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REVIEW

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# The role of exosomes in liquid biopsy for cancer diagnosis and prognosis prediction

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## Abstract

Liquid biopsy is a revolutionary strategy in cancer diagnosis and prognosis prediction, which is used to analyze cancer cells or cancer-derived products through biofluids such as blood, urine and so on. Exosomes play a crucial role in mediating cell communication. A growing number of studies have reported that exosomes are involved in tumorigenesis, tumor growth, metastasis and drug resistance by delivering cargos including nucleic acids and protein. Thus, exosomes, as a new type of liquid biopsy, have the potential to be diagnostic or prognostic biomarkers. Herein, we elaborate on the current methods and introduce novel techniques for exosome isolation and characterization. Moreover, we elucidate the advantages of exosomes compared to other biological components in liquid biopsy and summarize the different exosomal biomarkers in cancer diagnosis and prognosis prediction.

KEYWORDS

diagnosis, exosomes, liquid biopsy, prognosis

## 1 | INTRODUCTION

Up to now, the most common method for cancer diagnosis is based on tissue biopsy, which means the extraction of tumor tissues for further histological analysis.[1] However, this invasive method is time consuming and has the potential risk in some patients, making it unfit for monitoring tumor processes. Moreover, tissue biopsy will increase the potential of metastasis, and some tumors are not always accessible for a biopsy.[2] Liquid biopsy, as an emerging method for cancer diagnosis, has drawn considerable attention in recent years. Currently, the main types of biological components in liquid biopsy include circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), extracellular

**Abbreviations:** AML, acute myeloid leukemia; AR-V7, androgen receptor splice variant 7; AUC, area under the curve; BC, breast cancer; cfDNA, cell-free DNA; CIN, cervical intraepithelial neoplasia; CKAP4, cytoskeleton-associated protein 4; CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; DKK1, dickkopf1; ECL, electrogenerated chemiluminescence; EMT, epithelial-mesenchymal transition; EPI, ExoDx Prostate IntelliScore; EVs, extracellular vesicles; GC, gastric cancer; GPC-1, glypican-1; HCC, hepatocellular carcinoma; HSP, heat shock protein; Id-peptides, idiotype-binding peptides; Ig-BCR, immunoglobulin B-cell receptor; IS-NP, immunoaffinitive superparamagnetic nanoparticles; MM, multiple myeloma; MUC1, Mucin 1; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; PROX1, Prospero homeobox 1; PTENP1, PTEN pseudogene 1; qRT-PCR, quantitative reverse transcription-PCR; SEC, size-exclusion chromatography; SERS, surface-enhanced Raman scattering; SPR, surface plasmon resonance; TEPs, tumor-educated platelets; TIRF, total internal reflection fluorescence; tsRNAs, tRNA-derived small RNAs; TTF-1, transcription termination factor 1.

vesicles (EVs, including exosomes and ectosomes) and tumor-educated platelets (TEPs).[3] Compared to the conventional tissue biopsy, liquid biopsy is minimally invasive even noninvasive dependent on the sample origin and has the advantage of serial monitoring. Furthermore, it can reflect the comprehensive genome landscape, which is contributed by the tumor components from multiple sites.[4]

Exosomes are a subset of EVs with a diameter ranging from 40 nm to 160 nm. Exosomes are identified by their hallmarks, such as CD9, CD63, CD81, ALIX and heat shock protein 70 (HSP 70), which facilitate their capture and enrichment.[5] Through transferring specific cargos (nucleic acid or protein), exosomes can mediate cell communication under physiological and pathological conditions.[6] As increasingly exemplified in the research, exosomes play a crucial role in tumorigenesis, tumor growth, metastasis and drug resistance.[7] The cargos of tumor-derived exosomes are consistent with the genetic content of the parent tumor cells.[8] Thus, exosomes and their transferred cargos have been gradually regarded as novel biomarkers for cancer diagnosis and prognosis prediction. In addition, exosomes are stable in circulation and can protect their cargos from degradation.[9] Therefore, exosomes are excellent biological components in liquid biopsy. In this review, we elaborate on the techniques for exosomes isolation as well as characterization, elucidate the advantages of exosomes as a liquid biopsy and summarize the different biomarkers in exosomes in cancer diagnosis and prognosis prediction.

