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Exosomes and their importance in metastasis, diagnosis, and therapy of colorectal cancer

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Abstract

Extracellular vesicles are known as actual intermediaries of intercellular communications, such as biological signals and cargo transfer between different cells. A variety of cells release the exosomes as nanovesicular bodies. Exosomes contain different compounds such as several types of nucleic acids and proteins. In this study, we focused on exosomes in colorectal cancer as good tools that can be involved in various cancer-related processes. Furthermore, we summarize the advantages and disadvantages of exosome extraction methods and review related studies on the role of exosomes in colorectal cancer. Finally, we focus on reports available on relations between mesenchymal stem cell–derived exosomes and colorectal cancer. Several cancer-related processes such as cancer progression, metastasis, and drug resistance of colorectal cancer are related to the cargoes of exosomes. A variety of molecules, especially proteins, microRNAs, and long noncoding RNAs, play important roles in these processes. The microenvironment features, such as hypoxia, also have very important effects on the properties of the origin cell–derived exosomes. On the other hand, exosomes derived from colorectal cancer cells also interfere with cancer chemoresistance. Furthermore, today it is known that exosomes and their contents can likely be very effective in noninvasive colorectal cancer diagnosis and therapy. Thus, exosomes, and especially their cargoes, play different key roles in various aspects of basic and clinical research related to both progression and therapy of colorectal cancer.

KEYWORDS

colorectal cancer (CRC), exosome, long noncoding RNAs (lncRNAs), mesenchymal stem cells (MSCs), microRNAs (miRNAs)

1 | INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnozed cancer in both men and women worldwide.[1] CRC prevalence and death rates have been decreasing in developed countries for some decades due to a better control on risk factors especially changes in lifestyle, such

Abbreviations: CAF, carcinoma–associated fibroblast; CRC, colorectal cancer; CSC, cancer stem cell; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; EV, extracellular vesicle; HLSC, human liver stem cell; lncRNA, long noncoding RNA; miRNA, microRNA; MSC, mesenchymal stem cell; MVB, multivesicular body; MVP, major vault protein; NCK, non-catalytic region of tyrosine kinase adaptor protein; siRNA, small interfering RNA.

as decreased red meat consumption and smoking and increased use of aspirin, and also because of the introduction and availability of screening tests and advances in therapeutic strategies.[2,3] However, the trend observed in most developing countries and also some developed countries is different and due to growing population and aging effects, CRC incidence rates have a steep gradient.[4]

In the last decade, many studies have focused on extracellular vesicles (EVs) as important mediators for cellular communications, including the transfer of biological signals and various cargoes between cells, which result in regulation of several pleiotropic biological procedures.[5] Exosomes are small EVs with 30 to 200 nm in diameter that can include different materials, such as many types of nucleic acids and proteins (for example, messenger RNAs [mRNAs], microRNAs [miRNAs], long noncoding RNAs [lncRNAs], small interfering RNAs [siRNAs], tetraspanins, and tetraspanin-associated proteins) from their originating cells and thus, playing fundamental roles in intercellular communications.[6-8] In other words, these EVs play pleiotropic roles in cancer progression and metastasis, including invasion, angiogenesis, immune modulation, and even drug resistance.[9]

Studies have shown that exosomes are secreted by all cell types in culture and are also found to a great extent in body fluids, such as urine, blood, breast milk, and saliva.[10] Generally, exosomes are made during endocytosis. At first, endosomes are produced by internalization of the cell membrane. Then, multivesicular bodies (MVBs) are formed by generation of several small vesicles inside the endosomes. Finally, these large particles, fuse with the cell membrane, and exosomes are released into the extracellular space as the intraluminal endosomal vesicles.[11] Various EVs can be secreted in diverse methods, for instance, microvesicles are directly shed from the plasma membrane, while exosomes are released from different cell types by fusion with the cell membrane.[12] In fact, when MVBs fuse with the plasma membrane, it can be said that exosomes are released by exocytosis.[13]

In addition, it is known that several molecules act as a regulatory network for creation and secretion of exosomes in maternal cells. For example, both P53 and its downstream effector TSAP6 could enhance exosome creation.[14] In addition, it is revealed that syndecan-syntenin interacts directly with ALIX protein via Leu-Tyr-Pro-X(n)-Leu motif, which is an important interaction for exosome formation to support the intraluminal budding of endosomal membranes.[15] Another key example for this topic is the Rab27a and Rab27b, which are related to secretion of exosomes and knockdown of these molecules or SYTL4 and EXPH5, as their effectors could have a negative effect on exosome secretion.[16] However, because of differences in the cellular origins and modes of formation of the exosomes, it is suggested that even among exosomes themselves several subtypes can be defined.[17]

It should be noted that several methods with various advantages and disadvantages are used for exosome extraction. Generally, these methods should display high effectiveness in isolating exosomes from different sources.[18] Some of the widely used methods include ultracentrifugation, immune isolation, microfluidics-based techniques, exosome precipitation, filteration density-based separation, and chromatography.[19,20] Different methods lead to differences in the purity, concentration, and size of exosomes and exosomal contents.[21] Therefore, to promote the clinical applications of these particles, various isolation strategies must be optimized and validated. Some advantages and disadvantages of the more important exosome isolation techniques are shown in details in Table 1.

