Molecular Therapy
Nucleic Acids
Review

circRNAs and Exosomes: A Mysterious Frontier for Human Cancer

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Exosomes are nano-sized membrane-bound vesicles and contain active substances (DNA, noncoding RNA [ncRNA], protein), which provide a novel method of transferring effector messages between cells. Circular RNAs (circRNAs), a kind of ncRNA, have attracted increasing attention over the last decade given advances in whole-genome and transcriptome sequencing technologies. It has become increasingly clear that circRNAs regulate gene expression through various actions and play diverse roles in many fields of human cancer biology. Notably, several studies reported that circRNAs are enriched in exosomes and that exosomal circRNAs play an important role in cancer biology. Exosomal circRNAs can be taken up by neighboring or distant cells and affect many aspects of physiological and pathological conditions of the recipient cells, potentially promoting cell communication and tumor metastasis. Herein, we briefly review the molecular mechanisms of circRNAs and recent findings regarding exosomal circRNAs, and highlight the specific roles of exosomal circRNAs in human cancer.

Exosomes

Exosomes are defined as nano-sized phospholipid bilayer-bound vesicles that are secreted into the interstitial spaces and bodily fluids by all types of cells.[1] Exosomes range from 30 to 100 nm in diameter and display several specific surface molecular markers, such as CD9, CD63, and CD81.[2,3] Exosomes were discovered and defined by Harding et al.[4] and Pan and Johnstone[5] in 1983. Subsequent studies indicated that there are four stages in exosome formation: initiation, endocytosis, multivesicular body (MVB) formation, and exosome secretion.[6] During this process, the ESCRT (endosomal sorting complex required for transport) complex and relevant proteins play critical roles in exosomes biogenesis and material cargo sorting.[7,8] The complex can pick the protein labeled by ubiquitin, guide it to MVBs, and separate from the peripheral membrane through the process analogous to cytokinesis and virus budding.[8] After release, exosomes exist in intercellular spaces or circulate in biological fluids. These vesicles can be internalized by neighboring cells or remote recipient cells via target cell membrane fusion, thereby altering the behavior of the target cell.[9,10] In recent years, increasing data have begun to advance the idea that exosomes are not just "garbage bins" of cells but important mediators of interactions between different cells. In fact, it has recently been reported that exosomes enclose various types of bioactive cargos, including lipids, common or specific proteins, DNA fragments, and RNA molecules (mRNA and noncoding RNA [ncRNA]).[11] Hence, although effector messages are transferred between different cells, exosomes provide a novel method of intercellular communication and are involved in diverse physiological and pathological processes, such as angiogenesis, immune response, antigen presentation, cell differentiation, tumor cell migration, and invasion.[12] Intriguingly, researchers found that tumor cells secreted approximately 10-fold more exosomes than normal cells[13] and that cancer cells have an accurate targeting mechanism for the properties of exosomes, suggesting that exosomes could play an essential role in tumor formation and progression.[14]

Several review papers have summarized the pleiotropic roles of exosomal microRNAs (miRNAs) and lncRNAs (long ncRNAs) in cancer biology.[15,16] In addition to microRNAs and lncRNAs, circRNAs were also identified in exosomes, and the important role of exosomal circRNAs in human cancer cannot be neglected. Therefore, in the following sections, we briefly outline some of the underlying molecular mechanism of circRNAs and focus on the recent advances in exosomal circRNA research in cancer with an emphasis on the current significance of exosomal circRNAs in the biological function and clinical implications of cancer.

