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Original Article

Exosomal circRNAs: a new communication method in cancer

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Abstract: Exosomes are extracellular vesicles with unique membrane markers and components that participate in cellular communication. The contents of exosomes, including growth factors, microRNAs, long noncoding RNAs, and circular RNAs (circRNAs), have been recognized as prognostic biomarkers and promote cancer progression through cancer cell growth, metastasis, angiogenesis, and cancer development. One of the components of exosomes, circRNAs, are covalently closed and prevented from degrading, which results in their continually accumulating in exosomes. Evidence suggests that exosomal circRNAs are abundant and stable in body fluids and have been implicated in many diseases. In this article we summarize the biogenesis and function of circRNAs and explore the expressions of exosomal circRNAs in cancer, emphasizing the fact that exosomal circRNAs are a novel diagnostic biomarker in the early stages of cancer and/or a therapeutic target in further cancer treatment.

Keywords: Exosomes, exosomal circRNAs, cancers, biomarkers

Introduction

Exosomes were first found in sheep reticulocytes in 1983 and named in 1987 by Pan and Johnstone [1, 2]. They are the smallest extracellular vesicles (30-150 nm in diameter), with a phospholipid bilayer structure, specific surface molecules (CD9 and CD63), and inner components, and they can be secreted by most eukaryotic cells in physiological and pathological conditions [3]. Growing evidence suggests that exosomes represent a novel type of cellular communication in the context of cancer through their bioactive cargos, including proteins [4-6], lipids [7], mRNAs [5], miRNAs [5], and circRNAs [8].

CircRNAs, a novel class of endogenous noncoding RNAs, were discovered in 1993 and are considered by-products of linear RNA erroneously splicing [9]. However, through functional research and the help of deep sequencing and transcriptome technologies, circRNAs show a widespread expression and functional pattern in mammalian species. With the function of miRNA sponging, protein interaction, transcriptional regulation, and translation regulation, circRNAs play an essential role in cancer. Through the vehicle of the exosome, circRNAs are transported to cells in the tumor microenvironment, affecting tumorigenesis, progression, invasion, metastasis, the epithelial-mesenchymal transition, and apoptosis in various mechanisms. Due to the covalently closed-loop structure generated through back-splicing without 5'caps and 3'tails [10, 11], circRNAs are resistant to ribonuclease R (RNase R) and abundantly stable in the body fluid with a long half-life [12, 13]. Emerging evidence suggests that the content of circRNAs is about ten times richer than the corresponding linear RNAs [14, 15], and a large number of circRNAs exist in exosomes [8]. In addition, the exosomal circRNAs expression patterns are consistent with the corresponding tissues and cells, and they can serve a biological markers in various cancers diagnoses [16, 17].

In this review, we summarize the functions of exosomal circRNAs in various cancers, raise the question of exosomal circRNAs translocation to nuclear, clarify the biomarker function in

