Chapter

# Clinical Application of Exosome Components

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## Abstract

Exosomes belong to a subpopulation of EVs that carry different functional molecular cargoes, including proteins, nucleic acids, metabolites, and lipids. Notably, evidence has demonstrated that exosomes participate in bidirectional cell–cell communication and act as critical molecular vehicles in regulating numerous physiological and pathological processes. Since the specific contents within exosomes carry the information from their cells of origin, this property permits exosomes to act as valuable biomarkers. This chapter summarizes the potential use of exosome components in diagnosing, prognosis, or monitoring and treating multiple cancers and other non-neoplastic diseases. We also discuss the deficiency of basic applications, including the limitations of research methods and different research institutions and the differences generated by specimen sources. Thus, a better understanding of the problem of exosome detection may pave the way to promising exosome-based clinical applications.

**Keywords:** exosomes, cancer, dieases, diagnosis, prognosis, therapy

## 1. Introduction

Exosomes are a subpopulation of EVs secreted by the endocytosis process, with a diameter ranging from 30 to 150 nm [1]. Exosomes are naturally secreted by all kinds of cells and carry diverse functional molecular cargoes, including proteins, lipids, nucleic acids, enzymes, and metabolites to promote intercellular communication. As one of the types of cargo of exosomes, nucleic acids include DNA, messenger, and noncoding RNA. Among all the molecular cargoes, proteins, and nucleic acids are the most abundant contents in exosomes [2]. They have biological functions and are selectively packaged into exosomes. As exosomes are naturally secreted by all kinds of cells and are commonly detected in bodily fluids, including blood, urine, saliva, and cerebrospinal fluid [3], the application potential of exosomes in clinical tumor diagnosis and therapy is promising. In this chapter, we will discuss the bioactive exosomal contents, focusing on proteins, noncoding RNAs, and DNA to better clarify their roles in disease development and the potential application of exosome cargoes (**Figure 1**).

