Exosomal circular RNAs: A new frontier in the metastasis of digestive system tumors (Review)

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Received July 7, 2021; Accepted September 20, 2021

DOI: 10.3892/ol.2021.13087

Abstract. Exosomes are membrane vesicles with a diameter of 30-150 nm. Exosomes are secreted by various types of tumor cell and contain a variety of proteins, circular RNAs (circRNAs), microRNAs and DNA, depending on the host cells. Among them, circRNAs, which are long non-coding endogenous RNAs, form covalently closed and continuous loops that link the 3' and 5' terminals generated by back-splicing. circRNAs have become a hotspot of research. Exosomal circRNAs are in volved in the pathogenesis of cancer, especially metastasis, which is mainly ascribed to the frequently abnormal expression levels within neoplasms. Nonetheless, the functions and regulatory mechanisms of exosomal circRNAs in the progression of digestive system tumors (DSTs) remain unclear. More knowledge on the regulation and network interactions of exosomal circRNAs will help identify superior treatment strategies for the metastasis of DSTs. The present review aims to summarize the existing studies on the functions and mechanisms of exosomal circRNAs in tumorigenesis, and evaluate the associations between the dysregulation of exosomal circRNAs and tumor metastasis.

Contents

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Key words: exosomal circular RNAs, digestive system tumor, metastasis, epithelial-mesenchymal transition, microRNA sponge, pre-metastatic microenvironment

1. Introduction

Digestive system tumors (DSTs), such as colorectal cancer (CRC), hepatocellular carcinoma (HCC), gastric cancer (GC) and esophageal carcinoma, are the most common tumors in the world (1). DSTs also constitute a primary proportion of cancer-related deaths worldwide (2). As estimated by GLOBOCAN 2018 (2), there were ~1,000,000 new GC cases and 783,000 associated deaths worldwide in 2018; therefore, GC ranks 3rd in terms of cancer-related deaths, followed by liver cancer, which causes ~782,000 deaths annually. The prognosis of DSTs mainly depends on the cancer stage at diagnosis; however, a number of patients are not diagnosed until the advanced stage. Currently, the available treatment options for advanced DSTs are limited, and the 5-year survival rate remains low at <20% (3,4). Tumor metastasis contributes to high mortality; however, several biomarkers associated with tumor metastasis have been identified recently (5,6). Therefore, more knowledge concerning biomarker regulation and the underlying network behavior might help provide superior treatment options for DSTs.

Circular RNAs (circ/circRNAs) are long non-coding (lnc) endogenous RNAs in the form of covalently closed loops with no 3' polyadenylated tail or 5' cap (7). circRNAs are reported to be sponges for microRNAs (miR/miRNAs) in the cytoplasm, and they facilitate the translation of proteins and interact with the RNA-binding proteins (RBPs) to regulate transcription (8,9). Recently, the focus of research has been on the circRNA-related molecular pathways participating in tumor metastasis and progression (5,10). circRNAs may serve as novel tumor biomarkers.

Exosomes are vesicles with a diameter of 30-150 nm. Since they are released outside the cells, they are extensively present in a variety of body fluids such as plasma/serum, urine and saliva (11). Exosomes have been found to contain proteins, DNA, miRNAs and lncRNAs (12). Typically, exosomes eliminate the redundant cellular components. circRNAs have been identified in exosomes (13). When exosomes are released, they are absorbed by distant or neighboring cells, and those

containing circRNAs interfere with the processes that regulate the tumor microenvironment (TME), and may therefore, facilitate cancer occurrence, development and metastasis.

2. Biogenesis of exosomes

Exosomes were initially identified in the mature reticulocytes of sheep (14). Exosomes seem cup-shaped when observed using a cryo-electron and transmission electron microscope (15).

Three distinct processes are required to release exosomes (Fig. 1). Firstly, the selected cytosolic factors, such as proteins, are required to accumulate on the endosomal membrane for exosome biogenesis, to control the lipid bilayer invagination of endosomes to their luminal cavity, thus forming the intraluminal vesicle (ILV) (16). The protein sorting by ILVs is recognized as a highly regulated mechanism and it is dependent on the endosomal sorting complex required for transport (ESCRT) mechanism (17). Specifically, 4 complexes, namely ESCRT-0, -I, -II and -III, make up the ESCRT apparatus. This process is repeated to produce the ILVs that fill the multivesicular body (MVB). The MVB then fuses with the cell membrane using an adenosine triphosphate-dependent process, and exosomes are released into the extracellular space (18).

A protein complex comprising nucleic acids (miRNA, mRNA and DNA), lipids, extracellular matrix (ECM) proteins, transcription factors, enzymes and receptors is present either in the interior or on the exterior of the exosomes. These components contribute to the signal transmission between the recipient and the donor cells, and affect the recipient cells (19). An analysis of the exosomal protein composition revealed that certain proteins are present in specific cell- and tissue-derived exosomes, while others are commonly seen in all the exosomes (20). Lipids are involved in the biogenesis of exosomes and also maintain homeostasis in the recipient cells. For example, lipids with a high density, such as lyosbisphosphatidic acid (LBPA), on the inner MVB membrane lead to the formation of ILV and subsequent exosomes (21). LBPA interacts with the apoptosis-linked gene-2-interacting protein X, which contributes to the inward sprouting of the MVB membrane (22).

3. Role of exosomes in tumor metastasis

Several studies (23,24) have verified that tumor metastasis is related to various oncogenes and their diverse oncogenic pathways (24). Yang *et al* (25) found that FOXP3 can act as a co-activator to facilitate the Wnt-b-catenin signaling pathway, inducing epithelial-mesenchymal transition (EMT), tumor growth and metastasis in non-small cell lung cancer. It was suggested that the cell-to-cell communication in cancer together with the adjacent stroma facilitates tumor metastasis. Therefore, the present review further elaborates on the effect of the exosomes on tumor metastasis.

Promotion of tumor migration. When tumor cells metastasize, they need to break the basement membrane to penetrate the blood vessels. Such processes and tumor growth at the primary and metastatic sites are closely related to the TME. The exchange of information between the tumor cells and TME via the tumor-derived exosomes (TDEs) plays an important role in the promotion of cancer metastasis (26,27).