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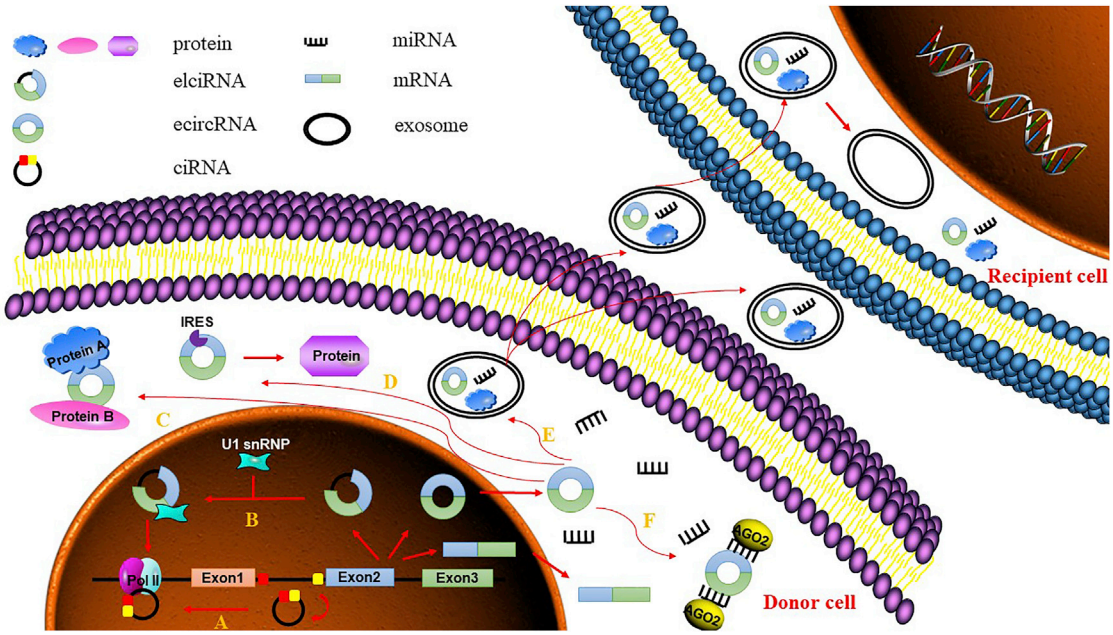

Figure 1. Paradigms for cellular molecular mechanisms of circRNAs

(A and B) circRNAs derived from introns (A) or contain retained introns (B) can bind to RNA pol II and play a key role in efficient gene transcription in *cis* or *trans*. (C) Certain circRNAs interact with proteins and form RNA-protein complexes to influence protein activity. (D) A few of the circRNAs exhibit translation ability. (E) circRNAs can be loaded into exosomes, released by donor cells, and enter recipient cells through endocytosis, thus modulating gene expression in recipient cells. (F) circRNAs act as miRNA sponges to affect miRNA activity and affect the ceRNA network.

circRNA

In recent decades, the extensive application of updated innovative technologies has promoted the comprehensive development of the RNA field. Evidence from next-generation sequencing suggests that the noncoding portions (for instance, lncRNAs) of the genome might regulate the complexity of an organism.[17] In the complicated landscape of ncRNAs, circRNAs have attracted growing attention given high biological and functional interest.

circRNAs were first identified in RNA viruses by electron microscopy in 1976.[18] However, circRNAs were once considered "splicing noise" or "dark matter" in organisms in the following decades given their low abundance and structure specificity.[19] As a result, only very few circRNAs (e.g., circular antisense non-coding RNA in the INK4 locus [ANRIL]) with little functional potential were reported.[20] In recent years, given the improvements in high-throughput sequencing technology and bioinformatics, circRNAs have become a research hotspot.[21,22]

As a new type of RNA molecule, circRNAs are single-stranded circularized molecules commonly generated from the precursor mRNA (pre-mRNA) backsplicing process, during which an upstream acceptor site is joined with a donor site.[23–25] The vast majority of circRNAs are derived from known protein-coding genes and contain one or several exons.[26] Based on the included structures, these RNA molecules are divided into four categories: exonic circRNAs (ecRNAs), intronic circRNAs (ciRNAs), exon-intron circRNAs (eIciRNAs), and intergenic circRNAs.[27,28] Unlike linear RNAs, circRNAs possess certain properties given their special structure.[29–31] circRNAs are stable and resistant to exonucleases (including RNase R) due to the lack of a poly(A) tail. The median half-life of circRNAs is 2.5-fold longer than that of their linear counterparts.[24,32] In addition, strict tissue, cell, developmental, and age expression specificity has been demonstrated for a number of circRNAs, supporting the hypothesis that these transcripts are of functional importance.[33] Indeed, numerous studies recently have shed light on the relationship between circRNAs and human diseases, including nervous system disorders,[34,35] cardiovascular diseases,[36–38] diabetic retinopathy,[39] and tumors.[40,41] As a result, our understanding of the function and molecular mechanism of circRNAs in human diseases is expanding rapidly.

