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Review

microRNA biomarkers in colorectal cancer liver metastasis

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Abstract

Liver metastasis is a primary factor of prognosis and long-term survival for patients diagnosed with colorectal cancer (CRC). Colorectal cancer liver metastasis (CRCLM), is a complex biological process involving multiple factors and steps, and its mechanisms are yet to be discovered. In recent years, small noncoding RNAs, especially microRNAs (miRNAs) have been proven to play an important role in tumorigenesis, progression and metastasis in a variety of cancers, including CRC. Increasing evidence suggests that miRNAs, including those from exosomes secreted by tumor cells in circulation, could be used as promising biomarkers in early cancer detection, treatment, and prognosis. In this review, we focus on the functional roles and clinical applications of miRNAs, especially those from circulating exosomes secreted by tumor cells related to CRCLM.

Key words: Colorectal cancer (CRC), liver metastasis, miRNAs, exosome

Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide [1, 2]. CRC is typically treated by surgical resection combined with modern chemo- and radiation- therapies, with a cure rate of ~20% [3]. About 70-80% of CRC patients will develop recurrence and/or metastasis, and more than 50% of the metastatic sites are in the liver [3, 4]. Colorectal cancer liver metastasis (CRCLM) is the leading cause of deaths for CRC [5]. Therefore, one of the main research focuses is on elucidating the mechanisms of CRCLM, and identifying potential biomarkers for both prediction and management of CRCLM [6]. Better understanding of the biological process of CRCLM will facilitate the development of early diagnostic and therapeutic methods. In recent years, small non-coding RNAs (sncRNAs) have been shown to play a key role in the regulation of gene expression and are involved in tumor initiation and development. These sncRNAs include small interfering RNAs (siRNAs), microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), etc., of which miRNAs are studied most extensively and in-depth [7, 8]. In this review, we will focus on the roles and clinical applications of miRNAs in CRCLM, including those in circulating exosomes secreted by tumor cells.

miRNAs are endogenous non-coding RNAs of 20-25 nucleotides that regulate gene expression at the post-transcriptional level through interaction with 3'-untranslated regions (3'UTRs) of the target messenger RNAs (mRNAs) [9]. Each miRNA may target a number of mRNAs, and each mRNA can be regulated by multiple miRNAs. Studies have shown that miRNAs play an important regulatory role in all aspects of CRCLM, including angiogenesis, tumor

invasion, epithelial-to-mesenchymal transition (EMT), and stemness of cancer cells [10]. Recently miRNAs have been used in the diagnosis and prognosis of cancer and other diseases [11]. However, metastasis of cancers, such as CRC, is not only challenging to predict, but also has limited and ineffective treatment options. Therefore, it is crucial to discover and validate novel biomarkers that can predict CRCLM in order to improve the survival rate of patients. A number of publications demonstrated that miRNAs, either oncogenic miRNAs (OncomiRs) or tumor suppressor miRNAs, are involved in CRCLM (Table 1).

Table 1. A list of miRNAs and their validated targets involving CRCLM

miRNAs	Validated targets	Functional pathways	References
OncomiRs			
miR-19	TG2	metastasis	[12]
miR-885-5p	Cpeb2	EMT	[13]
miR-20a-5p	Smad4	EMT	[14]
miR-21	PPCD4	Proliferation, invasion	[16]
miR-155	TP53INP1	Lymph node metastasis	[16]
miR-181a	WIF-1	EMT, metastasis	[17]
miR-429	SOX2	Apoptosis	[18]
Tumor suppressors			
miR-200c	Bmi1, ZEB1, ZEB2	ERK1/2MAPK/GSK-2β/β-catenin signaling, fas signaling	[19]
miR-26a/26b	FUT4	metastasis	[20]
miR-30e-5p	ITGA6,ITGB1	Proliferation, metastasis	[21]
miR-125	HIF-12, VEGF	angiogenesis	[22]
miR-127	BCL6	differentiation	[22]
miR-145	c-Myc, N-RAS	angiogenesis	[22]
miR-194	BMP1, CDKN1B	Proliferation	[22]
miR-199a-30	HIF-1α, VEGF	Proliferation, motility, invasion	[22]
miR-15b	MTSS1, Klotho	Invasion, metastasis	[23]
miR-30a-5p	TM4SF1	EMT	[24]