Mediators of EMT. Zomer *et al* (28) demonstrated for the first time that exosomes derived from highly invasive tumor cells enhance the invasiveness of lowly invasive tumor cells. Tight junctions are the main components in the adhesion complex of endothelial and epithelial cells. The natural barrier limits the metastasis of tumor cells; however, this natural barrier is destroyed by TDEs, which promote penetration of blood vessels by tumor cells. It was confirmed through *in vivo* experiments in mice that TDEs with high miR-105 expression increase the incidence rates of brain and lung metastases, accompanied by the downregulation of zonula occludens-1 (ZO-1) in endothelial cells and an increase in vascular permeability (29).

EMT involves the loss of intercellular adhesion and polarity, which exacerbates the processes of invasion and metastasis of various cancer cell types, such as GC, HCC and CRC (30). By contrast, EMT facilitates tissue remodeling and is regarded as a prerequisite for tumor metastasis (31). Under the regulation of the TME, epithelial tumor cells undergo EMT, characterized by low proliferation, high invasion and metastasis. When tumor cells reach the metastatic microenvironment in distant organs, a mesenchymal-epithelial transition occurs, thus recovering their high proliferation status, which is beneficial for the growth of metastatic tumors (30). TDEs have been proposed as the conduits to initiate EMT signaling. For instance, in the bladder cancer 5637 cell line with high expression of lncRNA-urothelial carcinoma-associated 1 (UCA1), EMT was used to transport lncRNA-UCA1 to low-expression sites through exosomes to promote tumor invasion and growth (32). Moreover, in the exosomes collected from nasopharyngeal carcinoma (NPC) cells infected with Epstein-Barr virus, high levels of hypoxia-inducible factor 1 (HIF1) and latent membrane protein 1 are detectable; HIF1 interacts with the Snail pathway to upregulate Twist, which triggers EMT in NPC cells (33). Co-culturing NPC cells with exosomes isolated from these cells induced EMT in an autocrine manner, which was supported by upregulated vimentin and N-cadherin levels, and downregulated E-cadherin levels (34). miR-23a in exosomes enhance the effect of TGF-1 to promote EMT by suppressing the synthesis of E-cadherin in melanoma and lung carcinoma cells (35). Yang *et al* (4) reported that hepatoma-derived exosomal miR-92a-3p plays a critical role in EMT progression and the promotion of metastasis by inhibiting PTEN and activating Akt/Snail signaling.

Involvement in angiogenesis. In tumorigenesis, exosomes activate neovascularization and deliver more nutritious blood to avoid tumor necrosis. TDEs mediate the exchange of information between the tumor cells and the TME, which also plays important roles in promoting angiogenesis (36). The NPC-derived exosomes contain miR-23a, which acts on endothelial cells, regulates target gene expression of testis-specific 10 and promotes angiogenesis (37). On the other hand, TDEs containing miR-25-3p enter endothelial cells to target Kruppel-like factor (KLF)2 and KLF4, regulate the expression of vascular endothelial growth factor (VEGF) receptor 2, ZO-1 and atrein, improve vascular permeability and promote neovascularization (38). Further animal experiments confirmed that miR-25-3p in exosomes markedly increased the occurrence rates of liver and lung metastases in mice (38).

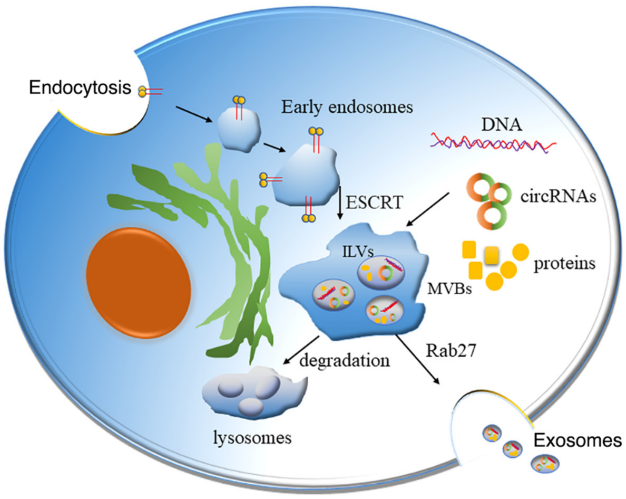

Figure 1. Function and processing of exosomes. Substances are encapsulated using endocytosis forming the early endosomes, which further mature into ILV-containing MVBs. The formation of ILVs is mediated by mechanisms that depend on the ESCRT. ILVs subsequently merge with the cell membrane via an adenosine triphosphate-dependent process, thus releasing exosomes outside the cells. These exosomes travel through biological fluids. circRNA, circular RNA; ILV, intraluminal vesicle; MVB, multivesicular body; ESCRT, endosomal sorting complex required for transport.

Involvement in immunosuppression. TDEs suppress both cellular and humoral immune responses to maintain tumor cell proliferation, and are linked to most immune cells. Specifically, they interfere with the functions of immune cells through a variety of pathways, including suppressing proliferation of immune cells, transmitting tolerance signals to immunocytes, suppressing natural killer (NK) cell activities, mediating the apoptosis of $CD8^+$ T cells and interfering with the differentiation of monocytes (39). For example, exosomes derived from epithelial ovarian cancer cells inhibit the cytotoxicity of NK cells by downregulating the natural killer group 2 member D receptor on NK cells (40).

Assisting tumor cells in escaping immune cell attack. The circulating tumor cells in the blood must escape the surveillance of the immune system for survival. Platelets help in protecting the tumor cells from the immune cells; they adhere to the circulating tumor cell surface to assist it to escape the destruction by obstructing the interactions between NK cells and tumor cells (41).

In addition, platelets also enhance the aggressiveness of tumor cells. Pang *et al* (42) demonstrated that the thrombin-activated platelets induced tumor cells to become more aggressive, and that the platelet membrane proteins p-selectin and glycoprotein IIb/IIIa were beneficial for the tumor cells, as they helped them to exude from blood vessels. Moreover, activated platelet-derived microparticles (PMPs) also contain p-selectin and glycoprotein IIb/IIIa. Co-culturing PMPs with tumor cells increased the proliferation, invasion and metastasis abilities of tumor cells, such as prostate and gastric cancer cells (43,44). The upregulation of PMPs is related to a poor prognosis and cancer development (45).

