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Review Article

Exosome: a new perspective in colorectal cancer therapy

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Abstract: Exosomes refer to the mediators between tumor cells and matrix information exchange. With the transfer of proteins and RNA mediated by exosomes, the microenvironment of tumors varies. Tumor-derived exosomes (TEXs) affect the progress, the treatment and the prognosis of colorectal cancer (CRC). In the present review, the latest research results of CRC-related exosomes in recent years are summarized, covering exosome-induced epithelial-mesenchymal cell transformation of cancer cells. They can potentiate angiogenesis, tumor cell movement, metastasis and migration and suppress immune response, and cancer chemotherapy resistance. Besides, the use of exosomes as carriers in cancer targeting therapy, and the safety and reliability of developing cancer vaccines with exosomes separated from body fluids are discussed as well. Lastly, the application prospects of exosomes as biomarkers in CRC diagnosis and prognosis are prospected.

Keywords: Exosomes, colorectal cancer, treatment, drug resistance, metastasis

Introduction

The first discovery of exosomes in rat reticulocytes [1] dates back more than 50 years. We have found that considerable diseases are associated with exosomes, and we believe that exosomes are only excreted in cells, but recently discovered exosomes involve considerable biological pathways, covering the body's immune response [2], antigen presentation [3], cell migration [4], cell differentiation [5], as well as tumor invasion [6]. Exosomes play important roles especially in the tumor cell genetic information exchange, angiogenesis, invasion and metastasis.

Recently, a growing number of research on the relationship between exosomes and cancer has been more heated, because exosomes carry proteins, RNA, LncRNA, microRNAs and lipids in tumor cells to regulate the functions of normal receptor cells, to complete the process of epithelial-mesenchymal cell transition (EMT) and to potentiate the progress of cancer [7]. Moreover, studies have shown that shed microbubbles (SMVs) can also be involved in drug resistance and spread, leading to multidrug resistance that has become a major obstacle to clinical cancer treatment [8]. In the tumor microenvironment, tumor markers mediated by exosomes are constantly discovered, which helps for a new understanding of the occurrence and development of tumor. It opens up a new way to explore the pathogenesis of cancer and provides great help for the diagnosis and treatment of cancer.

CRC ranks third among the world's male tumors and second among the world's female tumors [9]. Environment, diet and heredity are the major factors affecting the occurrence of CRC. At present, CRC is primarily treated by surgery and chemotherapy after operation. 5-fluorouracil and oxaliplatin have always been conventional chemotherapy regimens. Recently, targeted drugs have been clinically used. Nevertheless, new clinical trials confirmed that there was no value in adding targeted drugs based on some standardized therapeutic regimens (e.g., bevacizumab and cetuximab) [10]. Thus, for patients with advanced CRC, early detection of cancer, search for new biomarkers, reduction of side effects of chemotherapy and improvement of prognosis have always been our goals. Recently,

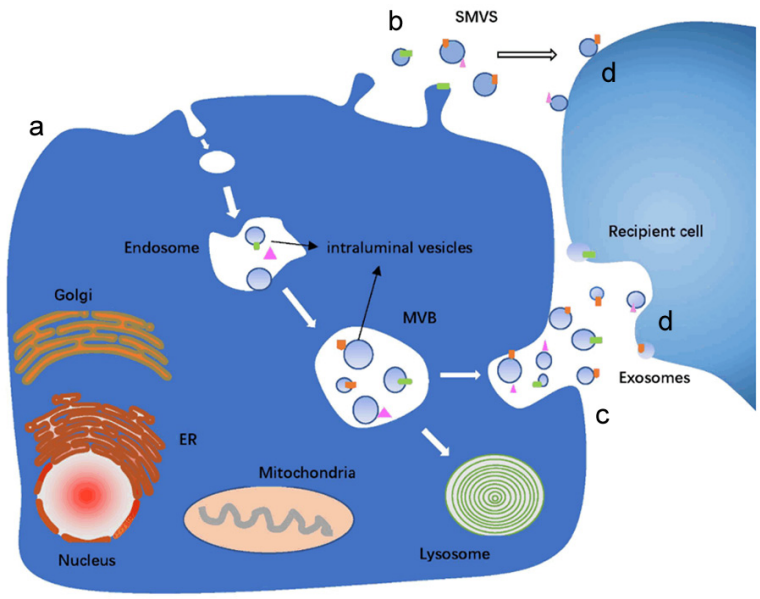

Figure 1. Schematic diagram of release of SMVs and exosomes. a. Plasma membrane budding into small vesicles, and then enters the endosomes and MVB to form intraluminal vesicles. Most MVB fuses with the plasma membrane to form exosomes and release them to the outside of the cell, while the rest MVB fuses with lysosomes. b. SMVS is discharged directly from the plasma membrane after bud. c. Exosomes carry proteins and genetic information into recipient cells via lymph nodes, saliva, intestinal fluid, ascites and blood. d. SMVS and exosomes entering the extracellular environment can stay on the surface of the target cell, or fuse directly with the plasma membrane, enter the target cell, or formation of endocytic vesicles into target cells by endocytosis.

with the further study of exosomes, we have found that the exosomes secreted by cancer cells carry their own proteins and nucleic acids, become antigens of cancer cells, can induce tumor immune response, but also suppress the immune system, and create a suitable microenvironment for the growth and metastasis of cancer cells [11]. Harada et al. found that Wnt5b-related exosomes potentiated CRC cell migration and proliferation [12]. CEA is a common diagnostic marker for CRC, but its specificity is low. Lee et al. found that TSPAN1 positive extracellular vesicles have 75.7% diagnostic value. Combining with CEA or CA-199 can significantly improve the diagnostic efficiency [13]. Recently, increasing studies have shown that exosomes play a critical role in the diagnosis and treatment of CRC.

Origin, composition and function of exosomes

Extracellular vesicles (extracellular vesicles EVs) is a phospholipid bilayer structure formed by the vesicular fragments. It has been found in all cells and can also be detected in blood, saliva, pleural and ascites, lymph, urine, intestinal fluid and synovial fluid [14-16]. EVs include exosomes, SMVs and apoptotic bodies, differentiated in line with the secretory mechanism and size [17]. The exosome is a discoid vesicle with a diameter of 40-100 nm [17]. Considerable types of cells can secrete, which is formed by the fusion of MVB and plasma membrane and released to the outside of the cell. The diameter of SMVs is about 100-1000 nm, and the size of SMVs varies. SMVs are released directly from the plasma membrane by germination [18]. Apoptotic bodies are released after the disruption of dead cells, nearly 50-5000 nm in diameter [19]. Due to current technical constraints, there has been no clear distinction between EVS subgroups, so EVS includes all extracellular vesicles in this review (The mechanism of exosome formation is shown in **Figure 1**).

Exosomes are composed of proteins, nucleic acids, lipids, etc. The main components are lipids and cholesterol [20], covering triglycerides, cholesterol, phospholipids [21]. However, exosomes from different cell contents are different, but the common protein is Rabs protein, covering Rab2b, Rab5, Rab11, Rab9a, Rab7 and Rab27. Moreover, studies have shown that the release of exosomes requires Rab27A and Rab27B to bind to effector proteins Slp4-a, Slac2-b, Munc13-4, regulate secretion, transport and fuse with receptor cell membrane [22-24]. Transmembrane proteins (e.g., CD9, CD63, CD82 and CD81) are characteristic tetramer membrane proteins in exosomes [25], while there are heat shock protein family (e.g., HSP60, HSP70, HSP20, HSC73). It has been reported that HSC73 can induce tumor immune response, presumably associated with the involvement of exosomes in anti-tumor effect [26]. Some adhesion molecules (integrin,