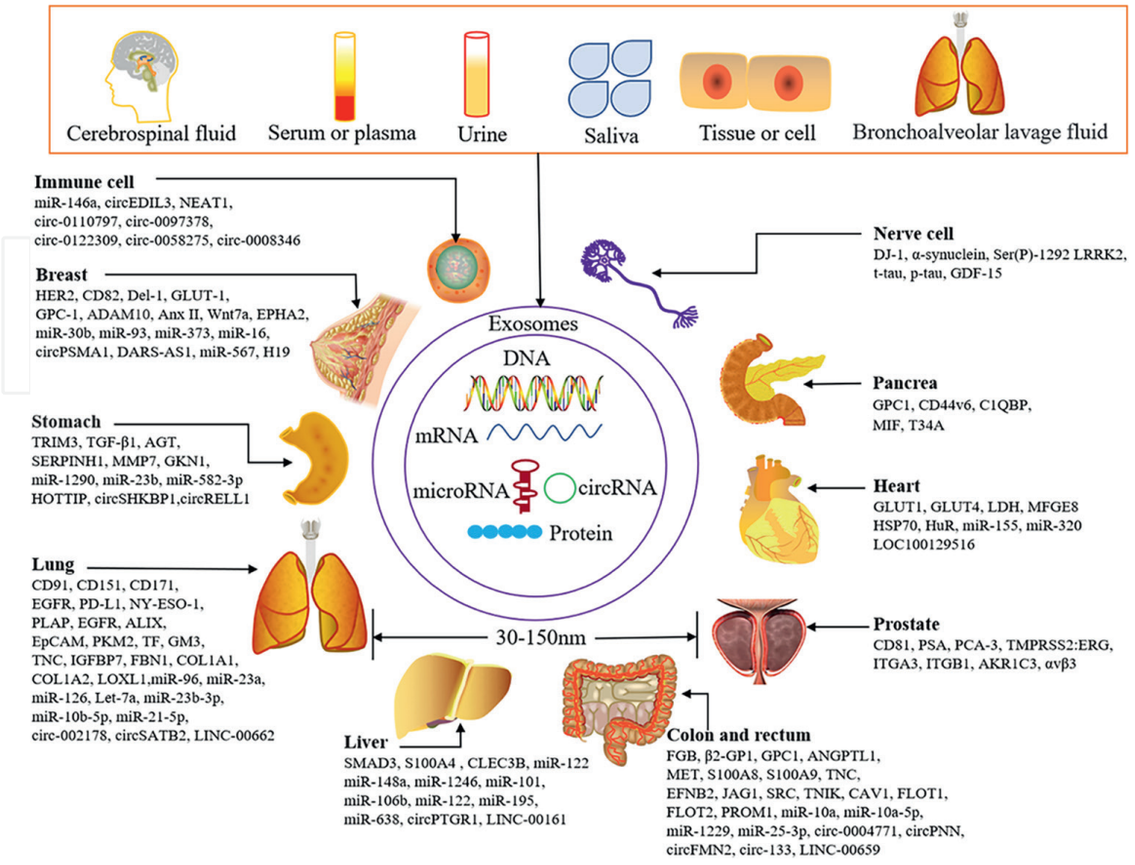


**Figure 1.**
*Application of exosome components in diseases.*

## 2. Exosome protein

With the recent development of isolation methods of exosomes and the applications of protein spectrum, the roles of exosome proteins in the diagnosis, prognosis, and treatment of diseases have been demonstrated in many medical fields, especially for cancer [4]. Exosomal proteins include (1) Membrane transport and fusion-related proteins, such as annexin (Anx II), Rab-GTPase, and heat shock proteins (HSPs), including Hsp60, Hsp70, and Hsp90; (2) Four-transmembrane cross-linked proteins, namely CD9, CD63, CD81, CD82, CD106, Tspan8, intercellular adhesion molecule-1 (ICAM-1); (3) Multi-vesicular bodies (MVBs)-related proteins, for instance, ALIX and TSG101; (4) Other proteins, like integrins, actin, and myosin [5].

### 2.1 Application of exosomal proteins in tumors: Diagnosis, prognosis, and treatment

#### *2.1.1 Lung cancer*

Numerous studies have focused on the clinical application of protein components in circulating exosomes from lung cancer patients. Yet, blood derivatives are the biofluids of choice for metabolomic clinical studies due to their low invasiveness and wealth of biological information [6]. Of note, some exosomal surface proteins, like CD91, CD151, and CD171, have been investigated to be used as biomarkers for early diagnosis of lung cancer [7, 8]. Of these, exosomal CD91 showed a high sensitivity

for diagnosing stage I and II lung adenocarcinoma (LUAD) patients [7]. Notably, exosomal CD151 and CD171 have been demonstrated to be upregulated in LUAD and can distinguish the subgroups of lung cancer [8]. Besides that, as a diagnostic biomarker, exosomal epidermal growth factor receptor (EGFR) and programmed death-ligand 1 (PD-L1) are considered to be the compact surface plasmon resonance (SPR) biosensor in lung cancer diagnosis [9]. Moreover, plasma exosomal proteins have also been reported to be served as prognostic and therapeutic biomarkers. As such, New York esophageal squamous cell carcinoma-1 (NY-ESO-1), placental alkaline phosphatase (PLAP), EGFR, ALIX (ALG-2-interacting protein X), and epithelial cell adhesion molecule (EpCAM) in circulating exosomes have been detected by extracellular vesicle array and correlated with overall survival (OS) of non-small cell lung cancer (NSCLC), which are recognized to be potential prognostic biomarkers for NSCLC [10]. Wang et al. also revealed that hypoxia-induced exosomes delivering pyruvate kinase M2 (PKM2) transmit cisplatin resistance to sensitive NSCLC cells. Additionally, Wang et al. found that exosomal PKM2 might be a potential biomarker and therapeutic target for cisplatin resistance in NSCLC [11]. Therefore, these exosomal proteins for lung cancer diagnosis, prognosis, and treatment must be further validated in larger cohorts.

### *2.1.2 Gastric cancer*

Gastric cancer (GC) is one of the most malignant cancers worldwide [12]. Importantly, exosomal proteins are specific diagnostic, prognostic, and therapeutic biomarkers for GC [13]. Various methods, such as liquid chromatography–tandem mass spectrometry (LC–MS/MS), have detected the proteomic profile of exosomes from the serum of GC patients. For example, a study reported that tripartite motif-containing protein 3 (TRIM3) protein in GC patients' serum exosomes was lower than in healthy donors using LC–MS/MS [14]. The higher exosomal transforming growth factor beta 1 (TGF-β1) expression of GC has been analyzed to be associated with tumor-node-metastasis (TNM) stage and lupus nephritis (LN) metastasis by enzyme-linked immunosorbent assay (ELISA) [15]. In addition, the high expression of exosomal TGF-β1 correlated with forkhead box protein $3^+$ ($FOXP3^+$) Treg cells in draining LNs, and the high percentage of $FOXP3^+$ Treg cells correlated with tumor size, Bormann type, tumor depth, and lymph node metastasis [15]. Furthermore, exosomal angiotensinogen (AGT), serpin family H member 1 (SERPINH1), and matrix metallopeptidase 7 (MMP7) have been demonstrated to perform well in predicting OS and be non-invasive prognostic biomarkers of GC [16]. Gastrokine 1 (GKN1) has also been identified to be secreted from HFE-145 gastric epithelial cells and can reduce tumor growth and tumor volume, which could be served as a therapeutic target for GC [17]. Likewise, these effective therapeutic proteins can be encapsulated into the exosomes and might prevent GC progression [18]. Thus, these exosomal proteins contribute to the development of GC.

