

Clinical Utility of Exosomal Micrnas for Colorectal Cancer

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Abbreviations:

miRNAs: microRNAs; CRC: Colorectal Cancer; CEA: Carcinoembryonic Antigen; ILVs: Intracavitary Vesicles; MVES: Multivesicular Endosomes; MWCO: Molecular Weight Cutoffs; EMT: Epithelial-Mesenchymal Transformation; RFS: Recurrence-Free Survival; OS: Overall Survival; LARC: Locally Advanced Rectal Cancer

1. Abstract

Exosomes are nanometer-sized membrane vesicles secreted by normal or cancer cells, which participate in intercellular communication by transporting RNAs and proteins. Recent studies have found that some differentially expressed microRNAs (miRNAs) in exosomes play key roles in the initiation and development of Colorectal Cancer (CRC) and are potential candidates for CRC detection. In this review, we describe the role of exosomal miRNAs in the development of CRC. We also briefly summarize the methods used for exosome extraction and enrichment. Finally, we examine the potential roles of exosomal miRNAs as biomarkers for the diagnosis of and prognostic assessment of CRC, as well as novel molecules for targeted therapy.

2. Introduction

Colorectal Cancer (CRC) is the third most frequently diagnosed cancer worldwide, with more than one million new cases diagnosed worldwide each year, and the mortality rate ranks second among malignant tumors [1]. In China, CRC is considered to be a gastrointestinal malignancy, and is one of the malignancies with the fastest rising incidence rates [2]. Due to the lack of symptoms in the early stage and that fact that it can rapidly metastasize in the advanced stage,

CRC is associated with a low detection efficiency and high mortality. This high incidence, poor detection, and unfavorable prognosis make it important to better understand the mechanisms underlying the development and progression of CRC and to identify ideal biomarkers for the early diagnosis of CRC [3, 4].

Given the importance of early detection, several strategies have been used for patient screening, primarily based on colonoscopy, fecal occult blood tests, serum Carcinoembryonic Antigen (CEA) and CA 19-9 levels, and sigmoidoscopy. Obtaining tissue biopsies is sometimes possible during colonoscopy or sigmoidoscopy, but this is limited by the location and size of the tumor, and biopsy sampling may require surgical intervention [5]. Compared with tissue samples, liquid-derived substances are easier to obtain, making it easier to conduct large-scale clinical trials for comparison [6]. Although serum tumor markers offer some diagnostic promise, their performance so far has been less than ideal [7,8].

Exosomes are 50-150 nm extracellular vesicles that can be isolated from diverse bodily fluids, including blood, urine, saliva, breast milk, amniotic fluid, ascites, cerebrospinal fluid, bile, and semen [9]. The level of exosomes is closely related to the environment of cells and

can reflect the health of the body to a certain extent. As tumor volume increases, cell renewal usually increases. Therefore, the exosome levels in cancer patients are much higher than in healthy individuals [10]. Exosomes that have escaped from CRC into the circulation were previously able to be detected in 'liquid biopsies', and might be able to characterize the tumor and help in follow-up monitoring [11]. Recently, some reports have shown that tumor exosomal miRNAs play a driving role in tumor metastasis [12,13]. Thus, monitoring exosomal miRNAs may be useful to diagnose and manage cancer patients, and these miRNAs may represent a novel target for cancer therapy [14]. In the present review, we focus on the potential clinical utility of exosomal miRNAs as key components of liquid biopsies for CRC.

3. The Origin and Function of Exosomes

Exosomes were originally thought to be waste products secreted by cells [15]. In fact, they contain a variety of substances that can mediate signal transduction, and play an important role in cell-to-cell communication [16,17]. Exosomes are Intracavitary Vesicles (ILVs) that bud from the endosome membrane during the Maturation of Multivesicular Endosomes (MVES). They are intermediate products of the endosome system and are secreted after the fusion of MVES with the cell surface [18,19]. The transfer of exosomes from cancer cells to other cells in the tumor microenvironment can promote or inhibit cancer-related proliferation, and may also affect metastasis [20,21]. As a carrier of various molecules, exosomes can protect their

contents from degradation and can also deliver many different signals at the same time [19].

