

Circulating exosomal miRNAs as potential biomarkers for Barrett's esophagus and esophageal adenocarcinoma

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Abstract

Exosomes, a class of extracellular vesicles, are small membrane-bound vesicles derived from almost all cell types that can play important roles in intercellular communication. Exosomes contain proteins, lipids, and nucleic acids that are obtained from the parental cells and participate in various pathophysiological processes, including cell growth, migration, inflammation, immune regulation, and tumor pathogenesis. Moreover, exosomes might be applied in clinical settings, such as diagnosis, treatment, and outcome prediction of diseases, including various cancers. The incidence rates of Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) have increased in recent decades, and studies have proposed specific factors that may contribute to the development and progression of these diseases. However, how exosomes play a role in this pathological process needs to be clarified. Studies have identified candidate microRNAs (miRNAs) that might be related to BE/EAC. Further studies are needed to ascertain whether circulating exosomal miRNAs are altered before or after disease onset, which could also help understand the pathophysiology of and find potential targets for prevention, diagnosis, and therapy in BE/EAC. This review summarizes recent findings on the features of circulating exosomal miRNAs in BE/EAC, which could be valuable for the early diagnosis, therapeutic approaches, and outcome prediction of BE/EAC.

Key words: Barrett's esophagus; Esophageal adenocarcinoma; Biomarkers; Exosomes; Circulation; MicroRNA

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Core tip: Barrett's esophagus (BE) is the only recognized precursor of esophageal adenocarcinoma (EAC), which is a common malignancy with a poor prognosis. Exosomes contain proteins, lipids, and nucleic acids and participate in various pathophysiological processes. The role of exosomes in BE/EAC progression cannot be ignored. Current studies on circulating exosomal microRNAs (miRNAs) in BE/EAC have mainly focused on miRNA profiling, and little is known about the associations between circulating exosomes and the pathogenesis of BE/EAC. This review focuses on the features of circulating exosomal miRNAs in BE/EAC, which might be potential biomarkers and play a causative role in BE/EAC pathogenesis.

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INTRODUCTION

Molecules performing specific functions are frequently separated and compartmentalized into organelles, and the components of organelles are exchanged dynamically with the rest of the cell to perform their physiological functions^[1]. The majority of organelles stay within the cell, while others, such as exosomes, are secreted into extracellular spaces. Exosomes, a class of membrane-bound extracellular vesicles, are released from their parental cell membrane by shedding or budding spontaneously^[2]. Exosomes are approximately 30-100 nm in diameter and can only be revealed by electron microscopy by their "cup shaped" morphology^[3]. Exosome secretion is a general phenomenon, and exosomes can be found in blood as well as other biological fluids^[4-9], such as saliva, breast milk, urine, and semen^[10-13]. Since differential ultracentrifugation enabled exosome isolation^[14], studies about exosomal components have appeared, and exosomes have already become an attractive topic in medical research. Differences in exosomal content have greatly facilitate their characterizations, indicating heterogeneity within the exosome population^[2,15]. Exosomes contain a set of proteins, lipids, and nucleic acids, such as mRNAs and noncoding RNAs (ncRNAs) (Figure 1)^[16]. Exosomal protein compositions vary depending on the cell origins and unique tissue types. However, a set of proteins were identified that were present or enriched in exosomes despite cell origins and tissue types, such as tetraspanins (CD9, CD63, and CD81), integrins, and adhesion molecules^[17]. The protein analytic approaches include Western blot, flow cytometry, and global proteomic analysis^[17]. Moreover, some metabolic enzymes, ribosomal proteins, and transmembrane and signal transduction molecules could also be detected in exosomes. Specific ligands present on the exosomal membrane could be recognized by the corresponding receptors^[2]; thus, exosomes could fuse with the recipient cell membrane directly and release their inner contents into the cytoplasm. However, not all cells internalize exosomes but instead interact with surface molecules to deliver signals as another communication method^[18]. The RNA molecules in exosomes are also cell-specific and tissue-specific. As a type of small ncRNA, microRNAs (miRNAs) can interact with the mRNA 3'-untranslated region *via* their seed sequence and then interfere with the expression of target genes post-transcriptionally to regulate physiological and pathological processes (Figure 2)^[19-24]. Thus, exosomes could carry and transfer miRNAs into recipient cells, and the transferred miRNAs may play a role in cell biological activities, such as cell proliferation, differentiation, migration, and apoptosis^[13,19-21]. Therefore, exosomes could regulate the functions of neighboring cells and distant cells by means of autocrine, paracrine, and endocrine mechanisms^[25], as a novel communication method between cells^[26].

