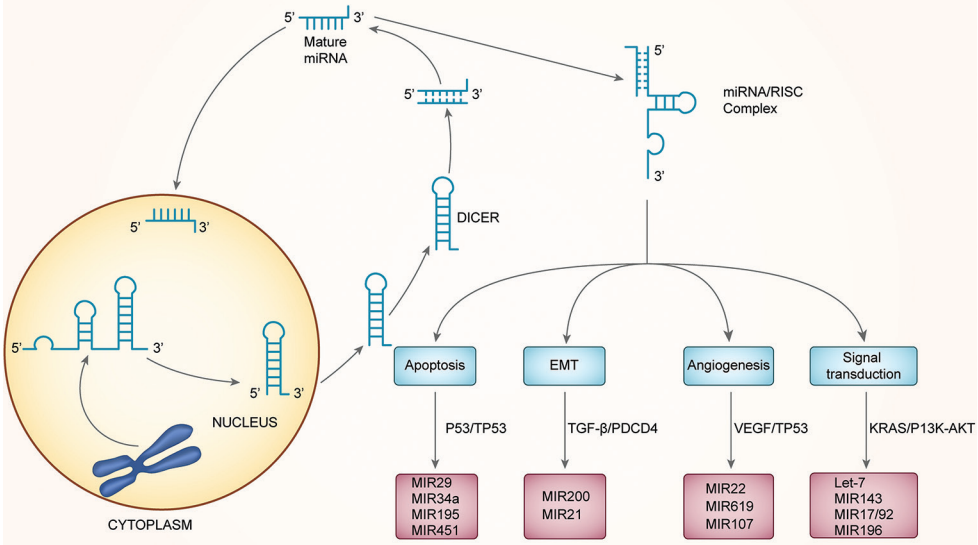

Figure 1. Biogenesis of microRNAs and miRNAs involved in the carcinogenesis of CRC. MiRNAs are encoded by genomic DNA and transcribed by RNA polymerase II or III into the primary miRNA (pri-miRNA). Pri-miRNA is cut by Drosha to form a precursor miRNA (premiRNA). Pre-miRNA is transported to the cytoplasm through exportin 5, and then processed by Dicer into double-stranded miRNA. One strand is called the mature miRNA (leading strand), and the other usually is degraded. Mature miRNA and other protein make up RNA-induced silencing complexes (RISC), interacting with target mRNA at its 3'UTR region through the complete or incomplete pairing way to induce the target mRNA degrade or inhibit translation. MiRNAs are related to the development and progression of colorectal cancer through several critical pathways involving apoptosis, epithelial-mesenchymal transition, angiogenesis and signal transduction.

and the normal tissue (37-39). Table 1 listed the types of miRNAs associated with CRC and its corresponding target gene. Some miRNAs were previously discovered function as promoting or suppressing colon cancer. For example, miR21 is one type of carcinogenic miRNAs, overexpressed at the advanced colon cancer organization (38). On the other hand, reduced expression of some miRNAs, such as miR-1, miR-30a-5p and etc., was confirmed in the colorectal cancer, and thus these miRNAs were considered to be anticarcinogenic miRNAs (37,39). Other important miRNAs in CRC are miR-17-92 cluster, miR-24, miR-31, miR-135a/b, miR-143, miR-145 and miR-200c (40-45).

5.1. Plasma miRNA

The research on miRNAs as diagnostic markers displayed that the carcinoembryonic Antigen (CEA) is the only molecular marker in colorectal cancer serum or plasma. However, poor specificity and low sensitivity limit clinical application of CEA, and is especially inadequate for early diagnosis of colorectal cancer (46). Part of the study revealed that human peripheral blood contained high concentration and high stability of free miRNAs, which participated in massive cell signal transduction and genetic transformation (47). Because carcinogenesis is closely related to abnormal miRNA expression, different types of tumors possess their own specific aberrant level of miRNAs, and some of these miRNAs are secreted into the blood cycle (47-48).