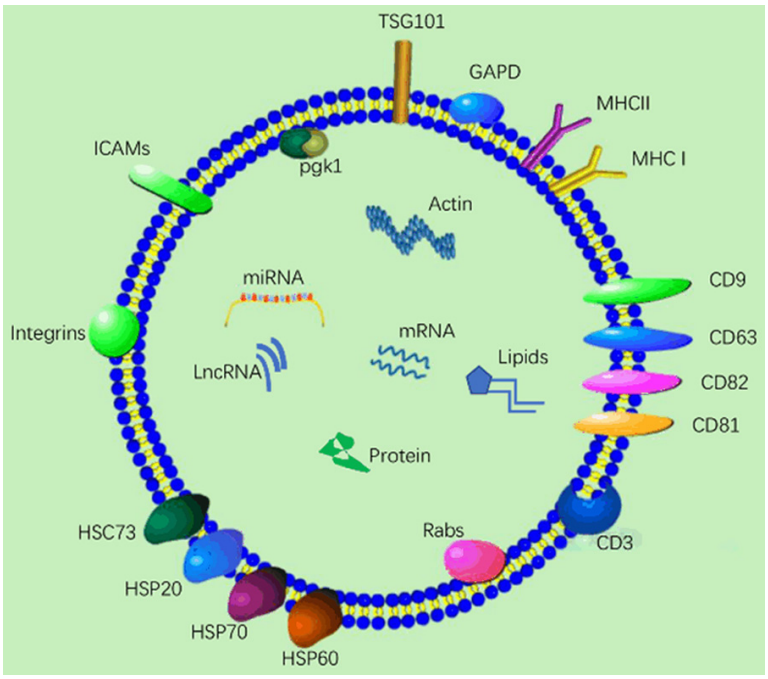

Figure 2. Contents of exosomes. Exosomes are membrane structures containing lipid bilayers, similar to plasma membranes. Exosomes from different cell sources contain different membrane proteins and genetic materials. Common proteins are Rabs protein family; transmembrane proteins CD9, CD63, CD82, CD81; heat shock proteins family, e.g., HSP60, HSP70, HSP20, HSC73. There are also adhesion molecules (integrin, ICAM-1), metabolic enzymes (GAPDH, PGK1). Some special molecules, e.g., CD3 molecules from T cells, MHC I and MHC II molecules from APC cells. Moreover, there are also genetic materials from host cells e.g., RNA, ncRNA.

ICAM-1) and metabolic enzymes (GAPDH, ATPase, pgk1) have been detected in exosomes also [27], and tumor susceptibility gene 101 (TSG101) participates in the formation and release of MVB [28]. Exosomes from different cell origins, protein expression is also different. For example, exosomes originating from T cells contain CD3, and APC exosomes contain MHC I and MHC II molecules [29]. The composition of exosomes is shown in **Figure 2**.

Besides lipids and proteins, exosomes are also rich in a large number of nucleic acids, covering RNA, lncRNA and microRNAs [30]. After the exosome is detached from the plasma membrane, the exosome carrying nucleic acid fuses with adjacent or distant cells and releases nucleic acid to regulate the function of target cells. Recent reports have shown that exosome-mediated ncRNA is closely associated with tumor effects, especially microRNA. Tumor tissue, normal tissue and tumor adjacent tissue exosome miRNAs also have differences [31]. This article reviews the effects of exosome-mediated microRNAs in microcirculation on the occurrence, metastasis and migration of CRC CRC-related microRNAs and their functions are shown in **Table 1**.

Tumor cell epithelial-mesenchymal cell transition

EMT is the process of transforming epithelial cells into mesenchymal cells through a certain process. It is characterized by loss of polarity of epithelial cells, disconnection from basement membrane, increased migration and invasion of cells and other characteristics of mesenchymal cells [57]. EMT not only is vital to cell development, but also potentiates angiogenesis, invasion, metastasis and metastasis niche establishment in tumor microenvironment [58]. TEXs enter receptor cells, potentiate matrix remodeling, form pre-metastasis niche, and potentiate the invasiveness and migration ability of receptor cells. Syn et al. suggested that these biological processes were primarily associated with caveolin-1, HIF1α, beta-catenin and transforming growth factor β (TGFβ) [6]. Gopal et al. [57] showed that, before the metastasis of tumor cells, the exosomes released protein to the adjacent or distant cells, via regulation of receptor intracellular signaling cascades, so as to establish the premetastatic niche and create a suitable environment for the growth of tumor. E.g., tumor cells secreted TGF-β protein in inducing Smad dependent signal transduction, regulation of fibroblast matrix, and induction of fibroblast differentiation into myofibroblast [59]. Huang et al. [60] studied the effects of hypoxia on CRC cells. Hypoxic CRC cells can transfer Wnt4 mRNA to normal CRC cells by exosome, which can activate β-catenin signal and potentiate the invasive ability of normal CRC cells. Most CRCs eventually develop liver metastasis. The study found that CRC cells secreted exosomes,

Table 1. Major miRNAs involved in CRC progress

miRNA	Target genes	Physiological Function
mir-193a [31]	Caprin1	cell cycle G1 arrest and cell proliferation repression
miR-125a-3p [32]	Fyn, FAK and paxillin3	biomarker for diagnosis and prognosis
mir-375 [33]	bcl-2	Inhibit cell progression and dissemination
miR-328 [34]	-	important predictor of metastases
miR-200c, miR-141 [35]	ZEB1, ZEB2	identify colon cancer patients with poor prognosis
miR-4772-3p [36]	RTN4, RAB9A	predictor of tumor recurrence
miR-21 [37, 38]	TLR7	induce an inflammatory premetastatic niche
	WNT/β-Catenin signaling	biomarker for diagnosis
miR-145-5p [39]	Cx43	transferring miRs from one endothelial cell to neighboring tumor cells
miR-1246 [40, 41]	Mutant p53, Smad 1/5/8	anti-inflammatory immunosuppression; potentiate angiogenesis
miR-183/96/182 [42]	-	a pleiotropic target for colon cancer therapy
miR-379 [43]	Cyclin-B1	control CRC cell metastasis
miR-19a [44]	-	potential marker of prognosis and a therapeutic target in CRC
miR-7641 [45]	CXCL1	biomarkers for the diagnosis of CRC
miR-210 [46]	E-cadherin	regulate epithelial-mesenchymal transition
miR-96-5p, miR-149 [47]	GPC1	biomarkers for the disease severity, prognosis
miR-92a [48]	Dkk-3	facilitate angiogenesis
miR-100 [49]	SMARCA5, E-cadherin	induce cell migration, inflammation, immune responses, invasion
miR-25-3p [50]	KLF2/4	involve in pre-metastatic niche formation
miR-21-5p, miR-155-5p [51]	BRG1	regulate migration and invasion of colorectal cancer cells
miR-486-5p [52]	PLAGL2/IGF2/β-catenin	potentiate CRC proliferation and migration
miR-200b [53]	p27, RND3	facilitate the CRC cells proliferation
miR-17-5p, miR-92a-3p [54]	-	noninvasive biomarkers for CRC and metastasis progression
miR-6869-5p [55]	TLR4	biomarker for the prediction of CRC prognosis
miR-203 [56]	SOCS3	potentiates the differentiation of monocytes to M2 macrophages

through miR-21-TLR7-IL-6 pathway, induced inflammatory metastatic niche, and potentiated liver metastasis [38, 61]. Moreover, CRC-related exosomes released microRNA-25-3p, which regulated the expression of Claudin 5, tight junction protein-1 (ZO-1), VEGFR2 and occludin in endothelial cells by targeting KLF2/4, which induced angiogenesis and up-regulated vascular permeability, potentiating the transfer before the formation of ecological niche [50]. In brief, by releasing exosomes, tumor cells can induce up-regulation or down-regulation of EMT-related genes, potentiate the transformation between cancer cells and normal cells, leading to invasion, migration and establishment of pre-metastasis niche (**Figure 3** for the specific mechanism of EMT).

Tumor movement, metastasis and migration

Human cells originally came from embryonic stem cells. Tumor cells are the same, it entrusts tumor cells with vitality, migration and metastasis, called tumor initiating cells (CIC) [62]. Specific markers are often found in CIC, and exosomes are often involved in the transport of these markers. CD44v6 was reported as a CIC marker of colorectal cancer and potentiated the movement and metastasis of cancer cells to non-CIC. The specific mechanism may be that CIC potentiated the movement of tumors by co-regulating the expression of non-CIC four-transmembrane protein 8 (Tspan8) through the synergism of CD44v6 secreted by exosomes with integrins and proteases. Besides, under hypoxia, CRC cells derived hypoxic CRC cells to normal CRC cells and potentiated normal CRC cell metastasis through the exosome-mediated Wnt4/beta-catenin signaling pathway [60].