The presence of exosomes in various biological fluids makes them easily accessible. Tumor cells have been found to release exosomes into the circulation and other biological fluids, and the levels of tumor-derived exosomes in cancer patients were higher than those in healthy controls^[27,28]. Taverna *et al*^[28] showed that exosomes play an important role in non-small cell lung cancer, especially exosomal miRNAs in lung

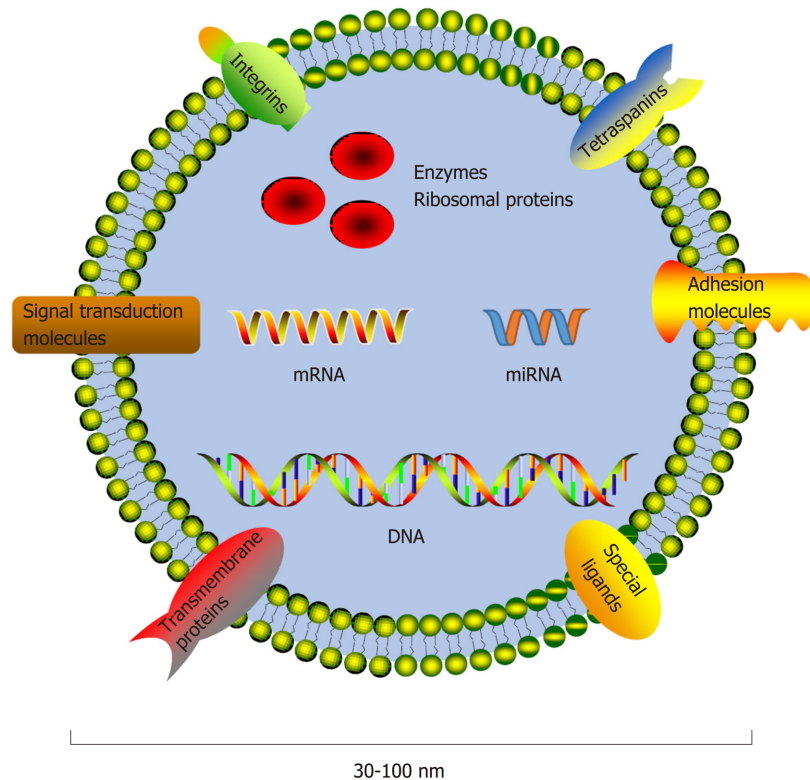


Figure 1 Components of exosomes. Exosomes are approximately 30-100 nm in diameter and contain a set of proteins, lipids, and nucleic acids. Exosomal protein compositions vary and include a series of common proteins that have been demonstrated to be present or enriched in exosomes in spite of cell origins and tissues, such as tetraspanins (CD9, CD63, and CD81), integrins, and adhesion molecules. Some metabolic enzymes, ribosomal proteins, transmembrane and signal transduction molecules, and special ligands could also be detected. miRNA: microRNA.

cancer diagnosis and prognosis^[29]. Moreover, cancer-specific exosomal miRNAs in ovarian cancer patients were detected, and the profiles varied between different disease stages^[30]. In other words, exosomes may have potential effects on disease pathology and represent a new potent noninvasive tool for disease diagnosis^[29,31-38], while their genetic content, such as mRNAs and miRNAs, allows easy screening for genetic markers of diseases. Therefore, more studies on the extensive characterizations of exosomal RNAs should be conducted in different disease settings, as well as in healthy human controls.

Esophageal cancer is a common malignancy worldwide, and esophageal adenocarcinoma (EAC) has experienced an incredibly rapidly increasing incidence in recent decades^[39-41]. The five-year survival rate of EAC patients is less than 20% because of the advanced stages when patients are first diagnosed with the disease^[42,43]. The incidence of EAC developing from Barrett's esophagus (BE) is much higher than that in the general population^[44,45]. The only well-recognized precursor of EAC^[46], BE, is defined as a metaplastic condition in which the stratified squamous epithelium lining of the lower esophagus is replaced by a columnar epithelium^[47]. However, the underlying mechanisms of the pathogenesis and carcinogenesis for the progression from BE to EAC have not yet been clarified^[48]. Thus, BE has become a focus of attention. Many researchers have shown that specific factors can contribute to the development of BE/EAC, and these identified risks include but are not limited to gastroesophageal reflux (GER), male gender, aging, obesity, tobacco consumption, and *Helicobacter pylori* eradication^[42,49,50]. Of note, studies on BE pathogenesis could provide approaches to prevent EAC development, and even inhibit carcinogenesis^[51]. However, preventive strategies are lacking. The roles of exosomes and exosomal miRNAs in BE/EAC progression cannot be ignored. Although studies of associations between miRNAs and BE/EAC are increasing, little is known about the relationships between circulating exosomes and the pathogenesis of BE/EAC. This review will focus on the features of circulating exosomal miRNAs in patients with BE/EAC, which could have the potential to promote disease development and be used as noninvasive biomarkers to help improve early diagnosis, therapeutic approaches, and outcome prediction in BE/EAC.