Accordingly, the study speculated that part of serum or plasma cyclic miRNAs secreted by tumor may be the biomarkers for clinical diagnosis of colorectal cancer. Ng *et al*. used miRNA microarray and quantitative PCR to verify and excavate differentially expressed miRNAs in plasma colorectal cancer. The study identified possible biomarkers for clinical diagnosis of CRC (49). Other studies showed that high levels of miR-17-3p and miR-92a in the miR-17-92 gene cluster were closely associated with CRC, and their level significantly decreased in the CRC patients who received surgery. These results confirmed that CRC cells could secrete abnormal expression of miRNAs into the blood cycle. Further investigation revealed that the sensitivity of diagnosing CRC with miR-92a is 89% and the specificity is 70%. MiR-92a can be used to distinguish colorectal cancer from inflammatory bowel disease and other gastrointestinal cancers. Studies speculated that miR-92a might be a potential marker for early diagnosis of CRC. Huang *et al*. further verified the diagnostic potential of miR-92a (50). The study found that plasma from CRC patients contained high levels of miR-92a, and the diagnostic sensitivity and specificity was consistent with the results from Ng *et al*. (49). Chen *et al*. used deep sequencing to excavate CRC serum miRNAs (51). There were 69 significantly differential expressions of specific miRNAs in colorectal cancer serum. Further reports showed that 80% of these miRNAs were also present in the serum from lung cancer patients, which left only a few of miRNAs to be developed into specific diagnostic markers for CRC.

Furthermore, the study speculated that common cancer-related miRNAs existed in different types of tumor serum. Some aberrant expressions of miRNAs could be

Table 1. Alterations of miRNAs in colorectal cancer

miRNA	Remarks	Target gene	Reference
let-7	Downregulated	KRAS	33
miR-1	Downregulated		39
miR-15b	Upregulated		73
miR-17-92 culster	Upregulated	E2F1	74
miR-18a	Downregulated		39
miR-21	Upregulated	PDCD4, RhoB	27, 39,75
miR-22	Downregulated	HIF-1α, VEGF	29
miR-24	Downregulated	DHFR	43
miR-28-3p	Downregulated	NM23-H1	76
miR-28-5p	Downregulated	CCND1, HOXB3	76
miR-29a/c	Downregulated		77
miR-30a-5p	Upregulated	DTL	37
miR-31	Downregulated		39
miR-93	Downregulated	BAMBI, CCND2, CDKN1A, HDAC8, KIF23, MAP3K11, MYCN, PPARD, TLE4, ZDHHC1	78
miR-95	Upregulated	SNX1	50
miR-101	Downregulated	COX2	79
miR-122a	Downregulated	APC/beta-catenin	80
miR-126	Downregulated	PI3K	81
miR-133b	Downregulated	MET	82
miR-135a/b	Upregulated	APC	44
miR-143	Downregulated	KRAS, DNMT3A, ERK5	42,83
miR-145	Downregulated	OCT4, SOX2, KLF4, IRS-1	42,84
miR-148b	Downregulated	CCK2R	85
miR-181b	Upregulated		73
miR-191	Upregulated		73
miR-192	Downregulated		86
miR-195	Downregulated	Bcl-2	87
miR-200c	Upregulated		73
miR-203	Downregulated		88
miR-211	Upregulated	CDKN1C/p57	89
miR-215	Downregulated	DTL	86
FiR-320a	Downregulated	NRP-1	90
miR-342	Downregulated	DNMT1	91
miR-365	Downregulated	CyclinD1, Bcl-2	92
miR-375	Downregulated		93
miR-451	Downregulated	MIF	94
miR-499-5p	Upregulated	FOXO4 and PDCD4	95
miR-675	Upregulated	RB	96

due to other tumor-induced sources, such as immune cells. Wang *et al*. used miRNA microarray to excavate differential expression of miRNAs between CRC patient and healthy human (52). The results showed that miR-10a, miR-19a, miR-24, miR-92a, miR-125a-5p, miR-141, miR-150, miR-188-3p, miR-192, miR-210, miR-221, miR-376a, miR-495, MiR-572, miR-601, miR-720, miR-760, HSA-let-7A and HSA-let-7E were downregulated in CRC patients plasma. Further qRT-PCR results verified that expressions of miR-601 and miR-760 in plasma of patients with CRC were significantly reduced. The study indicated that miR-601 and miR-760 could be potential markers for early diagnosis of CRC patients. Luo *et al*. considered the function of a single miRNA in CRC serum or plasma should be studied intensively and multiple combinations of serum or plasma miRNAs could be used as a specific diagnostic marker of CRC . (53).