### *2.1.3 Breast cancer*

Exosomal proteins play an essential role in diagnosis evaluation and prognosis prediction of breast cancer (BC) [19]. Notably, it has been demonstrated that the exosomal human epidermal growth factor receptor 2 (HER2) was significantly increased in BC patients compared with healthy donors [20]. In addition, exosomal

CD82 was significantly decreased in BC tissues compared with healthy donors and benign breast disease tissues [21]. Likewise, exosomes in preoperative plasma contained a higher level of developmental endothelial locus-1 (Del-1) than the post-operative plasma, and the high Del-1 level in postoperation was associated with early relapse [22]. Another report identified 1107 exosomal proteins between metastatic BC cell lines MDA-MB-231 and non-cancerous epithelial breast cell lines MCF-10A [23]. Moreso, 87 proteins were associated with BC, and 16 were correlated to BC metastasis [23]. Among them, exosomal glucose transporter 1 (GLUT-1), glypican 1 (GPC-1), and a disintegrin and metalloproteinase 10 (ADAM10) may be served as BC potential biomarkers [23]. Exosomes carrying proteins, including Anx II, Wnt7a, and ephrin type-A receptor 2 (EPHA2) could stimulate BC's invasive, angiogenesis, and metastasis abilities [24–26]. Exosomal Anx II has stimulated angiogenesis and BC metastasis [24]. Further, exosomal Wnt7a has been found to promote lung metastasis of BC [25]. Exosomal EPHA2 has also been found to activate the AMP-activated protein kinase (AMPK) signal pathway via the Ephrin A1-EPHA2 forward signal that promoted the angiogenesis and metastasis of BC [26]. Therefore, these exosome proteins may serve as potential BC therapeutic targets.

*2.1.4 Colorectal cancer*

Due to carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and CA24-2 having poor specificity for the diagnosis of colorectal cancer (CRC) [27], novel biomarkers need to be explored to diagnose CRC development. In recent years, exosomal proteins have been explored for their potential clinical values to serve as tissue and/or liquid biopsy biomarkers to diagnose early CRC. For example, Sun et al. extracted the exosomes in tissues and plasma. They validated that the fibrinogen beta chain (FGB) levels and beta-2-glycoprotein1 (β2-GP1) levels of exosomes in CRC tissue were significantly higher than those in paracancerous tissues [28]. Notably, the areas under the receiver operating characteristic (ROC) curve of plasma exosomal FGB and β2-GP1 as CRC biomarkers were 0.871 and 0.834, respectively, higher than those of CEA (0.723) and CA19-9 (0.614) [28]. Another oncogenic biomarker, exosomal GPC1 protein, was increased in tumor tissue and plasma of CRC patients [29]. However, GPC1 was also highly expressed in other tumor exosomes, which posed a challenge to the specificity of GPC1 in diagnosing CRC. In addition, some exosomal proteins were also proved to be carcinostatic biomarkers. For example, Jiang et al. verified that the level of exosomal angiopoietin-like protein 1 (ANGPTL1) was significantly decreased in CRC tissues compared with paired normal tissues and inhibited CRC metastasis to the liver [30]. Likewise, an exosome protein profile result demonstrated that the adherence-related proteins were enriched in the primary CRC cell (SW480) exosomes [31]. The metastatic factors (MET, S100 calcium-binding protein A8 (S100A8), S100A9, Tenascin-C(TNC)), signal transduction molecules (Ephrin B2 (EFNB2), Jagged1 (JAG1), SRC, Traf2- and Nck-interacting kinase (TNIK)), and lipid raft and lipid raft-associated components (Caveolin-1 (CAV1), Flotillin-1 (FLOT1), Flotillin-2 (FLOT2), Prominin 1(PROM1)) were enriched in the metastatic CRC cell (SW620) exosomes, which were associated with tumor progression and poor prognosis [31]. Interestingly, most exosomal proteins correlate with epithelial mesenchymal transition (EMT), migration, invasion, and angiogenesis. Therefore, exosomal proteins may be biomarkers for predicting CRC metastasis.