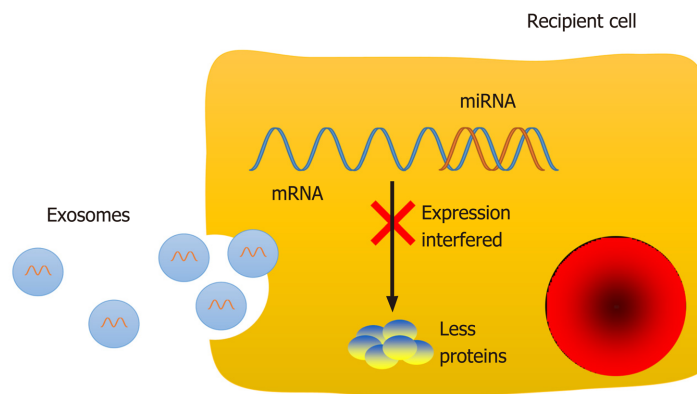


Figure 2 Function of exosomal microRNAs in recipient cells. The RNA molecules in exosomes are cell-specific and tissue-specific. MicroRNAs (miRNAs) can interact with the mRNA 3'-untranslated region *via* their seed sequence and then interfere with the expression of target genes post-transcriptionally to regulate physiological and pathological processes, such as cell proliferation, differentiation, migration, and apoptosis. miRNA: microRNA.

FUNCTIONAL ATTRIBUTES OF CIRCULATING EXOSOMES

Foremost among all exosomal functions is the information transfer from parental cells to recipient cells^[26]. Cellular communication can occur in several ways, including chemical receptor-mediated interaction, direct cell-cell contact, and synapses. Communication between cells is pivotal, and interactions between tumor cells are important for progression, angiogenesis, and invasiveness. Exosomes may represent a novel and essential way of intercellular communication and interaction, as exosomes not only participate in the local environment but also function at a distance by endocrine means^[25,52]. After fusion with the cellular membrane, exosomes are released into the extracellular microenvironment and then circulate to serve as communication agents. Their surface proteins, lipids, and inner contents, including soluble factors, enzymes, DNA and RNA, are biologically active and capable of influencing the functions of recipient cells^[2,53,54]. Exosomes can interact with surface membrane molecules, fuse with the recipient cell membrane directly, or be endocytosed in a nonselective way to transfer their inner components (Figure 3). In addition, exosomes can release small aggregates or be cleaved by proteases and then be taken up *via* a phagocytic mechanism by neighboring cells or act as ligands.

In addition to protein transfer, exosomes also contribute to the intercellular transmission of genetic information in the form of miRNAs^[3,55], which are protected from degradation by ribonucleases (RNase)^[31]. Some exosomal miRNAs can be detected in parental cells, while some can only be detected in either exosomes or parental cells, which may suggest programmed sorting of miRNAs into exosomes^[31,56]. A number of studies have proposed several potential mechanisms by which exosomal miRNAs participate in human carcinogenesis through complicated interactions with the human host immune system and signaling pathways^[2,10,15]. Tumor-derived exosomes can carry immunosuppressive molecules and factors that interfere with immune cell functions and then affect the development and maturation of immune cells^[2]. Moreover, exosomes may reprogram the recipient cell functions to promote tumor progression after delivering genomic miRNAs^[2]. Circulating exosomes definitely have the potential to be noninvasive biomarkers of disease progression and exert certain effects on disease pathophysiology. Therefore, the interactions and underlying mechanisms between exosomes and various diseases should be determined.