5.2. Faecal miRNA

In recent years, feces and other dejections based as a non-invasive screening test for CRC has been used widely in clinical application. The fecal occult blood test (FOBT) with guaiacis is currently the most widely used clinically screening test for CRC (54). However, irregular colorectal bleeding leads to low specificity and sensitivity of faecal occult blood test for CRC. Different situation may cause gastrointestinal bleeding, including residues from red meat. Due to this reason, some advanced tumors even greater than 1cm in diameter cannot be detected by FOBT (54-55).

In addition, some studies collected colonic epithelial cells that fell from the gut and site of tumor in the feces to detect CRC. Theoretically, these colonic epithelial cells residues should carry large amounts of important genetic and epigenetic information. Follow-up tests could be taken, including mutation or abnormal expression of mRNAs, proteins and miRNAs detection (56). Relative to the degradable properties of mRNAs and proteins, endogenous miRNAs are packed and protected by RNase enzymes, and are much easier to be detected. However, internal environment in feces is more complex and worse than in serum or plasma. Study indicated that the amount of human RNA is less than 1% in fecal compared with total RNA. Studies noted that clinical diagnostic marker of CRC must meet several criteria, including measurability, reproducibility, predictability and etc (56-57).

In 2009, Ahmed *et al*. innovatively attempted to isolate and screen miRNA biomarkers of CRC from fecal specimens (58). In their study, a new fecal miRNA detection procedure was created, including feces separation, miRNAs extraction and quantitative analysis from feces. Their results revealed that some abnormal expression of miRNAs could distinguish between samples from healthy people and ulcerative colitis from CRC patients. The test also could differentiate different Dukes' CRC stages. For example, feces of the CRC patients with late Dukes' staging contained higher levels of miR-21, miR-106a, miR-96,

miR-203, miR-20a, miR-326 and miR-92, meanwhile lower levels miR-320, miR-126, MiR-484-5p, miR-143, miR-145, miR-16 and miR-125b were expressed in feces (58). Due to limited samples in Ahmed's study (58), statistical significance was not observed from the screening of colorectal cancer miRNA biomarkers. The results were controversial (58). For instance, Link *et al*. used miRNA microarray to screen and compare miRNAs between healthy feces and colonic mucosal epithelial tissues (56). The result showed high similarity between 284 types of miRNA expression patterns within two samples. The fecal miRNA expression patterns were similar regarding individuals and time of sample collections. Further studies showed that high levels of miR-21, miR-106a in the feces of CRC patient, but levels of miR-21, miR-106a were negatively correlated with tumor stages. No significant differences were found in the expression of miR-143, miR-17, miR-622 and miR-65-3p between feces of CRC and healthy control group. Koga *et al*. separated and purified colonic epithelial cells in feces through combination of immunomagnetic beads and epithelial cell adhesion molecule antibody (EpCAM) (59). Timing and quantitative qPCR results showed there was no statistical significance in the expression of miR-21 expression in feces between CRC patient and healthy group. High expression levels of miR-135, miR-17-92 cluster in feces of patients with CRC suggested some specific differentially-expressed miRNAs in the feces could be used as early non-invasive biological diagnostic markers for CRC.

In addition, in tissues samples and cell lines, expression of quantitative reference RNU6B of miRNAs had no association with fecal total RNA concentration. The rapid degradation and low detection level of fecal RNU6B suggested other sustained expression of small molecule miRNAs could be used as a standardized reference gene in the expression test of fecal miRNA, such as miR-16 and 18s rRNA (7). Ahlquist *et al*. speculated that early CRC fecal tumor cells and most of tumor molecular markers were more likely to be detected in the blood (60). Fecal miRNA markers test had great advantage in screening for CRC precancerous lesion. However, small amount of clinical samples or preserved specimens were due to the long path. If these mechanisms are universal phenomenon in fecal miRNAs, it is not applicable to use feces miRNAs clinically to diagnose the CRC. Although miRNA biomarkers in clinical practice are still in its early stage, the progress related miRNA as potential diagnostic markers is promising. These findings suggested further investigation of method to screen or detect CRC is urgently in need. Discovering the mechanisms of miRNA in CRC will lead to the development of high specific, sensitive and reproductive miRNA markers in clinically diagnosis.