4. Isolation of Exosomes

Since exosomes are not concentrated in body fluids, isolation of high-purity exosomes is a necessary prerequisite for all related research. Isolation procedures include ultracentrifugation, co-precipitation-based separation, ultrafiltration, and immunoprecipitation. The methodologies currently used to isolate exosomes are shown in (Table 1). Ultracentrifugation, which can be divided into differential centrifugation and density gradient centrifugation, is the most widely used extraction method and the gold standard for exosome separation and purification at present [22]. The co-precipitation method mainly uses polyethylene glycol to capture exosomes and sediment them, and then the exosomes are separated by low-speed centrifugation [23]. At present, most commercial exosome extraction kits are based on this principle. The filtration method uses a series of ultrafiltration membranes with different Molecular Weight Cutoffs (MWCO) to filter the sample, and gradually separates exosomes from samples containing a large number of proteins and other biological macromolecules according to their size [24, 25]. Currently, ultracentrifugation results in high-purity exosomes, but it is associated with high costs, cumbersome steps, is time-consuming, and can affect the structure of exosomes, which is not conducive to subsequent experiments.

Table 1: Methodologies used for isolating exosomes

Method	Advantage(s)	Disadvantage(s)
Ultracentrifugation	High separation purity	High cost, tedious operation, time-consuming, affects the exosome structure
Co-precipitation-based separation	Easy to perform, can be performed using small sample volumes	Low separation purity, contamination of exosomes
Ultrafiltration	Easy to perform	Low separation purity, affects the exosome structure
Immunoprecipitation	High separation specificity and purity	High cost, not widely available

5. Clinical Application of Exosomes in CRC

Exosomes contain a variety of biomolecules, including proteins, DNA, and RNA [26], which are released from the tumor microenvironment. These may promote cancer progression at critical stages of the process through cell-to-cell communication with distant cells [27]. It appears that miRNAs may be of particular significance as biomarkers, and may be useful for both the diagnosis and prognostic monitoring of CRC. Additionally, these miRNAs may represent novel targets for CRC prevention or therapy.

5.1. Diagnostic Biomarkers

In view of the fact that tumor cells selectively package specific molecules into exosomes, which are released into body fluids, they likely play a role in the recipient cells. Because exosomes are detectable both in the circulation and are stored within cells, and because they accurately reflect the characteristics of their cells of origin, researchers have proposed that circulating exosomes may provide effective tools for the non-invasive diagnosis and prognostic determination of

human cancers. Moreover, several reports on CRC have also revealed that specific circulating exosomes in the plasma of patients are related to a poor prognosis and can be reliable biomarkers for the diagnosis of this cancer [27]. The major studies related to the functions of different miRNAs in CRC - derived exosomes are summarized in (Table 2).

Exosomes have been targeted as potential biomarkers for the diagnosis of CRC at initial stages due to their wide availability and the high specificity of certain molecules to CRC [28]. According to the literature, in 102 serum samples (90 from patients with CRC and 12 from healthy volunteers) miR-19a and miR-92a were significantly increased in both early and advanced stages of CRC patients compared with normal controls [29]. In an analysis of plasma samples from 159 cancer patients and 50 healthy controls, researchers found that miR-483-5p was up-regulated in the plasma exosomes of stage I CRC patients, and the same was true in stage II and IV CRC patients. miR-1246 was up-regulated in stage II, III and IV CRC patients. These

two may be useful as broad biomarkers for CRC. Several exosomal miRNAs were found to be up-regulated only in advanced CRC patients, such as let-7b-3p, miR-27a-5p, miR-182-5p, miR-192-5p and miR-486-5p [30]. The exosomal miR-6803-5p level was significantly increased in serum samples from patients with CRC (AUC=0.7399), particularly those at later TNM stages or with lymph node and/or liver metastasis [31]. Compared with the healthy controls, higher levels of miR-193a, miR-126 and miR-148a and lower levels of miR-196b were found in the exosomes isolated from the peripheral plasma of colon cancer patients, and this phenomenon was also noted in colon cancer patients with liver metastasis [32]. In a trial that prospectively followed colon cancer patients for 6 months after the initial diagnosis, patients with high levels of exosomal miR-193a in the peripheral blood were at higher risk of metastases [32].