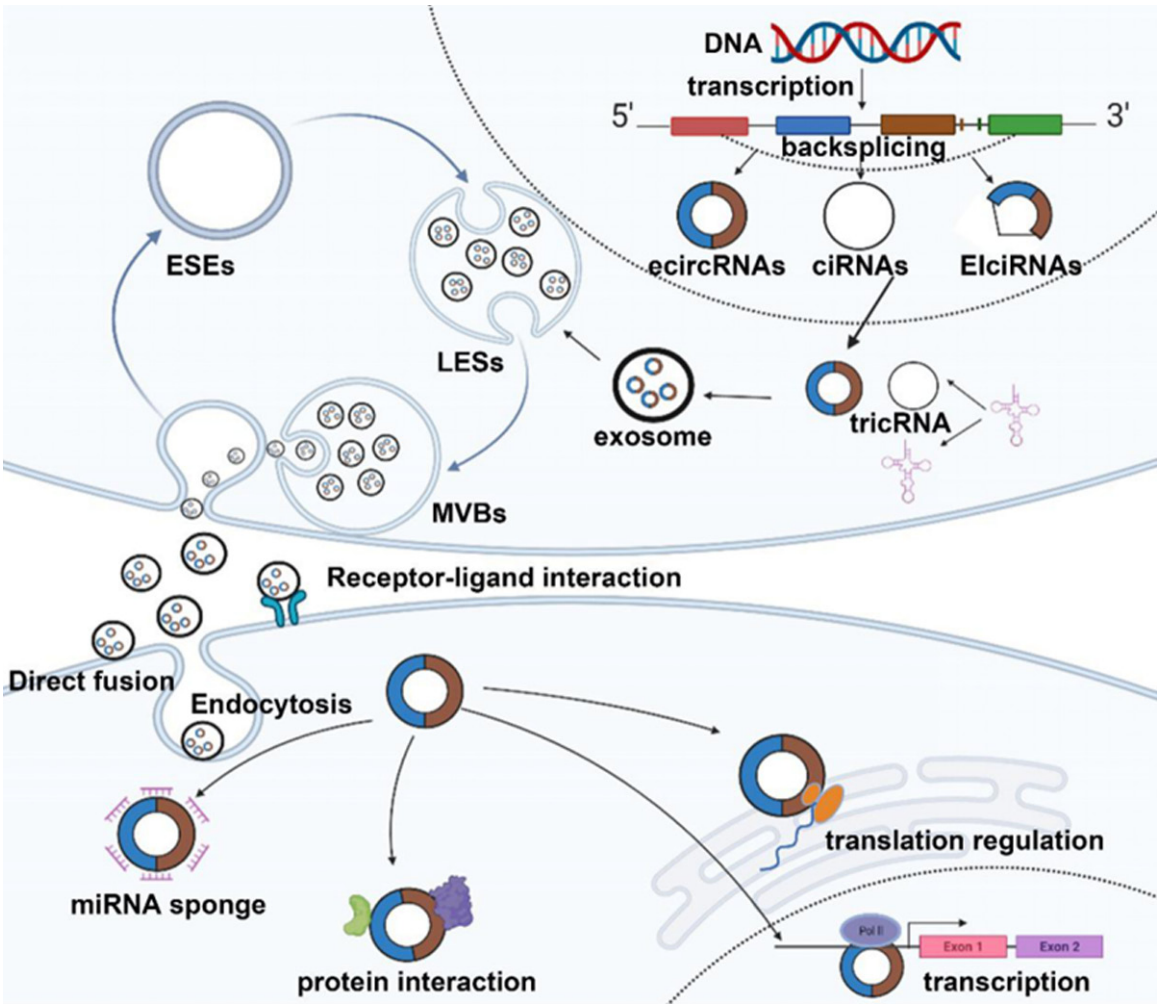

Figure 1. Biogenesis and delivery of exosomal circRNAs. CircRNAs are generated from pre-mRNA by back-splicing. According to the structural component, circRNAs are mainly divided into three types, including ecircRNAs, ciRNAs, and EIciRNAs. EcircRNAs are located in the cytoplasm and exert a regulatory function at the post-transcriptional level, while ciRNAs and EIciRNAs gather in the nuclei and interact with U1 snRNP to dominate the transcription. Exosomes are nanoscale extracellular vesicles of endocytic origin. The early endosome dissociates in the cytoplasm by folding the plasma membrane. With the help of the Golgi apparatus, the early endosome develops into the late endosome through the folding of the endosomal membrane. Vesicles accumulate in the late endosome to generate MVBs. MVBs are released in vitro by fusing with the plasma membrane to form exosomes. In this process, proteins, lipids, miRNA, mRNA, and non-coding RNAs are packaged into vesicles. Exosomes transfer circRNAs to recipient cells through direct fusion, receptor-ligand interaction, and endocytosis to perform the miRNA sponging, protein interaction, transcription, and translation regulation functions.

cancer detection and the underlying mechanism of exosomal circRNAs in promoting invasion, migration, metastasis, and drug resistance, and discuss clinical translation relevance-especially the potential of using liquid biopsies in the future.

Biogenesis of circRNAs

Hsu and Coca-Prados examined the circular structure of RNA in the cytoplasms of eukaryotic cells using electron microscopy in 1979 [18]. Traditionally, circRNAs are transcribed by RNA polymerase II and derived from precursor messenger RNAs (pre-mRNAs) through back-splicing (**Figure 1**) [19]. Alternative splicing and alternative circulation can produce tissue-specific circRNAs in the different cell cycles [15, 20, 21]. According to the structural components and the circulation mechanisms, there are five categories: exonic circRNAs (ecircRNAs), intronic circRNAs (ciRNAs), exonic-

intronic circRNAs (EIciRNAs), tRNAs (tricRNAs), and intergenic circRNAs or fusion circRNAs (f-circRNAs) [22-26]. The term "circRNAs" mainly refers to the ecircRNAs located in the cytoplasm [27]. Intronic circRNAs and exonic-intronic circRNAs predominantly gather in the eukaryotic cell nuclei that can interact with U1 snRNP and promote the transcription of their parental genes [28].

The biogenic process of circRNAs is ambiguous. However, three relatively mature models that permit the back-splicing procession have been broadly acknowledged: intron-pairing-driven circularization [15], lariat-driven circularization (also called exon-skipping) [29, 30], and RNA-binding proteins (RBPs) that induce circularization [19, 31, 32]. Zhang discovered that the ALU repeat sequence can promote the base pairing of the intron to induce circularization [27, 33, 34]. Supposing that there are some particular nucleic acid sequences in introns, such as the seven nucleotides GU-rich motif near the 5'splice site and the 11 nucleotides C-rich motif near the branching point, the introns will escape the activity of the debranching enzyme and form circRNAs [24]. Two intronic circRNA fragments (ICFs) are bound by the GT-AG splicing signals, forming intergenic circRNA [35]. Another subtype of circRNA named tricRNAs comes from the precursor tRNA [26, 36]. Also, the head and tail genes of the exons from different genes fuse using chromosomal translocations, thus giving rise to f-circRNAs [23]. The origination of the circRNAs is mainly caused by back-splicing that brings the downstream splice donor into proximity to the upstream splice acceptor, which is controlled by *cis* and *trans*-acting elements [13, 37].