Liver metastasis is the most common metastasis organ of advanced colorectal cancer, and also the main cause of CRC death. Moreover, the role of EMT in inducing pre-metastasis niche and potentiating tumor cell migration has been described previously. Exosomes can also mediate protein and RNA regulation of related signaling pathways to potentiate CRC cell metastasis. Integrin is a common adhesion molecule in exosomes. Hoshino et al. [63]

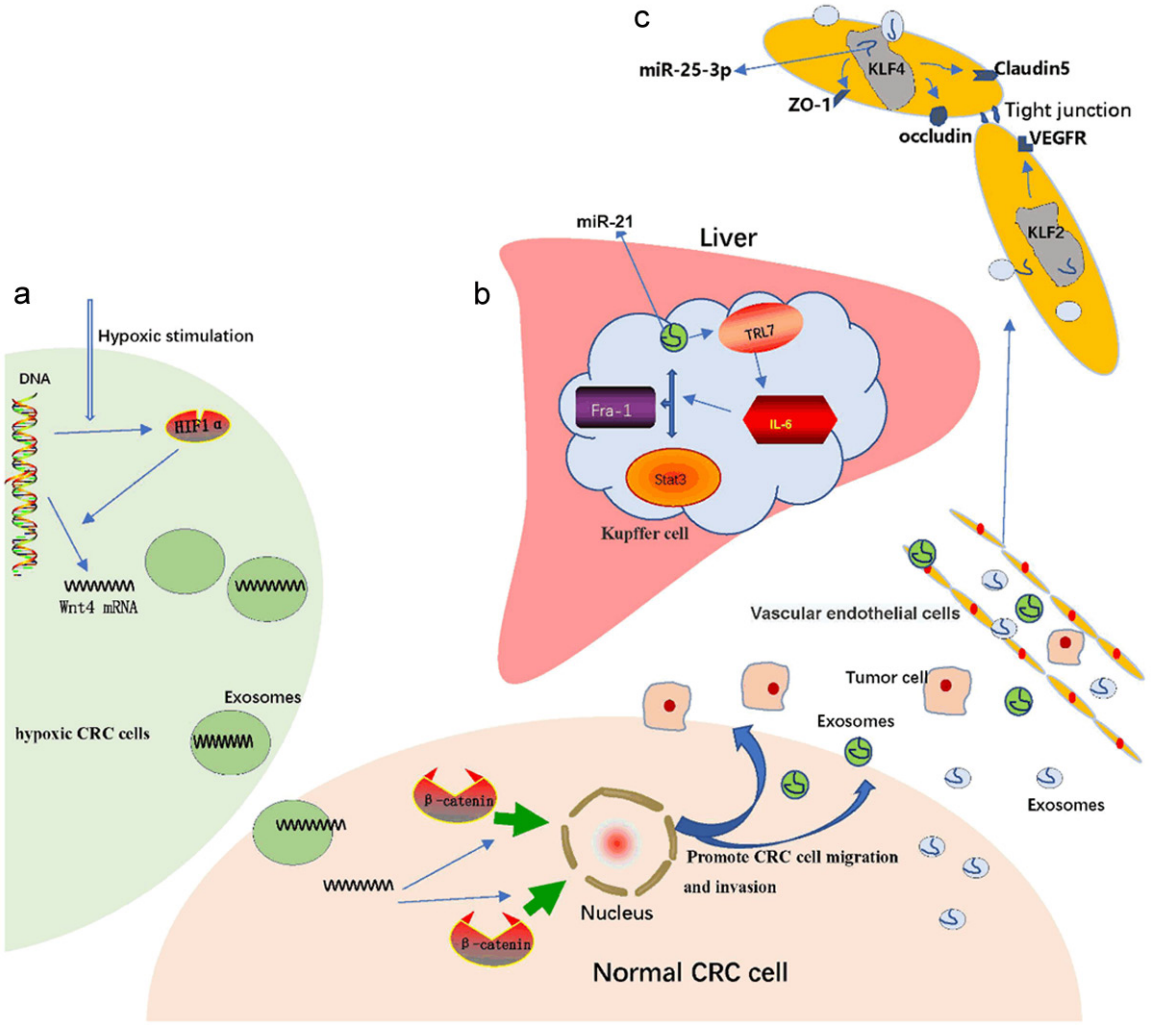

Figure 3. Schematic diagram of EMT. a. In hypoxic environment, the expression of HIF-1a in CRC cells is up-regulated, then HIF-1a potentiates Wnt4 gene transcription. Wnt4 gene transfers to normoxic CRC cells by exosome, activates the signal of beta-catenin, potentiates the aggregation of beta-catenin to the nucleus, regulates the expression of related genes, and leads to the migration and metastasis of normal CRC cells. b. CRC exosome carries microRNA-21 into the blood circulation, which is engulfed by Kupffer cell in the liver, and then releases microRNA-21. Mir-21 acts on TRL-7 to potentiate the synthesis and release of IL-6. Lastly, permissive inflammatory premetastatic niche was formed. Moreover, IL-6 induces the binding of microRNA-21 with signal transduction factor Stat3, which in turn potentiates the production of IL-6 and eventually forms a positive feedback pathway in the liver. c. CRC-mediated microRNA-25-3p enters vascular endothelial cells by silencing KLF2 and KLF4, down-regulating ZO-1, occludin, and Caladin 5, and increasing VEGFR2, ultimately leading to tumor angiogenesis and potentiating CRC metastasis.

showed that tumor metastasis has organ specificity, and integrin αvβ5 can predict whether the tumor cells have hepatic metastasis risk. S100P and A8 up-regulated gene in Kupffer cells, decreased cell adhesion, potentiated liver metastasis of tumor cells. Besides, the expression of Ras gene can regulate integrin subunits and their complexes, suppress ITGB1 glycosylation and reduce cell adhesion. The up-regulation of ITGA6-ITGB4 complex in considerable cancer cells was associated with the prognosis of invasive tumors [64]. Ji et al. [65] showed that compared with primary CRC cells, metastatic CRC cells were up-regulated with cell transfer factors: S100A9, TNC S100A8, MET; signal transduction molecules: EGFR, SRC, TNIK, JAG1, EFNB2, which finding would help us to study the microenvironment of cancer and target therapy of CRC.

The ncRNA carried by CRC exosomes plays a key role in tumorigenesis and progression.

Familial adenomatous polyposis gene (APC) is currently recognized as a cancer suppressor gene closely associated with CRC. Recently, Wang et al. [66] reported that APC can reduce the secretion of CRC-related exosomes by up-regulating lncRNA-APC1 expression, which regulates the stability of Rab5b RNA, and ultimately reduces the angiogenesis of tumors, so as to suppress the growth and metastasis of tumors. Mi-193a was a tumor suppressor, which can suppress the proliferation of CRC cells and bind with major vault protein (MVP) to form a complex of microRNA-193a-MVP, which was then transported to exosomes, resulting in the decrease of intracellular microRNA-193a and potentiating the proliferation and metastasis of tumors. 3'UTR of cell cycle-related protein-1 (Caprin-1) mRNA, which was closely associated with the development of tumors and drug tolerance, can bind to microRNA-193a and reduce the production of Caprin-1, thus arresting the cell cycle in G1 phase and suppressing the proliferation of CRC cells [31]. KRAS gene mutation often occurs in CRC. It was found that microRNA-100 was up-regulated in the exosome of KRAS mutant CRC, and it could down-regulate LGR5 in CRC cells, thus suppressing cell metastasis and migration. However, other studies have shown that mir-100 can induce EMT by targeting SMARCA5 to down-regulate E-cadherin level, and it can also target HOXA1 to suppress tumor progression [49, 67]. Accordingly, there was still no consensus on the dual biological effects of microRNA-100. It has been reported that microRNA-21-5p and microRNA-155-5p were closely associated with breast cancer and ovarian cancer invasion and chemotherapy resistance. However, recent studies have found that M2 macrophages released exosomes containing microRNA-21-5p and microRNA-155-5p, which were then transferred to CRC cells. MicroRNA-21-5p and microRNA-155-5p down-regulate BRG1 expression in CRC cells. BRG1 is capable of potentiating the metastasis of CRC cells via Wnt pathway and potentiating the migration of tumor cells to adjacent blood vessels, whereas the mechanism of CRC cells on M2 macrophages remains unclear [51]. Polymorphic adenoma gene-like protein 2 (PLAGL2), as a transcription factor, regulated gene expression and participated in the occurrence of considerable tumors (e.g., respiratory, blood, digestive and circulatory systems). Liu et al. [52] reported that the release of microRNA-486-5p from CRC exosomes potentiated CRC migration through the microRNA-486-5p-PLAGL2-IGF2/β-catenin signal axis. In brief, exosome-mediated ncRNA, especially microRNAs, is vital to regulate the movement and metastasis of CRC cells.