CIRCULATING EXOSOMES AS BIOMARKERS FOR BE AND EAC

The diagnosis of BE and EAC is mainly based on gastrointestinal endoscopic procedures and histopathological examinations. To investigate tissue miRNAs for BE diagnosis, Li *et al*^[57] analyzed the miRNA expression profiles of two independent sets of patients with BE and without BE. After microarray and Nanostring nCounter assays and target prediction using the related database and microarray datasets, the increased expression of miR-192 and miR-194 attracted their attention. The authors

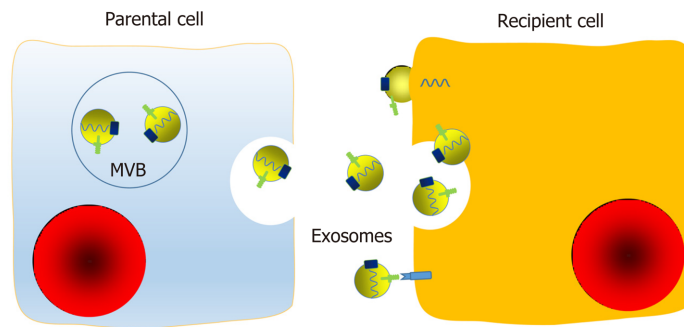


Figure 3 Mechanisms of transfer of exosomal components between cells. Exosomes are membrane-bound extracellular vesicles that are released from their parental cell membrane by shedding or budding of multivesicular bodies. Exosomes contain a set of proteins, lipids, and nucleic acids. Exosomes can interact with surface membrane molecules, fuse with the recipient cell membrane directly, or be endocytosed in a nonselective way to transfer their inner components. In addition, exosomes can release small aggregates or be cleaved by proteases, and then be taken up *via* a phagocytic mechanism by neighboring cells or act as ligands. Therefore, exosomes may regulate functions of neighboring cells and distant cells by means of autocrine, paracrine, and endocrine mechanisms, as a novel communication method between cells. MVB: Multivesicular bodies.

indicated that miR-194 negatively regulated *GRHL3* expression, which can result in the development of squamous cell carcinoma and is known to activate *PTEN* transcription^[58]. Thus, the miR-194-GRHL3-PTEN network may regulate normal esophageal cell growth and be involved in BE pathogenesis^[57]. Luzna *et al*^[59] indicated that the combination of upregulated miR-192 and miR-196a and downregulated miR-203 in esophageal tissues could be the biomarker to distinguish BE patients from the general population and independently diagnose patients with BE in spite of the fact that the histological examinations were unclear. Their data also showed that miR-196a could be considered a BE molecular biomarker, demonstrating that increased expression of miR-196a correlated with the progression from intestinal metaplasia to EAC^[59]. MiR-196a was also proposed to be a potential marker of progression from BE to EAC by Maru *et al*^[60], and they suggested that miR-196a could suppress the protein translation of *KRTS*, *SPRRZC*, and *S100Ag* to promote BE proliferation. Moreover, miR-145 could alter BMP4 signaling by inhibiting the translation of the transcription factor *GATA6* to promote columnar epithelial differentiation and proliferation, which may be important for BE formation, development, and malignant transformation^[61,62]. More studies have been conducted, and current data indicates that the expression levels of miR-21, miR-25, miR-143, miR-145, miR-192, miR-194, miR-196a, miR-215, and miR-223 are increased in BE/EAC tissues, while miR-136, miR-203, and miR-205 have been reported to be downregulated^[57,59-72]. Moreover, the upregulated levels of miR-194-5p and miR-215-5p were shown to be specific to BE epithelium and have a high accuracy in BE diagnosis^[57,73]. Furthermore, the miRNAs were differentially expressed among tissues from controls, BE, low-grade dysplasia, high-grade dysplasia (HGD), and EAC, indicating altered expression in the metaplasia-dysplasia-adenocarcinoma sequence^[64]. However, Garman *et al*^[74] indicated that miRNAs differ between squamous esophageal epithelium and BE/EAC but could not differentiate between BE and EAC patients. Nevertheless, it is clear that miRNAs play an important role in BE and EAC pathogenesis.

The studies above were mainly focused on esophageal tissue samples, and the sampling procedures were not pleasant, convenient, or economical. Researchers and medical doctors have made great efforts to seek a potent diagnostic tool to identify patients with BE/EAC from patients with other esophageal diseases. Due to the nature of chronic inflammatory diseases with progressive stages, searching for economic and convenient miRNA biomarkers for BE/EAC has been a challenge.