Emerging Functions of circRNAs in Cancer

circRNAs Serve as miRNA Sponges. The distinguishing function of circRNAs is its miRNA decoy ability. To date, accumulating evidence has revealed that numerous circRNAs regulate gene expression by functioning as miRNA sponge molecules (Figure 1F).[42] Compared with other competing endogenous RNAs (ceRNAs) (such as lncRNA or pseudogenes), circRNAs exhibit a greater preference to bind miRNAs and are called "super sponges." This is perhaps best exemplified by ciRS-7 (circular RNA sponge for miR-7). ciRS-7, also termed CDR1as (antisense to the cerebellar degeneration-related protein 1 transcript), is approximately 1.5 kb in length and contains more than 70 conserved miR-7 binding sites.[30,42] Most binding sites combine with the RNA-induced silencing complex (RISC), which is formed by Ago2 protein and miR-7.[43] Additionally, studies have

confirmed that the phenotypes caused by overexpression of miR-7 or knockdown of ciRS-7 are analogous, indicating that ciRS-7 might play profound roles in pathophysiology processes via the miR-7/ciRS-7 axis.[42,44] Analogously, circ-SRY is a circular RNA originating at sex determining region Y (SRY) and has a similar function to CDR1as.[45] It contains 16 miR-138 binding sites and modulates the expression of miR-138 target genes through sponging miR-138.[46] In addition, some circRNAs are composed of several kinds of miRNA response elements (MREs). For example, Li et al.[47] found that circ-ITCH has miR-7, miR-17, and miR-214 adsorption functional capabilities. circHIPK3 serves as a regulator of cell growth through sponging varieties of miRNAs (9 miRNAs with 18 binding sites) in human cancer.[48]

However, it is worth noting that although the mechanism of ceRNA is similar to the classical function of circRNA, the controversy surrounding circRNA is inevitable. Militello et al.[49] conducted a survey to verify the function of circRNAs as miRNA sponges that were screened in the published data and databases. Unfortunately, the data proved that types of circular RNAs could not sever as "bona fide" miRNA sponges.[50] Hence, further studies are still needed to elucidate the networks of circRNAs, miRNAs, and mRNAs.

circRNAs Interact with Protein. In addition to the effect of circRNAs on miRNAs, another new function of circRNA is its interaction with protein (Figure 1C). It can function as protein sponges by adsorbing one or some proteins via the binding sites, thus regulating gene expression. The best experimentally supported example of a circRNA protein decoy is circMBL. Given the presence of functional MBL binding sites in the sequence of circMBL, MBL protein could strongly and specifically bind to circMBL, which is essential for circRNA biogenesis.[51] Thus, circMBL plays a key role in balancing MBL mRNA and circMBL expression levels through sequestering excess MBL protein. In addition, circPABPN1 also regulates the PABPN1 protein expression level.[52] High circPABPN1 levels prevent HuR binding to PABPN1 mRNA and suppress PABPN1 translation. Moreover, another interesting example is circ-Foxo3, which is encoded by the Foxo3 gene and serves as a tumor suppressor.[53] Du et al.[54] reported that ectopic expression of circ-Foxo3 arrests cell cycle progression in the G_1 phase by forming the circ-Foxo3-p21-CDK2 ternary complex. In addition to p21 and CDK2, circ-Foxo3 interacts with ID-1, E2F1, FAK, and HIF1, and these proteins are retained in the cytoplasm, resulting in the loss of their anti-senescent and anti-stress efficacy and increased cellular senescence.[55]

circRNAs Regulate Gene Transcription. Compared with the important role of circRNAs mentioned above, the involvement of circRNA in transcriptional regulation is not extensively characterized. Recently, some circRNAs have been shown to be abundant in the nucleus, where they might interfere with transcription, for example, eIciRNAs and ciRNAs (Figures 1A and 1B). Researchers discovered that upon circEIF3J and circPAIP2 knockout, EIF3J and PAIP2 gene transcription efficiency is significantly reduced, respectively.[56] Particularly, these two eIciRNAs combine with U1 snRNP (small nuclear ribonucleoprotein) and promote the interaction of RNA polymerase II (RNA pol II) with the promoter region of the parental gene. Thus, the transcription of genes is increased. Additionally, certain ciRNAs, such as ci-ankrd52 and ci-sirt7, accumulate at sites of their parental gene transcription and interact with RNA pol II to form positive feedback regulation.[57] Intriguingly, both eIciRNAs and ciRNAs not only localize to their transcriptional regions but also accumulate in other sites of chromatin, indicating that they may regulate gene expression in *trans*. Taken together, these findings indicate that intron-derived circRNAs play a transcriptional regulatory role in the nucleus.