### *2.1.5 Pancreatic cancer*

Pancreatic cancer (PC) is one of the most lethal malignant neoplasms worldwide [32]. Existing tumor markers, such as CA19–9, cannot reasonably predict the occurrence and progression of PC, while exosomal proteins may play decisive roles in the occurrence and development of PC [33]. Excitingly, exosomal GPC1 has also been found in PC. Notably, the area under the ROC curve of GPC1 circulating exosomes (from the serum of pancreatic ductal adenocarcinoma (PDAC), benign pancreatic disease patients, and healthy donors) was 1.0, and the sensitivity and specificity of exosomal GPC1 were 100% [33]. Moreover, the level of circulating GPC1 exosomes was associated with tumor burden and the OS of PC patients [33]. Besides that, Xie et al. also observed that the high expression of exosomal CD44v6 (CD44 variant isoform 6) and C1QBP (complement C1q binding protein) was associated with a poor prognosis and a higher risk of postoperative liver metastasis of PDAC [34]. Costa-Silva et al. also found that exosomal migration inhibitory factor (MIF) was highly expressed in PDAC patients, which promoted liver pre-metastatic niche formation and metastasis [35]. The expression of exosomal MIF in PDAC liver metastasis was significantly higher than those without liver metastasis [35]. Furthermore, exosome survivin-T34A (T34A) enhances the sensitivity of gemcitabine to PC cells [36]. Importantly, these biomarkers illustrate their potential value in predicting the occurrence and development of PC.

### *2.1.6 Liver cancer*

Hepatocellular carcinoma (HCC) is one of the most common forms of cancer [37]. Although serum α-fetoprotein (AFP) has been widely applied as a biomarker for diagnosis and dynamic monitoring of HCC, it is not elevated in each HCC patient [38]. As a result, exosomal proteins have been searched for diagnosis, prognosis, and treatment of HCC. Fu et al. reported that high exosomal small mother against decapentaplegic family member 3 (SMAD3) protein level was positively related to tumor size and TNM stage and correlated negatively with disease-free survival (DFS) [39]. Sun et al. also found that HCC patients with high plasma exosomal S100A4 had a poor prognosis, which promoted HCC cell metastasis by activating the signal transducer and activator of transcription 3 (STAT3)/OPN signal pathway [40]. Moreso, downregulated C-Type Lectin Domain Family 3 Member B (CLEC3B) in HCC-derived exosomes promoted migration, invasion, and EMT of HCC cells via AMPK and vascular endothelial growth factor (VEGF) signals. Furthermore, the downregulation of CLEC3B in exosomes suppressed VEGF secretion in both HCC cells and endothelial cells (ECs), eventually inhibiting angiogenesis [41]. Therefore, these studies suggest that exosomal proteins play different roles in HCC development.

### *2.1.7 Prostate cancer*

Prostate cancer (PCa) is a life-threatening disease among men worldwide [42]. Notably, prostate-specific antigen (PSA) has often been used as a diagnostic biomarker for PCa [43]. However, due to PSA's lack of sensitivity and specificity, new biomarkers are urgently needed to assist in diagnosing, prognosis, and treating PCa [43]. A study reported that plasmatic exosomes expressing CD81 and PSA reached 100% specificity and sensitivity in distinguishing PCa patients from healthy donors [44]. Except for

the blood exosomes, urine exosomal prostate cancer antigen 3 (PCA3) and transmembrane protease serine 2:ERG (TMPRSS2:ERG) derived from PCa patients can also be used as non-invasive diagnostic biomarkers and monitor cancer patients' PCa status [45]. Another study reported that the urine exosomes integrin alpha-3 (ITGA3) and integrins beta-1 (ITGB1) in metastatic PCa patients were higher than PCa and benign prostatic hyperplasia (BPH) [46]. Plasma exosomal aldo-keto reductase family 1 member C3 (AKR1C3) have also been demonstrated to be associated with the OS of PCa patient, which is recognized to be a potential prognostic biomarker for PCa [47]. Furthermore, Krishn et al. demonstrated that αvβ3 integrin was transferred to β3-negative recipient cells by exosomes derived from PCa patient plasma and can be identified as a therapeutic target for PCa [48].