Biological fluids have a complicated extracellular milieu and include several kinds of RNA transporters^[17], such as serum and plasma. Extracellular RNAs are bound to different carriers, including protein complexes, lipoproteins, and exosomes^[17,75]. Numerous studies on extracellular miRNAs have indicated that circulating miRNAs can be potential biomarkers and reflect different aspects of pathophysiological conditions in a vast array of diseases^[9,65,75-77]. Moreover, studies identifying the correlations between circulating and tissue miRNAs could support the hypothesis that circulating miRNAs could serve as biomarkers for various diseases^[66]. In 2016, Bus *et al*^[65] performed miRNA expression profiling using plasma from 8 EAC patients, 8 BE patients, and 6 controls, and validation of the array data (6 selected miRNAs) was conducted in 115 plasma samples of another set of 59 EAC patients, 41 BE

patients, and 15 controls. They proposed that circulating miRNAs were differentially expressed in EAC and BE. Specifically, their data showed that the expression levels of miR-194-5p and miR-451a were significantly increased while miR-136-5p was significantly decreased in BE. In addition, miRNA-382-5p was significantly upregulated and miR-133a-3p was significantly downregulated in EAC^[65]. After comparison with their previous data using tissue samples^[61], the authors indicated that miR-194-5p and miR-451a were upregulated in both tissue and plasma samples from BE patients compared to controls, and miR-194-5p was more highly expressed in both tissue and plasma samples from EAC patients compared to controls^[65]. Moreover, miR-451a was shown to be downregulated in both tissue and plasma samples from EAC patients compared to BE patients^[65]. Fassan *et al*^[78] collected a series of 280 gastroesophageal biopsies and 80 plasma samples from patients with a spectrum of phenotypic changes involved in the gastroesophageal carcinogenetic process, and the results indicated that miR-223 was overexpressed in tissue samples at an early stage in BE patients and that its level in plasma samples was also upregulated in EAC patients. Another study conducted by Cabibi *et al*^[66] showed that the expression levels of miR-143, miR-145, miR-194, and miR-215 were increased in BE tissues compared to controls, and indicated that circulating serum miR-143, miR-194, and miR-215 were also upregulated in BE patients. Compared to the current routine surveillance and management endoscopy program with histopathological examination of tissue biopsies, a blood-derived test could be considerably more convenient, less invasive, and more cost-effective. Several studies^[79,80] analyzed the miRNA expression profiles in both plasma and serum samples and found little differences in the expression of circulating miRNAs; however, further validation is required.

Circulating miRNAs are present in various forms, and circulating exosomal miRNAs could be detected more stably and accurately because of the membranous envelope and protection from RNase degradation^[8,9,81,82]. Researchers have investigated whether exosomal miRNAs could be more related to disease states than total miRNAs in the circulation. Ashby *et al*^[75] separated different miRNA carriers in serum using asymmetrical flow field flow fractionation and demonstrated that accurate quantification yielded different distribution profiles in each fraction. Distribution profiling could reveal more distinct differences in miRNA expression between healthy controls and cancer patients^[75]. Zhao *et al*^[83] compared the miRNA expression profiles in bovine serum and exosomes and showed that the profiles of total miRNAs in serum and exosomes were quite different, such as miRNA numbers, types, and expression levels. Exosomes had fewer miRNAs and represented about 78% of the total miRNAs detected in serum. In the same year, Castoldi *et al*^[84] investigated the characteristics of vesicular and nonvesicular miRNAs in hepatectomized rats, and their results suggested that the altered expression profile of either vesicular or non-vesicular miRNAs may be more related to diseases and could be novel disease-associated biomarkers instead of total miRNAs isolated from serum/plasma.

Many existing studies have indicated that circulating exosomes in biological fluids could play a role in cellular communication and pathogenesis by delivering specific molecules from the parental cells to distant target cells. Research on miRNA profiling of disease-related circulating exosomes may help determine the potential use of exosomes as diagnostic biomarkers through noninvasive blood tests^[1]. Moreover, the pathological state of cancer cells may be reflected by exosomal shuttle profiling^[85]. Thus, circulating exosomal miRNAs have attracted great attention and could be a potential approach in biomarker exploration^[8].