Another point to consider about exosomes is their function. Generally, it is revealed that exosomes are components involved in intercellular communications and are messenger vesicles, which purposefully deliver several signaling macromolecules between very particular cells.[10] Especially in malignancies, it is shown that these vesicles have conflicting roles such that depending on different conditions, they can play a role as promoters of tumor progression or have antitumor properties. However, to explore the properties of exosomes in different cancer types, more studies are required to determine the complication and heterogeneity of these particles. On the other hand, today exosomes and especially their contents have developed as a potentially suitable tool in the field of diagnosis and treatment of various types of cancers.[10,33]

In recent years, some studies have confirmed that exosomes play roles in cancer pathogenesis via several strategies, such as formation of metastatic niche, epithelial-to-mesenchymal transition, hypoxia, and transforming growth factor beta (TGF-β) and Wnt-β-catenin signaling. In addition, it is specified that the isolation, quantification, and further scrutiny of tumor-derived exosomes have enormous and valuable clinical significance toward the development of cancer diagnosis and therapy, especially personalized therapy of these diseases.[34]

Recently, these vesicles have increased intense interest in scientific society as probable diagnostic biomarkers and therapeutic vehicles for cancer, infectious diseases, neurodegenerative illnesses, and several other different diseases.[6] One of the most important features of these vesicles is that they have the lipid bilayer membrane, which protects their cargo from RNases and proteases

TABLE 1 The advantages and disadvantages of the exosome isolation techniques

Technique		Advantage(s)	Disadvantage(s)	Reference(s)
Ultracentrifugation		– More suitable for protein analysis – High purity	– Poor recovery of exosomes from highly viscous biofluids such as plasma and serum – Time consuming and requires next multiple steps – High cost	18,22
Immune isolation		– Can be tissue specific – Rapid and simple (relatively)	– Not suited for large sample volumes – Captured extracellular vesicles may not retain biological functionality – High cost	23-25
Microfluidics-based techniques		– Rapid and simple – Can work with small amounts of starting material	– Contamination with microvesicles of smaller size – Not suited for large sample volumes – High cost – Set-up for academic research laboratories	26
Exosome precipitation		– Time saving – Appropriate with no need for expensive tools	– Unable to determine particle heterogeneity – Not specific for exosomes or other EVs	18,21,27,28
Filteration	– Microfiltration (pore size 0.1 to 10 μm) – Ultrafiltration (pore size 0.002 to 0.1 μm)	– Rapid and simple – Maintains vesicle structure – Nonspecific binding of soluble proteins and vesicles	– Low purity grade and purification of protein aggregates – Low exosome yield	23
	– Tangential-flow filtration	– Readily scalable – High-enrichment isolation	– Contamination with other microvesicles – Needs additional steps including ultracentrifugation with density gradients to attain high purity of microvesicles	24
Density-based separation	– Sucrose gradient	– No mechanical stress – No morphological changes	– Inefficient in separating exosomes from other particles such as HIV-1 due to similarities in their size/diameter and buoyant density	29
	– Iodixanol gradient	– Efficient in separating exosomes from particles such as HIV-1 due to similarities in their size/diameter and buoyant density	– The need for further supplementary tests	29-31
Chromatography	– Size-exclusion chromatography	– Time saving – High yield – Biologically active vesicles	– Final sample may require concentration – Variable level of impurities	24,32
	– Affinity chromatography	– Allows characterization of surface phenotype – Can be tissue specific – Easily scalable	– Nonspecific bindings – High cost	32