## 2.2 Application of exosomal proteins in other diseases: Diagnosis, prognosis, and treatment

### *2.2.1 Cardiovascular disease*

In recent years, many studies have paid more attention to the roles of exosomal proteins in cardiovascular diseases (CVD). Notably, exosomal proteins from different cell origination play critical roles in cardiac cell development [49]. For instance, GLUT1, GLUT4, and lactate dehydrogenase (LDH) functioned in ECs for glucose transport and metabolism in cardiac cell-derived exosomes [50]. Proteins carried by exosomes using *in vitro* cultures of neonatal cardiac fibroblasts under normoxic conditions are known to be associated with the extracellular matrix, cytoskeleton, mitochondrial, and nucleotide-binding [51]. Exosomal milk fat globule epidermal growth factor VIII (MFGE8) could activate phagocytic signaling and efficiently clear dead cells, promoting cardiac recovery after injury [52]. Additionally, *ex vivo*, *in vivo*, and *in vitro* studies using settings of ischemia-reperfusion found that exosomal HSP70 transmitted cardioprotective signals to the heart by activating the toll-like receptor 4 (TLR4) downstream signal pathway [53]. Exosomal-associated human antigen R (HuR) has also been recently reported to increase inflammatory and profibrogenic responses *in vitro* and *in vivo* using diabetic heart models [54]. Thus, exosomal HuR might be a therapeutic target to alleviate cardiac inflammation and fibrosis in diabetes [54]. Hence, exosomal proteins can affect CVD by regulating metabolism, macrophage engulfment, and inflammatory and profibrogenic responses.

### *2.2.2 Respiratory system disease*

Exosomes released from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection induced tissue factor (TF) expression that may drive thrombosis [55]. The EVs TF activity was related to disease severity and mortality and could be a prognostic biomarker and therapeutic for coronavirus disease 2019 (COVID-19) [55]. Monosialodihexosyl ganglioside (GM3)-enriched exosomes may contribute to the pathological processes related to COVID-19 and provide the most significant repository on the plasma lipidome and metabolome distinct to COVID-19 [56]. Moreso, *in vitro* studies have demonstrated that EV proteins, such as TNC, insulin-like-growth-factor-binding protein 7 (IGFBP7), fibrillin-1 (FBN1), alpha-2 collagen chain (I) (COL1A2), alpha-1 collagen chain (I) (COL1A1), and lysyl oxidase homolog 1 (LOXL1), secreted by fibroblasts might contribute to idiopathic pulmonary fibrosis (IPF) [57]. Furthermore, exosomes also transferred proteins to distant recipient cells

and were expected to be a new drug delivery system and a novel therapeutic target [58].

*2.2.3 Nervous system diseases*

Parkinson's disease (PD) and Alzheimer's (AD) are classic chronic neurodegenerative diseases [59]. Previous studies have reported few specific blood biomarkers in diagnosing PD and AD [60]. This is, to some extent, likely attributed to the lack of biomarker specificity [60]. For example, exosomal DJ-1 and α-synuclein in plasma failed to distinguish between PD patients and healthy donors [60]. However, the two proteins in plasma neural-derived exosomes could distinguish PD patients and healthy donors [60]. Besides the circulating specimen, the urine exosomal Ser(P)-1292 LRRK2 (leucine-rich repeat kinase 2) was considered a biomarker associated with PD progression [61]. In addition, the t-tau and p-tau levels derived from neuron exosomes of mild-AD groups were significantly higher than age-matched controls and mild cognitive impairment groups [62]. Likewise, exosomal growth differentiation factor-15 (GDF-15) derived from bone mesenchymal stem cells (MSCs) was confirmed to alleviate SH-SY5Y cell damage of AD by activating AKT/GSK-3β/β-catenin pathway [63]. Thus, exosomal proteins from different specimen sources may be used as potential biomarkers for diagnosis and prognosis of nervous system diseases.

## 3. Exosome RNA

Exosomal RNAs include miRNAs, circRNAs, lncRNAs, and mRNAs [64]. Additionally, evidence has established that exosomes play a significant role in tumorigenesis and tumor progression by transferring miRNAs, circRNAs, and LncRNAs [1]. These exosomal RNAs are biomarkers and therapeutic targets for human diseases, particularly malignant tumors.

### 3.1 Application of exosomal RNAs in tumors: diagnosis, prognosis, and treatment

*3.1.1 Lung cancer*

It has been reported that free RNA molecules secreted by tumor cells will degenerate in the bloodstream [65]. The exceptions are cell-free microRNAs that can be detected in cancer patients' blood plasma or serum [65]. Relevant RNA molecular information, such as exosomes, may also be obtained in EVs [66]. The existing literature also shows that exosomal RNAs play critical roles in different stages of the development cascade of cancer. For instance, serum exosomal miR-96 and miR-23a were upregulated in lung cancer and could be used as a biomarker for diagnosing lung cancer [67, 68]. Besides circulating exosomal miRNAs, exosomes released from bronchoalveolar lavage fluid could also serve as biomarkers for early lung cancer diagnosis. For instance, exosomal miR-126 and Let-7a from bronchoalveolar lavage fluid were significantly higher in LUAD patients than in healthy donors [69]. Exosomal miRNAs could be used as a prognostic biomarker for NSCLC development, such as miR-23b-3p, miR-10b-5p, and miR-21-5p were found to be independently associated with poor OS of NSCLC [70]. Moreso, exosomal circ-002178 was enriched in plasma exosomes from LUAD patients and could be delivered into $CD8^+$ T cells to induce PD1 expression [71]. Exosomes from NSCLC patient serum were enriched with circSATB2,

which has high sensitivity and specificity for clinical detection and is related to lung cancer metastasis [72]. Furthermore, Lv et al. verified that exosomal long intergenic non-coding 00662 (LINC-00662) promoted proliferation, invasion, and migration of NSCLC by the miR-320d/E2F1 axis, indicating that LINC-00662 may be a potential therapeutic target for lung cancer [73].