Tumor immunity

Exosomes are mediators of cell-to-cell communication. They contain a large number of immunoreactive proteins and RNA, e.g., heat shock protein family, transmembrane proteins, MHC I and MHC II molecules. They play a role in immune suppression, immune surveillance, immune activation and immune tolerance, and regulation of antigen expression. In particular, TEXs are capable of weakening immune surveillance, enhancing immune evasion, increasing drug resistance and weakening specific T-cell response, thereby potentiating the progress of cancer [68].

TEXs varied the function of receptor cells by signal transduction or internalization absorption on the surface of receptor cells. The main manifestation was the suppression of immune response. According to existing studies, SMVs isolated from plasma of melanoma patients are capable of suppressing the differentiation of monocytes into dendritic cells and destroying bone marrow immune response [69]. Moreover, TEXs interfered with DC cell maturation, induced macrophages to transform into tumor cell phenotype, potentiated regulatory T cell function by releasing TGF-beta, and suppressed effector T cell activity by Fas/FasL binding [70]. In CRC cells, myeloid-derived suppressor cells (MDSC) aggregated close to tumors, as differentiated into tumor-related macrophages, and then induced T-lymphocyte apoptosis [71]. Tumor-associated macrophages are macrophages infiltrating into tumor tissues, which suppress immunity and potentiate tumor proliferation, metastasis and angiogenesis. Takano et al. found that miR-203 potentiated monocyte differentiation into M2 macrophages and established pre-metastasis niche in CRC cells, presumably associated with Suppressor of Cytokine Signaling 3 (SOCS3), but the specific mechanism was unclear [56]. But not all TEXs suppressed immune response, and exosomes can also transfer tumor cell antigens to dendritic cells through MHC-I molecule

to induce cytotoxic T cell immune response and suppressed the growth of tumors in vivo. HSP70 transformed Tregs into highly effective Th17 cells through IL-6 to exerted a powerful anti-tumor effect [72].

Tumor resistance to chemotherapy

At present, CRC patients preferred 5 fluorouracil and oxaliplatin chemotherapy. Because of the lack of targeting to tumors, the emergence of a large number of drug-resistant tumors, the low concentration of drugs in tumors and serious systemic side effects, the quality of life of patients in the course of treatment is significantly reduced. In recent years, with the application of targeted drugs (e.g., bevacizumab, cetuximab and regorafenib), though the median survival time of metastatic CRC has increased to over 30 months, the overall effect of metastatic CRC is still very poor, especially the drug resistance of malignant tumors is increasing [73]. Given the current situation, a large number of recent studies have reported that exosomes play a critical role in chemotherapeutic resistance, drug resistance and targeted treatment of tumors.

Cancer stem cell (CSC) refers to the source of life of cancer, which has been in constant renewal and infinite proliferation. CSC can be constantly in a "dormant" state, and it contains various drug-resistant molecules, which are insensitive to chemotherapeutic drugs. Wnt activity can reflect the characteristics of CSC. Cancer-associated fibroblasts (CAFs) release Wnt protein, bind to Wnt target gene in CRC cells, aggregate β-catenin and activate Wnt signal. Exosome-mediated Wnt secreted by fibroblasts is capable of inducing CRC reprogramming, as dedifferentiated into CSC, and potentiating CRC resistance to chemotherapy. It has also been reported that fibroblast-derived Wnt also potentiates cell metastasis and migration in lymphoma and breast cancer. In vivo and in vitro experiments showed that suppressing Wnt release could potentiate the drug sensitivity of CRC cells and reduce cell proliferation [74]. Besides, CAFs release of exosome-mediated H19 also potentiated the stemness and resistance of CSC to oxaliplatin. LncRNA H19 was highly expressed in CAFs and up-regulated in exosomes. H19 activated Wnt/β-catenin signaling pathway and potentiated the proliferation and drug resistance of CSC cells. microRNA-141, as a competitive inhibitor of H19, targets β-catenin and significantly prevented CRC from transforming into CSC [75] (see **Figure 4** for details).

Urothelial carcinoma associated 1 (UCA1) gene has been demonstrated to be associated with bladder cancer, breast cancer, gastric cancer, lung cancer, CRC and other types of cancer invasion. UCA1 was up-regulated in the exosomes of CRC tissues and down-regulated in the serum of patients. It was found that UCA1 was associated with cetuximab and 5-fluorouracil resistance in CRC tissues [76-78]. According to Bian et al., UCA1 could up-regulate the target genes BCL2, RAB22A and CRB1 of microRNA-204-5p by directly binding to microRNA-204-5p and suppressing the expression of microRNA-204-5p, thus potentiating the proliferation of CRC cells and inducing drug resistance [76]. However, it was also found that microRNA-204 potentiated the sensitivity of CRC cells to 5-fluorouracil by targeting down-regulation of high mobility group protein A2 (HMGA2) [79]. HSP70 was a critical component of exosome membrane. A chaperone protein A8 can specifically bind HSP70 and prevent the exosome from activating MDSC. MDSC is often concentrated in spleen, blood, lymph nodes and tumor tissues. It inhibited immune response by suppressing the activity of NK cells. A8 can also combine with 5-fluorouracil to slow down the growth of tumors and potentiate the anti-tumor immune response [80]. In brief, exosomes are vital to resist chemotherapy. By suppressing the release of exosomes, they can suppress the progress of tumors and reduce drug resistance, revealing the direction for finding new targeted drugs.

Biotherapeutic vector

In the process of drug transportation in vivo, it has been a difficult problem for us to ensure that drugs are not degraded, combine safely and effectively with targets, and reduce side effects. In recent years, the idea of exosome-mimics as delivery vehicles for transporting biological products has been proposed. Exosome has a lipid bilayer structure formed by plasmalemma invagination, exhibiting low toxicity and immunogenicity. When combined with drugs, it can cross the biofilm barrier and avoid drug