Participation in the establishment of a pre-metastatic microenvironment. Organotropic metastasis indicates that the metastatic site is a result of the complicated interaction between tumor and stroma within the host organ, rather than being selected at random. Paget first explained this by proposing the 'seed and soil hypothesis' in 1889. The 'seed and soil hypothesis' suggested that metastases only formed when the seeds (circulating tumor cells) and the soil (host organ) were compatible (46). Based on this, the establishment of a microenvironment before metastasis is essential for tumor cells to colonize the metastatic site. For example, TDEs containing miR-122 act on the non-cancerous cells in the pre-metastatic microenvironment, downregulating the expression of alanine kinase in the non-cancerous cells, decreasing glucose uptake and utilization, and thereby elevating nutritional support for the tumor cells (47). TDEs from pancreatic ductal adenocarcinoma induce the formation of the microenvironment before liver metastasis in mice and elevate the occurrence of liver metastasis (48).

4. Classification and biological functions of circRNAs

circRNAs are generated through splicing events during the maturation of the corresponding precursor mRNAs subject to RNA polymerase II transcription (49). According to the parental gene components, circRNAs are classified into 3 subtypes (50): Exon-intron circRNAs (elciRNAs), circular intronic RNAs and exonic circRNAs (ecircRNAs). Among them, only ecircRNAs contain back-spliced exon sequences, which are mainly distributed in the cytoplasm and function as miRNA or RNA-binding protein sponges. Today, the term circRNA is commonly applied to describe ecircRNAs, in which downstream donor-exons splice to the upstream acceptor-exons (51). Further studies have uncovered the functions of circRNAs, and the biological functions of each circRNA subtype are suggested to be different across different types of cells (52,53).

circRNAs play a role in the processes regulating human physiology and pathology. Several biological functions of circRNAs have been demonstrated; for instance, they act as miRNA sponges and affect the downstream target genes of miRNAs, regulate parental gene transcription, modulate alternative splicing and interact with RBPs (Fig. 2). The present study attempts to provide an overview of the molecular mechanisms underlying the regulation of various biological processes by circRNAs.

Competitive endogenous RNA and miRNA sponges. Some RNAs with corresponding miRNA response elements competitively bind to miRNA sites to affect their target genes, thereby affecting tumor behavior. This process is referred to as 'miRNA sponging'. Additionally, circRNAs have also been shown to competitively bind to miRNA sites, and affect the expression of miRNA and downstream target genes (54,55). Furthermore, circRNAs serve as tumor suppressors or oncogenes in tumorigenesis as they act as miRNA sponges through response elements. circRNAs also affect cancer development. As early as 1993, Capel *et al* (56) showed through use of sequence analysis that circRNA sex-determining region Y (Sry), which was derived from the sex-determining region of the mouse Y chromosome, possessed 16 binding sites for miR-138, and it inhibited the expression of miR-138 and its

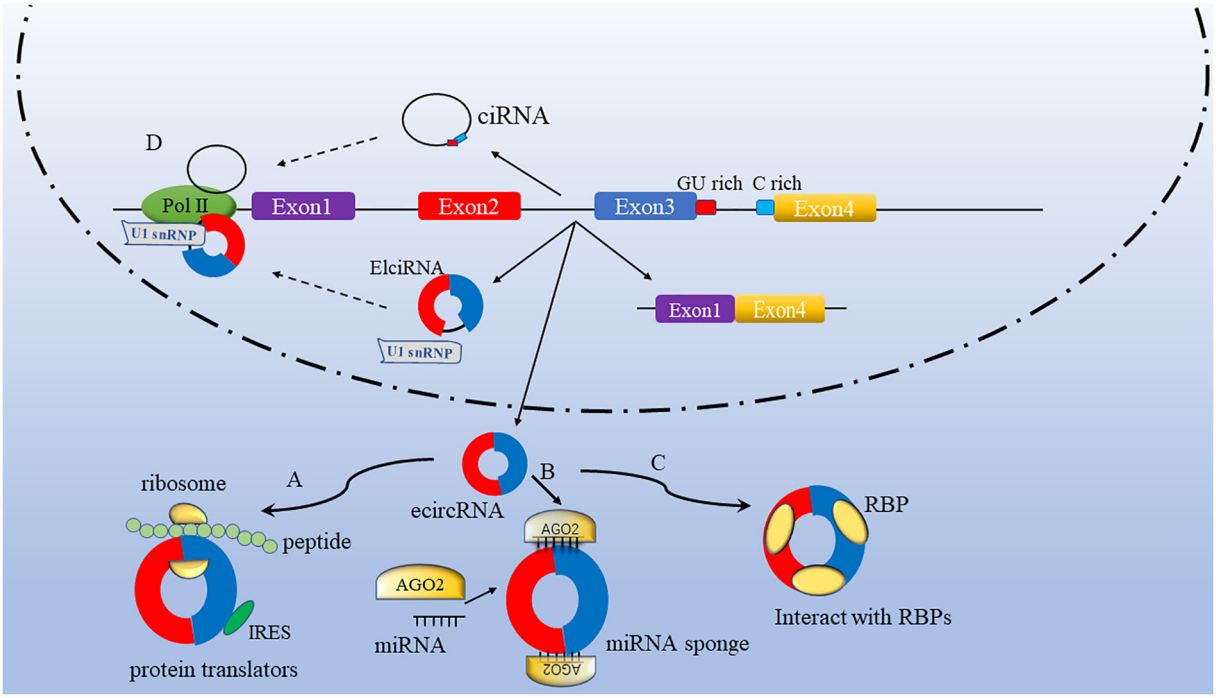

Figure 2. Multiple functions of circRNAs. (A) A subset of circRNAs are involved in protein coding. Protein coding by circRNAs is also possible with an IRES. (B) A majority of circRNAs function as miRNA sponges that interact with miRNA-Ago2 (representative protein) complexes to suppress the functions of miRNAs. (C) circRNAs bind with RBPs to form RNA-protein complexes, thus affecting their functions and translocation. (D) For transcriptional regulation, elcircRNAs and ciRNAs induce the transcription of parental genes by binding transcription complexes to the host promoters. circRNA, circular RNA; IRES, internal ribosome entry site; miRNA, microRNA; Ago2, argonaute 2; RBP, RNA-binding protein; elciRNA, exon-intron circRNA; ciRNA, circular intronic RNA; ecircRNA, exonic circRNA; snRNP, small nuclear ribonucleoprotein; Pol II, RNA polymerase II.

downstream target genes. In 2013, Hansen *et al* (8) found that circRNA ciRS-7, which is also known as the circRNA sponge for miR-7, possessed >70 miR-7-specific binding sites. The miR-7 sponge suppressed miR-7 activity, thus increasing miR-7 expression and that of its downstream target genes. At present, bioinformatics analysis has helped identify tens of thousands of circRNAs capable of miRNA sponging (57,58).