circRNAs Can Be Translated into Proteins. In consideration of the lack of a poly(A) tail as well as a 5′ 7-methylguanosine cap structure, most researchers thought that circRNAs represent a distinct category of endogenous ncRNAs. Nonetheless, convincing evidence has revealed that circRNAs can be used as templates for protein synthesis similar to linear mRNAs (Figure 1D). As early as 1986, Kos et al.[58] identified the first circRNA that can be translated to protein. It is a single-stranded circRNA transcribed from the genome of the hepatitis δ virus and generates 122 aa. In addition, some reports showed that circRNAs can be translated *in vivo* and *in vitro* when engineered with the initiation codon ATG or internal ribosome entry site (IRES).[59] For example, circ-ZNF609 contains a 753-nt open reading frame (ORF) and encodes a protein in a splicing-dependent and cap-independent manner.[39] Additionally, Zhang and colleagues[60,61] demonstrated that circ-SHPRH, circ-FBXW7, and proteins coded by them are abundantly expressed in normal human brains with decreased expression in glioma. All of the above studies suggest that endogenous circRNAs might generate proteins, which provides a new direction for research on circRNAs.

circRNA and Exosomes

In recent years, growing evidence has demonstrated that the transfer of ncRNA-enriched exosomes is involved in various biological processes of cancer, particularly malignant tumor metastasis. The presence of abundant circRNAs in exosomes was first reported by Li et al.[62] They characterized circRNA transcripts from MHCC-LM3 liver cancer cells and cell-derived exosomes via genome-wide RNA sequencing (RNA-seq) analyses. The result reveals that exosomal circRNAs were concentrated by at least 2-fold in exosomes compared with parental cells, providing novel avenues for the study of circRNAs. Given that the circRNA species in exosomes differ from those noted in multiple cell types, the mechanism of circRNA sorting was investigated. circRNAs that are integrated into exosomes are selective, and based on overexpression analyses, Li et al.[62] found that the process by which circRNAs enter exosomes was controlled, at least in part, by modulation of related miRNA levels in parental cells. Besides, other possible mechanisms include RNA-associated proteins binding to circRNAs.[63] Indeed, in another study, Dou et al.[64] identified circRNA expression profiles in both cells and exosomes from KRAS mutant (DKO-1), combined mutant/wild-type (DLD-1), and wild-type (DKs-8) cells. In accordance with the results of Li et al.,[2] Dou et al.[64] found that circRNA levels are far greater in exosomes than

those in cells. More importantly, the authors compared the expression level of two colon cancer-related circRNAs and their corresponding linear mRNA in mutant and wild-type KRAS-derived exosomes. Surprisingly, the shift in the two circRNA levels was not consistent with that noted for their linear mRNA host genes. Combined with the results from the proteomic analysis, the specifically exosomally localized, enriched RNA-binding proteins might be responsible for the relative differences in circRNA and linear RNA. Nonetheless, the precise mechanism of circRNA sorting remains largely unknown.

Although the biological function of exosomal circRNAs remains incompletely elucidated, increasing studies have focused on exosomal circRNAs in recent years. New studies show that exosomal circRNAs originating from tumor cells or other cells (such as activated human platelets and adipose cell) can transfer biological information to the specific cells to achieve the efficient transmission of phenotypical changes and thereby promote cancer (Figure 1E).

Roles of Exosomal circRNAs in Cancer Biology

circ-IARS. circ-IARS is a novel circRNA involved in pancreatic cancer progression.[65] Li and colleagues[65] showed that circ-IARS is upregulated in pancreatic cancer, and circ-IARS expression levels correlate positively with tumor metastasis and negatively with postoperative survival time. It should also be mentioned that pancreatic cancer is a gastrointestinal cancer that exhibits strong metastasis and high mortality rates.[66–68] The function of endothelium, which serves as a barrier, is a vital factor that controls the exchange between the surrounding tissues and blood and prevents the invasion of pancreatic cancer cells.[69] Therefore, maintenance of this function might represent a good method to restrain tumor invasion and metastasis. Previous studies reported that an increase in RhoA expression and activity in human umbilical vein endothelial cells (HUVECs) upregulates F-actin levels and decreases tight junction protein ZO-1 expression,[70,71] resulting in the increase in cell inward contractile force, endothelial barrier function injury,[72–74] and endothelial monolayer permeability enhancement.[75,76] Li et al.[65] demonstrated that circ-IARS is transferred from pancreatic cancer cells to HUVECs via exosomes and modulates changes in endothelial permeability through the absorption of miR-122 and activation of the RhoA signaling pathway, indicating that exosomal circ-IARS are key factors involved in cancer cell-endothelium communication.