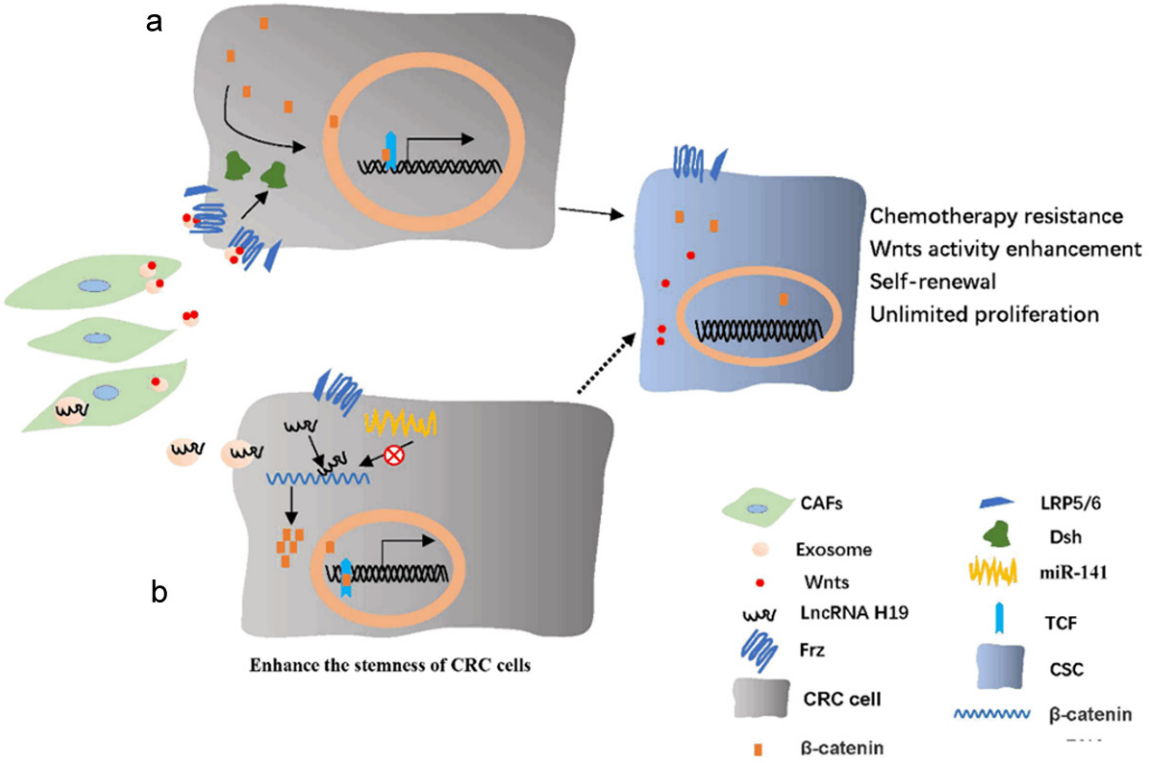

Figure 4. CAFs induce CRC differentiation into CSC. a. CAFs release the Wnt protein exosome, then interact with LRP5/6 and Frizzled (Frz) on the surface of CRC cells, activate the intracellular signal Frz family transmembrane receptor protein Dishevelled (Dsh), lead to the accumulation of beta-catenin in the cell nucleus, induce target gene transcription via T-cell factor (TCF), reprogramming CRC and dedifferentiate into CSC. b. CAFs carry LncRNA H19 exosome into CRC cells. H19 competes with microRNA-141 to potentiate the transcription of beta-catenin, and beta-catenin aggregates and locates in the nucleus. Gene expression is induced by TCF. Potentiate the stemness of CRC cells.

degradation by enzymes, in particular small interfering RNA. Exosome pertains to nanostructure, and it can also evade rapid clearance of mononuclear phagocytes [81]. Alvarez-Erviti et al. initially demonstrated that exosomes are successful in transporting siRNA by artificially assembling key parts of exosome lipid molecular layer in vitro and constructing exosome-mimics [82]. A A33-azithromycin complex was constructed by combining exosomes originating from A33-positive LIM1215 cells with azithromycin. It was employed to treat A33-positive mice with colon cancer. The results revealed that the complex had higher uptake rate, stronger targeting ability, significantly suppressed the growth of tumors, prolonged survival time, and exerted less cardiac side effects [83]. Besides acting as delivery vehicles, TEXs was also a new type of cancer vaccine. Heat stress can induce cells to produce MHC-I and HSP. Isolation of exosomes from heat stress CEA-positive tumor cells is immunogenic and can produce anti-tumor effects [84]. Phase I clinical trials of exosomes have shown that the combination of exosomes originating from ascites and granulocyte-macrophage colony stimulating factor was safe, feasible and effective in the treatment of advanced colon cancer. As a cancer vaccine, it was non-toxic, safe and tolerable [85]. In brief, exosomes are a double-edged sword, capable of inducing tumors, potentiating the proliferation and migration of tumors, resisting chemotherapeutic drugs, and acting as drug carriers to deliver biological products. In the meantime, TEXs can be prepared into tumor vaccines for cancer immunotherapy.

Tumor diagnostic and prognostic markers

Fiberoptic colonoscopy is the gold standard for CRC diagnosis. Because of the need for intestinal preparation before operation, long operation time and pain during operation, it cannot

act as an early screening method in routine physical examination. At present, CEA is commonly adopted as a non-invasive method to predict the prognosis and recurrence risk of CRC, but its specificity is low, and it often increases in other malignant tumors. CA199 is highly specific in CRC, but its sensitivity is low. It is primarily found in pancreatic tumors [86]. TEXs are released from tumors and are often detected in blood, ascites, saliva and feces. A study found that in the serum of CRC patients, some exosome microRNAs were significantly higher than those in the control group, and the level of microRNAs decreased after surgery [87]. This study suggests that exosome-mediated microRNA may be a marker for CRC diagnosis and prognosis.

MicroRNA-125a-3p expression independent of CEA increased significantly in CRC exosome, and the increase in left colon cancer was more obvious than that in right colon cancer, associated with the infiltration of CRC nerve. Analysis of serum microRNAs in 50 cases of early CRC showed that microRNAs-125a-3p were highly up-regulated in exosomes, and the area under ROC curve was 85.5% combined with CEA [32]. The recurrence group had a higher level of microRNAs, and the low level group had a better prognosis, according to a comparative study of serum exosome microRNAs in patients with recurrent and non-recurrent CRC. MicroRNAs-19a could act as a biomarker for predicting recurrence [44]. Circulating exosome microRNA-17-5p and microRNA-92a-3p were closely associated with the pathological grading and clinical staging of CRC and may act as prognostic markers for non-invasive assessment [54]. In brief, the formation and release of TEXs is a complex biological process. Exosome microRNAs are important for early diagnosis and prognosis assessment of CRC. There are considerable relevant microRNAs (**Table 1**). However, there has been no consensus on the diagnosis and prognosis of exosome microRNAs in the world.

Conclusion

Exosomes, a communication medium between cells, are capable of carrying proteins, RNA and lipids to transmit information between cells, regulating the microenvironment of CRC, potentiating the progress of cancer and affecting the prognosis of patients. TEXs are capable of establishing pre-metastasis niche by EMT, inducing angiogenesis and potentiating tumor migration. Exosome-mediated proteins and microRNAs potentiate the movement, metastasis and migration of cancer cells by activating receptor cell target genes and regulating downstream signaling pathways. Besides, TEXs suppress immune response, potentiate immune evasion and increase drug resistance, leading to chemotherapy resistance. Given this, the growth and metastasis of tumors can be inhibited by suppressing the release of exosomes. We can also use the biological characteristics of exosomes to construct exosome mimics that can be transported to target cells after carrying drugs. The exosomes isolated from TEXs can be used to prepare cancer vaccines, and they are safe and effective. Lastly, exosome microRNAs make up for the deficiencies of CEA and CA199, which is of great significance in assisting CRC in early diagnosis and prognosis assessment. It is noteworthy that the study of exosomes remains in the experimental stage, and there are numerous unknown mechanisms. There is still a long way ahead to guide the diagnosis and treatment of CRC.

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Disclosure of conflict of interest

None.

Abbreviations

TEXs, Tumor-derived exosomes; CRC, the treatment and the prognosis of colorectal cancer; EMT, Epithelial-mesenchymal cell transition; SMVs, shed microbubbles; TGFβ, transforming growth factor β; Dsh, protein Dishevelled; TCF, T-cell factor; UCA1, Urothelial carcinoma associated 1; HMGA2, high mobility group protein A2; CAFs, Cancer-associated fibroblasts; CSC, Cancer stem cell; MDSC, myeloid-derived suppressor cells; APC, adenomatous polyposis gene; PLAGL2, Polymorphic adenoma gene-like protein 2.

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References

[1] Harding C, Heuser J and Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. J Cell Biol 1983; 97: 329-339.

[2] Prattichizzo F, Micolucci L, Cricca M, De Carolis S, Mensa E, Ceriello A, Procopio AD, Bonafe M and Olivieri F. Exosome-based immunomodulation during aging: a nano-perspective on inflamm-aging. Mech Ageing Dev 2017; 168: 44-53.

[3] Smith VL, Cheng Y, Bryant BR and Schorey JS. Exosomes function in antigen presentation during an in vivo mycobacterium tuberculosis infection. Sci Rep 2017; 7: 43578.

[4] Sung BH and Weaver AM. Exosome secretion promotes chemotaxis of cancer cells. Cell Adh Migr 2017; 11: 187-195.

[5] Liu F, Bu Z, Zhao F and Xiao D. Increased T-helper 17 cell differentiation mediated by exosome-mediated microRNA-451 redistribution in gastric cancer infiltrated T cells. Cancer Sci 2018; 109: 65-73.