## 2 | METHODS FOR EXOSOMES ISOLATION AND CHARACTERIZATION

### 2.1 | Methods for exosomes isolation

Exosomes isolation needs to ensure the structural integrity and biological activity of exosomes to accurately infer their functions. The choice of isolation method has a profound influence on the identification of enriched pathways and gene sets. Therefore, choosing a

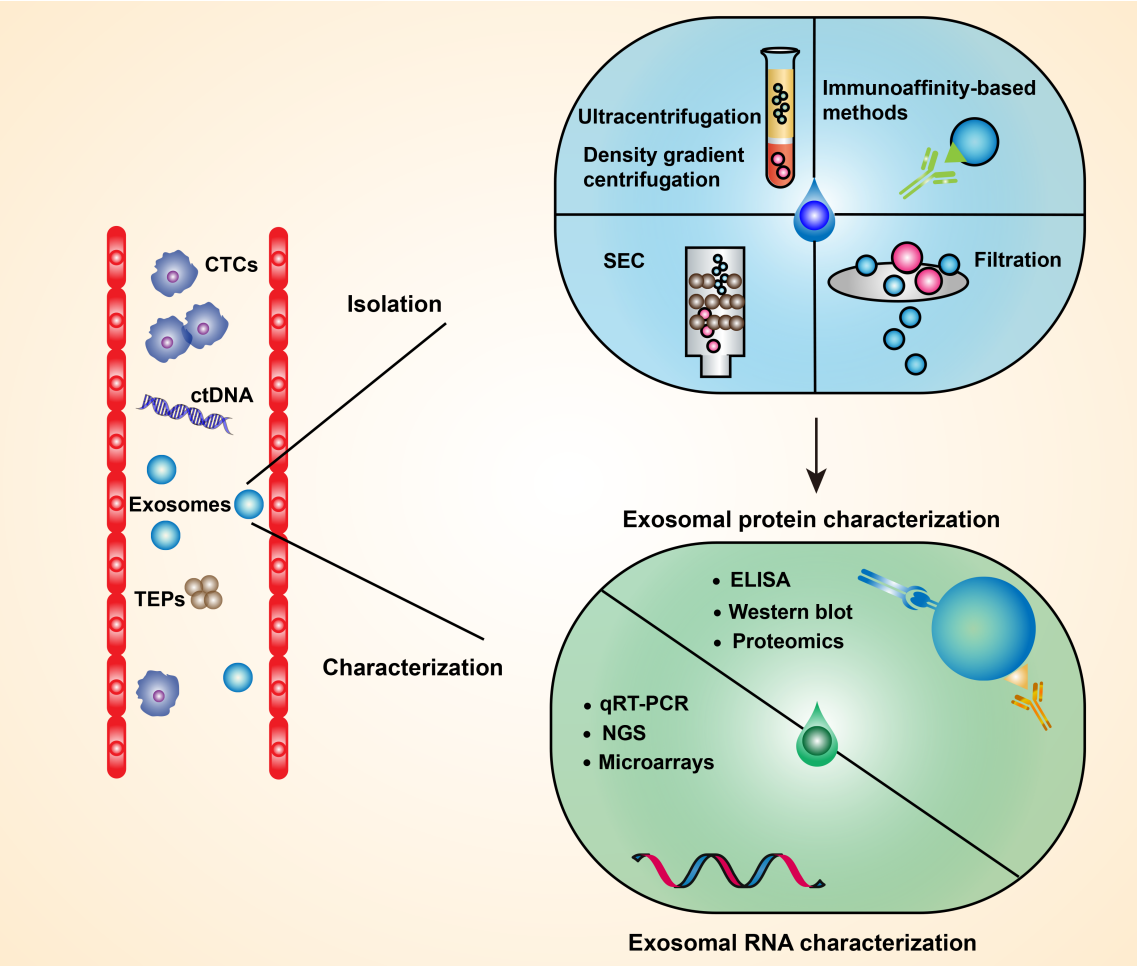


**FIGURE 1** The commonly used methods for exosomes isolation and characterization. Liquid biopsy mainly includes circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes and tumor-educated platelets (TEPs). Currently, the commonly used methods for exosomes isolation consist of ultracentrifugation, density gradient centrifugation, filtration, size-exclusion chromatography (SEC) and immunoaffinity-based methods like ELISA. As for exosomes characterization, qRT-PCR, next-generation sequencing (NGS) and microarrays are employed in exosomal RNA while ELISA, western blot and proteomics are applied to exosomal protein [Color figure can be viewed at wileyonlinelibrary.com]