For the first time, Warnecke-Eberz *et al*^[85] compared the exosomal miRNA profiles of EAC patients with those obtained from healthy controls in a study of matched primary tumor and normal tissues from EAC patients. They proposed that "exosomal onco-miRNAs", including miR-126-5p, miR-146a-5p, miR-192-5p, miR-196b-5p, miR-223-3p, miR-223-5p, miR-409-3p, and miR-483-5p, were overexpressed in exosomes and tumor tissues compared with the corresponding normal tissues. Moreover, miR-22-3p, miR-23b-5p, miR-27b-3p, miR-149-5p, miR-203-5p, miR-224-5p, miR-452-5p, miR-671-3p, miR-1201-5p, and miR-944-5p were significantly downregulated in EAC tissues or were undetectable in serum exosomes isolated from EAC patients. Hence, the authors suggested that the "exosomal onco-miRNAs" may play a major role in carcinogenesis and could be applied for diagnosis and therapy monitoring of EAC patients as a noninvasive tool. While Chiam *et al*^[86] first compared the expression profiles of circulating exosomal miRNAs among 18 patients with locally advanced EAC, 10 patients with BE, and 19 healthy controls to investigate the value of circulating exosomal miRNAs to discriminate EAC patients from BE patients and healthy controls. The researchers mainly focused on the miRNA ratios and all possible permutations of miRNA ratios for each subject and indicated that a multi-biomarker

panel, which included RNU6-1/miR-16-5p, let-7e-5p/miR-15b-5p, miR-17-5p/miR-194-5p, miR-25-3p/miR-320a, and miR-30a-5p/miR-324-5p, had a higher sensitivity and specificity over single miRNA ratios to identify EAC patients from BE patients and healthy controls^[86]. The ratio method that they used can be potentially used to overcome the limitations associated with global normalization approaches, which is vital for accurate assessment of miRNA data^[86]. Despite the exciting results, the number of studies on circulating exosomal miRNAs in BE/EAC is limited to date, and further studies on exploration and validation are needed to identify specific and unique exosomal miRNAs in BE/EAC. Furthermore, there remains a critical need to develop molecular biomarkers to stratify cancer risks in BE patients as well^[87].

Among the studies mentioned above, some focused on tissue miRNAs, while others focused on total circulating miRNAs or isolated exosomal miRNAs, some of which used the miRNA expression panels for differentially expressed miRNAs. Circulating biomarkers could provide an exciting approach to overcome sampling issues and be more convenient, stable, and reliable to translate into a clinically useful test. The ideal biomarker for clinical application should become positive at the early stage of a disease process and remain positive throughout the rest of the disease stages for surveillance^[87]. The diagnosis of BE/EAC at an early stage could improve prognosis, and circulating exosome analysis may be implicated in diagnosis and therapy monitoring. MiRNAs that are dysregulated in both tumor tissues and circulating exosomes may satisfy this diagnostic criterion^[85]. The value of circulating exosomal miRNAs as biomarkers for early disease detection in the clinic has to be examined and verified in a prospective study. Since the exact origins and functions of circulating miRNAs and circulating exosomal miRNAs are unknown and the study subjects and methods for detecting miRNA data are diverse, we could not fully explain the obtained results or clearly elucidate the underlying mechanisms. Additionally, data normalization remains an important issue because of variations between studies, and miRNAs have not been implemented in the clinical setting because of conflicting results from various studies. The reproducibility of circulating miRNAs is quite important, and their changes in expression over time also need to be clarified^[2,77].

CIRCULATING EXOSOMES AS THERAPEUTIC APPROACHES FOR BE AND EAC

Since exosomes carry cell-specific cargoes that could regulate recipient cell functions, is there any possibility that exosomes could be used as a therapeutic approach that can be applied in the clinic?

The findings of RNA-containing exosomes in serum/plasma suggest the possibility of delivering RNAs to distant cells at least theoretically. Valadi *et al.*^[31] indicated that exosomes isolated from cell supernatants of a mouse and a human mast cell line, as well as primary bone marrow-derived mouse mast cells, contain RNAs, many of which are not present in the cytoplasm of exosome-derived cells. These RNAs, including mRNAs and miRNAs, could be transferred into recipient cells and could function in these cells^[31]. In addition, exosome-mediated transfer of RNA information from human liver stem cells could stimulate liver regeneration in a model of 75% hepatectomy^[88]. Camussi *et al.*^[89] also suggested that exosomes could transfer selected proteins, mRNA, and miRNAs between stem cells and differentiated cells and act as paracrine signaling mediators. Exosomes derived from injured tissues may also reprogram the phenotype of bone marrow or resident stem cells by transferring genetic information^[89]. Additionally, stem cell-derived exosomes may reprogram cells that survive injury and favor tissue regeneration^[89]. The mRNA and miRNA cargoes may raise the possibility of genetic information transfer and functional alteration in recipient cells^[90]. The ability of exosomes with different origins to modulate different functions suggests that exosomes may be used as a therapeutic method^[89].