Table 2. A list of miRNAs with their respective clinical applications in CRCLM

miRNAs	sample	Source of sample	Clinical application/biological function	References
miR-29a	Serum	CRCLM	Early detection	[26]
miR-126	Serum	CRC/CRCLM	Early detection	[27]
miR-141	Serum	CRC/CRCLM	Early detection	[27]
miR-21	Serum	CRC/CRCLM	Early detection	[27]
miR-29	Tissue mouse model)	CRC/CRCLM	Therapeutic target	[28]
miR-622	Tissue/cell lines	CRC/CRCLM	Therapeutic target	[29]
miR-21	Cell lines/CAM module	CRC/CRCLM	Therapeutic target	[30]
miR-655-3p	Tissue (mouse model)	CRC/CRCLM	Therapeutic target	[31]
miR-424-3p,503,1292 (combine together)	Tissue	CRC/CRCLM	Therapeutic target	[32]
let-7i	Tissue/serum	CRC/CRCLM	Prognostic	[33]
miR-10b	Tissue/serum	CRC/CRCLM	Prognostic	[34], [38]
miR-885-5p	Serum	CRC/CRCLM	Prognostic	[33]
miR-214	Tissue	CRC/CRCLM	Prognostic	[35]
miR-625	Tissue	CRC/CRCLM	Prognostic	[36]
miR-122	Tissue	CRC/CRCLM	Prognostic	[37]
miR-196b-5p	Tissue	CRC/CRCLM	Prognostic	[39]
miR-203	Serum	CRC/CRCLM	Prognostic	[40], [41]
Exo-miR-19a, 19b, 23a, 92a, 320a, 4437,19a	Serum	CRC/CRCLM	Predict liver metastasis	[45]
Exo-miR-19a	Serum	CRC/CRCLM	Prognostic	[45]
Exo-miR-7,181a-5p,192,194,375	Serum/cell culture medium	CRC/CRCLM	Inflammation, fibrosis, angiogenesis	[48]
Exo-miR-210	Tissue	CRC/CRCLM	carcinogenesis, migration	[50]
Exo-miR-193a	Tissue (mouse model)	CRC/CRCLM	Migration	[51]

Oncogenic miRNAs (OncomiRs) in CRCLM

OncomiRs, which act like oncogenes by downregulating tumor suppressors and other regulatory genes, have been found to be either amplified or overexpressed in many types of cancers, including CRC. Cellura et al. reported that miR-19, significantly overexpressed in CRC cells, acts as an oncomiR, leading to altered invasive ability by inhibiting the expression of Transglutaminase-2 (TG2). TG2 is a critical crosslinking enzyme in the extracellular matrix (ECM) and tumor microenvironment (TME) [12] and plays an important role in colorectal cancer metastasis. Another study showed that up-regulation of miR-885-5p had a significant impact on EMT and the overexpression of miR-885-5p significantly induced cell migration, invasion and liver metastasis by targeting cpeb2, which is required for cell cycle progression [13]. miR-20a-5p promotes invasion and metastasis by suppressing Smad4 in CRCLM [14], while miRNA-21 and miRNA-155 were shown to be overexpressed in many malignant solid tumors, including CRC, thoracic tumors and lung cancer [15]. Shibuya et al[16] found that expression of miR-21 and miR-155 is much higher in tumor tissue compared to the adjacent normal tissue in 156 CRC patients, further demonstrating the correlation between miRNA-21 and miRNA-155, along with their respective target genes PDCD4 and TP53INP1. Interestingly, highly expressed miRNA-21 is positively correlated with CRCLM, which is most likely due to the fact that miRNA-21 promotes tumor cell infiltration and metastasis by inhibiting PDCD4, while miR-155 is associated with distant metastasis of lymph nodes. Ji et al. [17] demonstrated that miR-181a promotes tumorigenesis and EMT by inhibiting WIF-1 in CRCLM. miR-429, as an oncomiR, was shown to inhibit apoptosis and induce EMT in CRC by targeting SOX2 [18]. Overexpression of oncomiRs in CRC may promote liver metastasis; therefore, targeting oncomiRs may open a new avenue to prevent CRCLM and improve survival rate of CRC patients.