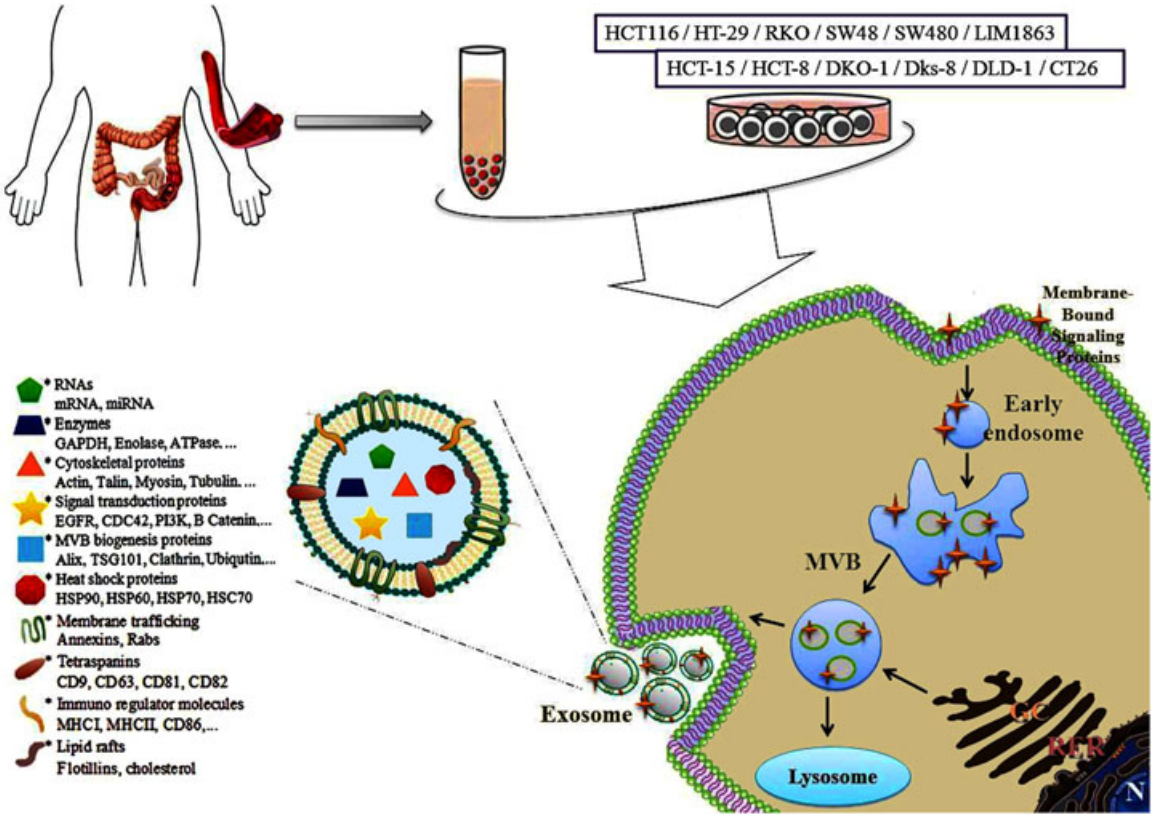

FIGURE 1 Schematic showing that exosomes are enclosed by a lipid bilayer membrane and contain different molecules derived from their cell of origin, such as many types of RNAs and proteins with different structural and functional roles. GC, Golgi complex; MVB, multivesicular body; N, nucleus, RER, rough endoplasmic reticulum

and also allows them to act as preferable delivery transporters in therapeutic processes (Figure 1).[35]

2 | EXOSOMES IN CRC PROGRESSION

Tumor-released exosomes may contribute to cancer progression in crucial stages of the process by contributing to the intercellular relationships. Cell-cell communication via these EVs in the tumor microenvironment and with distant cells appears to play a key role in the progression of cancer.[36] Tumor cells from different sites or organs may interconnect via transporting genetic information by cancer cell–derived exosomes and, in this way, increase tumor progression during multiorgan tumorigenesis.[37] It is also noted that exosomes have the potential to facilitate tumor progression both locally and systemically by supplying the tumor niche and with genetic contents and molecules that stimulate related processes, such as proliferation, invasion, metastasis, and drug resistance.[38]

In addition to the release of soluble proteins and other biological particles, CRC cells have been shown to release a variety of EVs including exosomes. EVs have been known as capable vehicles for simplifying intercellular communication in this type of cancer.[38] It is noted that exosomes derived from the CRC cells have been enriched for cell cycle–related molecules such as mRNAs and can stimulate proliferation of endothelial cells, thus suggesting that these cancer cell–derived microvesicles could be involved in CRC progression by simplifying some processes including angiogenesis.[39]

Moreover, it has been noted that proteins also play important roles in this regard. Although detailed functions of most of these molecules in various processes associated with exosomes such as CRC progression are not completely clear, it was shown that some related proteins such as CD44, ADAM10, macrophage migrating inhibitory factor and TAGLN2 play a role in CRC progression. Nevertheless, some of these proteins were not identified in CRC cells, HT-29-, and LIM1215-derived EVs. Some other proteins through exosome-related strategy play a role in cytoskeleton organization (ACTR2; actin related protein 2/3 complex subunit 1B; actin related protein 2/3 complex subunit 2; capping actin protein of muscle Z-line alpha subunit 2; capping protein (actin filament) muscle Z-line, beta; CORO1B; copine 3;

TABLE 2 The list of CRC-derived exosomal microRNAs and their features

CRC-derived exosomal microRNA	Description/ functions/ importance	Sources of extracted exosomes (sample type/cell line)	Methods					References
			qRT-PCR	miRNA microarray	Deep sequencing of RNA	High-throughput sequencing	miRNA in situ hybridization	
miR-18a	An oncogene in CRC/ the level in the exosomes from either primary colon tumour tissue or metastatic liver of colon tumour is higher than in their donor tumour tissues/biomarker for CRC	Serum, CT26, SW620, LIM1863	*	*	*			38,57–59
miR-19a	Prognostic biomarker for recurrence in CRC	Serum, LIM1863	*	*	*			38,52,58,59
miR-21	Suppression of apoptosis/promoting cell proliferation and cancer progression/Induction of CRC metastasis/Poor prognosis of CRC	Plasma, SW480, WiDr	*	*				56
miR-29a miR-92a	Noninvasive screening tools for colorectal adenomas	Serum	*					60
miR-34a miR-145	Intracellular low expression of these antioncomiRs in human colon cancer cells can contribute to the protection of cancer cell growth and drug resistance	DLD-1	*					61
miR-96-5p miR-149	Markers for the diagnosis of CRC	Plasma, HT-29, HCT-116	*					62
miR-100	Upregulated in exosomes derived from mutant KRAS cell lines/ potential biomarker for CRC	Serum	*			*	*	63
miR-125a-3p	Biomarker for early-stage colon cancer	plasma	*					64
miR-150	Biomarker for CRC/enhanced cell migration in HMEC-1 cells	Serum, Normal human fetal colon-derived FHC cells, HCT-116, HT-29, RKO, SW48, SW480	*	*				58,65,66
miR-192	Downregulated in CRC/induction of cell-cycle arrest/induction of chemoresistance mechanisms of fluoropyrimidine and antifolates/ inhibition of CRC metastasis	LIM1863, HT-29, SW480		*	*			55,59,66,67