Research has also focused on using panels of exosomal miRNAs, whereby the simultaneous measurement of multiple exosomal miRNAs might offer improved diagnostic accuracy compared to any lone exosomal miRNA. A four-miRNA panel (high expression levels of miR-19a-3p, miR-223-3p and miR-92a-3p, low expression of miR-

422a) with high diagnostic accuracy for CRC (AUC = 0.810) could differentiate stage I/II CRC patients from controls, where the AUCs of these four individual miRNAs were 0.849, 0.871, 0.890 and 0.843, respectively [33]. The serum exosomal levels of seven miRNAs (let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a) were significantly higher in primary CRC patients than in healthy controls, with significant increases observed even for the early stage (TNM stage I) CRC samples [34]. Among these markers, miR - 21 was significantly overexpressed (five-fold) in the exosomes derived from patients with colon cancer, particularly in patients at the time of their initial diagnosis or before any treatments. Decreased expression of miR-4772 was significantly associated with the recurrence of CRC [35]. The exosomal miR-17-92a cluster expression level in serum was correlated with the recurrence of CRC [24]. Although a single miRNA is not likely to be sufficiently sensitive or specific for diagnosing or monitoring CRC alone, these results suggest that the combination of a panel of serum exosomal miRNAs may be useful for the detection of CRCs or for monitoring for disease recurrence or progression.

Table 2: The functions of different miRNAs in CRC - derived exosomes

Name	Source	Expression	Function	Reference
miR-19a	Serum	Upregulated	Potential biomarker for both early and advanced stages of CRC	29
miR-92a	Serum	Upregulated	Potential biomarker for both early and advanced stages of CRC	29
miR-483-5p	Plasma	Upregulated	Potential biomarker for stage I, II and IV CRC	30
miR-1246	Plasma	Upregulated	Potential biomarker for stage II, III and IV CRC	30
miR-6803-5p	Serum	Upregulated	Potential biomarker for post-TNM stage or lymph node metastasis and liver metastasis	31
miR-193a, miR-126 and miR-148a	Plasma	Upregulated	Potential biomarker for CRC with liver metastasis	32
miR-196b	Plasma	Downregulated	Potential biomarker for CRC with liver metastasis	32
miR-19a-3p, miR-223-3p and miR-92a-3p	Serum	Upregulated	Potential biomarker for stage I/II CRC	33
miR-422a	Serum	Downregulated	Potential biomarker for stage I/II CRC	33
let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a	Serum	Upregulated	Potential biomarker for primary CRC, especially in the early stage (TNM stage I)	34
miR - 21	Serum	Upregulated	Potential biomarker for the initial diagnosis or before any treatments	35,36
miR-4772	Serum	Upregulated	Correlated with the recurrence of CRC	35
miR-17-92a	Serum	Upregulated	Correlated with the recurrence of CRC	29

5.2. Prognostic Biomarkers

There is also evidence the detection of exosomal molecules levels could have strong prognostic value in CRC, and the findings are directly related to the disease burden. For example, elevated exosomal miR-130a was associated with a poorer 5-year survival [36] and miR-30 was associated with the metastatic progression of CRC [35]. A group of CRC patients with high exosomal miR-19a expression showed a poorer survival than the low expression group, and the disease-free survival rate was significantly lower in patients with high exosomal miR-19a expression [29]. A study of plasma samples from