circRNA_100284. Another exosomal circRNA that is involved in carcinogenesis is circRNA_100284, which exhibits increased levels in liver cells subject to chronic arsenite exposure.[77] Dai et al.[77] found that exposure to arsenite could induce the malignant transformation of normal liver cells, which was accompanied with the overexpression of circRNA_100284. To examine the influence of circRNA_100284 on arsenite-related neoplasia, knockdown and overexpression analyses were performed. The results showed that circRNA_100284 increased the colony formation, invasion, and migration of arsenite-transformed cells. Surprisingly, arsenite-transformed cells interact with neighbor cells involved in the progression of cancer development. Medium from transformed liver cells upregulated circRNA_100284, promoted proliferation, and accelerated the cell cycle of normal liver cells. Using electron microscopy, Dai et al.[77] demonstrated that exosomes that carry circRNA_100284 are transferred from malignant cells to nonmalignant cells. The nonmalignant cells take up exosomes, and circRNA_100284 increases EZH2 and cyclin-D1 by acting as a sponge of miRNA-217, leading to the malignant transformation of nonmalignant cells.

circ-PDE8A. Pancreatic ductal adenocarcinoma (PDAC) is the most malignant human cancer. The high mortality is partly attributed to the tumor's metastatic potential and high risk of recurrence.[78] To explore the molecular mechanisms of PDAC progression and metastasis, Li et al.[79] identified the exosomal circRNAs expression profile of liver-metastatic PDAC cells by microarray. Combined with qRT-PCR results, they found that three circRNAs, including circ-PDE8A, exhibit distinctly high expression in exosome from Hs766T-L2 cells, which are second-generation primary cells from liver metastatic tissue of Hs 766T. circ-PDE8A functions as a ceRNA for miR-338 to regulate MACC1 and MACC/MET/ERK or AKT pathways, leading to invasive growth. Fluorescence microscope analysis showed that exosome communication indeed occurs in PDAC cells. Moreover, the high plasma expression of exosomal circ-PDE8A is an independent risk for overall survival.

ciRS-133. Cancer-related cachexia, a metabolic syndrome, is a negative risk factor for cancer survival.[80] Compared with healthy individuals, the defining characteristic of cachexia is excessive energy consumption.[81,82] However, little is known about the molecular mechanism of cancer-related cachexia. Zhang et al.[83] identified a cachexia-related plasma exosomal circRNA named ciRS-133. They found that ciRS-133 was increased in gastric cancer tissues and in plasma of gastric cancer patients. Further analysis revealed that ciRS-133 was positively associated with the mass of brown adipose tissue and body fat rate in gastric cancer patients. Gastric cancer cell-derived exosomes delivered ciRS-133 into pre-adipocytes. In pre-adipocytes, ciRS-133 promotes PRDM16 expression via sponging miR-133, promoting the differentiation of pre-adipocytes into brown-like cells. In an animal model, ciRS-133 suppression substantially restrained brown adipose tissue browning and generated tumor-associated cachexia.

circPTGR1. Although emerging evidence has uncovered the role of exosomal circRNA in intercellular communication, the interplay between cancer cell lines with different levels of metastatic potential remains poorly understood. Wang et al.[84] compared divergent exosomal RNAs in hepatocellular carcinoma (HCC) with different metastatic potentials. The result revealed that three isoforms of circPTGR1 were upregulated in metastatic HCC cells. Surprisingly, knockdown of circPTGR1 could suppress metastasis in LM3 cells through competing with the seed sequence of miR449a, facilitating MET expression. However, knockdown of PTGR1 mRNA did not affect HCC cell metastasis. More importantly, the increased metastatic potential of HCC cells can confer this potential on cells with

Table1. Exosomal Circular RNAs Act as ceRNAs to Regulate Human Cancer Progression

