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REVIEW

Circulating exosomes and exosomal microRNAs as biomarkers in gastrointestinal cancer

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The most important biological function of exosomes is their possible use as biomarkers in clinical diagnosis. Compared with biomarkers identified in conventional specimens such as serum or urine, exosomal biomarkers provide the highest amount of sensitivity and specificity, which can be attributed to their excellent stability. Exosomes, which harbor different types of proteins, nucleic acids and lipids, are present in almost all bodily fluids. The molecular constituents of exosomes, especially exosomal proteins and microRNAs (miRNAs), are promising as biomarkers in clinical diagnosis. This discovery that exosomes also contain messenger RNAs and miRNAs shows that they could be carriers of genetic information. Although the majority of RNAs found in exosomes are degraded RNA fragments with a length of < 200 nucleotides, some full-length RNAs might be present that may affect protein production in the recipient cell. In addition, exosomal miRNAs have been found to be associated with certain diseases. Several studies have pointed out miRNA contents of circulating exosomes that are similar to those of originating cancer cells. In this review, the recent advances in circulating exosomal miRNAs as biomarkers in gastrointestinal cancers are discussed. These studies indicated that miRNAs can be detected in exosomes isolated from body fluids such as saliva, which suggests potential advantages of using exosomal miRNAs as noninvasive novel biomarkers.

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INTRODUCTION

The microRNAs (miRNAs) are a class of small non-coding RNAs of size 19–25 nucleotides, which play an important role in biological processes.[1–4] The first miRNA, called Lin-4, was identified in 1993 in the *Caenorhabditis elegans*organism. It is involved in the growth and development of the organism.[2] In 2000, the second miRNA, called let-7, was discovered in the same organism. For the first time, homologous let-7 was identified in humans in 2000, and very soon other miRNAs were also identified in humans. This process of discovery is still ongoing.[5] Recent assumptions suggest that one-third of human genes' expression is regulated by miRNAs. The ability of miRNA to regulate multiple RNAs is very high because it does not need a complete pairing between miRNA and the end sequences of 3'-untranslated region (3'-UTR)-messenger RNA (mRNA). Recent studies show that pairing six or seven nucleotides of one miRNA to 3'-UTR of mRNA is essential for the selection of target mRNA. Recently, miRNAs have attracted a great deal of attention in the context of the investigation of molecular pathways involved in cancer development and progression. In addition to their important cellular functions, it is possible that secreted miRNAs embedded in exosomes may be diagnostic biomarkers for cancer detection. In summary, some attempts have recently been made to use miRNAs in serum or plasma as diagnostic biomarkers of various cancers, but there is no general view so far with regard to which miRNAs should be selected as biomarkers. The unique properties of exosomes, including their ability to embed specific miRNAs, their stability in circulation, their reproducible detection, and most importantly, the fact that they reflect the properties of cancer cells, may make them useful for the development of highly sensitive diagnostic strategies for rapid and noninvasive monitoring of the pathological condition of cancer patients. Moreover, it has been reported that miRNA level in serum/plasma is not associated with vesicles, and sustainability is different compared with treatment by RNAaseA.[6] This indicates that exosomal miRNAs are preferable as biological specimens for developing diagnostic biomarkers because of their stability in serum/plasma. The values of exosomal miRNAs demonstrate that they also reflect cancer development and pathological changes in patients. Initially it was thought that exosomes serve only as 'garbage bags' for cells to dispose of unwanted constituents. However, evidence has shown that exosomes have an important role in cell-to-cell communication and influence both physiological and pathological processes. Furthermore, researchers have found that the molecular constituents of exosomes are associated with certain diseases and treatment responses. This indicates that