Tumor suppressor miRNAs in CRCLM

Tumor suppressor miRNAs function by targeting genes with oncogenic activity. Inactivation or downregulation of tumor suppressor miRNAs was widely reported in many types of cancers including CRC. Bmi1 plays an essential role in Fas-signaling induced stem cell properties in CRC specimens. It was shown that Fas ligand treatment promoted Bmi1 expression by inhibiting miR-200c, a tumor suppressor miRNA [19]. Li et al. [20] discovered that forced expression of miR-26a and miR-26b not only modulates migratory behavior of CRC cells but also inhibits FUT4 expression in CRC cells, resulting in tumor progression. Laudato et al. [21] found that miR-30e-5p inhibits CRCLM by directly targeting both ITG6 and TGF-B1. Another study showed that there are 939 human miRNAs related to CRCLM. Among them, miR-125, miR-127, miR-145, miR-194 and miR-199a-30 act as tumor suppressors and could serve as biomarkers for early diagnosis of CRCLM [22]. Two other recent studies demonstrated that miR-15b inhibits CRCLM by regulating MTSS1 and Klotho [23], while miRNA-30a-5p (miR-30a) regulates cell motility and EMT by directly targeting oncogenic protein TM4SF1 in CRCLM [24]. Downregulation of tumor suppressor miRNAs may lead to CRCLM and detection of these miRNAs may help manage the progression of CRCLM.

miRNA biomarkers CRCLM

It is crucial to identify and validate novel biomarkers that can predict CRCLM in order to improve patients' survival. Many miRNAs are associated with early diagnosis and staging of CRCLM as discussed. Therefore, miRNAs can be used as biomarkers for early CRCLM detection [25], particularly circulating miRNAs given their minimal invasiveness and easy accessibility. One study showed that miR-29a is significantly elevated in the serum of CRCLM patients compared to those without metastasis [26]. In fact, miR-29a can discriminate CRCLM patients from non-metastatic CRC patients with 75% sensitivity and specificity, which is more sensitive than serum CEA, with only 60% sensitivity and 34% specificity. Therefore, serum miR-29a could be a biomarker for CRCLM early detection. In a study of 224 serum samples, Yin et al[27] evaluated the expression of 11 metastasis-associated miRNAs in 116 localized CRC, 72 synchronous liver-metastatic CRC and 36 other organ-metastatic CRC. They found that the expression of serum miR-126, miR-141 and miR-21 in liver metastatic CRC were significantly different from that in localized CRC. Therefore, these three miRNAs may be used as novel biomarkers for the clinical diagnosis or prediction of early stage CRCLM. With the identification and validation of more circulating miRNA markers, early detection methods using miRNAs / miRNA signatures may be developed for clinical use in CRCLM.

Therapeutic potentials of miRNAs

It is a new strategy to treat CRCLM by targeting miRNAs, or in combination with other well-established strategies. Clinically, high expression

of miR-29 may be predictive for the development of CRCLM. APOBEC3G, involved in CRCLM in a mouse model, was modulated by miR-29-mediated suppression of matrix metalloproteinase-2 (MMP2) to promote CRCLM [28]. Therefore, miR-29 may serve not only as a predictive marker for CRCLM, but could also be a therapeutic target. miR-622, which is overexpressed in CRC tissues and cell lines, affects metastasis and invasion of CRC cells by regulating DYRK2 and its downstream signaling pathways[29]. Nedaeinia et al. [30] transfected LS174T human colorectal adenocarcinoma cells with locked nucleic acid (LNA)-anti-miR-21 *in vitro* to determine the effect of miR-21 in cell growth and colony formation. At the same time, they constructed the LS174T cell chick embryo chorioallantoic membrane (CAM) module. The results showed that the LNA-anti-miR-21 in the CAM module inhibits the expression of colorectal cancer LS174T cells by regulating the PDCD4 gene, thereby reducing cell migration and liver metastasis. This provides new ideas for the development of novel miRNA therapeutics and a new direction for the treatment of CRCLM. One recent study [31] showed that an oligometastatic miR-655-3p was successfully delivered to metastasized liver using nanoscale coordination polymers (NCPs) for a targeted and prolonged distribution of miRNAs. miRNA-655-3p suppressed the tumor growth when co-delivered with oxaliplatin, suggesting additive or synergistic interactions between miRNA and platinum drugs. This was the first time the researchers systemically administered nanoparticles to deliver an oligometastatic miRNA into advanced metastatic hepatic tumors and demonstrated their tumor-suppressive effects. Their findings suggest a potential therapeutic strategy that combines tumor-suppressive miRNAs with conventional cytotoxic chemotherapies for the treatment of CRCLM. However, a single miRNA for CRCLM therapeutic target may not be effective as expected. According to Torres et al. [32], there are 38 miRNAs that were differentially expressed between highly metastatic (KM12SM/SW620) and poorly metastatic (KM12C/SW480) CRC cell lines. They found that three miRNAs (miR-424-3p, miR-503 and miR-1292) were overexpressed in both metastatic CRC cell lines and patient samples. Down-regulation of these three miRNAs shared same targets, CKB and UBA2, which increased cell adhesion and proliferation in CRC cells. This three-miRNA panel along with their two targets may constitute therapeutic biomarkers for CRCLM, suggesting that targeting multiple miRNAs may be a more efficient therapeutic strategy.