(Continues)

TABLE 2 (Continued)

CRC-derived exosomal microRNA	Description/ functions/ importance	Sources of extracted exosomes (sample type/cell line)	Methods					References
			qRT-PCR	miRNA microarray	Deep sequencing of RNA	High-throughput sequencing	miRNA in situ hybridization	
miR-193a	Inhibition of cell proliferation and tumor progression, cell cycle G1 arrest, biomarker for predicting progression of colon cancer	CT26, SW620	*	*				57
miR-200	CRC prognosis/predict distant metastasis/cellular differentiation chemoresistance	Serum, CCL227, LIM1863	*		*		*	59,60,68,69
miR-210	Upregulated in exosomes derived from HCT-8 cell line/help in metastasis and homing	HCT-8	*					70
miR-215	Potential biomarker/induction of chemoresistance mechanisms of fluoropyrimidine and antifolates	LIM1863, HCT-15	*		*			59,66,67
miR-223	Potential biomarkers for CRC	Serum, LIM1863	*	*	*			58,59,71
miR-375	Downregulated in CRC/ via the Bcl-2 pathway, play an important role in inhibition of CRC progression and invasion	Serum, HCT-116, CT26	*	*				57,72
miR-379	Decreases cell proliferation and migration	HT-29, HCT-116	*					38,55
miR-1229	Potential biomarker for CRC	Plasma, Serum, Normal human fetal colon-derived FHC cells HCT-116, HT-29, RKO, SW48, SW480	*	*				64,65
miR-1224-5p	Potential biomarker for CRC	Serum, HCT-116, HT-29, RKO, SW48, SW480	*	*				65
miR-4772-3p	Prognostic biomarker for recurrence of cancer in stage II and stage III CRC patients	Serum	*					52
let-7a	Potential biomarker for CRC	Serum, HCT-116, HT-29, RKO, SW48, SW480, LIM1863	*	*	*			36,71,73

Abbreviations: CRC, colorectal cancer; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

and FSCN1) and cancer progression (CD276, epithelial cell surface adhesion molecule [EpCAM], and intercellular adhesion molecule 1).[40-42] Serine/threonine kinase receptor–associated protein, upregulated in CRCs, is also referred to as an enhancer of tumorigenicity by promoting anchorage-independent growth.[43] Furthermore, tetraspanins, as integral transmembrane proteins, play key roles in cell proliferation, migration, adhesion, and apoptosis. These proteins have diverse roles via activities through their corresponding interactions, for example, CD151 and tetraspanin-8–containing exosomes support tumor progression by stimulating matrix metalloproteinases and induction of angiogenesis, respectively.[7,44-46] On the other hand, it is shown that alteration in surface proteins of cancer cells such as alteration in O-GlcNAc extent on EV proteins of metastatic cells may be of notice as a prognostic biomarker for metastasis and progression in CRC.[47]

It has been made clear that exosomes derived from hypoxic cancer cells, such as oral squamous cell carcinoma cells,[48] glioma cells,[49] and leukemia cells,[50] promote tumor progression by regulating phenotypic modulation of endothelial cells or normoxic tumor cells. In the case of exosomes derived from hypoxic CRC cells, it has been reported that these exosomes stimulate the proliferation and migration of endothelial cells. The cellular role and fundamental mechanisms of hypoxic exosomes have not been well described.[48]

Another thing to consider is that exosomal miRNAs play an important role in cancer progression via two mechanisms: first, because exosomes have the capability to protect miRNAs in circulation from degradation and also function as carriers to transfer these molecules from donor cells to recipient cells; and second, investigations of miRNA profiles in tumor versus normal tissues and in the circulating blood have resulted in the detection of several miRNAs that are related to cancer progression and patient survival.[51,52] CRC cells secrete some miRNAs inside exosomes that may lead to inhibition of this cancer. For example, miR-375 targets genes that play a key role in regulating signaling pathways, such as mitogen-activated protein kinase, Wnt, TGF-β, and by these ways, it results in inhibition of CRC progression.[53,54] Moreover, miR-379 in HT-29 and HCT-116, as two well-known CRC cell lines, also decreases cell proliferation and migration.[55]

Furthermore, the study of plasma samples from patients with CRC and also SW480 and WiDr cell lines in vitro has shown that miR-21, as a CRC-derived exosomal miRNA, can promote cell proliferation and progression and also can induce CRC metastasis and, therefore, it can be used as a poor prognosis CRC biomarker.[56] Studies related to the functions of different miRNAs in CRC-derived exosomes are summarized in Table 2.