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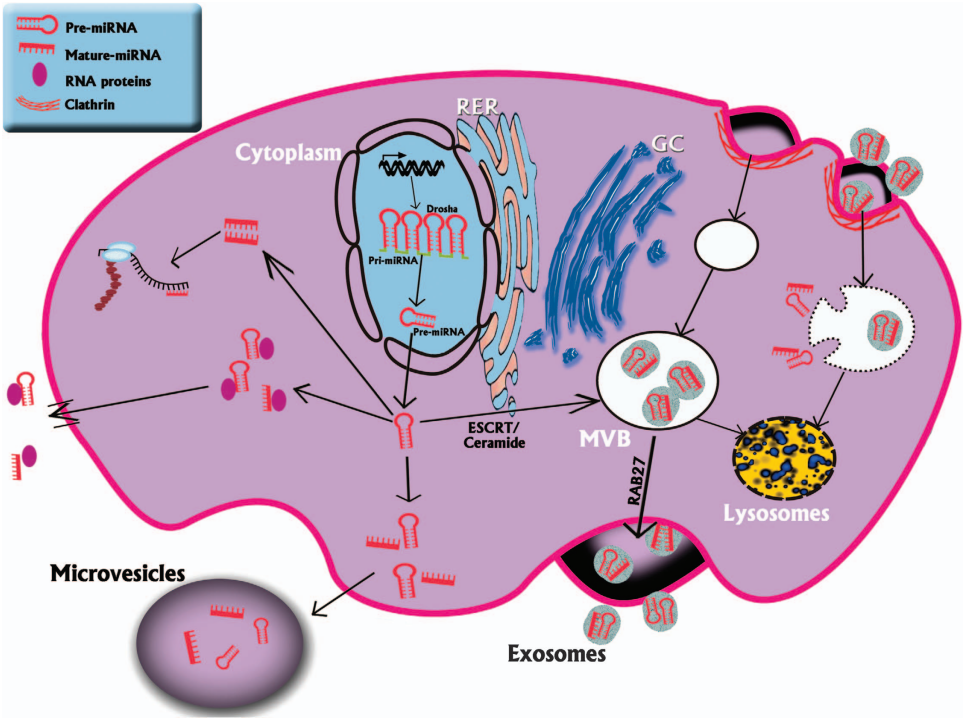

Figure 1. Biogenesis of exosomal miRNAs and microvesicles. The miRNA transmission mechanism of exosomes and endosomal systems showed in endocytic pathway. GC, Golgi complex; miRNA, microRNAs; MVB, multivesicular body; RER, rough endoplasmic reticulum.

they may also be used as a diagnostic tool.[7] Exosomes are actively released from cancer cells and their specific constituents are dependent on the cells in which they were created. In essence, cancer cells may use some unidentified mechanism to put a subset of miRNAs into exosomes. Recently, some efforts have been made to use exosomal miRNAs in serum or plasma as diagnostic biomarkers of various types of cancers in both early and advanced stages.[8] This review is mainly focused on the recent advances in circulating exosomal miRNAs as biomarkers in gastrointestinal (GI) cancers. The aim of the current review is to give an overview about origin and trafficking of exosomes between cells, techniques to isolate exosomal miRNAs as well as the potential applications of exosome-encapsulated miRNAs as diagnostic markers in clinical settings in GI cancers.

EXOSOME

Extracellular vesicles (exosomes, prostasomes, microvesicles (MVs), ectosomes and oncosomes) function as a model of intercellular communication and molecular transfer. These are released as blebbing from the plasma membrane (MVs) or as multivesicular endosomes through an exocytosis process.[9] The term 'ectocytosis' is used to describe the beginning of the release of right-side-out membrane vesicles from the plasma membrane, but the term 'ectosome' is more commonly related to vesicles released by neutrophiles. Extracellular vesicles can be classified into two general groups—MVs released directly from the cell membrane and exosomes released by exocytosis when multivesicular bodies (MVBs) fuse with the plasma membrane. Here, the main focus is on the second group of vesicles, that is, exosomes. Exosomes are generally organelles derived from endosomes within the diameter range of 50–100 nm that can be released actively through exocytosis pathway, and can only be pelleted after ultracentrifugation at 100 000 *g*. Vesicles with a diameter of 120–1000 nm are called endosomes. Exosomes released from sheep reticulocytes were first reported in 1983. The transferring receptor and some other membrane-associated elements are selectively released in MVB-derived circulating vesicles called exosomes, which were identified in 1989 by R Johnstone. The miRNA transmission mechanism of exosomes was confirmed in 2007.[10] MVBs are, in fact, late endosomes that carry intraluminal endosomal vesicles. Some MVBs are broken down exclusively into lysosomes, whereas the others are fused with the cell membrane and release internal vesicles in extracellular space (Figure 1). Exosomes are formed by proteins containing endosomal sorting complex required for transport (ESCRT), such as Alix and Tsg101. Proteomic analyses of purified exosomes from various cell types show the presence of ESCRT components (Tsg101, Alix) and ubiquitinated proteins.[11] Exosome is better described in immune cells, where small vesicles stimulate the immune system to eliminate tumor. Exosomes, in particular exosomes of specific cells of the immune system such as dendritic cells and B cells, may be mediators of adaptive immune responses to pathogens and tumors. Exosomes derived from tumor cells have an active role in carcinogenesis, metastasis and response to treatment through the transfer of oncogenes and onco-miRNAs between cancer cells and tumor stroma cells.[12] After finding that exosomes are derived from B lymphocytes, the first break through in practical usage in the treatment of human was the *in vivo* demonstration in mice with tumors that could be suppressed or eradicated by the suppressor activity of exosomes.[13] It was shown that the dendritic cells have the capacity to secrete exosomes. As Valadi *et al.*[14] reported that exosomes also contain RNAs, the compound and functions of exosomes have been evaluated to a large extent.[7] Exosomes can be shown to possess a cup-shaped morphology using transmission microscopy. Several mechanisms are involved in the formation of endosomes. The regulatory molecules in exosomes' secretion were recognized by Ostrowski *et al.*[15] who indicated that Rab27a and Rab27b are related to the release of exosomes in parent cells. Rab27 knockdown or their effects—SYTL4 and EXPH5—could inhibit secretion of exosomes in HeLa cells. Furthermore, Yu *et al.* found that both the tumor repressor