[6] Syn N, Wang L, Sethi G, Thiery JP and Goh BC. Exosome-mediated metastasis: from epithelial-mesenchymal transition to escape from immunosurveillance. Trends Pharmacol Sci 2016; 37: 606-617.

[7] Sleeman JP. The metastatic niche and stromal progression. Cancer Metastasis Rev 2012; 31: 429-440.

[8] Bebawy M, Combes V, Lee E, Jaiswal R, Gong J, Bonhoure A and Grau GE. Membrane microparticles mediate transfer of P-glycoprotein to drug sensitive cancer cells. Leukemia 2009; 23: 1643-1649.

[9] Siegel R, Desantis C and Jemal A. Colorectal cancer statistics, 2014. CA Cancer J Clin 2014; 64: 104-117.

[10] Dienstmann R, Salazar R and Tabernero J. Personalizing colon cancer adjuvant therapy: selecting optimal treatments for individual patients. J Clin Oncol 2015; 33: 1787-1796.

[11] Zhao L, Yu J, Wang J, Li H, Che J and Cao B. Isolation and Identification of miRNAs in exosomes derived from serum of colon cancer patients. J Cancer 2017; 8: 1145-1152.

[12] Harada T, Yamamoto H, Kishida S, Kishida M, Awada C, Takao T and Kikuchi A. Wnt5b-associated exosomes promote cancer cell migration and proliferation. Cancer Sci 2017; 108: 42-52.

[13] Lee CH, Im EJ, Moon PG and Baek MC. Discovery of a diagnostic biomarker for colon cancer through proteomic profiling of small extracellular vesicles. BMC Cancer 2018; 18: 1058.

[14] Szatanek R, Baj-Krzyworzeka M, Zimoch J, Lekka M, Siedlar M and Baran J. The methods of choice for extracellular vesicles (EVs) characterization. Int J Mol Sci 2017; 18.

[15] Schorey JS and Bhatnagar S. Exosome function: from tumor immunology to pathogen biology. Traffic 2008; 9: 871-881.

[16] Mathivanan S, Fahner CJ, Reid GE and Simpson RJ. ExoCarta 2012: database of exosomal proteins, RNA and lipids. Nucleic Acids Res 2012; 40: D1241-1244.

[17] Yanez-Mo M, Siljander PR, Andreu Z, Zavec AB, Borras FE, Buzas EI, Buzas K, Casal E, Cappello F, Carvalho J, Colas E, Cordeiro-da Silva A, Fais S, Falcon-Perez JM, Ghobrial IM, Giebel B, Gimona M, Graner M, Gursel I, Gursel M, Heegaard NH, Hendrix A, Kierulf P, Kokubun K, Kosanovic M, Kralj-Iglic V, Kramer-Albers EM, Laitinen S, Lasser C, Lener T, Ligeti E, Line A, Lipps G, Llorente A, Lotvall J, Mancek-Keber M, Marcilla A, Mittelbrunn M, Nazarenko I, Nolte-'t Hoen EN, Nyman TA, O'Driscoll L, Olivan M, Oliveira C, Pallinger E, Del Portillo HA, Reventos J, Rigau M, Rohde E, Sammar M, Sanchez-Madrid F, Santarem N, Schallmoser K, Ostenfeld MS, Stoorvogel W, Stukelj R, Van der Grein SG, Vasconcelos MH, Wauben MH and De Wever O. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles 2015; 4: 27066.

[18] Stahl AL, Johansson K, Mossberg M, Kahn R and Karpman D. Exosomes and microvesicles in normal physiology, pathophysiology, and renal diseases. Pediatr Nephrol 2019; 34: 11-30.

[19] Gyorgy B, Szabo TG, Pasztoi M, Pal Z, Misjak P, Aradi B, Laszlo V, Pallinger E, Pap E, Kittel A, Nagy G, Falus A and Buzas EI. Membrane vesicles, current state-of-the-art: emerging role of

extracellular vesicles. Cell Mol Life Sci 2011; 68: 2667-2688.
[20] Record M, Poirot M and Silvente-Poirot S. Emerging concepts on the role of exosomes in lipid metabolic diseases. Biochimie 2014; 96: 67-74.
[21] H Rashed M, Bayraktar E, K Helal G, Abd-Ellah MF, Amero P, Chavez-Reyes A and Rodriguez-Aguayo C. Exosomes: from garbage bins to promising therapeutic targets. Int J Mol Sci 2017; 18.
[22] Pfeffer SR. Two Rabs for exosome release. Nat Cell Biol 2010; 12: 3-4.
[23] Ostrowski M, Carmo NB, Krumeich S, Fanget I, Raposo G, Savina A, Moita CF, Schauer K, Hume AN, Freitas RP, Goud B, Benaroch P, Hacohen N, Fukuda M, Desnos C, Seabra MC, Darchen F, Amigorena S, Moita LF and Thery C. Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat Cell Biol 2010; 12: 19-30.
[24] Fan Q, Yang L, Zhang X, Peng X, Wei S, Su D, Zhai Z, Hua X and Li H. The emerging role of exosome-derived non-coding RNAs in cancer biology. Cancer Lett 2018; 414: 107-115.
[25] Khushman M, Bhardwaj A, Patel GK, Laurini JA, Roveda K, Tan MC, Patton MC, Singh S, Taylor W and Singh AP. Exosomal markers (CD63 and CD9) expression pattern using immunohistochemistry in resected malignant and non-malignant pancreatic specimens. Pancreas 2017; 46: 782-788.
[26] Thery C, Regnault A, Garin J, Wolfers J, Zitvogel L, Ricciardi-Castagnoli P, Raposo G and Amigorena S. Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73. J Cell Biol 1999; 147: 599-610.
[27] Jia Y, Chen Y, Wang Q, Jayasinghe U, Luo X, Wei Q, Wang J, Xiong H, Chen C, Xu B, Hu W, Wang L, Zhao W and Zhou J. Exosome: emerging biomarker in breast cancer. Oncotarget 2017; 8: 41717-41733.
[28] Ha D, Yang N and Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharm Sin B 2016; 6: 287-296.
[29] Tran TH, Mattheolabakis G, Aldawsari H and Amiji M. Exosomes as nanocarriers for immunotherapy of cancer and inflammatory diseases. Clin Immunol 2015; 160: 46-58.
[30] Zhang J, Li S, Li L, Li M, Guo C, Yao J and Mi S. Exosome and exosomal microRNA: trafficking, sorting, and function. Genomics Proteomics Bioinformatics 2015; 13: 17-24.
[31] Teng Y, Ren Y, Hu X, Mu J, Samykutty A, Zhuang X, Deng Z, Kumar A, Zhang L, Merchant ML, Yan J, Miller DM and Zhang HG. MVP-mediated exosomal sorting of miR-193a promotes colon cancer progression. Nat Commun 2017; 8: 14448.
[32] Wang J, Yan F, Zhao Q, Zhan F, Wang R, Wang L, Zhang Y and Huang X. Circulating exosomal miR-125a-3p as a novel biomarker for early-stage colon cancer. Sci Rep 2017; 7: 4150.
[33] Zaharie F, Muresan MS, Petrushev B, Berce C, Gafencu GA, Selicean S, Jurj A, Cojocneanu-Petric R, Lisencu CI, Pop LA, Pileczki V, Eniu D, Muresan MA, Zaharie R, Berindan-Neagoe I, Tomuleasa C and Irimie A. Exosome-carried microRNA-375 inhibits cell progression and dissemination via Bcl-2 blocking in colon cancer. J Gastrointestin Liver Dis 2015; 24: 435-443.
[34] Monzo M, Santasusagna S, Moreno I, Martinez F, Hernandez R, Munoz C, Castellano JJ, Moreno J and Navarro A. Exosomal microRNAs isolated from plasma of mesenteric veins linked to liver metastases in resected patients with colon cancer. Oncotarget 2017; 8: 30859-30869.
[35] Santasusagna S, Moreno I, Navarro A, Martinez Rodenas F, Hernandez R, Castellano JJ, Munoz C and Monzo M. Prognostic impact of miR-200 family members in plasma and exosomes from tumor-draining versus peripheral veins of colon cancer patients. Oncology 2018; 95: 309-318.
[36] Liu C, Eng C, Shen J, Lu Y, Takata Y, Mehdizadeh A, Chang GJ, Rodriguez-Bigas MA, Li Y, Chang P, Mao Y, Hassan MM, Wang F and Li D. Serum exosomal miR-4772-3p is a predictor of tumor recurrence in stage II and III colon cancer. Oncotarget 2016; 7: 76250-76260.
[37] Uratani R, Toiyama Y, Kitajima T, Kawamura M, Hiro J, Kobayashi M, Tanaka K, Inoue Y, Mohri Y, Mori T, Kato T, Goel A and Kusunoki M. Diagnostic potential of cell-free and exosomal microRNAs in the identification of patients with high-risk colorectal adenomas. PLoS One 2016; 11: e0160722.
[38] Shao Y, Chen T, Zheng X, Yang S, Xu K, Chen X, Xu F, Wang L, Shen Y, Wang T, Zhang M, Hu W, Ye C, Yu X, Shao J and Zheng S. Colorectal cancer-derived small extracellular vesicles establish an inflammatory premetastatic niche in liver metastasis. Carcinogenesis 2018; 39: 1368-1379.
[39] Thuringer D, Jego G, Berthenet K, Hammann A, Solary E and Garrido C. Gap junction-mediated transfer of miR-145-5p from microvascular endothelial cells to colon cancer cells inhibits angiogenesis. Oncotarget 2016; 7: 28160-28168.
[40] Cooks T, Pateras IS, Jenkins LM, Patel KM, Robles AI, Morris J, Forshew T, Appella E, Gorgoulis VG and Harris CC. Mutant p53 cancers reprogram macrophages to tumor supporting macrophages via exosomal miR-1246. Nat Commun 2018; 9: 771.