Participation in protein translation. Although circRNA is defined as a non-coding RNA, some studies (59,60) report that circRNAs possess open reading frames, which are presented in the form of proteins or polypeptides. In 1995, Chen and Sarnow (61) found that circRNAs containing internal ribosome entry sites (IRESs) might potentially induce the recruitment of ribosomes together with the initiation of translation, but no protein was encoded in the IRES-lacking circRNAs.

Regulation of transcription and alternative splicing. circRNAs are abundant in the nuclei and can bind to RNA polymerase II, thus regulating the process of transcription. For example, Ci-ankrd52 is mainly located near the transcription site and is a positive regulator of the RNA polymerase II (Pol II) complex, which exerts the cis-regulation of its parent gene elciRNA in combination with U1 small nuclear ribonucleoprotein (snRNP) to form the elciRNA-U1snRNP complex using a special binding method. Specifically, this complex binds to Pol II to enhance the transcription of the parent gene through cis-regulation (62). circRNAs are stably expressed in cells, display tissue specificity and are present predominantly in the cytoplasm. Studies have shown that circRNAs also participate in alternative splicing (63). circMBL is a circRNA formed from exon 2 of the muscleblind gene that encodes the splicing factor MBL. The flanking introns of circMBL possess the conservative binding sites of muscleblind and display strong and specific MBL binding. Regulating the MBL content potently impacts the biosynthesis of circMBL, which depends upon the binding sites of MBL (64).

Interaction with RBPs. RBPs are capable of binding to circRNA, and they also participate in nearly all cellular processes, such as differentiation, apoptosis and proliferation. circRNAs act as protein sponges, which promote the regulatory effects of RBPs on protein expression. For instance, human and drosophila circMBL binds to the MBL protein at a variety of binding sites (64). Also, Forkhead box O3 (Foxo3) is a protein encoded by the *FOXO3* gene, while circFoxo3 is one of the most researched circRNAs at present, and has been found to serve as an adapter to connect cyclin-dependent kinase 2 (CDK2) with p21 (65). circFoxo3 also induces p21 to release CDK2, and p21 phosphorylates cyclin E and cyclin A while promoting the cell cycle. Furthermore, circFoxo3 promotes the combination of p53 and the oncogene murine double minute 2, and accelerates p53 degradation (66).

5. Exosomes and circRNAs

circRNAs in exosomes are mainly 200-600 bp in length. Li *et al* (13) performed circRNA sequencing analyses using the MHCC-LM3 HCC cell line and exosomes secreted by

these cells, and discovered 5,484 and 6,751 circRNAs in the cells and exosomes, respectively. The circRNA content in the exosomes was nearly twice as high as that in the cells. The sequencing analyses on the normal human serum exosomes identified a total of 1,215 circRNAs, indicating the abundance of circRNAs in serum exosomes. Tumor tissue also continuously releases exosomes during its growth (67). circRNAs are stable in exosomes, which are also stable at room temperature. Therefore, the detection of circRNAs in humoral exosomes is easy and facilitates the diagnosis and prognosis of patients. In addition, Li *et al* (13) performed circRNA sequencing analyses on patients with CRC and found that, compared with that in healthy individuals, 67 types of circRNA were missing from the exosomes of patients, and an additional 257 types of circRNA were detected. Most of the circRNAs are housekeeping genes. PCR results showed that circ-kelch domain containing 10 can be used to distinguish between tumor patients and the non-tumor population, serving as a potential tumor biomarker. Exosomes are secreted by most cells in the body and contain multiple endogenous active substances (13). Exosomes can be stably stored in peripheral body fluids, making it easy to obtain and store exosomes (68). Importantly, they reflect the physiological and pathological changes in cells or tissues from which they are derived. When taken up by the recipient cells, exosomes impact the functionality of the recipient cell in a variety of physiological ways, especially since miRNA can mediate RNA interference (69). Numerous studies (70-72) have been performed on exosomal proteins, nucleic acids and other biologically active substances, aimed at finding new potential biomarkers, but there are still few studies on the circRNAs in exosomes. Among the thousands of circRNAs discovered using sequencing, microarray analysis and other methods, only a small proportion have been validated using PCR; therefore, further research to check whether the circRNAs in plasma exosomes can be used as biomarkers is warranted.

The exosomes secreted by cells come in contact with recipient cells and fuse to release their contents into recipient cells, thereby achieving the transfer of intracellular proteins, RNA and lipids (73). In such exosome-mediated non-contact cell communication, the TDEs play a dual regulatory role in either suppressing or promoting tumors using the paracrine and endocrine pathways. Moreover, exosomes derived from non-tumor cells also participate in tumor growth (74). Typically, the differences in the functions of exosomes are ascribed to the different types of cells and their contents. Generally, exosomes derived from tumor cells promote tumors (27), in contrast to those obtained from non-tumor cells; for instance, exosomes obtained from the immune cells mainly play a role in tumor inhibition (75). In general, exosomes affect tumor initiation and progression. In recent years, it has been suggested that exosome-derived circRNAs are closely related to tumor metastasis. For example, Li *et al* (76) found that the expression of circ_0044516 in exosomes from patients with prostate cancer was upregulated and inhibition of expression could inhibit the metastasis of tumor cells. In the current review, studies on the novel mechanisms and functions of exosomal circRNAs for regulating the initiation of DSTs are summarized. In addition, the association between the dysregulation of exosomal circRNAs and DST metastasis is also assessed.