patients with CRC showed that miR - 21, as a CRC - derived exosomal miRNA, can promote cell proliferation and progression and can also induce CRC metastasis [37]. These results were consistent with findings using SW480 and WiDr cell lines in vitro [37]. High expression of miR-106b-3p in exosomes from highly metastatic CRC cells promoted cell migration, invasion and the Epithelial-Mesenchymal Transformation (EMT), which was correlated with a poor prognosis and an increased rate of lung metastasis of CRC cells in vivo [38]. Exosomal miR - 548c - 5p significantly inhibited the proliferation of HCT116 cells, while a univariate analysis revealed that decreased lev-

els of serum exosomal miR - 548c - 5p were related to poorer overall survival in patients with CRC (HR = 3.69, 95% CI, 1.14 - 11.88; P = 0.029) [39]. The downregulation of exosomal miR - 548c - 5p in serum predicted a poor prognosis in patients with CRC [39]. Another study showed a strong association between a high level of exosomal miR-6803-5p and the Recurrence-Free Survival (RFS) and Overall Survival (OS) in patients with CRC irrespective of age, sex, TNM stage, and lymph node or liver metastasis [31]. Low plasma levels of exosomal miR-486-5p and miR-181a-5p were associated with organ-invasive primary tumors (p = 0.029) and lymph node metastases (p = 0.024), respectively, both attributes of an adverse prognosis of Locally Advanced Rectal Cancer (LARC) [40]. CRC patients with low miR-548c-5p expression in serum exosomes had poorer outcomes, lower Overall Survival (OS) (P = 0.008) and poorer disease-free survival (P = 0.007) than those with increased expression [41]. The CRC patients with high exosomal miR-19a expression showed poorer prognoses than the low expression group (P < 0.001) [42]. Together, these data suggest that exosomal miRNAs can be used as prognostic biomarkers for CRC.

5.3. Targeting Exosomal miRNAs for the Treatment of CRC

In addition to their potential applications as diagnostic or prognostic biomarkers, exosomal miRNAs have also been suggested as therapeutic targets for several cancers. The wide variety of miRNAs carried by exosomes suggests that they likely affect diverse processes ranging from immune surveillance to cell metabolism to the response to cancer therapy [43-45].

In one study, exosomes were extracted from colorectal cancer cells (CT26) to treat a nude mouse model of colorectal cancer. The rate of tumor growth in the exosome-treated group significantly decreased, and the flow cytometry results showed a significant reduction in the spleen regulatory T cell (Treg) counts of the exosome-treated group compared with the untreated group [46]. Therefore, tumor-derived exosomes can be used as potential targets for cancer immunotherapy. In another study, the overexpression of miR-96-5p and miR-149 significantly decreased the secretion of Glypican-1+ exosomes from colorectal cancer cells and xenograft tumors, decreasing the cell viability and increasing cell apoptosis, and inhibiting the growth of xenograft tumors [47].

Studies have also shown that exosomes have antigen - presenting capability, which suggests that they may represent an ideal vehicle for cancer immunotherapy [48]. For example, miR-128-3p delivery via exosomes up-regulated the E-cadherin levels and inhibited oxaliplatin-induced epithelial-mesenchymal transition by suppressing Bmi1 expression in chemoresistant cells, thus enhancing the chemosensitivity of CRC [49].

However, there are many challenges and complications associated with exosome - based approaches for cancer therapy, and more studies are needed to improve the quality and accuracy of these types of treatments.

6. Future Prospects and Conclusions

There has been increasing interest in the role of exosomes in the pathogenesis of CRC. Exosomes can dynamically reflect the status of living cells, and tumor cells secrete more exosomes per day than normal cells, suggesting that tumor-derived exosome represent a practical target. However, as mentioned above, most exosome purification schemes currently lack standardization and optimization, which is problematic for exosomal research and a major challenge for clinical diagnostics. While it is clear that many exosomal miRNAs are involved in CRC, their effects are complex [50], and the miRNAs may have different effects based on the specific context. Given the increasing popularity of research in the field, the next few years will hopefully yield more information that can help to clarify the specific roles of the different miRNAs in CRC initiation, progression, metastasis, and drug resistance, leading to applications in the diagnosis and treatment of CRC.

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