Functions of circRNA

A sponge of miRNA

MiRNA, an endogenous non-coding RNA with a length of 20-25 nucleotides, can bind to the miRNA response elements (MREs) sequence of mRNA and the suppression or activation target genes at the post-transcriptional level [38, 39]. CircRNAs can act as competitive endogenous RNAs (ceRNAs) to regulate the expression of miRNA in various tumors [40-42] through absorbing miRNA with MREs and influence the target gene through its tumor suppressor or oncoprotein functions [43]. For example, MiR-7 is a tumor-suppressive miRNA in many malignant tumors. The circRNA, *Cdr1as,* harbors a total of 73 conserved miR-7 interaction sites [44], which keeps miR-7 stable and reverses the miR-7 target gene expression, activating the downstream signaling pathways to promote tumor proliferation, differentiation, migration, and invasion [29, 45]. CircRNA-0000442 is down-regulated in breast cancer tissues compared to adjacent normal tissues. It restrains cancer growth by serving as a sponge for miR-148b-3p, contributing to PTEN inhibition and further PI3K/Akt activation [46]. In addition to the linear function model, circRNA and miRNA are network interplays for the multi-miRNA response elements found in single circRNA and multi-circRNA sponging for the same miRNA family [19, 47]. A class of non-coding RNA established the network of circRNA, miRNA, and mRNA, for instance, the circUBE2D2/miR-512-3p/CDCA3 axis, the circRNF20/miR-487a/HIF-1α axis, and the circKDM4C/miR-548p/PBLD axis [48]; however, the precise regulatory mechanism remains unclarified.

Protein interaction

CircRNAs also have many binding sites for RNA-binding proteins (RBPs), which are also known as "protein decoys" with a scaffolding function [10]. Circ-Foxo3, generated from the forkhead box three genes, can interact with cyclin-dependent kinase inhibitors 1(p21) and cyclin-dependent kinase 2(CDK2) to form a circ-Foxo3-p21-CDK2 tertiary circRNAs protein complex to retard cell cycle progression [49, 50]. CircMbl originates from the second exon of the splicing factor muscleblind (Mbl) with many Mbl binding sites [19]: the over-expression of Mbl, binding to the flanking introns, can promote the formation of circMbl and reduce the linear Mbl formed by conventional splicing [51]. Therefore, the circMbl levels are negatively correlated with mature linear mRNA [19]. CircACC1 can bind directly to the regulatory β and γ subunits, promoting AMPK holoenzyme activity [52]. In summary, circRNAs can directly or indirectly bind to the protein in an RNA-protein binding manner to activate or restrain the functions of those proteins.

Transcriptions regulation

CircRNAs regulate gene transcription and expression mainly through circRNA transloca-

tion to nuclear and binding to cis-regulatory elements (CREs). Previous research has shown that ciRNAs and EIciRNAs exist in the nuclei and regulate RNA polymerase II transcriptional activity (Pol II), thus controlling the transcription of the parental genes [28]. Ci-ankrd52 and Ci-sirt7 can interact with pol II to improve the expression of the parental gene [24]. In addition, Li Z *et al.* found that two exon-intron circRNAs, circEIF3J, and circPAIP2 can interact with U1 snRNP to form an RNA-protein complex, further connecting with RNA polymerase II (pol II) in the promoter region of the host gene to enhance transcription [28]. In general, the lines of evidence suggest that circRNA can control the expressions of the parent genes at the transcription and post-transcription levels [53]. However, the exosomal derived circRNA indirect nuclear transcription regulation remains unclear. MicroRNA and siRNA can translocate to nuclear through the importin and Argonaute mechanisms [54]. There is still a lack of clear conclusive evidence that exosomal circRNA are translocated to recipient cells' nuclei even after the Argonaute binding possibility was found [44, 55]. This fundamental evidence of RNA biology provides a possibility that exosomal derived circRNA can also participate in transcription regulation in recipient cells potentially.

Translation regulation

It was considered that circRNAs were short of 5'-3' polarity and polyadenylated tails and lacked internal ribosome entry sites, because circRNAs could not translate into proteins [56]. However, Chang-you Chen and Peter Sarnow demonstrated that the synthesized circRNAs containing internal ribosome entry sites could be translated into short proteins or peptides in 1995 [57]. After that, it was proved that more and more circRNAs could be translated into proteins or peptides [58]. The translation mechanisms of circRNAs are divided into two types: dependent internal ribosome entry sites and independent internal ribosome entry sites [59, 60]. For example, circSHPRH contains a small open reading frame driven by an internal ribosome entry site, translated into a 17kDa SHPRH-146aa protein in the internal ribosome entry site-dependent machinery, acting as a tumor suppressor in Glioblastomas [61]. While some circRNAs are translated into peptides through internal ribosome entry sites in an independent manner. Those circRNAs recruit YTHDF3 through the m6A modification and then combine with translation initiation factor eIF4G2 to initiate translation [62, 63]. These findings show that circRNAs can take place in protein translation by IRES or m6A, but the roles of those peptides are still unknown.

Immunity moderation

CircRNAs have recently been implicated in the differentiation of immune cells and regulate the immune response. Macrophages express circRasGEF1B upregulated under the stimulation of lipopolysaccharide (LPS) and positively regulate the expression of intercellular adhesion molecule 1 (ICAM-1) through the LPS/toll-like receptor 4 (TLR4) signaling pathway [64]. ICAM-1 is an adhesion molecule expressed by endothelial cells and is crucial for promoting lymphocyte homing and inflammation [65, 66]. By regulating the stability of mature ICAM-1 mRNA, the endothelial and immune cells increase the cell-cell and cell-matrix interactions to promote antigen presentation and signal transduction [67]. Upon viral infection, the 2'-5'-oligoadenylate synthetase (OAS) is activated, which stimulates endonuclease RNase L to degrade the endogenous circRNAs, releasing PKR to bind with the virus dsRNA to inhibit replication [68]. In addition, those exogenous circRNAs without m6A modification are recognized by the immune receptor RIG-I, which triggers the immune response. However, the mechanism by which RIG-I recognizes the exogenous circRNAs is still not clear [69].