6. Role of exosomal circRNAs in tumor proliferation

Exosomal circRNAs and HCC. HCC is the seventh most frequently occurring cancer in the world and the second most common cause of cancer mortality (77); it has a high degree of malignancy and a 5-year survival rate of <10% (78). HCC is a primary tumor originating in hepatocytes and is mainly caused by chronic cirrhosis and the gradual progression of hepatitis B or C. The pathogenesis of HCC is not fully elucidated yet, but it is characterized by rapid progression, a poor prognosis, a high mortality rate and early intrahepatic metastasis (79). Therefore, early detection and early intervention are particularly important to improve the prognosis and long-term survival rate of patients with HCC.

Zhang *et al* (80) demonstrated that the exosomal circRNAs released by adipocytes promoted HCC growth by sponging miR-34a and activated the ubiquitin-specific protease 7 (USP7)/Cyclin A2 pathway. Also, exosomes derived from adipose tissue upregulated USP7, which decreased oncogene ubiquitination.

Exosomal circRNAs also exist that suppress HCC cell proliferation. Chen *et al* (81) reported that circ0051443 level was significantly lower in the plasma and tissue exosomes in patients with HCC compared with that in healthy controls. circ_0051443 was transmitted from normal cells to HCC cells via exosomes and suppressed malignant behavior by promoting cell apoptosis and arresting the cell cycle. Consequently, exosome circ_0051443 could be used as a predictor and potential therapeutic target of HCC.

Exosomal circRNAs and CRC. Studies have shown that the pathology of CRC is characterized by multiple steps and stages, from benign polyps to invasive adenocarcinoma and distant metastasis (82). Such pathological changes are caused by the inactivation or abnormal activation of the protein-encoding oncogenes and tumor suppressor genes.

circ-formin 2 (circFMN2) may serve as a new treatment target and biomarker for CRC. Li *et al* (83) demonstrated for the first time that exosomal circFMN2 promoted the proliferation of CRC by directly binding with miR-1182 and subsequently decreasing the inhibition of human telomerase reverse transcriptase. A dual-luciferase reporter assay was performed to further validate this result and it suggested that the knockdown of circFMN2 inhibited the growth of CRC *in vivo*. In addition, circFMN2 expression was detectable in the extracted serum exosomes derived from patients with CRC, and circFMN2 expression was inversely correlated with miR-1182 in serum exosomes (83).

7. Role of exosomal circRNAs in tumor metastasis

Exosomal circRNAs are involved in the regulation of tumor metastasis. This review section emphasizes the functions of various aberrantly expressed exosomal circRNAs during tumor metastasis (Table I).

Exosomal circRNAs and HCC. Several studies have reported the effect of circRNAs on HCC (84,85), and the presence of circRNAs in exosomes has also been verified. However, the effects of exosomal circRNAs on highly metastatic HCC cells

Table I. Exosomal circRNAs involved in tumor metastasis: Signaling pathways and functions.

First author, year	Exosomal circRNA	Cancer type	Regulation	Signaling pathway	Function/clinical association	(Refs.)
Wang *et al*, 2019	circPTGR1	HCC	Upregulated	circPTGR1/ miR-449a/ MET	Increases invasive and migratory capacities in poorly metastatic and non-metastatic cells	(86)
Liu *et al*, 2020	circMMP2	HCC	Upregulated	circMMP2/ miR-136-5p	Promotes tumor metastasis. Associated with low overall survival of patients with HCC	(87)
Huang *et al*, 2020	circRNA _100338	HCC	Upregulated	Not mentioned	The metastatic ability of HCC cells can be enhanced by influencing the proangiogenic activity by regulating angiogenesis	(88)
Li *et al*, 2020	circZNF652	HCC	Upregulated	circZNF652/ miR-29a-3p/ GUCD1	Contributes to HCC cell proliferation, migration, invasion and glycolysis	(89)
Li *et al*, 2018	circPDE8A	Pancreatic cancer	Upregulated	circPDE8A/ miR-338/ MACC1/ MET/ERK or AKT	Plays a vital role during cancer metastasis. Serves as an efficient biomarker for predicting the prognosis and diagnosis of pancreatic cancer	(92)
Li *et al*, 2018	circIRAS	Pancreatic cancer	Downregulated	circIRAS/ miR-122/ZO-1/ RhoA/F-actin	Decreases ZO-1 and miR-122 expression, increases and the permeability of the endothelial monolayer, enhances tumor metastasis and invasion	(93)
Wang *et al*, 2019	circ_ 0000284	Cholangio carcinoma	Upregulated	circ_0000284/ miR-637/ *LY6E*	Upregulates the circ-0000284 level and stimulates cell proliferation, invasion and migration	(100)
Zhang *et al*, 2019	circNRIP	GC	Upregulated	circNRIP1/ miR-149-5p/ AKT1/mTOR	Sponges miRNA and promotes the proliferation, migration and invasion of GC cells	(102)
Xie *et al*, 2020	circSHKBP1	GC	Upregulated	circSHKBP1/ miR-582-3p/ HUR/VEGF	Suppresses HSP90 degradation and promotes cell proliferation, migration, invasion and angiogenesis	(103)
Lu *et al*, 2020	circRanGAP1	GC	Upregulated	circRanGAP1/ miR-877-3p/ VEGFA	Enhances GC cell migration and invasion. Suppresses tumor growth and metastasis	(104)
Zhong *et al*, 2021	circ_ 0032821	GC	Upregulated	circ_0032821/ miR-515-5p/ SOX9	Boosts the proliferation, migration, and invasion	(107)
Yao *et al*, 2021	circPVT1	GC	Upregulated	circPVT1/ miR-30a-5p/ YAP1	Modulates autophagy, invasion, and apoptosis	(108)

Table I. Continued.