Although studies on how exosomal miRNAs alter target cell functions are rare in BE/EAC, there are still some exciting and promising clues that could encourage continuing research. For instance, miR-223-overexpressing EAC cells had significantly higher migratory and invasive potential than transfected control cells, and miR-223 may also modulate sensitivity to chemotherapy by targeting *PARP1*^[63]. In addition, because miR-145 could alter BMP4 signaling by inhibiting the translation of the transcription factor GATA6 to promote columnar epithelial differentiation and proliferation, manipulation of BMP4 signaling by targeting miR-145 may be of great use to prevent BE development and even progression in BE patients at high risk^[61,62]. Future validation work is needed to explore the possible therapeutic role of miR-145^[61], and whether mRNAs and miRNAs entering target cells can activate

translational control mechanisms remains to be further investigated. However, several clinical trials have tested the use of exosomes with limited success^[91-93]. Despite these ambiguous data, targeting exosomes or generating exosomes containing requisite miRNAs may be a viable method to enhance therapeutic activity, and the reduction of exosome release or depletion of certain circulating exosomes could also be another method for therapy^[94].

CIRCULATING EXOSOMES FOR OUTCOME PREDICTION IN BE AND EAC

The dismal outcome of EAC patients highlights the need for novel prognostic biomarkers. In the interest of exploring the predictive value of miRNAs in the progression from BE to EAC, Revilla-Nuin *et al*^[95] conducted the first long-term follow-up study to our knowledge. MiR-192, miR-194, miR-196a, and miR-196b were shown to have significantly higher expression in tissues from BE patients with progression to EAC compared to that from other BE patients without progression to EAC^[95], which suggested that altered miRNA expression could identify BE patients with higher risks for progressing to EAC and indicated the potential use of miRNAs for surveillance and management of this malignancy. Leidner *et al*^[87] compared the miRNA expression profile in esophageal tissues, including disease tissues and paired normal esophageal squamous tissues from patients with BE, HGD, and EAC using next-generation sequencing. They found that the majority of dysregulated miRNAs were commonly expressed in HGD and EAC lesions^[87]. However, the authors indicated that the reduced expression of miR-31 and miR-375 may be candidate biomarkers of multistep malignant progression after comparison of BE, HGD, and EAC lesions and suggested that these two downregulated miRNAs may be associated with a poor prognosis in patients with EAC after analysis of survival data from EAC patients^[87]. In addition, Mathe *et al*^[68] indicated that low expression of miR-375 in cancerous tissues was strongly associated with a worse prognosis in EAC patients with BE. MiR-223 was also significantly upregulated along with the metaplasia-dysplasia-adenocarcinoma progression sequence^[63]. Moreover, Saad *et al*^[71] showed that the expression of miR-21, miR-31, miR-192, and miR-194 was upregulated in BE adjacent to HGD compared to isolated BE samples. In addition, downregulated miR-203 was significantly associated with tumor progression of EAC, while the expression of miR-192, miR-194, and miR-200a was higher in stage I than in advanced stages of EAC, which suggested that these miRNAs might serve as biomarkers for disease progression and outcome prediction.

These data suggested that the miRNAs could be used as biomarkers for BE/EAC to screen and monitor patients at high risk of progression to EAC. However, it is still uncertain whether these miRNAs have a role in the progression sequence or are merely biomarkers. Additionally, these results were based on tissue samples, not circulating exosomal miRNAs. Current studies have established the role of circulating exosomal miRNAs in BE and EAC, but associations between miRNA profiles and the outcome of BE/EAC patients are still under investigation.

A molecular biomarker that predicts the progression in the metaplasia-dysplasia-adenocarcinoma progression sequence of BE and EAC should be of high value in identifying individuals who have higher risks and need closer surveillance and would be of great use to formulate rational therapeutic interventions^[87]. The most promising surveillance and prediction programs should contain risk stratifications with a variety of biomarkers. MiRNA expression profiling could expand the current knowledge on molecular pathology and BE/EAC carcinogenesis, and enable the identification of molecular biomarkers for the early detection and stratification of BE and prediction of progression to EAC^[64]. A large-scale multi-center study is important for clinical application to rationalize these strategies in BE/EAC patients.