*3.1.2 Gastric cancer*

As discussed earlier, several circulating RNAs are excellent as potential diagnostic markers of GC. Circulating exosomal miR-1290 has been reported to be upregulated in various malignant cancers, including GC [74], LUAD [75], epithelial ovarian cancer [76], and PCa [77]. Kumata et al. observed that the miR-23b in exosomes from the plasma of GC patients was significantly lower than that of the healthy donors [78]. Many studies have also confirmed that exosomal circRNAs act as molecular sponges of miRNAs to regulate the proliferation, invasion, metastasis, and angiogenesis of GC cells [79–81]. Moreso, the circSHKBP1, elevated in GC tissues and serum, promoted GC progression by sponging miR-582-3p to increase HuR expression and suppress HSP90 degradation [79]. In contrast, exosomal circRELL1 has been reported to inhibit the progression of GC via the circRELL1/miR-637/EPHB3 axis [80]. Furthermore, exosomal lncRNA HOTTIP was also found to be a GC diagnostic biomarker associated with poor OS of GC patients [82]. In summary, these exosomal RNAs may serve as potential biomarkers for GC.

*3.1.3 Liver cancer*

Much evidence has demonstrated that exosomal RNAs are involved in the growth, metastasis, and angiogenesis of HCC cells and could be used as diagnostic and prognostic biomarkers and therapeutic tools in HCC [83, 84]. Notably, exosomal miRNAs have been studied as the potential diagnostic biomarker for HCC. For example, serum exosomal miR-122, miR-148a, and miR-1246 were significantly higher in HCC patients than in liver cirrhosis and healthy donors [85]. Interestingly, exosomal miR-101, miR-106b, miR-122, and miR-195 were significantly lower in HCC serum than in chronic hepatitis B (CHB) [86]. A study also reported that high serum exosomal miR-638 was associated with HCC recurrence, suggesting the potential of exosomal miR-638 as a reliable biomarker for prognostic monitoring [87]. Another study reported that serum exosomal circPTGR1 was upregulated in HCC patients and associated with the TNM stage and OS. Moreover, exosomal circPTGR1 has promoted the proliferation, invasion, and migration of HCC via the miR-449a-MET axis [88]. Beyond miRNAs, some lncRNAs in exosomes may also be served as the promising diagnostic marker for HCC. Sun et al. indicated that the serum exosomal LINC-00161 was higher in HCC patients than in healthy donors, suggesting that LINC-00161 could be a potential diagnostic biomarker for HCC [89].

*3.1.4 Breast cancer*

Most BC patients are hormone-dependent [90]. Increasing evidence demonstrated that exosomes play an essential role in breast tumorigenesis and progression by transferring miRNAs and LncRNAs [1]. Triple-negative breast cancer (TNBC) refers to the expression of estrogen receptor (ER), progesterone receptor (PR), and HER2

being negative in BC tissue [91]. Notably, miR-30b was associated with recurrence, and miR-93 was abundant in ductal carcinoma in situ (DCIS) [92]. Circulating exosomal miR-373 has been enriched in TNBC, and serum exosomal miR-373 was higher in ER-negative and PR-negative patients than in patients with hormone-receptor-positive patients [93]. In contrast, exosomal miRNAs, such as miR-16, were particularly enriched in estrogen-positive BC patients [92]. These studies remind us that the expression level of miRNAs in exosomes may contribute to the luminal classification of BC. Beyond miRNAs, circPSMA1 in exosomes acted as a "miRNAs sponge" to absorb miR-637 [94]. It could transmit migration and proliferation capacity to recipient cells to promote TNBC cell proliferation, migration, and metastasis via the miR-637/Akt1/β-catenin (cyclin D1) axis [94]. The lncRNA DARS-AS1 delivered by exosomes has been found to effectively inhibit TNBC cell growth and liver metastasis [95]. Thus, different RNA types can play different roles in TNBC development. To date, drug resistance is a significant obstacle to BC treatment [96]. Many pieces of evidence have demonstrated that exosomes regulate drug resistance for BC by delivering RNA. For instance, Han et al. demonstrated that miR-567 delivered by exosomes increased the sensitivity of BC cells to trastuzumab [97]. Similarly, lncRNA H19 could be transferred via exosomes to sensitive cells, leading to doxorubicin resistance in BC [98]. Thus, these studies strongly suggest that exosomal RNAs are known to act as biomarkers for BC development and drug resistance.