First author, year	Exosomal circRNA	Cancer type	Regulation	Signaling pathway	Function/clinical association	(Refs.)
Liu *et al*, 2021	circ_0026611	ESCC	Upregulated	Not mentioned	Is significantly upregulated in ESCC with lymph node metastasis and is a predictor of ESCC prognosis	(109)
Zang, 2021	circ_0000337	ESCC	Upregulated	circ_0000337/ miR-377-3p/ JAK2	Promotes DDP resistance and metastasis in DDP-sensitive esophageal cancer cells	(110)
Zhao *et al*, 2020	circABCC1	CRC	Upregulated	circABCC1/ β-catenin/ Wnt pathway	Mediates cell stemness and metastasis	(112)
Yang *et al*, 2020	circ_133	CRC	Upregulated	circ_133/ miR-133a/ GEF-H1/ RhoA	Transports to normoxic cancer cells and promotes cell migration	(113)
Shang *et al*, 2020	circPACRGL	CRC	Upregulated	circPACRGL/ miR-142-3p/ miR-506-3p-TGF-β1	Promotes cell proliferation, migration and invasion. Plays an oncogenic role in CRC proliferation and metastasis	(114)

circ/circRNA, circular RNA; miR, microRNA; DDP, cisplatin; ESCC, esophageal squamous cell carcinoma; GC, gastric cancer; HCC, hepatocellular carcinoma; CRC, colorectal cancer.

are rarely investigated. Wang *et al* (86) found that exosomal circ-prostaglandin reductase 1 (circPTGR1) enhanced the metastasis of HCC through the miR-449a-MET signaling pathway. In the study, exosomal circRNAs obtained from highly metastatic (LM3), poorly metastatic (97L) and non-metastatic (HepG2) cells were sequenced. The highly metastatic cells transferred their contents to the poorly metastatic and non-metastatic cells via exosomes, leading to increased invasive and migratory capacities in the recipient cells. During this process, circPTGR1 affected the poorly metastatic recipient cells by decreasing the interaction between miR-449a and MET within, destroying TME homeostasis and promoting HCC metastasis.

Liu *et al* (87) speculated that exosomal circMMP2 may be a novel biomarker for HCC treatment. The study found that circMMP2 was delivered by 97H- or LM3-secreted exosomes to L02 and HepG2 cells, and promoted metastasis in HCC by sponging miR-136-5p to enhance MMP2 expression. A high level of circMMP2 and a low level of miR-136-5p was associated with low overall survival rates in patients with HCC.

Huang *et al* (88) showed for the first time that circ_100338 was highly expressed in both metastatic HCC cells and their secreted exosomes. The study reported that the overexpression or knockdown of exosomal circ_100338 significantly enhanced or decreased the invasive abilities of the HCC cells, respectively. The results suggested that the metastatic ability of HCC cells could be enhanced by transferring exosomal circRNA-100338 to receptor human umbilical venous endothelial cells (HUVECs) and influencing their proangiogenic activity by regulating angiogenesis.

In addition to the aforementioned studies, Li *et al* (89) explored the function of exosomal circ-zinc finger protein 652 (circZNF652) in HCC. The study found that exosomal circZNF652 was upregulated in the serum and HCC cells of patients with HCC. Exosomal circZNF652 contributed to HCC cell proliferation, migration, invasion and glycolysis via the miR-29a-3p/guanylyl cyclase domain containing 1 axis.

Exosomal circRNAs and pancreatic cancer. Pancreatic cancer has the worst prognosis among the gastrointestinal system cancers, and it ranks fourth in the world in terms of number of cancer-related deaths (90). Metastasis is a primary cause of death among patients with pancreatic cancer (91). Pancreatic cancer occurrence, development and metastasis involve intricate biological processes comprising several signaling pathways and target genes. Therefore, it is important to identify and verify the specific gene and its molecular mechanism that shows a causal relationship with pancreatic cancer metastasis in the diagnosis and treatment of pancreatic cancer.

circ-phosphodiesterase 8A (circPDE8A), which was discovered using microarray analyses of pancreatic ductal adenocarcinoma (PDAC) cells in liver metastasis, plays an

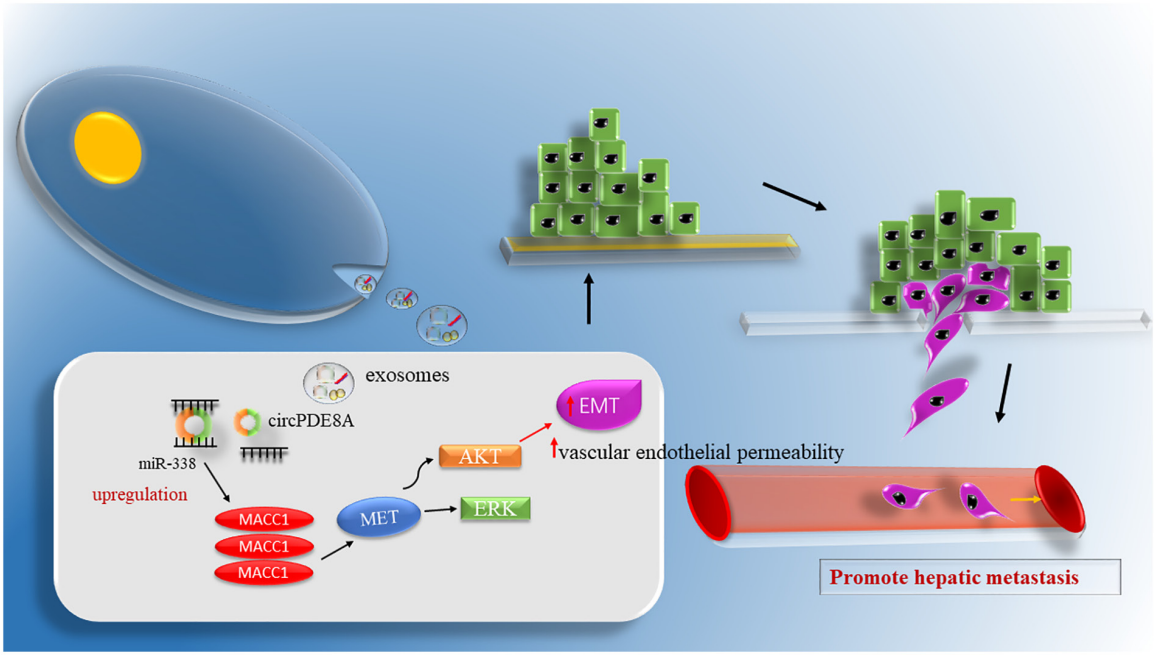

Figure 3. Exosomal circRNAs affect tumor metastasis. Exosomal circPDE8A plays the role of a competing endogenous RNA, sponging miR-338 and inducing the invasive growth of tumors via the AKT, ERK, MET and MACC1 pathways. Finally, such processes result in EMT, increase the permeability of vascular endothelial cells and promote liver metastasis of pancreatic cancer. circ/circRNA, circular RNA; miR, microRNA; EMT, epithelial-mesenchymal transition; MACC1, MET transcriptional regulator MACC1; PDE8A, phosphodiesterase 8A.