6. ROLE OF MIRNAS IN PROGNOSIS OF CRC

Although surgical techniques have improved so much in recent years, operable CRC patients still have a relatively high risk of postoperative local recurrence. The effect of clinical adjuvant therapy depends on individuals. Currently, more institutions carry out the clinical screening for CRC miRNAs as prognostic markers for CRC patients. Some preclinical and clinical studies showed that some miRNAs with specifically differential expression could be used for CRC prognosis. MiR-21 was considered to be an important CRC prognostic molecular marker (24,61). Slaby *et al*. revealed that upregulated expression of miR-21 in CRC patients was closely related to lymph node positive and metastases (61). Subsequently, two research teams used miRNA microarray to screen and identify colorectal cancer prognosis miRNA molecular markers of different ethnic groups in different regions. Schetter *et al*. excavated 37 miRNAs with abnormal expressions for CRC prognosis (62). The result showed that five highly expressed miRNAs (miR-20a, miR-21, miR-106a, and miR-181b, miR-203) were associated with low survival rate in prognosis of CRC patients. Further studies revealed that the poor prognosis of asian CRC patients is significantly correlated with high levels of tumor miR-21, and that high levels of miR-21 in CRC patients was closely related to poor response to clinical adjuvant therapy and ealier recurrence in phase III CRC patients. Kulda *et al*. confirmed that early high level of miR-21 was related to a shorter disease-free interval (DFI), but not related to the overall survival (OS) in CRC patients (24). Further investigation revealed that DFI and OS didn't correlate with the levels of miR21, regardless of liver metastases.

MiR-31 is an important factor controlling tumor metastasis and is abnormally expressed in CRC cell lines and tissues. Bandrés *et al*. showed that miR-31 expression was significantly higher in patients with phase IV than with phase II (63). Wang *et al*. confirmed miR-31 expression was closely related with CRC stage, including local invasion (64). Some studies indicated that microsatellite status was related to CRC processes and responses for adjunctive therapy in patients with CRC. In 2007, Lanza *et al*. first reported microsatellite status was associated with miRNA microarray of multiple tumors (65). 14 differentially expressed miRNAs were identified between tumor microsatellite stability (MSS) and microsatellite high instability (MSI-H). Schepeler *et al*. used miRNA microarray to analyze 49 clinical patient samples with CRC TNM phase II, and showed that miR-142-3p, miR-212, miR-151 and miR-144 could be assembled to differentiate significant tumor microsatellite instability (MSI) from tumor microsatellite stability (MSS) (66). The sensitivity of separation was 92% and the specificity was 81%. Further study showed that 17 miRNAs out of the microarray results were capable of differentiating microsatellite stability (MSS) subtype from CRC metastasis and recurrences state with a 81 % accuracy, 77% sensitivity and 83% specificity. In these 17 miRNAs, high expression levels of miR-320 and miR-498 were closely related with a longer progression-free survival (PFS) in patients with CRC. Subsequently, Earle *et al* showed that upgraded expression of miR-92, let-7A, miR-145 were associated with microsatellite low

instability (MSI-L), but elevated expression levels of miR-155, miR-223, miR-31 and miR-26b were associated with microsatellite high instability (MSI-H) (67).

7. ROLE OF MIRNAS IN CRC THERAPY

At present, the adjuvant therapy based on fluorouracil medicament has been widely used in clinical treatment for CRC patients (68). Studies showed that the expression levels of miR-181b and let-7g were relatively low in patients with CRC responsive to the fourth generation of fluorouracil medicament S-1. However, miR-181b and let-7g didn't indicate the survival time of patients with CRC (69). Song *et al*. found that miR-215 could improve the expression of cell cycle gene p53 and p21, through the downregulation of no dentate homologues (DTL). Furthermore, miR-215 also induced inhibition of cell proliferation at G2 phase and enhanced HCT116 drug resistance of methotrexate (MTX) and raltitrexed (TDX). However, miR-215 had no effect on cisplatin and adriamycin (70). The study further noted that CRC stem cells had a high level of miR-215 even though the miR-215 was downregulated in patients with CRC.