[41] Yamada N, Tsujimura N, Kumazaki M, Shinohara H, Taniguchi K, Nakagawa Y, Naoe T and Akao Y. Colorectal cancer cell-derived microvesicles containing microRNA-1246 promote angiogenesis by activating Smad 1/5/8 signaling elicited by PML down-regulation in endothelial cells. Biochim Biophys Acta 2014; 1839: 1256-1272.

[42] Zhang Q, Ren W, Huang B, Yi L and Zhu H. MicroRNA-183/182/96 cooperatively regulates the proliferation of colon cancer cells. Mol Med Rep 2015; 12: 668-674.

[43] Clancy C, Khan S, Glynn CL, Holian E, Dockery P, Lalor P, Brown JA, Joyce MR, Kerin MJ and Dwyer RM. Screening of exosomal microRNAs from colorectal cancer cells. Cancer Biomark 2016; 17: 427-435.

[44] Matsumura T, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, Sawada G, Ueda M, Uchi R, Ueo H, Takano Y, Shinden Y, Eguchi H, Yamamoto H, Doki Y, Mori M, Ochiya T and Mimori K. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. Br J Cancer 2015; 113: 275-281.

[45] Chen M, Xu R, Rai A, Suwakulsiri W, Izumikawa K, Ishikawa H, Greening DW, Takahashi N and Simpson RJ. Distinct shed microvesicle and exosome microRNA signatures reveal diagnostic markers for colorectal cancer. PLoS One 2019; 14: e0210003.

[46] Bigagli E, Luceri C, Guasti D and Cinci L. Exosomes secreted from human colon cancer cells influence the adhesion of neighboring metastatic cells: role of microRNA-210. Cancer Biol Ther 2016; 17: 1062-1069.

[47] Li J, Li B, Ren C, Chen Y, Guo X, Zhou L, Peng Z, Tang Y, Chen Y, Liu W, Zhu B, Wang L, Liu X, Shi X and Peng Z. The clinical significance of circulating GPC1 positive exosomes and its regulative miRNAs in colon cancer patients. Oncotarget 2017; 8: 101189-101202.

[48] Yamada N, Nakagawa Y, Tsujimura N, Kumazaki M, Noguchi S, Mori T, Hirata I, Maruo K and Akao Y. Role of intracellular and extracellular microRNA-92a in colorectal cancer. Transl Oncol 2013; 6: 482-492.

[49] Cha DJ, Franklin JL, Dou Y, Liu Q, Higginbotham JN, Demory Beckler M, Weaver AM, Vickers K, Prasad N, Levy S, Zhang B, Coffey RJ and Patton JG. KRAS-dependent sorting of miRNA to exosomes. Elife 2015; 4: e07197.

[50] Zeng Z, Li Y, Pan Y, Lan X, Song F, Sun J, Zhou K, Liu X, Ren X, Wang F, Hu J, Zhu X, Yang W, Liao W, Li G, Ding Y and Liang L. Cancer-derived exosomal miR-25-3p promotes pre-metastatic niche formation by inducing vascular permeability and angiogenesis. Nat Commun 2018; 9: 5395.

[51] Lan J, Sun L, Xu F, Liu L, Hu F, Song D, Hou Z, Wu W, Luo X, Wang J, Yuan X, Hu J and Wang G. M2 macrophage-derived exosomes promote cell migration and invasion in colon cancer. Cancer Res 2019; 79: 146-158.

[52] Liu X, Chen X, Zeng K, Xu M, He B, Pan Y, Sun H, Pan B, Xu X, Xu T, Hu X and Wang S. DNA-methylation-mediated silencing of miR-486-5p promotes colorectal cancer proliferation and migration through activation of PLAGL2/IGF2/beta-catenin signal pathways. Cell Death Dis 2018; 9: 1037.

[53] Zhang Z, Xing T, Chen Y and Xiao J. Exosome-mediated miR-200b promotes colorectal cancer proliferation upon TGF-beta1 exposure. Biomed Pharmacother 2018; 106: 1135-1143.

[54] Fu F, Jiang W, Zhou L and Chen Z. Circulating exosomal miR-17-5p and miR-92a-3p predict pathologic stage and grade of colorectal cancer. Transl Oncol 2018; 11: 221-232.

[55] Yan S, Liu G, Jin C, Wang Z, Duan Q, Xu J and Xu D. MicroRNA-6869-5p acts as a tumor suppressor via targeting TLR4/NF-kappaB signaling pathway in colorectal cancer. J Cell Physiol 2018; 233: 6660-6668.

[56] Takano Y, Masuda T, Iinuma H, Yamaguchi R, Sato K, Tobo T, Hirata H, Kuroda Y, Nambara S, Hayashi N, Iguchi T, Ito S, Eguchi H, Ochiya T, Yanaga K, Miyano S and Mimori K. Circulating exosomal microRNA-203 is associated with metastasis possibly via inducing tumor-associated macrophages in colorectal cancer. Oncotarget 2017; 8: 78598-78613.

[57] Gopal SK, Greening DW, Rai A, Chen M, Xu R, Shafiq A, Mathias RA, Zhu HJ and Simpson RJ. Extracellular vesicles: their role in cancer biology and epithelial-mesenchymal transition. Biochem J 2017; 474: 21-45.

[58] Greening DW, Gopal SK, Mathias RA, Liu L, Sheng J, Zhu HJ and Simpson RJ. Emerging roles of exosomes during epithelial-mesenchymal transition and cancer progression. Semin Cell Dev Biol 2015; 40: 60-71.

[59] Webber J, Steadman R, Mason MD, Tabi Z and Clayton A. Cancer exosomes trigger fibroblast to myofibroblast differentiation. Cancer Res 2010; 70: 9621-9630.

[60] Huang Z, Yang M, Li Y, Yang F and Feng Y. Exosomes derived from hypoxic colorectal cancer cells transfer Wnt4 to normoxic cells to elicit a prometastatic phenotype. Int J Biol Sci 2018; 14: 2094-2102.