important role in tumor invasion. Li *et al* (92) discovered that exosomal circPDE8A enhanced metastasis through the miR-338/MET transcriptional regulator MACC1/MET signaling pathway in pancreatic cancer (Fig. 3). In addition, circPDE8A was identified to be a miR-338 sponge and regulated MET (one of the tyrosine kinase receptors). miR-338 is a critical oncogene in the epithelial tumor subset. Li *et al* (92) discovered an association between circPDE8A upregulation and lymphatic metastasis, poor prognosis and high TNM stage in patients suffering from pancreatic cancer. Furthermore, circPDE8A secreted by the tumor was introduced to the circulation via exosomes, and the exosomal circPDE8A in the plasma showed an association with metastasis and a poor prognosis in PDAC cases. Therefore, the aforementioned results suggested that exosomal circRNA potentially serves as an efficient and suitable biomarker for PDAC.

As shown in previous studies, exosomes are rich in circRNAs and take part in intercellular communication. However, it remains unclear whether the exosomal circRNA-associated mechanisms contribute to cancer metastasis and invasion. As discovered by Li *et al* (93), circIARS accessed HUVECs through pancreatic cancer cell-derived exosomes, which elevated the permeability of the endothelial monolayer, thereby promoting tumor metastasis and invasion. Specifically, circIARS sponged miR-122, which elevated the activity of the downstream target gene Ras homolog family member A, upregulated F-actin levels, downregulated ZO-1 levels and increased the permeability of the endothelial monolayer. Therefore, these processes boost the metastasis and invasion of the tumor. Exosomal circRNAs serve as vital biomarkers to diagnose and predict tumor prognosis early.

Several studies have shown that exosomes derived from bone marrow mesenchymal stem cells (BM-MSCs) are crucial regulators of the progression of various tumors, such as colorectal cancer and multiple myeloma (94,95). Yao *et al* (96) reported for the first time that circ_0030167 derived from BM-MSCs regulated miR-338-5p, enhanced Wnt inhibitory factor 1 expression and inhibited the Wnt8/β-catenin pathway, ultimately inhibiting the invasion, migration, proliferation and stemness of pancreatic cancer cells.

Exosomal circRNAs and cholangiocarcinoma. Cholangiocarcinoma is a cancer originating from the epithelial cells of the bile duct that has a high malignancy grade (97). The morbidity and mortality rates of cholangiocarcinoma have increased over time (98). Currently, radical surgical resection is the only curative method that achieves the long-term survival of patients with cholangiocarcinoma (99). However, as cholangiocarcinoma is not associated with chronic liver disease, the early symptoms are not obvious in patients, and diagnostic markers with high specificity are lacking. Therefore, a number of patients are affected by intrahepatic or extrahepatic lymph node metastasis (LNM), while only 30% are eligible for radical surgery, yielding a low surgical resection rate. The postoperative recurrence rate is up to 50%, even for patients who have the chance of surgery, and the 5-year survival rate is 20-45%. Therefore, it is of great clinical significance to investigate the molecular mechanism underlying cholangiocarcinoma invasion and metastasis, and to identify novel therapeutic targets. Wang *et al* (100) found that the upregulation of circ_0000284 promoted cholangiocarcinoma cell proliferation, invasion and migration both *in vitro* and *in vivo*. Additionally, circ_0000284 was upregulated within the cholangiocarcinoma cell-derived exosomes.

Even more unexpected was that the cholangiocarcinoma cell-derived exosomes upregulated the circ-0000284 level, and stimulated the proliferation and migration of adjacent normal cells.

Exosomal circRNAs and GC. GC has been identified as the second leading cause of cancer-related deaths in the world, and is associated with a poor prognosis, a high relapse rate, a decreased cure rate and a decreased survival rate (101). GC treatment is complicated due to heterogeneity in the tumor tissue resulting from both epigenetic and genetic alterations. Gastric cancer easily develops metastasis due to changes in the TME. Therefore, it is important to understand the mechanism of genetic links in GC metastasis. The functions and mechanisms of exosomal circRNA in GC are not known.

Zhang *et al* (102) reported for the first time that exosomal communication across GC cells was able to transmit circ-nuclear receptor interacting protein 1 (circNRIP1). Specifically, circNRIP1 was significantly upregulated within human GC tissues, where it promoted GC cell invasion, proliferation and migration by acting as a miR-149-5p sponge. The knockdown of exosome-derived circNRIP1 blocked GC cell invasion, migration and proliferation, and downregulated AKT serine/threonine kinase 1. In addition, by injecting a luciferase label into circNRIP1-containing exosomes in nude mice through the tail vein, Zhang *et al* (102) also proved that exosomal circNRIP1 enhanced tumor metastasis. circNRIP1 expression was higher in GC clinical samples compared with that in adjacent normal tissues. The study verified that circNRIP1 transmitted by exosomes upregulated EMT markers to promote GC metastasis via EMT.

Xie *et al* (103) demonstrated that exosomal circ-SH3KBP1 binding protein 1 (circSHKBP1) regulated the miR-582-3p/HUR/VEGF pathway, suppressed heat shock protein 90 degradation, and promoted GC cell proliferation, migration, invasion and angiogenesis *in vitro* and *in vivo*, while the suppression of circSHKBP1 had the opposite effect. Liquid biopsy of serum exosomes targeting circSHKBP1 helped diagnose and predict the prognosis of GC. Lu *et al* (104) identified that circ-Ran GTPase activating protein 1 (circRanGAP1) was markedly upregulated in exosomes from the plasma of patients with GC. Plasma exosomes in these patients enhanced GC cell migration and invasion. The study found that silencing of circRanGAP1 markedly suppressed tumor growth and metastasis of GC *in vivo*. In terms of mechanism, circRanGAP1 sponged miR-877-3p to upregulate VEGFA expression and promote GC progression.