Circular RNA	Biological Functions	miRNA	Downstream Pathways	Tumor Types	The Expression Level
circ-IARS	permeability of the endothelial monolayer and tumor metastasis	miR-122	RhoA/F-actin/ZO-1	pancreatic cancer	up
circRNA_100284	cell cycle and proliferation	miR-217	EZH2	hepatocellular carcinoma induced by arsenite	up
circ-PDE8A	invasive growth	miR-338	MACC/MET/ERK or AKT pathway	pancreatic cancer	up
ciRS-133	differentiation of pre-adipocytes	miR-133	PRDM16	gastric tumor	up
circPTGR1	migration, invasion	miR-449a	MET pathway	hepatocellular carcinoma	up
circ-DB	deubiquitination	miR-34a	USP7/Cyclin A2 signaling pathway	hepatocellular carcinoma	up
circPRMT5	metastasis	miR-30c	SNAIL1/E-cadherin pathway	urothelial carcinoma of the bladder	up
circRASSF2	proliferation and migration	miR-302b-3p	IGF-1R	laryngeal squamous cell carcinoma	up
Hsa_circ_0109046	unknown	unknown	unknown	endometrial cancer	up
Hsa_circ_0002577	unknown	unknown	unknown	endometrial cancer	up
Hsa_circ_007293	unknown	unknown	unknown	papillary thyroid carcinoma	up
Hsa_circ_031752	unknown	unknown	unknown	papillary thyroid carcinoma	up
Hsa_circ_031752	unknown	unknown	unknown	papillary thyroid carcinoma	up

lower or no metastatic ability via exosomes with circPTGR1, thus promoting HCC cell metastatic abilities and progression. In particular, since circPTGR1 is highly abundant in serum exosomes from HCC patients and is associated with clinical stage and prognosis, it could act as a therapeutic target and prognostic biomarker in HCC.

circ-DB. In addition to cancer cell-derived exosome circRNAs, some studies focused on the role of exosomal circRNAs secreted by other cells. A recent article published in *Nature* identified adipose-derived exosomes that regulate gene expression in other tissues, suggesting that exosomes might be crucial carriers mediating signal conduction between adipocytes and target organs.[85] circ-DB (circ-deubiquitination, Has_circ_0025129) is an adipose-secreted exosomal circRNA and is upregulated in HCC patients with higher body fat ratios.[86] circ-DB is transcribed from TNFRSF1A (TNFR1) that is located on chromosome 12.[87] Given that exosomal circ-DB was negatively related with miR-34a expression levels, which is negatively associated with USP7 in HCC patients, the researchers subsequently explored the potential molecular mechanisms of exosomal circ-DB, miR-34a, and USP7.[86] It was shown that adipose-derived exosomes acted as carriers of exosomal circ-DB, which promotes HCC growth and inhibits DNA damage by adsorbing miR-34a and activating the USP7/CyclinA2 pathway *in vitro* and *in vivo*. Interestingly, a previous study revealed that deubiquitination of nuclear factor κB (NF-κB) by USP7 is necessary for TNFR-induced gene expression. Hence, whether positive feedback regulates USP7 and circ-DB expression needs to be elucidated in future studies.

Exosomal circRNAs as Potential Cancer Biomarkers

Numerous cancer patients are diagnosed at an advanced stage partly because of the lack of accurate biomarkers. Common indicators, such as CEA, AFP, and CA125, might be within normal limits at early stages. Therefore, it is urgent to identify sensitive and specific biomarkers for early detection and treatment of neoplasia, particularly for high-risk patients. Noninvasive early detection of malignancy is a perennial hot topic in tumor research, and liquid biopsy is a very attractive solution for this important issue. Most of the studies have focused on the exosomal lncRNAs and miRNAs that function as diagnostic biomarkers.[15,88,89] Given their special circular structure, circRNAs are more stable in human liquid than are linear RNAs. Serum exosomal circRNAs seem to be more promising noninvasive cancer biomarkers. Herein, we summarize the clinical significance of exosomal circRNAs in human cancer.