Prognostic values of miRNAs

It is suggested that miRNAs can not only assess the risks of tumor metastasis and recurrence, but also evaluate the responses of therapeutics and predict the chemo-resistance to specific drugs in CRCLM. Hur et al. [33] experimentally verified a number of miRNAs specifically associated with CRCLM in tissue and serum, which could be used to determine the prognosis. They found twenty-three miRNAs that are aberrantly expressed by comparing CRC with- and without- metastasis. Among them, four miRNAs (let-7i, miR-10b, miR-221 and miR-320a) were down-regulated in the liver metastasis group, and one (miR-885-5p) was up-regulated in the non-metastatic CRC group. It is suggested that low let-7i expression predicts possible distant metastasis and worse prognosis, whereas high miR-10b expression indicates a high incidence of distant metastasis [34]. High expression of miRNA-885-5p in serum is associated with lymph node and distant metastasis. The results were subsequently validated by RNA *in situ* nucleic acid hybridization. A similar study [35] revealed that miR-214 was downregulated in CRCLM, which is associated with the increased expression of FGFR-1, a target of miR-214. The data suggested that miR-214 could be used as a biomarker for poor prognosis of CRCLM patients. Lou et al. [36] found that miR-625 was significantly down-regulated in CRC tissues and cell lines, which is associated with lymph node metastasis and liver metastasis. Both *in vitro* and *in vivo* experiments demonstrated that the expression of ectopic miR-625 inhibited the invasion and metastasis of the HCT116 CRC cell line. At the same time, low miR-625 expression is associated with low survival rate, indicating that miR-625 can be used as an indicator of poor prognosis of CRC patients. miRNA-122 [37] was reported to be differentially expressed in CRCLM clinical specimens by laser capture microdissection (LCM). As a target of miR-122, cationic amino acid transporter 1 (CAT1) is expressed significantly lower in CRCLM compared to that in CRC. The analysis of 132 primary tumors revealed that the expression level of CAT1 was negatively correlated with liver metastases, and the overexpression of miRNA-122 was associated with downregulated CAT1 expression. Taken together, these data suggest that CAT1 and miR-122 could predict the prognosis of liver metastasis in CRC patients. According to some other recent studies, miR-10b [38], miR-196b-5p[39] and miR-203[40, 41] are independent risk factors for overall survival in CRC patients with liver metastases. Despite the progress being made, there is much more work that needs to be done in both animal models and clinical trials in order

to uncover the full potential of miRNA biomarkers in CRCLM.

Circulating exosomal miRNAs in CRCLM

Exosomes are associated with cancer progression and can act in a paracrine or endocrine manner to affect cell behaviors [42]. With the progression of exosome studies in recent years, especially the discovery of the intercellular communications through exosomes and other extracellular vesicles, the potential clinical application of miRNAs and/or proteins from secreted exosomes has generated a lot of interest in the cancer research field. An exosome is a type of lipid nanoparticle with a diameter of 30-100 nm that are released and secreted by various living cells, containing internal proteins, mRNAs, miRNAs and other substances [43]. They can be transmitted in the circulation system and eventually absorbed by other cells. Cancer-secreted exosomes influence the tumor microenvironment and affects the growth and metastasis of cancer cells. Exosomes are a newly discovered communication medium for intercellular transporters and nucleic acid substances. They are involved in a variety of cellular processes, such as intercellular communication, cell migration, angiogenesis and immune regulation. Compared to direct cell contact or secretory factors, exosomes are more targeted and can affect the function of recipient cells more efficiently. They play an important role in cancer, infectious diseases and neurodegenerative diseases. Currently, there are no standard agency-approved, exosome-based diagnostic, therapeutic, or prognostic clinical tests for CRCLM. miRNAs contained in exosomes derived from CRC cells are closely related to oncogenes and tumor metastasis-related genes, suggesting that they may be related to CRCLM, and play a role in the initiation and development of CRCLM [44].