The formation and function of exosomal circRNAs in cancer

Exosomes are intraluminal vesicles (ILVs) generated within the payload that sprout inward containing components (nucleic acids, proteins, lipids, *etc.*) (**Figure 1**). Due to the invagination of the plasma membrane, the early endosomes dissociate in the cytoplasm [2]. The ILVs are accumulated in the late endosomes to generate multivesicular bodies (MVBs) [70]. Then the MVBs fuse with the cell membrane and release ILVs (exosomes) into the external environment [71]. As components of the exosome, the circRNAs exist stably in exosomes, resulting in highly abundant exosomes. However, different or tissue-specific cir-

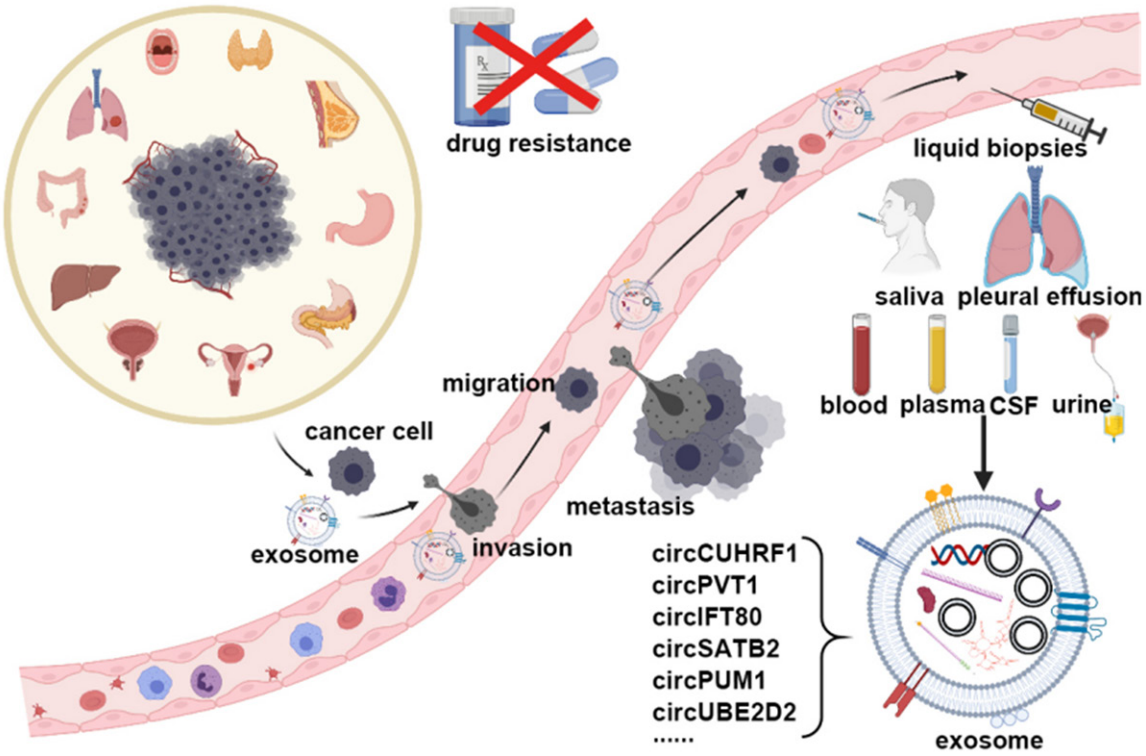

Figure 2. The pathway and detection of the exosomes. Cancer cells originating from different tissues can invade blood vessels and migrate to a particular destination with the help of exosomes and circRNAs. Then the cancer cells can settle in a new place, becoming a metastatic lesion. During those processes, exosomal circRNAs can be detected in the bodily fluids, such as the blood, saliva, pleural effusions, plasma, cerebrospinal fluid, and urine. Through liquid biopsies, the expressions of the circRNAs, for example, circCUHRF1, circPVT1, circIFT80, circSATB2, circPUM1, circUBE2D2 et al. can reflect cancer progression.

cRNAs are found in the exosomes originating from the different cells, indicating that the sorting of circRNAs is selective and not random [72]. For example, hnRNPA2B1 can transport miRNA and lncARSR into vesicles by combining with exosome motifs [6, 73]. It is speculated that exosomes may be with characteristic motifs that can combine with circRNAs to transfer them into vesicles. Li Y *et al.* introduced the ectopic expression of miR-7 into cells to down-regulate CDR1as/cirS-7 in exosomes, so the miRNA levels in the production cells can affect the sorting of circRNAs [74]. In addition, cooperation between the endosomal sorting complex required for transport (ESCRT) -0, -I, -II, and -III protein complexes promote the sorting of cargoes, the accumulation of ILVs, and the release of MVBs [75, 76]. Other ESCRTs such as Alix and vps4A are expressed on the membrane of MVBs and contribute to the sorting of miRNA [77, 78].