Recently, a study reported circRNA profiles in extracellular vesicles (EVs) that are isolated from serum of patients with endometrial cancer.[90] In total, 275 circRNAs with a p value <0.05 were identified in these patients, including 209 upregulated circRNAs and 66 downregulated circRNAs. Through KEGG pathway analysis, they identified that 12 of 275 differentially expressed circRNAs were enriched in five pathways (focal adhesion, regulation of actin cytoskeleton, extracellular matrix [ECM]-receptor interaction, amoebiasis, and arrhythmogenic right ventricular cardiomyopathy). Additionally, another study identified altered exosome circRNAs in the serum of patients with papillary thyroid carcinoma using high-throughput sequencing.[91] The results showed that 22 circRNAs were differentially expressed, including 3 upregulated circRNAs and 19 downregulated

circRNAs. These differentially expressed circRNAs were associated with 16 signaling pathways via Gene Ontology (GO) enrichment analysis. Moreover, the biological function and molecular mechanism of these exosomal circRNAs, which were confirmed using qRT-PCR, should be assessed in future studies.

Chen et al.[92] reported that tumor-released exosomal circular RNA PRMT5 is overexpressed in both urinary and serum exosomes derived from patients with urothelial carcinoma of the bladder (UCB). Additionally, a positive correlation was noted between circPRMT5 expression levels in serum or urinary exosomes and lymph node metastasis and advanced tumor progression, implying that circPRMT5 is a promising prognostic biomarker for UCB patients. Further *in vitro* analysis indicated that circPRMT5 accelerated the epithelial-mesenchymal transition (EMT) process in tumor cells and resulted in an aggressive phenotype via the circPRMT5/miR-30c/SNAIL1/E-cadherin pathway. In addition, circRASSF2 levels were increased in extracted serum exosomes derived from LSCC (laryngeal squamous cell carcinoma) patients.[93] Further analysis revealed that circRASSF2 expression levels exhibit a significant inverse relationship with miR-302b-3p in serum exosomes derived from LSCC patients. Unfortunately, the authors of these two articles did not calculate the sensitivity and specificity of specific circRNAs.

All of the data regarding the roles of exosomal lncRNAs are summarized in Table 1.

Other

In addition to the established role of exosomal circRNAs in cell-to-cell communication, unneeded cell circRNA clearance is another role of extracellular vesicles (including exosomes and microvesicles). Although considerable attention has focused on circRNA expression levels and biogenesis,[22,94] little is known about the metabolism of circRNA within normal and tumor cells. Since circRNAs lack 5′ and 3′ ends, they are resistant to major mRNA-degrading enzymes and more stable than linear RNAs, leading to circRNA accumulation.[95,96] It is currently unknown whether such accumulation is toxic. Lasda and Parker[97] reported one mechanism by which cells could deal with circRNAs. They found that cells could eliminate circRNAs into extracellular space via extracellular vesicles. Nevertheless, the conclusions are relatively new, and further studies are needed to verify the process of circRNA degradation in extracellular space.

Conclusions

Continuing advances in the RNA field indicate that circRNAs fulfill crucial functions in physiological and pathological processes.[98] As nanoscale biological vesicles, exosomes carry particular protein and RNA repertoires and act as novel methods of exchanging information between cells, thus contributing to cell-to-cell communication. In recent years, exosomal RNAs have been in the spotlight of exosomal research.[15,16] Thus, in this review, we highlighted the diverse mechanisms of circRNAs and reported the current status of knowledge regarding the functions of exosomal circRNAs in human cancer development and their clinical significance. Taking advantage of the stability and high specificity of exosomal circRNAs, these molecules might serve as promising cancer biomarkers with early detection and powerful prediction for patients to receive the most suitable therapy and might have potential for monitoring disease progression or recurrence. Moreover, to successfully develop progressive therapeutic methods for the treatment of cancer, in particular precision medicine, manipulating exosomal circRNAs should not be disregarded as an option because of the excellent biodistribution and biocompatibility of exosomes. Nonetheless, compared with exosomal lncRNA and miRNA, many gaps in our current understanding of the connection of circRNAs with exosome remain, for instance, the mechanism by which exosomal circRNAs travel in bodily fluids and the roles of exosomal circRNAs in lung cancer. Upon complete elucidation of exosomal circRNA functionality and molecular mechanisms relevant to human cancer, avenues of new insight will be opened, providing novel therapeutic approaches in malignant tumors.

AUTHOR CONTRIBUTIONS

X.S. and B.W. wrote the manuscript; X.F. retrieved literature; M.S. and Y.X. designed this manuscript; and K.L. revised the manuscript. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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