In addition, a few studies that focus on exosomal lncRNAs revealed that these molecular particles have key roles in CRC-related processes (Table 3). For example, it is noted that exosomal colon cancer associated transcript 2 lncRNA stimulates cell proliferation and migration and promotes angiogenesis in vivo and tube formation in vitro.[74,75] Furthermore, several studies have also shown that some specific lncRNAs such as H19 and UCA1 are strongly associated with proliferation and tumor growth.[76-84] Other studies reported that upregulated lncRNA CRNDE-h (colorectal neoplasia differentially expressed-h) is related to cell proliferation.[85-87] Moreover, studies revealed that exosomal Hox antisense intergenic RNA, as one of the best frequently reported lncRNAs implicated in cancer progression, is an activator of the Wnt pathway in intestinal cells.[88,89]

3 | EXOSOMES IN CRC METASTASIS

Since cancer cells release exosomes into the extracellular environment, these microvesicles as the most efficient

TABLE 3 The list of CRC-related exosomal lncRNAs

CRC-drived exosomal lncRNA	Description/function/importance	References
CCAT2	Stimulates cell proliferation and migration/promotes angiogenesis in vivo and tube formation in vitro	74,75,90
HOTAIR	Activator of the Wnt pathway/cancer development	88,89
BCAR4	Downregulated in the serum of patients with colon adenoma/suitable as part of panel for diagnosis of CRC. (BCAR4, KRTAP5-4, and MAGEA mRNAs)	91
CRNDE-h	Increased in patients with CRC/positively associated with regional lymph node metastasis and distant metastasis/correlated with shorter overall survival of patients with CRC	92

Abbreviations: CCAT2, colon cancer associated transcript 2; CRC, colorectal cancer; CRNDE-h, colorectal neoplasia differentially expressed-h; HOTAIR, hox antisense intergenic RNA; lncRNA, long noncoding RNA; mRNA, messenger RNA.

intercellular communicators play a critical role in metastasis.[7] According to current knowledge, there is reasonable evidence that tumor-derived exosomes play a key role in the communication between cancer and host, and in this regard, it is noted that the transmembrane 4 superfamily or tetraspanins are probably effective on the functional activity of exosomes involved in metastasis.[93] Of course, this should be noted that tetraspanins have a diversity of the functional roles in metastasis process, for example, some of them such as CD9, CD63, and CD82 mediate metastasis inhibition, whereas some others including CD151 and tetraspanin-8 have increasing effects on this process by several mechanisms.[7,39,44-46,51]

Cancer cells lose their cellular adherence properties as they develop into malignant forms. Hence, metastatic cancer cell–derived EVs may not represent the cell adhesion features of the cell of origin.[40] In the case of the CRC cells, specifically about the secretome protein profiles released in vitro from primary SW480 cell line and its lymph node metastatic variant SW620, it has been shown that many of the proteins that are selectively enriched in metastatic CRC cell–derived exosomes can act both as secreted modulators of the metastatic niche such as main metastatic factors (MET, S100-A8, S100-A9, and tenascin C) and in key signaling pathways (Ephrin B2, epidermal growth factor receptor [EGFR], jagged1, SRC, and TRAF2 and non-catalytic region of tyrosine kinase adaptor protein (NCK) interacting kinase) relative to primary CRC cell exosomes.[94] Furthermore, by using high-throughput proteomic analysis to compare EVs derived from these two related cell lines, it was found that despite the importance of both types of EVs in the field of metastasis, SW620 EV-enriched proteins have more complex effects and can act as metastatic factors and also play roles in essential signaling pathways.[9,43] Moreover, other proteomic studies focused on the discovery of candidate protein markers for CRC metastasis and have shown that some proteins such as jagged 1 protein, ephrin-B2, cadherin-17, met-proto-oncogene, TRAF2, tenascin C, and NCK-interacting protein kinase are important in this regard.[95]

MiRNAs are another type of specific metastatic factors and signaling pathway components related to colon cancer cell–derived exosomes and these molecules have a cross-talk between tumor and stromal cells in the tumor microenvironment. Nowadays, the number of identified miRNAs with a variety of incremental or decreasing effects in related to metastatic colon and rectal cancers is constantly growing. Studies have shown that miR-135b was increased in CRC metastatic and tumor tissues, whereas some other miRNAs, such as miR-375, miR-215, miR-378, and miR-422a, were significantly decreased in these tissues. It has been shown that miR-375 via the Bcl-2 pathway plays a key role in governing the pathways responsible for inhibition of CRC.[72] Moreover, downregulated miR-375 in several solid tumors, found in primary tumor specimens as well as in circulating fluids via exosomes, may subclinically detect the existence or the persistence of cancer, underlining a poor prognosis.[72,96] Furthermore, in vitro analyses on DLD-1 and HCT-116, as two colon cancer cell lines, showed that ectopic expression of miR-215 increases apoptosis, stimulates cell cycle arrest, and also reduces migration and viability. In other words, miR-215 could be used as a potential therapeutic target for prevention of metastasis and also as a new main biomarker in the pathogenesis of CRC.[97] In contrast, a study on HCT-8 cell line has shown that miR-210 was upregulated in exosomes derived from these cells and this miRNA resulted in cancer cell metastasis and homing processes[70] (Table 2). Furthermore, it is shown that exosomal CRNDE-h lncRNA levels in patients with CRC were higher and it was positively associated with distant metastasis and especially regional lymph node metastasis (Table 3).[92]