protein P53 and its downstream effectors TSAP6 could raise exosome production. Baietti *et al.*[16] found that syndecan-syntenin interacted directly with ALIX proteins via Leu-Tyr-Pro-X (n)-Leu motif to support the intraluminal budding of endosomal membranes, which is an essential step in exosome formation. All these studies illustrate that a set of molecules act as a regulatory network responsible for the formation and secretion of exosomes in mother cells.[10] The exact entering mechanism of exosomes is unclear. However, receptor-mediated endocytosis (for example, LFA 1, TIM 1 and TIM 4), phagocytosis and direct fusion with the plasma membrane are proposed. It is shown that low pH value in tumor micro-environment is essential to attract exosomes to human metastatic melanoma at *in vitro* conditions. This suggests that an increase in the stability and lipid/cholesterol content of exosomal membrane in an acidic environment is related to the arrival of exosomes.[17] The exosome compounds of different cell types are collected from ExoCarta database. Vesicle pedia and extracellular vesicle pedia are two other databases that contain complex information published on exosomes and MVs.[9] Information on the internal contents of exosomes can be investigated through mass spectrometry. A valid method for the investigation of small amounts of isolated exosomes is the fluorescence-activated cell sorting optimal method, which is based on labeling the vesicles released by fluorescent probes. More detailed studies show that exosomes carry non-coding RNA parts with high diversity.[9] Exosomes appear to have a role in T-cell activity and possibly carry out immunosuppressive activities. They are formed by the bilayer membrane of a specific lipid composition that is very rich in sphingomyelin, cholesterol and GM3 glycolipid.[18] Several reports suggest that certain lipid components of exosomes, such as phosphatidylinositol, serine and prostaglandins, may have an important role in the function of exosomes. Proteins that have been identified with a higher frequency are fusion proteins, membrane transport proteins (GTPases, annexins and flotillin), heat-shock proteins (for example, HSC70), tetraspanins (for example, CD9, CD63 and CD81), MVB bio-proteins (for example, Alix and TSG101), lipoproteins and phospholipids. Several proteins are known as markers of exosomes, among which tetraspanins, CD63 and CD81 are the most common. Nowadays, we know that exosomes carry different materials of parent cells such as proteins, lipids, mRNA and miRNAs. Exosomes are released from different cells such as CDs, lymphocytes, platelets, mast cells, epithelial cells, endothelial cells and neurons. They can be found in all body fluids including blood, urine, saliva, amniotic fluid, breast milk, hydrothoracic fluid, ascitic fluid and the culture medium of most cells.[7] Interestingly, the release of exosomes from the plasma membrane is not only a feature of mammalian cells, it is also exploited by microbial life, bacteria, viruses, fungi and parasites in which intraluminal vesicle (within MVBs) production occurs not only *in vitro* but also *in vivo* during infection.[19] For decades, it has been reported that mammalian cells discard fragments of their plasma membrane in the form of MVs.[20] Therefore, miRNA of exosomes can be associated with cancer development. Recent studies show that identification of particular exosomes can provide a new diagnostic tool. In fact, numerous studies are investigating the use of exosomes as biomarkers and capsules to deliver content and transfer medication therapy. Therefore, miRNAs in the exosomes may both provide biomarkers and achieve therapeutic potential goals. According to exosomes' function, cell-to-cell communication by exosomes may have an important role in the development of cancer. Several reports have shown that particular exosomal miRNAs are associated with certain types of cancer phenotypes and hence may be used as important biomarkers. In addition, many studies have examined the use of miRNA in therapies to treat the molecular level of GI cancer. Metastasis of cancer to distant places through the dissemination of cancer cells in the blood is directly associated with poor prognosis in patients with GI cancer. Moreover, GI cancer cells release exosomes into the blood stream. Therefore, diagnosis of miRNAs within the serum exosomes is in the form of biomarkers of recurrence in GI cancer.