CONCLUSION

Almost all cells release exosomes, and exosomal miRNAs can either repress the translation of or induce the degradation of multiple target mRNAs, and thus regulate the expression of certain genes in recipient cells^[96,97]. Circulating exosomes from patients with different cancers carry cancer-specific miRNAs that might be correlated with cancer progression and therapeutic reactions^[98,99]. BE provides a good mechanism to study carcinogenesis of EAC^[48], and the number of studies on how circulating exosomes relate to BE/EAC is increasing. However, only a few studies have focused on the effects of exosomes from specific cell types *in vivo*^[100], and little attention has

been paid to the etiology and molecular mechanisms. Exploring and understanding how circulating exosomes derived from specific cells function to influence pathophysiology and progression could improve the early diagnosis, prevention, and therapeutic approaches of BE and EAC.

Despite the progress made by studies on the feasibility and safety of using circulating exosomal miRNAs of BE and EAC in the clinic, their application in diagnosis, therapeutic utility, and outcome prediction remains a difficult problem to solve. Moreover, there are some limitations of exosomal analysis based on biological fluids. First, the techniques for extraction and isolation of circulating exosomes are not widespread, and they are mainly used in scientific research. Moreover, the experimental conditions should be standardized in such studies, and the results should be reproducible. Second, the cellular origins of exosomes are important, so further investigations are needed to determine the cells that secrete exosomes containing functional RNAs. Although exosomes are mainly derived from platelets in the circulation of healthy controls, exosomes can originate from a wide array of cells under pathological conditions^[89], and normal cells could also secrete exosomes into biological fluids, which may affect the purity of biological fluid-extracted exosomes. To understand the molecular specialty of cell-derived exosomes in diseases, exosomal preparation free of normal cell-derived exosomes is needed. Therefore, the cell-specific and tissue-specific signatures should be verified, and the purification strategies should be well defined. A new valuable tool is ExoCarta^[101,102], a database of previously conducted exosomal proteomic and transcriptomic studies. Tissue-specific proteins and RNAs in exosomes from certain diseases are identified and listed in ExoCarta^[101,102]. Third, the functions of circulating exosomal miRNAs are largely unknown, and how the direct and indirect interactions between exosomal miRNAs and esophageal epithelium regulate local inflammation is still not completely understood. Thus, functional studies on potent miRNAs in the extracellular microenvironment and recipient cells should be conducted, which could provide important information on the mechanisms of the pathophysiology and progression of BE/EAC. Additionally, the timing of the changes in exosomal miRNAs in the circulation during disease progression should be elucidated. Since exosomes in biological fluids could be easily screened for cancer genetic markers^[31], the removal of harmful plasma exosomes might be beneficial in certain pathological conditions^[94]. Moreover, if engineering exosomes by specific nucleic acids could be achieved, the delivery of nucleic acids by exosomes would make exosomes ideal candidates as vectors for gene therapy^[31]. Further investigation should also be helpful in defining the different regulatory activities of exosomes and the mechanisms of their targeting to recipient cells. Last, the sample sizes of related studies were not large enough, and current data are too limited to draw definitive conclusions. Study results are not completely reliable and consistent, and no consensus about the changes in circulating exosomal miRNAs has been researched. Thus, circulating exosomal miRNAs that could differentiate BE/EAC patients from other subjects have not been identified. Of note, miRNA panels have shown a better sensitivity and specificity than one single miRNA, and we still need to identify the distinct exosomal miRNAs that could be biomarkers for BE/EAC^[90]. Without doubt, confirming the underlying mechanisms is complicated but also crucial, and the identification of a panel of miRNAs responsible for BE/EAC requires considerable effort.

Currently, endoscopic procedures and histopathological examinations are still the gold standard for BE/EAC diagnosis, and endoscopic therapy and surgery continue to be the optimal therapeutic approaches. The cost-effectiveness and feasibility of gastrointestinal endoscopy application urge us to seek a more effective and less invasive method to screen BE and EAC^[103]. Since early detection of EAC will improve patient survival rates and quality of life^[42], individuals with high risks for BE/EAC will benefit from screening and early diagnosis, and medical resources could be distributed more effectively. The associations between circulating exosomal miRNAs and BE/EAC have been indicated in the discussed studies, and the exciting roles of exosomal miRNAs give us an opportunity to explore carcinogenesis from another perspective. Future prospective studies are needed to determine when circulating exosomal miRNAs change during disease onsets, identify potential targets for prevention, early diagnosis, and therapy, and improve our understanding of pathogenesis.

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