Cetuximab is a monoclonal antibody to epidermal growth factor receptor (EGFR), which can be used to treat wild type (KRAS) metastatic CRC (71). Ragusa *et al*. showed that miR-146b-3p and miR-46-5p in mutant KRAS metastatic CRC were more abundant than those in wild type KRAS. They also proposed that downregulation of let-7b, let-7e and upregulation of miR-17-3p upregulation might be predictive for the combined treatment where potential cetuximab plus chemotherapy were used (71).

Some miRNAs may be involved in the regulation of radiation sensitivity. For example, Svoboda *et al*. showed that capecitabine chemoradiotherapy (CRT) in patients with rectal cancer induced downregulation of miR-125b and miR-137 and upregulation of miR-125b and miR-137, which were associated with poor therapeutic effect (72). Ahmed *et al*. showed that the models of miRNAs expressing in HT29 were different in CRC cell lines from HT29 irradiated by two groups of X-ray. The research of temporary practice value needs further investigation (58).

8. CONCLUSIONS

With the rapid progress in miRNA research, many studies revealed that miRNAs could not only play a role of oncogene, but also cast an important effect on anti-oncogene in the generation, development and metastasis of tumors. MiRNAs provide us with novel possibilities of molecular markers and therapeutic targets for cancer diagnosis and therapy , including colorectal cancer. This paper reviewed the research progress in some specific differentially expressed miRNAs as molecular markers for clinically early diagnosis, prognosis and treatment of rectal cancer. However, there are few rectal cancer miRNA biomarkers with clinical practical value. Recent studies suggested a large number of potential miRNA biomarkers for rectal cancer, but further investigation is needed for clinical diagnosis and treatment. In addition, some studies speculated that colorectal cancer fecal miRNAs might potentially be high specific, sensitive and reproducible diagnostic markers. However, research on CRC fecal miRNAs is still in its infancy. Currently, investigation on colorectal cancer fecal miRNA is a hot spot to explore the complex molecular mechanisms and to identify CRC diagnostic miRNA markers in the future. Many studies on relationship of rectal cancer and miRNAs have revealed that miRNAs were so important in CRC oncogenesis that further mechanism needs to be elucidated. Currently, it is unknown how to massively screen and indentify the possible CRC miRNA biomarkers at full gene level, what factors regulate the abnormal expression of miRNAs or what is the function of certain miRNAs in colorectal cancer. It is also unclear what gene regulatory mechanisms and pathways are involved in CRC miRNAs in blood circulation or how to establish a suitable standardized clinical detection system for CRC miRNAs in clinic. With the establishment of colorectal cancer miRNAs mechanism, there will be a wide clinical application in colorectal cancer diagnosis, prognosis and treatment. Intervention of specific CRC miRNAs may improve the sensitivity of chemotherapy drugs for primary tumors and metastases in CRC, and help develop new types of miRNA gene therapy drug.

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Abbreviations: CRC, colorectal cancer; miRNAs, microRNAs; pri-miRNA, primary miRNA; premiRNA, precursor miRNA; RISC, RNA-induced silencing complex; cyt C, cytochrome C; TP53, tumor protein p53; MIF, macrophage migration inhibitory factor; EMT, Epithelial-mesenchymal transition; TGFβ,Transforming growth factorβ; ZEB, zinc finger binding protein; PDCD4, programmed cell death factor 4; VEGF, vascular endothelial growth factor; HIF-1,hypoxia-inducible factor 1; KRAS gene, Kirsten rat sarcoma viral oncogene homolog; Tsp1, thrombospondin-1; CTGF, connective tissue growth factor; CEA, carcinoembryonic Antigen; FOBT, fecal occult blood test; EpCAM, epithelial cell adhesion molecule antibody; DFI, disease-free interval; OS, overall survival; MSS, microsatellite stability; MSI-H, microsatellite high instability; DTL, dentate homologues; EGFR, epidermal growth factor receptor

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