Accumulating evidence has shown that circRNAs play a critical role in the development of nervous system diseases [79], cardiovascular diseases [80], and cancer [23], especially in the processes of tumorigenesis [81], invasion [82], metastasis [83], angiogenesis [83], immune modulation [84] and drug resistance through protein-coding, miRNA sponging, and the cancer-related signaling pathways (**Figure 2**) [56]. Hsa_circ_0006528 [43], Hsa_circ_0001982 [85], and circ_ABCB10 [86] are upregulated in breast cancer to promote tumor growth and inhibit apoptosis. The knockdown of circDENND4C suppresses invasion and migration in breast cancer under hypoxia by increasing miR-200b and miR-200c [87]. CircPRRC2A promotes angiogenesis and metastasis through EMT and upregulates TRPM3 in renal cell carcinoma [88]. On the other hand, circ_0000442 [46], circ_Foxo3 [49], and circ_VRK1 [89] are down-regulated in

breast cancer and decrease the sensitivity of cancer cells to chemotherapeutic drugs, playing tumor inhibitory roles.

Exosomal circRNAs as a novel biomarker in cancer diagnosis and treatment

Cancer is a high-mortality disease characterized by dysregulation gene expression, genetic alternation, heterogeneity, invasiveness, and atypical clinical manifestations. Finding biomarkers with high sensitivity, high specificity, minimal invasiveness, and low cost is the basic requirement of liquid biopsies, especially in detecting cancer and finding the other risk factors contributing to cancer progression. Numerous works have shown that certain circRNAs are ectopically expressed in cancer cells and in the tumor microenvironment, promoting tumorigenesis and progression. Exosomal circRNAs exist stably in bodily fluids (**Figure 2**), and their phospholipid bilayer can protect circRNAs from degradation and exert their biological function through plasma membrane fusion, so the detection of exosomal circRNAs can be used as a new, minimally invasive biological marker in various cancers. CircRNA can be potentially employed in tumorigenesis detection in situ and in progression (**Table 1**).

Hepatocellular carcinoma

Exosomal circ-deubiquitination (circ-DB) establishes a relationship between adipose tissue and hepatocellular carcinoma. Zhang *et al.* found that exosomal circ-DB secreted from adipose tissue can promote the progression of hepatocellular carcinoma and reduce DNA damage by reducing the miR-34a mediated USP7 suppression [90]. Also, the overexpression of circ-DB activates the USP7/cyclin-2 signal pathway, which promotes the synthesis of DNA in the S phase and transmits the cell cycle from the G2 phase to the M phase [91]. The knockout of exosomal circ-DB secreted by adipose tissue can inhibit the development of HCC. Reducing adipocytes may be an effective way to prevent HCC.

The serum exosomal circCUHRF1 expression is consistent with the HCC tumor load level. When the tumor is resected, the serum exosomal circCUHRF1 decreases [92]. In addition, there is a negative correlation between the serum exosomal circCUHRF1 level and the proportion of peripheral blood NK cells [93]. Exosomal circCUHRF1 acts as a sponge of miR-449c-5p to upregulate the expression of downstream TIM-3, which can reduce the anti-tumor immunity ability and the IFN-γ and TNF-α secretion functions of the NK cells to induce immune therapy failure [94].

Zhang *et al.* found that exosomal circTMEM45A plays a role in the progression of HCC through the circTMEM45A/miR-665/IGF2 axis [95]. Huang *et al.* had indicated that exosomal circRNA-100338 promotes hepatocellular carcinoma metastasis by enhancing invasiveness and angiogenesis [83]. Sun *et al.* found the upregulation of hsa_circ_0004001, hsa_circ_0004123, and hsa_circ_0075792 in the plasma of hepatocellular carcinoma patients, which can be utilized as a diagnostic biomarker [96].

Gastric cancer

Serum exosomal ciRS-133 is significantly increased in patients with gastric cancer [97]. Luciferase assays show that the function of miR-133 is inhibited by being exposed to exosomal ciRS-133, which contains more than ten miR-133 binding sites. Exosomal ciRS-13 is transported into preadipocytes to induce energy consumption and weight loss through the miR-133/PRDM16 axis [98]. PRDM16, upregulated after the loss of miR-133, can induce the conversion of white fat to brown fat, which speeds up the body's metabolism and energy expenditure [99]. The increased expression of PRDM16 leads to the browning of the white fat and the activation of UCP1 to promote the consumption of oxygen and glucose in brown fat and to energy metabolism disorders, leading to cachexia [97].

Exosomal circ-PVT1 promotes the progression, invasion, metastasis, and drug resistance of gastric cancer to cisplatin (DDP). Down-regulated exosomal circ-PVT1 can upregulate the expression of miR-30a-5p, negatively regulates the expression of its downstream target gene YAP1, and alleviates YAP1-mediated DDP resistance, reducing cell proliferation in gastric cancer [100].