4 | EXOSOMES IN CRC DRUG RESISTANCE

Another important role of the RNAs and proteins that have been extracted from the tumor-derived EVs is induction and/or enhancement of drug resistance in cancer cells.[47] Generally, two suggested patterns and fundamental mechanisms may contribute to drug resistance of cancer stem cells (CSCs) in the recurrent CRC tumors. One is related to specific properties of CSCs such as having a variety of ABC-transporters that make them intrinsically resistant to chemotherapy; and the second mechanism is related to the exosomes in tumor microenvironment, which lead to further drug resistance of CSCs.[98] There are a number of important components such as carcinoma-associated fibroblasts (CAFs) and especially CAF-derived exosomes, which are extensively involved in chemotherapeutic resistance.[99] It has also been revealed that CSCs resistant to chemotherapy via exosomes can spread their resistance to other cancer cells.[100]

Moreover, applying changes to microenvironment can have important effects on drug resistance properties of the tumor cells. For example, Lugini et al[101] showed that tumor exosomes induce a disorder in colon-derived mesenchymal stem cells (cMSC). Colon cancer cells might change the cMSC niche to preserve their stem cell element that consequently becomes resistant to chemotherapy.[101]

Furthermore, a study on DLD-1, a colorectal adenocarcinoma cell line, has shown that some other

CRC-derived exosomal miRNAs, such as miR-34a and miR-145, can contribute to the maintenance of CRC cell proliferation and also, especially, to the induction of drug resistance.[61] Moreover, other related studies on CRC-derived exosomal miRNAs have shown that miR-192 and miR-215 can induce chemoresistance to fluoropyrimidine and antifolates in CRC cells (Table 2).[59,66,67]

5 | EXOSOMES FOR DIAGNOSIS OF CRC

Despite progress in curative surgical interventions and adjuvant chemotherapies, 20% to 50% of all patients with CRC will eventually face disease recurrence from which 74% of them develop within the first 3 years of diagnosis.[102] Therefore, diagnosis of early-stage CRC appears to be an important factor to decrease its mortality and problems associated with the disease. Unfortunately, despite many progresses in the development of new methods for diagnosis of CRC, there is still no accurate biomarker or biomarker panel for early diagnosing purposes, and half of all patients with CRC are diagnosed at stage II or stage III of the disease.[102,103] In general, a perfect screening system should have high specificity and sensitivity for early-stage CRC diagnosis and it should be noninvasive and economical so that it can be easily accepted by patients.[103,104]

Furthermore, many factors have been used for diagnosis and prognosis of CRC, including carcinoembryonic antigen, EpCAM, EGFR, claudin-3, glycoprotein A33, galectin 4, S100-A8 calcium-binding protein, SRC, and S100-A9 calcium-binding protein.[95] Since all cancer cells produce exosomes from early stages and these EVs play efficient roles in recipient cells, the molecular content of these exosomes may provide unique molecular markers for early diagnosis and prognosis. Therefore, researchers propose that circulating exosomes may provide an effective tool for noninvasive diagnosis and prognosis of human cancers.[104,105] Moreover, several reports on CRC have also revealed that specific circulating exosomes in the plasma of patients are related to poor prognosis and can be reliable biomarkers for diagnosis of this cancer.[106-108]

Furthermore, proteome analysis of exosomes derived from colon cancer cell lines has led to the documentation of other candidate markers for this cancer, such as ephrin-B1 and cadherin-17.[41,109] It was also found that collapsin response mediator protein-2 (CRMP-2) was exclusively detected in the colon adenocarcinoma cell lines Colo205 and SW480 secretome. CRMP-2 was eventually confirmed in serum, indicating its importance for discriminating patients with CRC from healthy donors.[110]

Another study reported that the plasma glypican 1 (GPC1) positive exosomes could be used as a biomarker for CRC. The percentage of GPC1+exosomes and the GPC1 protein expression in exosomes from cancerous tissues and plasma of patients with CRC before the surgery were meaningfully higher than those in the plasma of healthy controls and the normal tissues. It was also shown that together with GPC1 expression and GPC1+exosomes, miR-96-5p and miR-149 could be suitable markers for diagnosis, evaluation of therapeutic efficacy, and likely targets for molecular therapy of CRC.[62]

Recently, more efforts have been made to use miRNAs in plasma or serum as diagnostic markers of different cancers, such that several miRNAs with high levels of expression in cancer tissues have been reported as suitable candidates for diagnosis of CRC.[111,112] For example, miR-21, miR-29a, and miR-92a as noninvasive screening tools in patients with colorectal adenomas can be incorporated into routine clinical practice in the not-so-distant future pending validation in large-scale potential trials.[60] On the other hand, nowadays, more research is focused on the exosomal miRNAs in body fluids that might be useful and specific as diagnostic biomarkers for the detection of various cancers such as CRC.[65] For example, several studies revealed that some of the identified exosomal miRNAs such as miR-100, miR-200, miR-223, miR-1229, miR-1224-5p, and let-7a could assist as noninvasive screening tools in patients with CRC and this approach may easily complement existing conventional invasive detection approaches.[59,60,63,68,69]

Another example for exosomal miRNAs as CRC biomarkers is the plasma exosomal miR-125a-3p that may provide a chance to distinguish between early-stage CRC and normal controls.[64] Furthermore, it was demonstrated that miR-21 is significantly overexpressed (five folds) in exosomes derived from patients with colon cancer, particularly in patients at the first diagnosis or before any treatments.[113] Generally, a unique miRNA like miR-21 is not an exact diagnostic marker in CRC; it requires to be accompanied with other miRNAs for improved specificity.[60]