EXOSOMAL miRNAS IN GASTRIC CANCER

In gastric cancer, previous studies have shown that increased expression of CD97 is associated with differentiation and invasion of tumor cells. CD97 was found first in hematopoietic cells and then in abundance in numerous cancers, including gastric, colorectal, thyroid, esophageal, pancreatic and oral squamous-cell carcinomas. Recently, it was observed that CD97 is associated with increasing invasion *in vitro* as well as high growth and metastasis of gastric cancer *in vivo*. However, the underlying role of the mechanism in simulating CD97 has not been determined yet. In gastric cancer, cancer cells produce many exosomes that contain abundant miRNAs. Studies conducted by using exosomes isolated from cells with high and low CD97 expressions show that CD97 promotes gastric cancer cell proliferation and invasion *in vitro*, at least in part, by exosome-mediated MAPK signaling pathway. Moreover, exosomal miRNAs may be involved in activation of the CD97-associated pathway. Since then, the number of miRNAs have been shown to be downregulated exosomal miRNA signatures in body fluids in gastric cancer, including miR-34b/c, miR-218 and miR-10b, whereas three miRNAs, including miR-21, miR-103 and miR-223 showed significant upregulation (Table 1).[21–24] Exosomes obtained from a gastric cancer cell line led to proliferation of that cell line and second gastric carcinoma cell line. Increased expression of the Akt pathway also leads to simulation of cell proliferation.[25] As mentioned above, exosomal modulation of apoptosis may affect tumor growth. The surviving antiapoptotic protein and its associated proteins—HSP70 and HSP90—may be present in and on exosomes. Furthermore, their concentrations may be increased

Table 1. Exosomal miRNAs summarizing miRNA expression as biomarkers in GI cancer

Disease	*Upregulation*	*Downregulation*	*Reference*
Gastric cancer	miR-21, -103, -223, -17/92	miR-34b/c, -218, -10b, let-7	21–24,29,108
Colorectal cancer	miR-1224-5p, -1229, -1246, -150, -21, -223, -23a, -19a, -17/92	miR-143, Let-7 family, -145, -195, -130a, -331, -124a, -145, -542-3p, -34a	48,51–57,108
Pancreatic cancer	miR-17-5p, -20, -92-1, -99a, -99b, -100, -103, -107, -125a, -125b, -130a, -132, -204, -211,-342, -155, -15b, -95, -186, -190, -196-a, -200b, -221, -222, -21,- 125b	miR-29c, -30a-3p, -96, -130b, -141, -148a, -148b, -216, -217, -375, -494, -34a	55,73,88,125–132
Esophagus cancer	miR-21, -1246, -3202, -23a, -718, -3610, -4271, -33a, -326, -16-5p	miR-34a, -144, -106b, -486-5p, -93, -451, -324-5p	24,57,102,103,108,109,133
Liver cancer	miR-21	miR-34a, -125b	24,134,135

Abbreviations: GI, gastrointestinal; miRNA, microRNAs.

by cellular stresses. It should be noted that surviving increases cellular proliferation and invasion as well. Therefore, exosomal survival is a strong micro-environmental stimulus for the development and metastasis of tumors.[26] Circulating tumor exosome level in the serum has been associated with cancer; it has been shown that the number of circulating MVs of no platelet origin ($CD61^-$) is higher in gastric cancer patients.[27] The role of exosomes in cancer progression and metastasis has been shown in recent studies on animal. Heterogeneity among tumor cells with distinct metastatic potential has been identified based on the intrinsic genetic instability of tumor cells.[20] Let-7 is generally considered to be a tumor suppressor miRNA; loss of the family members of let-7 is an indication of poor survival.[28] It has been indicated that the metastatic gastric cancer cell line—AZ-P7a—expresses let-7 miRNAs both at intra-cellular level and in the exosomes at high levels. In contrast, in other analyzed cancer cell lines, the level of let-7 miRNAs is high in the cells and low in the exosomes.[29] AZ-P7a cells release let-7 miRNA family into the extracellular environment in a selective and active manner through exosomes. This would decrease the anti-tumor effect inside cells and trigger them to continue their oncogenesis and invasiveness, as AZ-P7 is highly metastatic.[14]