Colorectal cancer

In colorectal cancer, the circRNA levels in the serum exosomes are related to the clinicopathological characteristics like tumor size, stage,

Table 1. Summary of the exosomal circRNAs in cancers

Cancer type	Exo-circRNAS	Expression	Mechanisms	Sample type	Isolation method	Ref.
HCC	circ-DB	UP	circ-DB/miR34a/Usp7	Medium and plasma	gradient centrifugation	[90]
	circCUHRF1		circCUHRF1/miR-449c-5p/TIM-3	Serum and medium	ExoQuick	[92]
	circTMEM45A		circTMEM45A/miR-665/IGF2	plasma	ultracentrifugation	[95]
	circ-100338		mTOR signaling pathway	medium and plasma	ultracentrifugation	[83]
	circ-0051443	Down	Circ-0051443/miR-331-3p/BAKI	plasma	ExoQuick	[127]
GC	ciRS-133	UP	ciRS-133/miR-133/PRDM16	medium and plasma	ultracentrifugation	[97]
	circ-PVT1		circ-PVT1/miR-30a-5p/YAP1	serum	ExoQuick	[100]
CC	circFMN2	UP	circFMN2/miR-1182/hTERT	serum	ultracentrifugation	[101]
	circIFT80		circIFT80/miR-1236-3p/HOXB7	plasma	ultracentrifugation	[102]
	ciRS-122		ciRS-122/miR-122/PKM2	medium and serum	ultracentrifugation	[105]
	has_circ_0000338		/	cell culture medium	QIAGEN exoRNeasy Midi Kit, ultracentrifugation	[106]
	has_circ_0004771		/	serum and medium	Invitrogen™ Total Exosome Isolation Kits	[104]
	circPACRGL		circPACRGL/miR-142-3p/miR-506-3p/TGF-	plasma	ultracentrifugation	[4]
PC	circ-PDE8A	UP	circ-PDE8A/miR-338/MACC1/MET/AKT or ERK	plasma	ultracentrifugation	[107]
	circ-IARS		circ-IARS/miR-122/RhoA	plasma	Total Exosome Isolation Kit	[108]
LC	circSATB2	UP	circSATB2/miR-326/FSCN1	cell culture medium	ultracentrifugation	[109]
	has_circ_0002130		has_circ_0002130/miR-498/GLIT1, HK2 and LDHA	Serum and medium	ultracentrifugation	[110]
	has_circ_0001492		/	plasma	Total Exosome Isolation Kit	[111]
	has_circ_0001346		/	plasma	Total Exosome Isolation Kit	[111]
OC	circWHSC1	Up	circWHSC1/miR-145/MUC1	cell culture medium	ultracentrifugation	[112]
	circPUM1		circPUM1/miR-6753-5p/MMP2; circPUM1/miR-615-5p/NF-B	cell culture medium	ultracentrifugation	[117]
BC	circ-UBE2D2	Up	circ-UBE2D2/miR-200a-3p	cell culture media	ultracentrifugation	[128]
	has_circRNA_0088088		hsa-circRNA-0088088/let-7a-2-3p	cell culture media	Total Exosome Isolation Kit	[119]
	circASS1		circASS1/miR-443/Hippo signaling pathway	serum	/	[119]
	has_circRNA_00005795	Down	hsa-circRNA-0005795/hsa-miR-1304-3p or hsa-miR-3154	cell culture media	Total Exosome Isolation Kit	[119]
OSCC	circGDI2	Down	circGDI2/miR-424-5p/SCAI	cell culture media	ExoQuick	[121]
PC	circ_0044516	Up	circ_0044516/miR-29a-3p	/	/	[124]
TPC	has_circ_007293	Up	/	serum	GSTM Exosome Isolation Reagent	[126]
	has_circ_031752		/	serum	GSTM Exosome Isolation Reagent	[126]
	has_circ_020135		/	serum	GSTM Exosome Isolation Reagent	[126]

HCC, hepatocellular carcinoma, GC, gastric cancer, CC, colorectal cancer, PC, pancreatic cancer, LC, lung cancer, OC, ovarian cancer, BC, breast cancer, OSCC, oral squamous cell carcinoma, PC, prostate cancer, TPC, thyroid papillary carcinoma.

and distant metastasis. The overexpression of exosomal hsa-circ-0005100 (circFMN2) and hsa_circ_0067835 (circIFT80) can promote the cell cycle from G0 to G1 and EMT through the circFMN2/miR-1182/hTERT axis and the circIFT80/miR-1236-3p/HOXB7 axis, leading to the progression of colorectal cancer [101, 102]. Knockout circIFT80 can also induce cell apoptosis [103].

Several studies on colorectal cancer indicate that tumor-driven circRNA serves as a potential biomarker for auxiliary diagnosis. Pan B showed that tumor-derived exosomal hsa-circ-0004771 is used as a diagnostic marker for colorectal cancer [104]. Colorectal cancer-derived exosomal circPACRGL is significantly upregulated and promotes tumor growth, metastasis, and neutrophil N1-N2 differentiation through the miR-142-3p/miR-506-3p TGF-β1 axis [4]. Oxaliplatin-resistant cells transmit ciRS-122 to drug-sensitive cells and can promote drug resistance through the miR-122/PKM2 axis. PKM2 increases the consumption of ATP and glucose and then generates lactic acid. At the same time, the ectopic expression of si-ciRS-122 in the exosomes down-regulating ciRS-122 can reverse the occurrence of drug resistance [105]. Hon KW *et al.* showed that HCT116 drug resistant cells selectively transduce hsa-circ-0000338 into the parent cells via exosomes, reducing the sensitivity of parent cells to drugs and prolonging the cell activity, so high exosomal hsa-circ-0000338 levels indicate chemoresistance in colorectal cancer [106].

Pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) was typically considered to have a poor prognosis, with a one-year survival rate of 25% and a five-year survival rate of 5%. Finding a PDAC biomarker for early detection is especially important for those with disease risk. Li Z *et al.* explained the intercellular communication of exosomes among PDAC cells using immunofluorescence. An RFP-tagged CD63 lentivirus was used to label the exosomes in Hs 766T cells, which were added to GFP-labeled HS 766T cells, finding a dot-like RFP signal in the cytoplasm of GFP-labeled Hs 766T cells. The phenomenon indicates that exosomes can transfer contents between PDCA cells. High expressions of exosomal circ-PDE8A in PDCA are associated with high tumor TNM stages, invasion, metastasis propensity, and poor prognoses. One multivariate analysis shows that exosomal circ-PDE8A is also negatively associated with the overall survival rate. Exosomal circ-PDE8A, acting as a competitive endogenous RNA, activates MACC1/MET/AKT or the ERK pathway by restraining miR-338 [107]. In addition, the circ-IARS levels in the serum exosomes also accelerate through the adsorption of miR-122 to upregulate the RhoA level, increasing the endothelial monolayer permeability to improve PDAC tumor invasion [108].

Lung cancer

Lung cancer is the most common cancer-related cause of death in humans. The manu subtype of lung cancer is defined according to its histological type. The expression of exosomal circSATB2 in non-small cell lung cancer is significantly higher than it is in normal bronchial epithelial cells. Exosomal circSATB2 can directly bind to miR-326, a tumor suppressor gene related to the progression of non-small cell lung cancer, and positively regulates the expression of FSCN1 to promote the proliferation, invasion, and metastasis of non-small cell lung cancer and the proliferation of the normal bronchial epithelia. One Pearson correlation analysis shows that exosomal circSATB2 is positively correlated with FSCN1, and both are negatively correlated with miR-326. An ROC curve analysis shows that exosomal circSATB2 have high sensitivity and specificity as a serum marker for diagnosing lung cancer. Serum exosomal circSATB2 is associated with the lymphatic metastasis of lung cancer, so it is helpful for judging the clinical stage and prognosis of lung cancer [109].

Exosomal hsa_circ_0002130 regulates GLUT1, HK2, and LDHA proteins by targeting miR-498 in Osimertinib-resistant NSCLC cells, affecting glycolysis and affecting tumor growth and progression [110]. The effects of upregulated exosomal hsa_circ_0002130 can be alleviated by the ectopic expression of miR-498. Chen *et al.* established a circular RNA expression profile of early-stage lung adenocarcinoma (LUAD) serum exosomes and found that 182 circRNAs expressed differently (105 upregulated and 77 downregulated) compared with the normal con-

trols. Among those, hsa_circ_0001492 and hsa_circ_0001346 were significantly upregulated in plasma exosomes and potentially served as new markers for the early diagnosis of LUAD [111].

Ovarian cancer

CircRNAs in ovarian cancer tissue or cells can induce the endothelial-mesenchymal transformation of recipient cells and promote the progression and metastasis of ovarian cancer through the circWHSC1/miR-145/MUC1 axis. *In vitro*, exosomal circWHSC1 extracted from ovarian cancer was used to incubate the HMrSV5 cells, then the MMT (mesothelial-to-mesenchymal transition) process had happened. The morphology of the cells changed to fiber-like, and the E-adhesion decreased while the N-adhesion and MUC1 increased, resulting in cell loss tight junctions [112]. MUC1 is a membrane-bound mucin, and it transforms peritoneal mesothelial cells to malignancy through intracellular communication and provides a tumor micro-environment for peritoneal implantation and diffusion [113-115]. Exosomal circWHSC1 positively regulates the expression of MUC1 and promotes peritoneal diffusion and adhesion, so it is beneficial to the metastasis of ovarian cancer to the abdominal cavity. In addition, Cho *et al.* found that detecting the expression of MUC1 in ascites can distinguish metastatic adenocarcinoma cells from normal mesothelial cells [116].

Guan X *et al.* extracted exosomal circPUM1 from the circPUM1 overexpressed CAOV3 ovarian cancer cell line, which they used to incubate HMrSV5 cells. CircPUM1 upregulated NF-κB and MMP2 proteins by inhibiting miR-615-5p and miR-6753-5p, resulting in the occurrence of MMT and the metastasis of the cancer cells [117].

Breast cancer

In a recent study, qRT-PCR showed that the expression of circ-UBE2D2 in MCF-7/TAM-R-Exo was more than 20 fold than that in MCF-7/Par-Exo. Further, the CCK-8 and Transwell assays showed that MCF-7/TAM-R-Exo can enhance cell viability, the IC50 values, and the invasion and migration abilities. Those experiments demonstrated that the transduction of circ-UBE2D2 through exosomes in vitro can enhance tamoxifen resistance in breast cancer. Dual-luciferase reporter and RNA immunoprecipitation assays showed that MiR-200a-3p is the target of circ-UBE2D2 [118].

Yang *et al.* extracted serum exosomes from breast cancer patients and healthy volunteers. Their bioinformatics analysis and RT-qPCR found that hsa-circRNA-00005795 and hsa-circRNA-0088088 act as competitive endogenous RNA in breast cancer [119]. Exosomal circASS1 increases in breast cancer, which promotes growth and metastasis by inhibiting miR-443 or activating the Hippo signaling pathway [119]. In the 2020 ASCO Annual Meeting (May 29-June 2, 2020), researchers reported that the bioinformatics analysis and RT-qPCR further pointed out that circHSDL2 is upregulated in triple-negative breast cancer patients and adsorbed let-7a-2-3p to promote the proliferation and invasion of tumors [120]. Few studies exist on exosome-mediated circRNAs in breast cancer, so a large number of studies is needed to verify whether exosome-mediated circRNAs can be used as a new biological marker and therapeutic target for the prediction and treatment of breast cancer.