### *3.1.5 Colorectal cancer*

Exosomes carry and deliver specific molecules and have been found to mediate crosstalk between primary cancer sites and metastatic cancer loci [31, 84]. Exosomal miR-10a derived from SW480 cells inhibits human lung fibroblast migration and inflammatory factors releases, transferring the metastasis suppression signal to primary CRC [99]. Further studies have established a pair of human liver fibroblast cell lines to confirm the regulation function of miR-10a-5p between primary CRC and metastatic liver loci [100]. As mentioned above, abnormal expression of exosomal RNAs in peripheral blood can also be considered emerging diagnostic, prognostic, and therapeutic biomarkers of CRC. For instance, exosomal miR-1229 is significantly upregulated in the serum exosomes from CRC patients and was associated with tumor size, TNM stage, lymphatic metastasis, and poor OS [101]. Zeng et al. also found that miR-25-3p could form a pre-metastatic niche via stimulating angiogenesis and vascular permeability in CRC [102]. Circulating exosomal circRNAs could also serve as strong diagnostic biomarkers for CRC. Serum exosomal circ-0004771, circPNN, and circFMN2 levels were significantly upregulated in CRC patients [103–105]. Exosomal circ-133 was also significantly upregulated in the plasma of CRC patients and associated with hypoxia and cell metastasis by miR-133a/GEF-H1/RhoA axis [106]. Moreover, the mechanisms for targeting dysfunctional exosomal LncRNAs are being developed to treat CRC. For example, exosomal LINC-00659 has been found to promote CRC cells proliferation, invasion, migration, and EMT by miR-342-3p/ANXA2 pathway, suggesting that LINC-00659 could work as a potential biomarker for selecting a suitable treatment strategy [107]. Essential to the development of improved therapeutic strategies is a mechanistic understanding of exosome-mediated cell communications. Furthermore, MSCs -derived exosomes have been used as carriers to deliver anticancer agents in CRC [108]. Notably, this therapeutic effect is based on direct cell–cell communications and indirect communications mediating by cell secretome [109].

### 3.2 Application of exosomal RNAs in other diseases: Diagnosis, prognosis, and treatment

#### *3.2.1 Cardiovascular disease*

As mentioned earlier, exosomal RNAs secreted from senescent cells are considered to be associated with CVD and vascular aging [110]. miR-155 contained in exosomes is transferred from smooth muscle cells to endothelial cells to induce endothelial injury and promote atherosclerosis [111]. In diabetic cardiomyopathy, miR-320 is enriched in diabetic patients and could inhibit Hsp20 in endothelial cells, exerting an anti-angiogenic function [112]. Additionally, exosomal LOC100129516 has been found to ultimately alleviate the progression of atherosclerosis and decrease the cholesterol level via the PPARγ/LXRα/ABCA1 pathway [113]. Therefore, these studies suggest that the pathologic role of exosomes involves RNA delivery and could contribute to developing diabetic cardiomyopathy via different cell signals.

#### *3.2.2 Autoimmune disease*

Autoimmune diseases may be related to genetic, environmental, hormonal, and immunological factors [114]. Mounting evidence indicates that exosomes play an important role in autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and ankylosing spondylitis (AS). For example, it was demonstrated that urinary exosome miR-146a level was significantly increased in SLE patients compared with healthy donors. Its expression level was closely related to renal injury index, like proteinuria, histological features, and lupus activity [115]. It was also verified that exosomal circEDIL3 suppresses inflammation-induced angiogenesis and ameliorates RA via the miR-485-3p/PIAS3/STAT3 pathway [116]. In addition, the expression of serum exosomal nuclear paraspeckle assembly transcript 1 (NEAT1) in RA patients was higher than that of healthy donors. Moreover, NEAT1 has promoted the progression of RA by downregulating miR-144-3p and upregulating Rho-associated protein kinase 2 (ROCK2), suggesting that NEAT1 may be a potential biomarker and therapeutic target for RA [117]. The levels of exosomal circRNAs, like circ-0110797, circ-0097378, circ-0122309, circ-0058275, and circ-0008346 in AS, were significantly down-regulated compared with healthy donors, providing more optional biomarkers for the early diagnosis of AS [118].