EXOSOMAL miRNAS IN COLON CANCER

Colorectal cancer (CRC) manifests in the lower part of the digestive system and is the most common malignancy involved in the GI system.[30–41] Despite surgical resection and chemotherapy before and after surgery, molecular and targeting therapy tumor recurrence is common in patients with CRC. Recurrence, especially liver metastasis, is clearly associated with the survival of patients with CRC. Many studies have examined the potential biomarkers of recurrence in CRC for prognosis.[42] The frequency in the occurrence of CRC has increased by about two to four times in recent decades worldwide. CRC in men is observed after lung cancer and prostate cancer, whereas in women it is observed after lung cancer and breast cancer. In the United States of America, 140 000 people each year are diagnosed with cancer, and this type of cancer is considered as the second leading cause of mortality in the country.[43] A systemic method for the detection of the pathology of the disease can increase systemic diagnosis of patients in the early stages of CRC, which reduces the mortality rate of the disease. Use of fecal occult blood test and flexible sigmoidoscopy as screening methods decreases the rate of CRC occurrence. However, these techniques have some limitations. Occult blood test detection sensitivity is very low and flexible; sigmoidoscopy is painful and uncomfortable for patients. In fact, 9–19 carbohydrate antigens (CA19-9) and carcinoembryonic antigens are widely used as tumor markers for the diagnosis of many types of cancers, including colon, liver, pancreatic and stomach cancer. Yet the sensitivity of this marker for CRC diagnosis is low, especially in the early stages of the disease. Thus, there is an urgent need to develop specific diagnostic markers for CRC, equipped with high sensitivity, less pain and fast diagnosis. In many studies, the biomarkers of CRC recurrence were tested. The direction of patients' prognosis and miRNA associated with cancer development are potential determinants in various types of cancer. Deregulation of miRNAs has been seen in different types of cancers, and expression profiles of endogenous miRNAs can be used to classify types of cancer. Several miRNAs that are greatly increased in tumor tissues are reported as diagnostic or prognostic markers of disease. Recent studies show that miRNAs are released via exosomes from different cells, including cancer cells, in body fluids such as blood, urine, milk and saliva. Depending on the nature of the secreting cells, exosomes contain proteins, lipids, mRNA and miRNA. Thus, miRNAs of exosomes in body fluids can be useful biological indicators for the diagnosis of cancer. Tumor cells use different methods, including exosomes, for the transfer of genetic information, including transfer of miRNAs, to surrounding cells supporting tumor growth and progression.[44] In addition, circulating miRNA may cause regulation of immune responses.[45] For example, MVs derived from human colon cancer cells can support tumor growth and evasion from immune system by differentiation of monocyte to transforming growth factor-β-secreting cells of the myeloid suppressor.[45] Circulating miRNAs were analyzed to detect cancer in the early stages of development. Huang *et al.*[46] investigated circulating profiles of miRNAs in a blood stream in the early stages of CRC. Interestingly, they found that circulating miRNA profiles differentiate adenoma from healthy controls with 73% sensitivity and 79% specificity. These data indicate that extracellular miRNAs are reliable markers for the early detection of tumors.[47] MiRNA-17-3p was confirmed as a diagnostic marker of colon cancer. This miRNA belongs to gene cluster of miRNA-17–92, which has a role in pathogenic cancer. In addition, stool is another biological material which can be studied as miRNAs are maintained in it. The most important miRNAs identified in the stool with an increased expression include miR-21, miR-203, miR-126 and miR-16, whereas miR-320 and miR-192 have decreased expression.[48,49] Low expression of miR-16 and miR-126b in stool with 91% sensitivity and 72% specificity can be used to detect the presence of CRC.[50] The presence of exosomes in cancer patients at different stages should necessarily be examined because it is likely that exosomes can be detected with high frequency in this population. Moreover, circulating kinetics miRNAs must be analyzed in detail to determine whether infectious diseases or lifestyle changes could lead to changes in a variety of circulating miRNAs within the exosomes. This leads to the correction of exosomes' biomarker potential in future studies.[47] In studies based on micro-array-based profiling of exosomal miRNA in the serum of primary CRC patients, eight miRNAs (let-7a, miR-1224-5p, miR-1229, miR-1246, miR-150, miR-21, miR-223 and miR-23a) significantly increased in serum exosomes[48,51–57] and were downregulated after surgery.[56] CRC cells release exosomes containing miRNAs into the blood stream in order to transmit signals to the recipient cells. The MiR-19a in serum exosomes can be a potential biomarker to predict the recurrence of CRC. Expression of exosomes of serum miR-19a was higher in patients with CRC than in the healthy control group. Moreover, miR-25-106b and miR-17-92a clusters are effective in raising their malignity grade. In particular, miR-17-92a, known as the tumor-maker miRNA cluster, was shown to increase different types of cancers such as colon cancer, lung cancer and lymphoma. These findings suggest that CRC cells release exosomal miRNAs to survive. Cells with overexpression of miR-19a for genomic changes may release exosomes rich in miR-19a. It is reported that miR-19a increases proliferation and invasion of cancer cells with gain or loss of function. The function of miR-19a is not yet entirely clear. However, various reports indicate that miR-19a, with suppressor gene PTEN that represses tumor growth in lymphoma cells, gastric cancer cells and bladder cancer cells, has a role in tumor formation. High expression of miR-19a exosomes is associated with potential marker of poor prognosis in CRC. In general, large quantities of miR-19a exosomes in the serum of CRC patients are associated with tumor recurrence. MiR-19a is increased in patients at early as well as advanced stages of CRC. The results suggest that miR-19a may be used in the early stages of colorectal oncogenesis[56,58] (Table 1). Other studies state that protease cargo in the MVs is functionally robust and increases matrix degradation. The interesting part of these studies is that they indicate that the proteolytic activities of MVs shed by tumor cells are directly correlated with malignancy and invasiveness.[59] However, it cannot be denied that cancer cells and other cells release both MVs and exosomes with related properties. This case can also be observed in platelets, endothelial cells and certain other cells.[59] The molecular composition of exosomes indicates physiology, tissue of origin, or pathophysiological changes in the