proper isolation method is important to illustrate the specific functions of exosomes. Currently, the common methods of exosomes isolation are shown in Figure 1 and Table 1. Ultracentrifugation (UC) is regarded as the "gold-standard" technique at present, which separates and concentrates exosomes from other constituents based on the different densities.[10] UC can reduce protein contamination.[11] However, it is low throughput and may isolate other particles with similar size.[12] Using density gradient centrifugation can overcome the impurity of using the method of UC while the throughput is still low.[10] Filtration is another commonly used method for isolating exosomes, which is based on the membranes with specific pore sizes to exclude other particles.[13] Filtration has the advantages of simple steps, effective purification and time efficiency, while the disadvantages are about the extrusion effects and low yield.[13] Size-exclusion chromatography (SEC) separates specimen components according to the hydrodynamic volume. SEC is gentler than centrifugation, which may damage the membranes of exosomes.[14] Nonetheless, it is limited by the low resolution because of the presence of other contaminants like viral particles and lipoprotein particles.[15] Immunoaffinity-based isolation strategies use antibodies to target the specific surface antigens of exosomes, which can significantly increase the purity of exosomes and save the time of isolation.[16] Generally, antibodies are immobilized in the ELISA plates or magnetic beads. But it is costly and sometimes plagued by nonspecific binding of antibodies.[17] Multiple methods are often combined to increase the purity of exosomes. For instance, UC followed by SEC or density gradient step has been reported.[18] Moreover, ultrafiltration followed by liquid chromatography has been applied as well to obtain a higher yield.[19]

There are several newly developed techniques for isolating exosomes, in which microfluidics-based and nanotechnology approaches have the potential to overcome the limitation of traditional methods for isolating exosomes. Microfluidic methods can isolate exosomes from a small volume of biofluids sample in a more efficient, high-purity and high-yield manner.[20] Immunoaffinity-based capture is a common approach in the microfluidics-based technique. Microbeads that were conjugated with anti-CD63 antibody were designed as trapping arrays in a microfluidic hydrodynamic device, which could significantly reduce the interference of background noise to enhance the purity of exosomes.[21] Fang et al used the microfluidic chip in a similar principle to capture tumor-derived exosomes to assist the clinical diagnosis of breast cancer.[22] Nanotechnology is also gradually applied to isolate exosomes in recent years. Cai et al designed immunoaffinitive superparamagnetic nanoparticles (IS-NP) for capturing exosomes with high purity and efficiency. Notably, the particle-to-protein ratio of IS-NP, which is used to evaluate the purity of exosomes, is eight times that of conventional UC and nearly two times that of PEG-based precipitation and commercial kit.[23] Nanotechnology usually incorporates other methods like microfluidics to isolate exosomes. For example, Davies et al developed a pressure-driven microfluidic separation system combined with in situ nanoporous membranes. By changing the ratio of prepolymer solution to porogenic diluent, the nanopore size can vary to filter particles of specific size.[24]

Once exosomes are isolated, they need to be further quantified and analyzed. ELISA, fluorescent-activated cell sorting (FACS) and nanoparticle tracking analysis (NTA) are the commonly used methods for quantifying exosomes. ELISA can capture specific protein and produce a color change, which is associated with the concentration of the target protein. CD9, CD63 and CD81 are identified as the usually used exosome-specific markers for the exosome quantification in ELISA method.[25] These exosome-specific markers can be used in FACS to quantify and sort the exosomes, too. However, FACS requires a relatively complex setup and expensive equipment, which is not feasible for clinical application. Another limitation of FACS is a lack of consistent results due to the different optical and laser settings for sensing the exosomes.[20] NTA is another fluorescent-based method for quantifying and sorting exosomes. The principle is using a laser beam to track the exosomes by their movement. NTA can detect the smaller size of exosomes than FACS, but it cannot be applied to the clinical use due to the long analysis time.[26] Thus, there emerge several novel strategies for detecting and quantifying exosomes more economically and efficiently. For example, Lv et al coated the nanoellipsoids with anti-CD63 antibody as the substrate of the biosensors based on localized surface plasmon resonance. The concentration of exosomes can be determined according to the peak wavelength. Compared to ELISA, this kind of biosensor only requires a quarter of sample volumes but can halve the processing time. Moreover, it is of low cost, which makes its potential to be applied to clinical work.[27]

**TABLE 1** The advantages and disadvantages of common methods for exosome isolation

| Method | Principle | Advantages | Disadvantages | Ref. |
|---|---|---|---|---|
| UC | Separation via differential centrifugation | "Gold standard", low protein contamination | Low throughput, the potential damage of exosomes, contamination of similar particles | 10-12 |
| Density gradient centrifugation | Isolation by further density discrepancy based on UC | Increased purity compared to UC | Low throughput | 10 |
| Filtration | Using a specific pore size membrane to isolate exosomes | Simple steps, time efficient | Low yield, extrusion effects | 13 |
| SEC | Isolation by hydrodynamic volume | Relatively gentle | Low resolution | 14,15 |
| Immunoaffinity-based techniques | Using antibodies to capture exosomes | High purity, time-saving | Costly, nonspecific binding of antibodies | 16 |

Abbreviations: SEC, size-exclusion chromatography; UC, ultracentrifugation.