Several studies have reported that exosome-mediated circRNAs are associated with tumor drug resistance (105,106). Zhong *et al* (107) reported that circ_0032821 contributed to oxaliplatin (OXA) resistance in gastric cancer cells. circ_0032821 was highly expressed in exosomes secreted by OXA-resistant GC cells. circ_0032821-containing exosomes secreted by OXA-resistant GC cells boosted the proliferation, migration and invasion of OXA-sensitive GC cells by acting as an miR-515-5p sponge to regulate SOX9 expression. Yao *et al* (108) highlighted a novel mechanism for the development of cisplatin (DDP) resistance in GC cells. It was discovered that exosomal circPVT1 accelerated DDP resistance by modulating autophagy, invasion and apoptosis via the miR-30a-5p/yes-associated protein 1 axis in GC cells. It was hypothesized that these exosomal circRNAs are promising diagnostic biomarkers for GC treatment.

Exosomal circRNAs and esophageal carcinoma. Esophageal carcinoma is an aggressive disease, with 5-year survival rates ranging from 15-25%. Due to this aggressiveness, 30% of esophageal tumors have already invaded adjacent tumors at the time of diagnosis (90). Therefore, early detection and diagnosis are important.

Recently, Liu *et al* (109) elaborated on the diagnostic and prognostic value of serum exosome circ_0026611 in esophageal squamous cell carcinoma (ESCC). The study found that the expression of circ_0026611 in serum exosomes from cases of ESCC with LNM was higher than that for ESCC without LNM. This suggested that circ_0026611 is a predictor of ESCC prognosis.

Exosome-mediated transfer of circRNAs is reported to be related to drug resistance in esophageal cancer. Zang *et al* (110) found that circ_0000337-containing exosomes secreted by DDP-resistant esophageal cancer cells promoted DDP resistance and metastasis in DDP-sensitive esophageal cancer cells *in vitro* through the miR-377-3p/Janus kinase 2 axis.

Exosomal circRNAs and CRC. CRC is one of the most prevalent types of cancer globally, causing multiple cancer-related deaths (111). A growing body of evidence suggests that circRNAs play a regulatory role in the initiation and development of cancer.

Zhao *et al* (112) attempted to explore the regulatory role of circ-ATP binding cassette subfamily C member 1 (circABCC1) in CRC for the first time. Exosomes from $CD133^+$ cells are known to regulate tumor progression in cancer types such as breast cancer and glioblastoma. A study found that exosomes from $CD133^+$ cells carrying circABCC1 mediated cell stemness and metastasis in CRC. circABCC1 was shown to bind with β-catenin in the cell nucleus, which activated the Wnt pathway.

Hypoxia is one of the important characteristics of solid tumors. The metastatic potential of hypoxic tumor cells is different from that of normoxic tumor cells. Yang *et al* (113) observed that hypoxia-derived exosomes transported circ-133 to normoxic cancer cells and promoted cell migration via the miR-133a/GEF-H1/RhoA axis in CRC.

Shang *et al* (114) found that circ-Parkin coregulated like (circPACRGL) was significantly upregulated in CRC cells after treatment with TDEs. Moreover, circPACRGL promoted CRC cell proliferation, migration and invasion, and the differentiation of N1 to N2 neutrophils via the miR-142-3p/miR-506-3p-TGF-β1 axis. The study suggested that cancer-derived exosomal circPACRGL plays an oncogenic role in CRC proliferation and metastasis.

8. Conclusions and future perspectives

Tumor metastasis is a crucial factor when accounting for the high mortality rate in cancer patients. The main purpose of therapy for cancer patients is to block tumor metastasis and

enhance the survival rate. Continuous efforts have been made to explore the mechanism of tumor metastasis. It is now known that, before the metastatic cells reach the metastatic site, the environment at the metastatic site, including the cell state, blood supply and ECM, needs to be altered to host and help proliferation of the metastatic cells (115). Similarly, the instability of the tumor cell genome is recognized as the driving force for tumor metastasis; although this is still a hypothesis, it is supported by most researchers in the field.

With rapid developments in biotechnology, particularly in RNA sequencing technology, circRNAs have now been confirmed to act as sponges of miRNAs. Researchers can control circRNAs to regulate miRNAs, and thereby further modulate the occurrence and development of gastrointestinal tumors. circRNAs are stable in serum exosomes, and consequently, circRNA sampling has the advantages of reduced trauma for patients, compared with surgery to remove tissues, and ease of access. Also, circRNAs are highly stable due to their special loop structures, and are widely distributed in various tissues and organs. Tissue expression is temporally and spatially different, which regulates tumor proliferation and metastasis. Thus, circRNAs are expected to serve as biomarkers for the early diagnosis of DSTs. Currently, clinical diagnosis of DSTs is mainly performed using imaging and biopsy. Further studies are required to demonstrate whether DSTs can be diagnosed by detecting the levels of circRNAs.

At present, an increasing number of studies are being performed to elucidate the role of circRNAs as tumor biomarkers, providing a clearer picture of their role in physiology. However, research on circRNAs in exosomes and their role as tumor biomarkers is lacking. Exosomes carry a large number of circRNAs, and they can transfer information from one cell to another. Exosomes also promote tumor metastasis by improving the invasion capacity of tumor cells, establishing the TME before metastasis and improving the tendency of organs to guide tumor metastasis. Serum circRNA levels are different in patients at various stages of cancer compared with in healthy patients, which increases the number of biomarkers available for diagnosis, treatment and evaluation of prognosis. The exploration of the mechanism of tumor occurrence and development is a long-term project, and exosomes represent a key method for the transmission of information for tumor cells; therefore, greater efforts should be made to further explore the limited information available, which will help to provide strong guidance for tumor diagnosis and treatment strategies in the next few years.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

BS searched the literature and wrote the manuscript. KS searched the literature and revised the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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