cell. This is due to the significant potential of exosomes as biomarkers for disease diagnosis. These results are the basic foundation of a hypothesis that has an active role in intercellular exosome communication, at least in the immune system, and they prompt the very first attempt at using them in a clinic as a new type of exosome-based cell-free vaccines. They are alternatives to dendritic cells-adoptive therapy for suppressing tumor growth.[59]

EXOSOMAL miRNAS IN PANCREATIC CANCER

Pancreatic cancer (PC) is one of the most lethal cancers and the fourth most common cause of cancer death in the world. The prognosis is poor, with 1-year and 5-year survival rates of only 20% and 6%, respectively, and a median survival rate of about 6 months.[60,61] Pancreatic ductal adenocarcinoma (PDAC) is the most common type that includes more than 90% of cases[62] due to a combination of factors, including difficulties in detecting early stage disease, its high metastatic potential and resistance to conventional therapies, which have made a poor prognosis of PDAC.[63] Therefore, a better understanding of the initial events in PDAC development is needed to improve early detection. Systemic chemotherapy administered either after tumor resection surgery[64] or in patients with metastatic disease has been shown to prolong survival; however, surgery is the only curative treatment.[65,66] Approximately 20% of patients with PDAC can be operated on with curative care because most have locally progressive or metastatic PC at the time of diagnosis.[62,67] More than 2500 human miRNA sequences are known today, and several specific miRNA profiles related to PC tissue.[68–70] The particular miRNA profiles from patients suspected of having PC can be identified with a sensitive, specific and noninvasive diagnostic blood test for PC. This would be very valuable because it is difficult to get useful biopsies of pancreas tissue. The number of miRNAs including miR-21, miR-155, miR-34, miR-17-5p and miR-138-5p has been shown to regulate PDAC growth, invasion and metastasis by targeting members of key signaling pathways.[71–73] Small reconsideration studies have demonstrated that expression of specific miRNAs in plasma or serum can distinguish patients with PC from healthy participants.[74,75] Whole-blood-derived miRNA profiles have been suggested as a new tool for the early detection of PC and other adenocarcinomas.[76] Studies conducted by using exosomes isolated from cells with PC metastasis show relation to liver pre-metastatic niche formation.[68,74] It has been demonstrated that exosomes derived from malignant pancreatic lesions have a key role in liver pre-metastatic niche initiation.[77] It has been found that macrophage migration inhibitory factor (MIF) is highly expressed in PDAC-derived exosomes and Kupffer cells in the liver when that uptake of exosomes causes activation of fibrotic pathways. Specifically, it has been shown that exosomal MIF prompts the release of transforming growth factor-β by Kupffer cells, which, in turn, stimulates fibronectin production by hepatic stellate cells.[78,79] An fibronectin deposit, consequently, induces the arrest of bone marrow-derived macrophages and neutrophils in the liver, concluding the formation of the pre-metastatic niche.[79,80] Significantly, studies on a mouse model of PC (PKCY mice) bearing either pancreatic intraepithelial neoplasia or PDAC lesions show that MIF is elevated in plasma exosomes.[81] Moreover, MIF is also highly expressed in plasma exosomes isolated from PDAC patients whose disease progressed post diagnosis compared with patients with no evidence of disease 5 years after diagnosis and compared with healthy control subjects. These observations suggest that exosomal MIF may be a biomarker of PDAC liver metastasis.[81,82] Thus, miRNAs have an important role in cell proliferation, apoptosis and differentiation.[83] Recently, several studies have shown the abnormal expression of serum miRNAs in PC, which might be useful for the early diagnosis of PC. More than 100 miRNAs are aberrantly expressed in PC.[84,85] Exosomal miRNAs in PC have not been observed, especially regarding the connection between serum exosomal miRNAs and clinical pathological characteristic of PC, which is still unknown.[86] Studies have demonstrated that serum exosomal miR-17-5p and miR-21 levels are significantly increased in PC patients compared with those in non-PC patients, suggesting that serum exosomal miRNAs, seldom reported in other types of cancer, may be used as potential biomarkers of PC, especially serum miR-17-5p.[87] The relationship between high levels of miR-17-5p with PC metastasis and advanced stage encouraged us to perform further research to identify the biological function of exosomal miR-17-5p as an intercellular messenger that mediates PC invasion and metastasis. Research results show that the foundation of the development of serum exosomal miRNAs can be used as novel biomarkers for PC detection.[88]