Another study in this area showed that exosomal miR-193a may be used as a biomarker for predicting the progression of colon cancer.[57] Furthermore, increased exosomal miR-19a in human serum samples correlates with early recurrence of CRC.[65] Moreover, a study showed that expression of serum exosomal miR-4772-3p is also a prognostic biomarker for recurrence of CRC at stage II and stage III.[52] In the same field, data from cultured cells and patient-derived samples suggest the presence of a set of exosome miRNAs that includes miR-18, miR-1229, let-7a, miR-150, miR-1246, miR-223,

miR-21, and miR-23a, which can be used as a reliable package for CRC diagnosis (Table 2).[71,73]

On the other hand, although the significance of circulating lncRNAs in predicting and diagnosis of various cancers is extensively investigated, and it is known that some of these molecules can also be probable biomarkers for CRC diagnosis, a few studies have considered the use of exosomal lncRNAs as suitable biomarkers for diagnosis of this cancer.[76-84,87] Moreover, several studies reported that some particular exosomal lncRNAs, such as UCA1, H19 and upregulated lncRNAs CRNDE-h and Zinc finger antisense 1 in CRC are related to poor prognosis and had specific advantages for diagnosis of this cancer.[76-87,92] Furthermore, a recent study, after a series of bioinformatic analyses, screening important pathways and GO terms that were related to upregulated and downregulated transcripts, noted that lncRNA BLACAT1 and four downregulated lncRNAs including LOC344887, LINC00675, DPP10-AS1, and HAGLR are all biomarkers for diagnosis of CRC.[114] In this regard, studies revealed that exosomal BCAR4 lncRNA is downregulated in the serum of patients with colon adenoma compared with normal subjects. In addition, the combination of two mRNAs, KRTAP5-4, and MAGEA, with this lncRNA provided a suitable panel for diagnosis of CRC (Table 3).[91] However, despite some challenges in the clinical application of exosomes, some specific properties of these EVs give the promise that exosomes can be novel diagnostic markers for CRC in the near future.

6 | EXOSOMES IN CRC THERAPY

Since CRC is the second important cause of cancer-related death in the world, more investigation to find better strategies for highly effective targeted therapy is necessary. Studies have shown that exosomes have specific properties such as antigen-presenting capability, which make them a theoretically smart vehicle for cancer immunotherapy.[115-117] Furthermore, because exosomes can easily cross biological barriers, they represent a fine potential delivery vehicle for targeted transfer of therapeutic molecules such as proteins and different types of RNAs, especially miRNAs into cancerous cells. Therefore, exosome engineering with the aim of loading specific molecules into the exosomes may overcome the problems associated with in vivo delivery of these molecules as the most major challenge in this type of therapy.[38]

Generally, miRNA-based therapy appears to be a very ingenious and promising new approach in gene therapy of CRC. Furthermore, it is known that exosomes can be engineered to overexpress these molecules and, thus, affect the recipient cancer cells. For example, it was noted that exosomes can be engineered to overexpress miR-379 and these exosomes could be transferred to recipient cancer cells and reduced CRC cell proliferation and migration (Table 2).[38]

Furthermore, major vault protein (MVP) that is overexpressed in multidrug-resistant cancer cells binds to tumor suppressor miR-193a, forming an MVP protein-miR-193a complex in the exosomes. It was found that patients with colon cancer at more progressive stages show higher levels of circulating exosomal miR-193a. MiR-193a causes tumor progression inhibition, cell cycle arrest at G1, and cell proliferation repression through targeting Caprin1, which upregulates Ccnd2 and c-Myc.[57,118,119] So, MVP protein-miR-193a complex is packed into exosomes leading to the reduction of cytoplasmic miR-193a and it is obvious that knockout of MVP leads to accumulation of miR-193a in the cytoplasm instead of exosomes and ultimately leads to inhibition of tumor growth.[57]

On the other hand, some studies have proposed that loading chemotherapeutic drugs into exosomes can be considered as one of the most effective strategies for exosome-based cancer therapy, especially to target CSCs in vivo. Furthermore, another study showed that chemotherapeutic drug delivery by exosomes has more anticancer effects than the free drugs in animal tumor models.[120] For example, exosome-delivered doxorubicin minimized tumor size much more efficient than free or liposome-delivered doxorubicin in a colon adenocarcinoma mouse model.[121]

Furthermore, understanding the molecular heterogeneity of tumor is of specific correlation to forecast medical consequence of targeted therapies, an example is an observation that anti-EGFR therapy in CRC does not have an effect in Kirsten rat sarcoma viral oncogene homolog (KRAS) mutant CRC.[122] The efficiency of EGFR-targeted therapy is meaningfully associated with KRAS and BRAF (B-raf serine/threonine kinase proto-oncogene) mutations in patients with CRC. However, a relative measurement for the presence of KRAS and BRAF mutations in the serum exosomes and primary tumor tissues from patients with CRC revealed that serum exosomal mRNA may be used as a unique and innovative source for rapid and noninvasive genotyping of patients with CRC.[123]