Compared to the general population, cancer patients have larger numbers of circulating exosomes [45]. Therefore, circulating exosomal miRNAs may be used for diagnosis or prognosis for CRC patients. Reliable blood test could be a perfect companion tool for routine imaging examination of CRCLM patients or for early detection of CRCLM. Exosomal miRNAs are considered the main source of circulating miRNAs isolated from serum or plasma [46]. Currently, there are a few studies showing the analysis of circulating exosomal miRNAs in early diagnosis of CRCLM. Matsumura et al. [44] analyzed the expression of exosomal miRNAs in 227 serum samples from CRC patients. Compared with patients without recurrence, expression of 18 exosomal miRNAs was increased while 46 exosomal miRNAs decreased. Among them, six exosomal miRNAs (miR-19a, miR-19b, miR-23a, miR-92a, miR-320a and miR-4437) were associated with the development of liver metastasis, rendering them to be potential biomarkers for CRCLM early detection. Further studies suggested that the CRC patients with high level of exosomal miR-19a expression were associated with poor prognosis compared to the low expression group. This may indicate that the exosomal miR-19a could be a potential marker of prognosis in CRCLM.

At present, there are a few studies that focus on the clinical applications of circulating exosomal miRNAs in CRCLM. The stability and reproducibility of exosomal miRNA detection and its independent features require more research to be evaluated and validated. Hence, the potential clinical impact on CRCLM cannot be ignored. As the research technology matures and deepens, the detection of circulating exosomal miRNAs may become a very promising field for identifying CRCLM biomarkers.

In recent studies, the functional studies in cancer cell lines or xenograft models have revealed the mechanisms of exosomal miRNAs in cancer cells along with another cell type of TME in supporting or inhibiting tumor growth, transferring drug resistance, and preparing for the metastatic niche [47].

Based on the study by Fu et al. [48], there are 25 miRNAs being up-regulated and 5 miRNAs being down-regulated in exosomes purified from SW620 culture supernatant. Candidate miRNAs were further evaluated for CRC diagnosis by using qRT-PCR. High expression levels of circulating exosomal miR-17-5p and miR-92a-3p were associated with the stages of CRC patients, indicating that these exosomal miRNAs could be biomarkers for CRC and / or CRCLM. The data further showed that exosomal miR-7, miR-181a-5p, miR-192, miR-194, and miR-375, which are related to multiple biofunctions such as inflammation, fibrosis, and angiogenesis, may also play an important role in CRCLM.

Valcz et al. [49] detected the expression of the Alix protein, which are stably present in exosomes, from normal intestinal epithelial tissue, adenoma tissue and colorectal cancer tissue. They discovered that exosomes play an important role in promoting the carcinogenesis of colonic epithelium and migration. Recent studies show that the expression of exosomal miR-210 was significantly higher compared with its intracellular levels in adherently growing HCT-8 cells, and correlated to anoikis resistance and EMT markers. Exosomes can induce cancerous growth of normal epithelial cells by promoting EMT through miRNA-210 [50] and can affect the adhesion and migration of CRC cells.

Teng et al. [51] examined different exosomal miRNA expression profiles in primary mouse colon

tumor, CRCLM and naïve colon tissues. They found that exosomal miR-193a interacts with major vault protein (MVP), causing cell cycle G1 arrest and cell proliferation repression by targeting Caprin1. This suggests that MVP-mediated selective sorting of tumor suppressor miRNA into exosomes promotes tumor progression and liver migration.

We are still in the early stages of exosomal research in CRCLM. Since exosomes and exosomal miRNAs can be detected from patients non-invasively through blood, urine or other body fluids, these microvesicles could be potential sources of diagnostic, prognostic or predictive biomarkers of CRCLM. It is likely that the studies focusing on circulating exosomal miRNAs analysis in patient blood, urine or other body as liquid biopsy research are a step forward towards precision medicine.

Discussion and conclusions

Liver metastasis is an important biological feature of CRC, and it is also an important factor of recurrence and poor prognosis of CRC patients. Combining molecular and traditional treatments may lead to an improved Overall Survival (OS), a reduction in intrahepatic recurrence, and a decreased toxicity of perioperative therapies. The process of liver metastasis of CRC depends on multiple complex interactions between cancer cells in the tumor and host-derived cells in the microenvironment in both the primary tumor and secondary organs. In contrast to the complex and diverse oncogenes and tumor suppressor genes involved in cancer, miRNAs with tumor promoting or tumor suppressing effects have many more advantages [52]. Identification of miRNAs, including exosomal miRNAs, involved in liver metastasis could lead to the development of more accurate detection methods for early diagnosis, treatment and prognosis of CRCLM. With the continued improvements of miRNA expression profiling, and the development of more stable, effective and low-toxic miRNA mimics and inhibitors, the number of drugs targeting miRNAs has increased at much faster pace. It seems that miRNA-based therapeutics as adjuvant tools of targets will be realized in the near future once we overcome the technical limitations. Although the research on miRNAs especially the exosomal miRNAs in CRCLM are full of challenges, the prospects are still very broad.

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Conflicts of Interest

The authors declare that they have no competing interests.

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