[61] Liu H, Ren G, Wang T, Chen Y, Gong C, Bai Y, Wang B, Qi H, Shen J, Zhu L, Qian C, Lai M and Shao J. Aberrantly expressed Fra-1 by IL-6/STAT3 transactivation promotes colorectal cancer aggressiveness through epithelial-mes-

enchymal transition. Carcinogenesis 2015; 36: 459-468.
[62] Marhaba R, Klingbeil P, Nuebel T, Nazarenko I, Buechler MW and Zoeller M. CD44 and EpCAM: cancer-initiating cell markers. Curr Mol Med 2008; 8: 784-804.
[63] Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, Molina H, Kohsaka S, Di Giannatale A, Ceder S, Singh S, Williams C, Soplop N, Uryu K, Pharmer L, King T, Bojmar L, Davies AE, Ararso Y, Zhang T, Zhang H, Hernandez J, Weiss JM, Dumont-Cole VD, Kramer K, Wexler LH, Narendran A, Schwartz GK, Healey JH, Sandstrom P, Labori KJ, Kure EH, Grandgenett PM, Hollingsworth MA, de Sousa M, Kaur S, Jain M, Mallya K, Batra SK, Jarnagin WR, Brady MS, Fodstad O, Muller V, Pantel K, Minn AJ, Bissell MJ, Garcia BA, Kang Y, Rajasekhar VK, Ghajar CM, Matei I, Peinado H, Bromberg J and Lyden D. Tumour exosome integrins determine organotropic metastasis. Nature 2015; 527: 329-335.
[64] Demory Beckler M, Higginbotham JN, Franklin JL, Ham AJ, Halvey PJ, Imasuen IE, Whitwell C, Li M, Liebler DC and Coffey RJ. Proteomic analysis of exosomes from mutant KRAS colon cancer cells identifies intercellular transfer of mutant KRAS. Mol Cell Proteomics 2013; 12: 343-355.
[65] Ji H, Greening DW, Barnes TW, Lim JW, Tauro BJ, Rai A, Xu R, Adda C, Mathivanan S, Zhao W, Xue Y, Xu T, Zhu HJ and Simpson RJ. Proteome profiling of exosomes derived from human primary and metastatic colorectal cancer cells reveal differential expression of key metastatic factors and signal transduction components. Proteomics 2013; 13: 1672-1686.
[66] Wang FW, Cao CH, Han K, Zhao YX, Cai MY, Xiang ZC, Zhang JX, Chen JW, Zhong LP, Huang Y, Zhou SF, Jin XH, Guan XY, Xu RH and Xie D. APC-activated long noncoding RNA inhibits colorectal carcinoma pathogenesis through reduction of exosome production. J Clin Invest 2019; 129: 727-743.
[67] Chen D, Sun Y, Yuan Y, Han Z, Zhang P, Zhang J, You MJ, Teruya-Feldstein J, Wang M, Gupta S, Hung MC, Liang H and Ma L. miR-100 induces epithelial-mesenchymal transition but suppresses tumorigenesis, migration and invasion. PLoS Genet 2014; 10: e1004177.
[68] Greening DW, Gopal SK, Xu R, Simpson RJ and Chen W. Exosomes and their roles in immune regulation and cancer. Semin Cell Dev Biol 2015; 40: 72-81.
[69] Valenti R, Huber V, Filipazzi P, Pilla L, Sovena G, Villa A, Corbelli A, Fais S, Parmiani G and Rivoltini L. Human tumor-released microvesicles promote the differentiation of myeloid cells with transforming growth factor-beta-mediated suppressive activity on T lymphocytes. Cancer Res 2006; 66: 9290-9298.
[70] Chen W, Jiang J, Xia W and Huang J. Tumor-related exosomes contribute to tumor-promoting microenvironment: an immunological perspective. J Immunol Res 2017; 2017: 1073947.
[71] Manning S and Danielson KM. The immunomodulatory role of tumor-derived extracellular vesicles in colorectal cancer. Immunol Cell Biol 2018; [Epub ahead of print].
[72] Guo D, Chen Y, Wang S, Yu L, Shen Y, Zhong H and Yang Y. Exosomes from heat-stressed tumour cells inhibit tumour growth by converting regulatory T cells to Th17 cells via IL-6. Immunology 2018; 154: 132-143.
[73] Geng F, Wang Z, Yin H, Yu J and Cao B. Molecular targeted drugs and treatment of colorectal cancer: recent progress and future perspectives. Cancer Biother Radiopharm 2017; 32: 149-160.
[74] Hu YB, Yan C, Mu L, Mi YL, Zhao H, Hu H, Li XL, Tao DD, Wu YQ, Gong JP and Qin JC. Exosomal Wnt-induced dedifferentiation of colorectal cancer cells contributes to chemotherapy resistance. Oncogene 2019; 38: 1951-1965.
[75] Ren J, Ding L, Zhang D, Shi G, Xu Q, Shen S, Wang Y, Wang T and Hou Y. Carcinoma-associated fibroblasts promote the stemness and chemoresistance of colorectal cancer by transferring exosomal lncRNA H19. Theranostics 2018; 8: 3932-3948.
[76] Bian Z, Jin L, Zhang J, Yin Y, Quan C, Hu Y, Feng Y, Liu H, Fei B, Mao Y, Zhou L, Qi X, Huang S, Hua D, Xing C and Huang Z. LncRNA-UCA1 enhances cell proliferation and 5-fluorouracil resistance in colorectal cancer by inhibiting miR-204-5p. Sci Rep 2016; 6: 23892.
[77] Yang YN, Zhang R, Du JW, Yuan HH, Li YJ, Wei XL, Du XX, Jiang SL and Han Y. Predictive role of UCA1-containing exosomes in cetuximab-resistant colorectal cancer. Cancer Cell Int 2018; 18: 164.
[78] Barbagallo C, Brex D, Caponnetto A, Cirnigliaro M, Scalia M, Magnano A, Caltabiano R, Barbagallo D, Biondi A, Cappellani A, Basile F, Di Pietro C, Purrello M and Ragusa M. LncRNA UCA1, upregulated in CRC biopsies and downregulated in serum exosomes, controls mRNA expression by RNA-RNA interactions. Mol Ther Nucleic Acids 2018; 12: 229-241.
[79] Wu H, Liang Y, Shen L and Shen L. MicroRNA-204 modulates colorectal cancer cell sensitivity in response to 5-fluorouracil-based treatment by targeting high mobility group protein A2. Biol Open 2016; 5: 563-570.
[80] Gobbo J, Marcion G, Cordonnier M, Dias AMM, Pernet N, Hammann A, Richaud S, Mjahed H, Isambert N, Clausse V, Rebe C, Bertaut A, Goussot V, Lirussi F, Ghiringhelli F, de Thonel A,

Fumoleau P, Seigneuric R and Garrido C. Restoring anticancer immune response by targeting tumor-derived exosomes with a HSP70 peptide aptamer. J Natl Cancer Inst 2016; 108.

[81] van den Boorn JG, Schlee M, Coch C and Hartmann G. SiRNA delivery with exosome nanoparticles. Nat Biotechnol 2011; 29: 325-326.

[82] Kooijmans SA, Vader P, van Dommelen SM, van Solinge WW and Schiffelers RM. Exosome mimetics: a novel class of drug delivery systems. Int J Nanomedicine 2012; 7: 1525-1541.

[83] Li Y, Gao Y, Gong C, Wang Z, Xia Q, Gu F, Hu C, Zhang L, Guo H and Gao S. A33 antibody-functionalized exosomes for targeted delivery of doxorubicin against colorectal cancer. Nanomedicine 2018; 14: 1973-1985.

[84] Dai S, Wan T, Wang B, Zhou X, Xiu F, Chen T, Wu Y and Cao X. More efficient induction of HLA-A*0201-restricted and carcinoembryonic antigen (CEA)-specific CTL response by immunization with exosomes prepared from heat-stressed CEA-positive tumor cells. Clin Cancer Res 2005; 11: 7554-7563.

[85] Dai S, Wei D, Wu Z, Zhou X, Wei X, Huang H and Li G. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. Mol Ther 2008; 16: 782-790.

[86] Toma SC, Ungureanu BS, Patrascu S, Surlin V and Georgescu I. Colorectal cancer biomarkers - a new trend in early diagnosis. Curr Health Sci J 2018; 44: 140-146.

[87] Ruiz-Lopez L, Blancas I, Garrido JM, Mut-Salud N, Moya-Jodar M, Osuna A and Rodriguez-Serrano F. The role of exosomes on colorectal cancer: a review. J Gastroenterol Hepatol 2018; 33: 792-799.