Furthermore, two separate phase I clinical trial studies for CRC revealed that ascite-derived exosomes combined with granulocyte-macrophage colony-stimulating factor, and plant exosomes to deliver curcumin, both have a useful tumor-specific antitumor cytotoxicity.[124,125]

However, some studies have shown that there are many challenges and complications in exosome-based

approaches for cancer therapy and thus more studies are needed to improve the quality and accuracy of these types of treatments. For example, it is known that Wnt/β-catenin is a typical pathway in cancer and without Wnt signaling, β-catenin is degraded in the cytoplasm. Whereas, β-catenin with Wnt signaling is accumulated in the cytoplasm and as a transcriptional cofactor translocates into the nucleus.[126,127] However, some exosomes by delivering Wnt mRNAs can affect on β-catenin levels in the recipient cells and help to change cell fate. Moreover, hypoxic tumor microenvironment is essential for effective angiogenesis-targeted therapy in metastatic CRC, and it was also shown that hypoxic CRC cell–derived exosomes by delivering Wnt4 mRNAs could stimulate β-catenin in endothelial cells and thus, facilitate endothelial cell proliferation and migration.[49]

7 | MSCs, EXOSOMES, AND CANCER

In recent decades, stem cell therapy as the advanced and promising therapeutic approach to treat different types of diseases has attracted much attention. Furthermore, because of MSCs unique features, they are one of the most reliable cells in therapy compared with other stem cells.[128] MSCs may be involved in several vital processes, such as regulation of immunological responses, homeostasis-related processes, tissue maintenance and repair, and also these cells can differentiate into mesodermal lineage and many other cell types with dissimilar embryonic origins, such as lung, muscle, liver, bone marrow, and skin cells.[129] Moreover, MSCs are easily isolated, and therefore, there is a high tendency to test MSCs and their components in various clinical applications.[128]

Recently, MSC-derived exosomes are being studied for their likely roles in stem cell–based therapy.[130] Several studies related to different strategies of MSC-based therapy suggest that the capacity of MSC-derived EVs can be used for treatment of some diseases. The therapeutic potential of MSC-derived EVs has been observed in different species, such as human, mouse, and rat, and for several types of disease models, including brain and neurological injury, myocardial diseases and kidney injury. Although the numbers of related studies are still restricted, but results powerfully support the idea that MSC-derived EVs are suitable vehicles for therapeutic applications in a wide range of diseases.[131-137] Furthermore, exosomes can reflect the phenotype of their parent cell and thus, therapeutic effects of MSC-derived factors, including cytokines and growth factors, can be relatively due to their released EVs. In addition, lung barrier is one of the main challenges for systemic administration of MSCs, thus exosomes may provide an advantage over the use of MSCs in that these microvesicles can pass through this barrier.[138]

However, cancer-related studies have shown that MSCs within the tumor microenvironment can display both pro- and antitumor activities. These cells may cause immunosuppression via their effects on tissue remodeling activity at inflammatory positions, which ultimately leads to tumor formation or progression.[139] In a study on T24, as a bladder cancer cell line, it has been made clear that human cord blood Wharton's jelly MSCs-EVs induced apoptosis and cell cycle arrest by upregulating caspase-3 cleavage while suppressing Akt phosphorylation pathways.[140] Furthermore, several studies have shown that conditioned medium derived from human liver stem cells (HLSCs) express the MSC-related phenotypes and some embryonic stem cell markers specifically Lefty A. Lefty A can disrupt Nodal signaling pathways, and thus, HLSCs have displayed antitumor effects in several specific cells, such as HepG2, MCF7, KP6, KS, and Jurkat, as cell lines with high level of Nodal signaling pathways.[141-143] Other studies have shown that subcutaneous coinjection of MSCs or MSC-EVs with SGC-7901 and SW480, as human gastric and colon cancer cells, respectively, increased tumor burden and growth. It was shown that only CXCR4 and VEGF mRNA and protein expressions were increased both in vivo and in vitro and no detectable changes in proliferation and cell cycle could be observed when compared with controls. Thus, despite the promoting effects of MSC-EVs on the angiogenic program, which affects on tumor seeding and tumor growth, the increased tumor burden observed in vivo is likely an indirect consequence of MSC-EVs.[144] Finally, selective overexpression of specific molecules, which are known as necessary for a special therapeutic effect, in the parental MSCs may lead to improvement of the therapeutic efficiency of the MSC-derived exosomes.[145-148]

8 | CONCLUSIONS

Exosomes are small EVs that can include different important cargoes such as mRNAs, miRNAs, IncRNAs, siRNAs, tetraspanins and tetraspanin-associated proteins, and many other structural and functional proteins.[6,7,149] Furthermore, these microvesicles, depending on their content and specific features, have a variety of key functional roles in CRC-related processes such as cancer progression, metastasis, drug resistance, and of course processes associated with the diagnosis and treatment of this malignancy. Despite many challenges to be addressed over time, the therapeutic strategies for CRC are still developing,[150] and due to very unique features of exosomes and growing studies—which almost every day prove the importance of using these microvesicles,

specially MSC-derived EVs—we believe that in the not too distant future, the importance of these nano-sized EVs will be understood which would lead to their applications in different cancer diagnosis and therapy, especially for CRC.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

This study does not contain any studies with human participants or animals performed by any of the authors.

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