Oral squamous cell carcinoma

The upregulation of exosomal circGDI2 can inhibit the proliferation, invasion, metastasis, and glycolysis of oral squamous cell carcinoma [121]. Oral squamous cell carcinoma cells transmit circGDI2 to neighboring cancer cells through exosomes. Then circGDI2 plays an inhibitory role in oral squamous cell carcinoma progression through the circGDI2/miR-424-5P/SCAI axis [122, 123]. The expression of serum exosomal circGDI2 is consistent with the expressions of the corresponding donor cells [122]. Therefore, exosomal circGDI2 can be used as a new non-invasive biological marker of cancer and a treatment target for inhibiting oral squamous cell carcinoma.

Other cancers

The serum exosomal circ-0044516 of prostate cancer patients is significantly upregulated, inhibiting the activity of miR-29a-3p and promoting the proliferation of cancer cells. MiR-29a-3p is a tumor suppressor gene, which has been proved to be involved in the formation of various tumors [124]. For example, the down-

regulation of miR-29a-3p in gastric cancer cells can promote cancer metastasis [125]. The increased expression of exosomal Hsa-circ-007293, Hsa-circ-031752, and Hsa-circ-020135 can be used as a diagnostic marker for thyroid papillary carcinoma [126].

Clinical suggestions and prospects

In the past decade, significant advances in the screening, diagnosis, treatment, and prognostic evaluation of cancer have been made. Due to cancer heterogeneity, pre-existing drug resistant cells and the continuous self-differentiation of cancer cells leads to increased mortality and drug resistance. Cancer-related exosomes are secreted by cancer cells and cells in the tumor microenvironment, which play a regulatory role in the progression, migration, metastasis, invasion, and deterioration of cancers. CircRNAs are a covalently closed circular structure, lacking 5'-3'ends and poly-A tail, with the characteristics of stability, conservation, a long half-life, tissue-specific expression, and easy detection in bodily fluids. The circRNA research field has revealed the essential role of circRNA in tumor biology. CircRNA can act as biomarkers for early cancer detection, diagnosis, prognosis, and therapeutic efficacy evaluation. We can develop a standard for biopsy detection for various cancer types and cancer progression.

Although many studies clarify the molecular mechanism of circRNA biogenesis and functions in the context of tumors, more efforts are still needed to elucidate the exosomal circRNA detail functions of circRNA besides the sponging for miRNA and the clinical reality for exosomal circRNA communication between cells. Functioning as a novel therapeutic target, circRNAs can be divided into tumor suppressors and oncogenes. Oncogenic circRNAs are highly expressed in cancer tissues and act as competitive endogenous RNA to retain miRNA activity by adsorbing miRNA. We can inhibit the formation of circRNAs and degrade circRNAs using enzymes or circRNA inhibitors (blocking the combination of circRNAs and miRNA) to delay cancer growth. Tumor-suppressing circRNAs should be increased in tissue to inhibit tumor progression and migration. Exosomes are vesicles secreted by many cell types, including miRNA, mRNA, circRNA, proteins, and other cargos. Many previous studies have shown that cancer cells can transfer circRNAs to the surrounding cells through exosomes to promote carcinogenesis. Therefore, the synthetic tumor-suppressing circRNAs can be transferred into exosomes, which are transported to specific target cells to inhibit tumor metastasis and invasion. For example, Chen *et al.* injected circ-0051443-Eoxes into the HuH7 and Hep3b cell lines, stopping the cell cycle at the G0/G1 phase through the circ-0051443/miR-331-3p/BAKI axis, which inhibited the progression of hepatocellular cancer [127]. Exosomal circRNAs can be used as an effective therapeutic target in cancer.

Liquid biopsy provides a non-invasive method for early detection to monitor cancer progression, evaluate genetic alternation and adopt the individual approach in cancer treatment. As extracellular vesicles in liquid biopsy, exosomes can be isolated or detected via micro NMR devices, nanoplasmonic chips, a magneto-electrochemical sensor, and the like. Exosomal circRNA has a built-in advantage during exosomal liquid biopsy elevation in its relative stability over miRNA and lncRNA and inconvenience over lipids or proteins. However, more clinical randomized controlled trials are needed to evaluate the effectiveness and safety of the diagnosis and treatment using exosomal circRNAs before pre-market approval. The application of exosomal circRNAs in the diagnosis and treatment of cancer needs to be further explored.

Conclusion

In this review, we summarized the biogenesis and functions of circRNAs, especially in exosome-mediated cellular communication. Scientists have mainly studied the sponging of circRNAs, which act as a competitive endogenous RNA and inhibit miRNA activity by binding to miRNA, thus affecting the downstream signaling pathway. The circRNAs/miRNA/mRNA axis plays an essential role in cancer progression and has become a research hotspot. With the development of high-throughput technology and bioinformatics analysis, many exosomal circRNAs have been found in bodily fluids which potentially serve as a biomarker for detecting tumorigenesis, progression, and drug resistance. Exosomal circRNA detection in liquid biopsies is an emerging application in cancer.

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Disclosure of conflict of interest

None.

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