EXOSOMAL miRNAS IN ESOPHAGUS CANCER

Esophageal cancer with distant metastasis and local invasion is associated with a poor prognosis. Esophageal squamous-cell carcinoma (ESCC) is a common pathological cancer subtype in non-Western countries, and can be caused by smoking or alcohol use. Combination therapy for ESCC includes surgery, chemotherapy and radiation that may improve clinical outcomes but are not curative. Early detection, including endoscopic and radiological examinations, is the key to improved prognosis. Some complicated diagnostic methods, such as blood and urine ESCC screening, are currently not available.[89–91] Several studies have shown that miRNAs have an important role in multiple aspects of carcinogenesis through their oncogenic or tumor-suppression functions.[92,93] MiRNAs in serum and plasma potentially are useful as clinical diagnostic or prognostic tools.[94–96] Exosomes are secreted by most cell types, including T cells, platelets and cancer cells.[97,98] Exosomes may act as a delivery system for cells, tissues and organs, and they may regulate various bioactivities associated with intercellular communication.[99,100] It remains unknown whether exosomes contain significant amounts of functional miRNAs in human samples. Tanaka *et al.*[101] reported that the exosomal miR-21 level was elevated in serum from patients with ESCC as compared with serum from patients with benign tumors without systemic inflammation. In addition, exosomal miR-21 is positively correlated with tumor progression and aggressiveness. In particular, miR-34a was epigenetically downregulated in ESCC.[102] Moreover, Takeshita *et al.*[103] reported in 2013 that serum miRNA-1246 showed a sensitivity of 71.3% and a specificity of 73.9% in ESCC diagnosis. Serum miRNA is found to be a strong independent risk factor for poor survival; it correlates significantly with the tumor, lymph node and metastasis stage.[104,105] Studies have examined the expression of miR-21 in normal and tumor tissues from patients with ESCC and found that it has a strong potential as a novel diagnostic and prognostic biomarker in ESCC.[106] These studies indicated that miR-21 targets programmed cell death protein 4 at the post-transcriptional level and regulates cell proliferation and invasion in ESCC.[107] A higher level of expression of serum miR-21 was detected in patients with ESCC, indicating that miR-21 may be a biomarker for initial ESCC diagnosis. The first research results show that exosomes with miR-21 enrichment was higher in serum from patients with ESCC and that exosomal miR-21 in the serum was correlated with younger age, advanced tumor (T) classification, positive lymph node status and metastasis. A few studies have shown that exosomes in serum from patients with ESCC contain large quantities of miRNA-21, which may potentially be used as biomarkers in clinical diagnostics. They further indicated that exosomal miR-21 may participate in ESCC progression, suggesting that it may be a useful therapeutic target or diagnostic biomarker of ESCC.[105] A recent study by Chiam *et al.*,[108] explored the value of circulating exosomal miRNAs in individuals with esophageal adenocarcinoma and non-dysplastic Barrett's esophagus.

They suggested that a multi-biomarker panel, containing RNU6-1/miR-16-5p, miR-25-3p/miR-320a, let-7e-5p/miR-15b-5p, miR-30a-5p/miR-324-5p and miR-17-5p/miR-194-5p had a higher specificity and sensitivity (receiver-operating characteristic = 0.99, 95% confidence interval 0.96–1.0) over single miRNA ratios in order to discriminate esophageal adenocarcinoma from controls and Barrett's esophagus.[108] Another study by Warnecke-Eberz *et al*.[109] showed that exosomal onco-miRs (for example, miR-223-5p, miR-223-3p, miR-483-5p, miR-409-3p, miR-196b-5p, miR-192-5p, miR-146a-5p and miR-126-5p) might be used as noninvasive diagnostic biomarker and monitoring of esophagus adenocarcinoma.