## 2.2 | Methods for characterization of RNA and protein in exosomes

### 2.2.1 | RNA

RNA is one of the most important molecules in exosomes research. In recent years, manifold exosomal ncRNAs, including miRNA, lncRNA and circRNA, have shown the potential to be specific biomarker candidates for cancer diagnosis and prognosis prediction.[28] Herein, we summarize some common methods for the characterization of RNA in exosomes.

Quantitative reverse transcription (qRT)-PCR is employed for quantifying the level of a particular sequence of DNA or RNA in exosomes.[29] Through the amplified fluorescent signal, qRT-PCR can have a low detection limit.[30] Nevertheless, this technique can only detect and quantify the specific and known sequences of RNA rather than the total amount of RNA.[31] Next-generation sequencing (NGS) is a powerful and advanced technique for comprehensively analyzing nucleic acids.[32] However, it is time consuming as well as costly and is susceptible to several factors, such as the preparation of libraries and bioinformatics pipelines.[33] Microarrays are based on the principle of hybridization of probes and complementary target gene sequences in the exosomes, which have been applied to the differential analysis of DNA/RNA samples.[34] Simultaneous analysis of thousands of RNAs is the major advantage of microarrays. The disadvantages include high cost and low specificity.[35] Therefore, a second verification step (eg, qRT-PCR) should be generally conducted to confirm the key RNAs.[31]

Current techniques, such as qRT-PCR analysis, are time consuming and laborious, which are relatively unsuitable for exosomal RNA detection for clinical diagnosis or prognosis prediction. Lee et al developed a novel exosomal miRNA detection method based on a nanosized fluorescent oligonucleotide probe. The high specificity and sensitivity of the probe had been verified via detecting exosomal miR-21 in breast cancer (BC) cells.[36] Besides, this method had been improved for simultaneously detecting and quantitatively analyzing exosomal RNA in a single reaction.[37] Notably, the US Food and Drug Administration (FDA) granted Breakthrough Device Designation to ExoDx Prostate IntelliScore (EPI) in 2019. EPI test utilizes urinary exosomal RNA expression levels of three genes (ERG, PCA3 and SPDEF) to predict the probability of having high-grade prostate cancer (≥grade group 2). EPI could avoid about 26% of unnecessary prostate biopsies by setting the cut-point at 15.6.[38] Recently, a biosensor-based method is developed for detecting and analyzing exosomal RNA. Aptamers are specific oligonucleotide molecules, which can be an effective and promising alternative to antibodies for targeted recognition. Based on the excellent specify and binding affinity, aptamers are gradually employed in biosensors, called aptasensors. Without RNA extraction, a thermophoretic sensor implemented with nanoflares was used for in situ detection of exosomal miRNAs. The nanoflares containing aptamers could be internalized by exosomes. The presence of target exosomal miRNA would induce the appearance of fluorescence. After localized laser heating, the fluorescence signal became amplified, leading to the high sensitivity of the thermophoretic sensor. The diagnosed accuracy was 85% of estrogen receptor-positive BC at an early stage by detecting miR-375.[39]

Currently, the majority of analytic methods provide the overall features and ignore the heterogeneities of exosomes. Thus, single-exosome analysis has emerged for providing additional information. A single-vesicle imaging assay based on a total internal reflection fluorescence (TIRF) was developed for the quantitative detection of exosomal miRNAs. Besides, the TIRF imaging assay could measure the precise stoichiometry of target exosomal miRNA in situ through delivering molecular beacon probes into exosomes. In terms of distinguishing cancer patients from healthy individuals by detecting exosomal miR-21, the TIRF imaging assay showed better performance than conventional real-time PCR assay.[40]