## 4. Exosome DNA

Circulating tumor DNA (ctDNA) fragments are released by tumor cells into the bloodstream. The information on genomic alterations identified in tumors, including point mutations, rearrangements, amplifications, and even gene copy variations, could be identified by analyzing ctDNA molecules [119]. Although cancer detection by monitoring ctDNA is an area of active investigation, identifying very low amounts of ctDNA in blood samples with variable amounts of free DNA (cfDNA) remains challenging. To date, the studies focused on exosome DNA (exoDNA) are fewer than those on exosome RNA and protein [120]. It is revealed that a variety of cancer-derived DNA markers in exosomes by high throughput genome and transcriptome comparative analyses, including copy number, point mutation, insertion, deletion, and gene fusion. ExoDNA has great potential for disease diagnosis, prognosis assessment, and treatment

monitoring. For instance, methylation tests of exoDNA and/or cfDNA derived from the gastric fluid can be used to diagnose GC. In fact, the difference between exoDNA and cfDNA is that the former derives from living cells, the latter from dead ones [121]. Urine from bladder cancer patients contained significant amounts of exoDNA compared with healthy donors [122]. Bernard et al. studied that the increased level of exoDNA was significantly associated with disease progression. Moreover, PC patients with metastases and detectable ctDNA at baseline status had poor progression-free survival (PFS) and OS compared with patients without detectable ctDNA [123]. Degli Esposti et al. also demonstrated that neuroblastoma patients with high tumor mutation load values in exoDNA had a worse outcome than those with lower values [124]. Importantly, the advantage of exoDNA is that they are stable enough to be analyzed retrospectively from frozen bio-banked samples [125]. Meanwhile, exoDNA widely exists in various body fluids and can be easily collected [126]. Therefore, exoDNA may be an ideal liquid biopsy method and a novel tumor marker. However, due to the uncertainty of technical methods and high cost, research on exoDNA is relatively limited.

## 5. Problems and prospects of exosomes

Exosomes can be isolated from multiple sources, including cell culture medium, body fluids, and tumor tissues [28, 127, 128]. Thus, the components of exosomes have potential applications in diagnosis and prognosis for cancer and other diseases. Nevertheless, the basic application of exosomes is at an early stage and restricts their clinical application. Furthermore, considering that the tissue collection method is a non-invasive procedure, patient compliance may also limit the clinical application of exosomes.

### 5.1 Various isolation methods of exosomes

Exosomes can be extracted by differential ultracentrifugation (UC), density gradient fractionation, polymeric precipitation, microfluidics techniques, and immunoaffinity isolation [129]. The major problem is the different methods will cause significant differences in the composition and content of exosomes. In addition, the low amounts of components in exosomes led to difficulties in quantification. To date, differential UC is the most commonly used isolation method to harvest highly purified exosomes from a cell culture medium [130]. Polymeric precipitation requires little hands-on time but produces the highest contamination [131]. Additionally, immunoaffinity isolation is based on the characteristic surface proteins on certain exosomes [132]. Antibodies conjugated with beads can select the desired exosomes (immuno-enrichment) or trap unwanted exosomes (immuno-depletion) [129]. This selection process makes it possible to clarify unique exosome populations while undoubtedly leading to lower yields [129]. Each method has advantages and limitations and varies in the quantification of exosome size [133]. As exosomes diagnostic and prognostic platforms become available, there will be requirements for clinical application and manufacturing standards development.

### 5.2 Differences in research institutions

A biomarker that can be used in clinical applications should meet the following premise: There should be no statistically significant differences between different

detection institutions, detection methods, and researchers. However, it is not the case in the current situation of studying exosomes. For example, a report indicated that surface protein CD47 in circulating exosomes was higher in healthy than BC patients [134]. However, exosomal CD47 in BC patients was reported to be significantly higher than those in healthy controls [19]. Thus, the standards of specimen source, collection, and process in different institutions should first be well established.

### 5.3 The differences generated by sources of specimen

Specimens for clinical application could be obtained from blood, tissues, and other biological fluids, like urine, hydrothorax, ascites, and cerebrospinal fluid [6, 28]. Among all these specimens, serum and plasma are the most suitable for reflecting healthy or diseased conditions, genetic variations, environmental factors, lifestyle, nutrition habits, and drugs [6]. They can also provide important information at a systemic level [6]. Nevertheless, the exosomal contents from plasma and serum simultaneously and place have been identified to differ in the stability and composition of metabolome and lipoproteome [6]. Thus, the criterion should be established for using different specimens, and collection tubes, even among the same blood matrix.

## 6. Conclusions

In summary, technological improvements and our understanding of exosomal proteins, nucleic acids, and their exosomal content profiles may provide diagnostic, prognostic, and therapeutic clues for diseases in the future.

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## Conflict of interest

The authors declare no conflict of interest.

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