EXOSOMAL miRNAS IN OTHER GI CANCERS

Abnormal regulation of miRNAs and applications of miRNA have been frequently reported in a variety of cancers, including GI cancers, lung cancer, breast cancer, hematologic malignancies, brain tumor, ovarian cancer and prostate cancer. In particular, miRNA has been studied as a biomarker for histological classification, disease prognosis, clinical response and the diagnosis of cancer.[110] In fact, MiRNA is being investigated as a noninvasive biomarker in human cancers because of the ease and repeatability of sample collection of serum, and plasma. Exosomes have been found in body fluids such as plasma, urine, saliva, breast milk and synovial fluid.[111,112] Exosomes are comprised of cell-specific protein, mRNA and miRNA.[83] Interestingly, a previous research has suggested that GI tumors can be strictly distinguished from non-GI tumors by the total analysis of miRNA expression profiles. There are two types of miRNAs—oncogenic and tumor-suppressive miRNAs—which are involved in the pathogenesis of GI cancers.[113–115] Interestingly, research studies have focused on the functional role of two types of cancer-related miRNAs—oncogenic miR-21 and the tumor-suppressive miR-34 family that is frequently deregulated in GI cancers.[116,117] These miRNAs' abnormal expressions are associated with the expansion and progression of GI cancers.[24]

The identification of biomarkers that can be routinely measured in easily accessible samples and that are able to diagnose cancer and predict treatment efficacy and the risk of progression or relapse is a major challenge in cancer research. For example, the role of miRNA in the diagnosis and therapy of hepatocellular carcinoma (HCC) and other malignancies has been studied.[111,118] The miRNAs are a well-known class of regulatory molecules that post-transcriptionally control the translation and stability of mRNA. A single miRNA can regulate the expression of mRNA from hundreds of genes.[119] Alterations of miRNA expression could be used for tumor classification, diagnosis and prognosis in cancer. Recent studies show that exosomal miRNA is stable in blood because exosomes have a protective function against degradation from enzymes such as RNAs and are resistant to unfavorable physiological conditions, including repeated freeze–thawing cycles or low/high pH. Thereby, miRNAs are tissue-specific and possibly correlate with tumor progression and recurrence.[120,122] Moreover, Weber *et al*.[121] tested the expression of miRNAs in plasma, saliva, tears, urine, amniotic fluid, colostrums, breast milk, bronchial lavage, cerebrospinal fluid, peritoneal fluid, pleural fluid and seminal fluid from normal individuals and found miR-335, miR-509-5p, miR-515-3p, miR-873 and miR-616 to be among the most abundant miRNAs present in all or most of the fluid types.[46] Of the 15 most frequent malignancies worldwide, five are cancers of the GI tract (colorectum, stomach, liver, esophagus and pancreas) and circulating miRNAs show potential as clinically relevant biomarkers in these cancers. Increasing evidence suggests that unique serum miRNA expression signatures may be used as new noninvasive biomarkers for cancer diagnosis, including HCC. Many studies investigated the feasibility of using serum exosomal miRNAs as novel serological biomarkers for HCC.[123] Later studies investigated the expression of miR-21, a significantly expressed miRNA in many human cancers including HCC, in exosomes isolated from serum samples from healthy volunteers and HCC patients. In addition, the potential of using serum exosomal miR-21 for early detection of HCC has been reported. As a result, it also highlights potential clinical applications for circulating miRNAs, exosomes and unique miRNAs as biomarkers for diagnostic and prognostic evaluation.

CONCLUSION

Exosomes are small membranous vesicles that originate from internal MVBs. Exosome-encapsulated miRNAs are being suggested as a new class novel biomarker as diagnostic and predictive marker in GI cancer. These particles are released from many cell types into the extracellular space upon fusion of MVBs with the plasma membrane. They contain a wide variety of information, including proteins, lipids, RNAs, non-transcribed RNAs and miRNAs, which can be circulated in various body fluids. Exosomes can be taken up by neighboring or distant cells and thereby modulate the functional of recipient cells and have a key role in disease progression or facilitate metastasis in cancers. This study is an attempt to investigate the molecular properties of exosomes and the importance of intra-exome miRNAs in the diagnosis of GI cancers. Exosomes carry proteins and nucleic acids of host cells that reflect an individual path of physiological conditions, and they are widely considered as good biomarkers for clinical diagnosis. For example, tumor cells release exosomes containing RNAs that are probably specific to the tumor and can be used to detect cancer. Recently, a growing number of proteins found in exosomes have been found to be potential biomarkers for various types of diseases such as cancer. There is some evidence that body fluids except for blood and urine may be used as alternative sources for exosomes' detectors. Lau *et al*.[124] showed that exosomes of saliva may provide symptomatic biomarkers for PC. Exosome application may be expanded as exosomes can be found not only in mammalian cells but also in pathological microorganisms such as Gram-negative bacteria, eukaryotic parasites of the kinetoplast family and opportunistic fungi pathogens. Theoretically, it seems that medicines targeting exosomes secreted by tumor cells can appear as a potential strategy to restore immunity response against the tumor and disruption of tumor progression. Further studies in larger and multi-center setting are warranted to investigate the clinical application of these miRNAs in GI cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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