Since being discovered in 1974, surface-enhanced Raman scattering (SERS) has been applied to a variety of fields, including pharmaceutical assay and bioanalytical chemistry. Recently, it has been developed for detecting and analyzing exosomes. SERS has the advantages of narrow spectral bandwidth, high sensitivity and resistance to photobleaching.[41] It has shown the superb performance in distinguishing healthy individuals from patients with non-small cell lung cancer (NSCLC) and pancreatic ductal adenocarcinoma (PDAC) by the quantitative analyses of exosomal miR-21 and miR-10b, respectively.[42,43] Notably, it has the potential for point-of-care testing in clinical analysis with a low limit of detection.[44] However, the dependable quantification of miRNAs via SERS is obstructed by the lack of reproducible and uniform SERS substrates. Therefore, a uniform plasmonic head-flocked gold nanopillar substrate has been developed for enhancing the SERS signal and accurately discriminated BC subtypes according to expressions of different exosomal miRNAs.[45] Besides, inspired by the beehive, Dong et al designed a SERE structure of $TiO_2$ macroporous inverse opal coated by gold. SERS method based on this structure is economical and time saving and can also significantly amplify the SERS signal.[46] Generally, the majority of studies focus on the SERS substrate rather than the SERS probe that is more convenient. Thus, Zhang et al developed a kind of SERS probe via assembling gold nanoparticles in triangular pyramid DNA. Through binding recognition DNA to one corner of the triangular pyramid DNA, this SERS probe could successfully distinguish healthy people from BC patients. Importantly, by switching other recognition elements, the SERS probe can also be employed for detecting other biological samples including toxin and bacterial.[41] Intriguingly, the combination of SERS and deep learning model achieved the superb performance for detecting early-stage NSCLC with the area under the curve (AUC) of 0.912.[47]

### 2.2.2 | Protein

Protein is an essential constituent of exosome structure and is also an important class of molecules transported by exosomes. Thus, the analysis of exosomal protein is crucial to comprehend its role in biological functions and pathological processes.

ELISA and Western blot are traditional immunoaffinity-based techniques for the characterization of specific exosomal protein. Western blot can provide information on the abundance and size of

protein while it needs significant processing time. On the contrary, ELISA is a method of relative high throughput owing to the less processing time and the usage of 96-well plates.[16]

As for the emerging techniques, Zhang et al constructed 3D porous serpentine nanostructures to achieve superior sensitivity of microfluidic chip. Importantly, it can simultaneously detect eight markers on a single exosome sample by a switchable microfluidic design. Compared to ELISA, this novel technique has a lower limit of detection and significantly reduces the experimental time.[48] Single-exosome analysis can also be applied to exosomal protein profiling. Liu et al introduced a novel single-exosome analysis for the digital qualification of target exosomes using droplet microfluidics based on immunosorbent assay. An exosomal membrane protein, glypican-1 (GPC-1), was chosen for differentiating BC patients from healthy people and patients with benign breast disease by this means and consequently achieve unprecedented accuracy.[49]

Biosensors also begin to be employed for the detection and profiling of exosomal protein. Electrogenerated chemiluminescence (ECL) biosensor was developed for the detection of exosomes by ECL nanoprobe, which consisted of aptamer-modified $Ti_3C_2$ MXenes nanosheets. Notably, the detection limit was over 100 times lower than that of ELISA.[50] Huang et al developed an electrochemical aptasensor based on a G-quadruplex-linked Mucin 1 (MUC1) protein aptamer, which was specifically expressed within gastric cancer (GC) exosomes. When GC exosomes with MUC1 bound the aptamer, it would induce rolling circle amplification and produce electrochemical signals, which exhibited high sensitivity and selectivity coupled with a low detection limit simultaneously.[51] Intriguingly, Lyu et al developed a luminescent nanosensor for multiplex differentiation of cancer exosomes, which was composed of a quencher-tagged aptamer complexed with a near-infrared semiconducting polyelectrolyte. The presence of targeted exosomes would turn on the afterglow signal. Importantly, by changing the sequence of aptamer, this method could be easily designed to detect various exosomal proteins.[52] Wang et al, Fan et al and Thakur et al designed different structures of gold nanoparticle, respectively, to amplify the surface plasmon resonance (SPR) signal so as to detect exosomal protein more sensitively. Through switching the gold nanoparticle -inked aptamers or antibodies, SPR biosensors can be applied to specific exosomal protein detection from different cancers.[53-55]

## 3 | THE ADVANTAGES OF EXOSOMES AS A LIQUID BIOPSY IN CANCER DIAGNOSIS AND PROGNOSIS

Traditionally, tissue biopsy has been regarded as the gold standard for the diagnosis of many diseases, particularly cancer. Besides, tissue biopsies and image-based assessment guide clinical decisions to a great extent. However, with the era of precision medicine around the corner, the limitations of tissue biopsy have emerged as it just provides a single snapshot of a tumor tissue, which ignores the tumor heterogeneity.[56] The tissue biopsy cannot systemically and dynamically reflect the response to the treatment because it needs time to deliver critical information and is unfit for frequent repetition.[57] With the introduction of novel techniques, the performance of liquid biopsy has been improved recently. Liquid biopsy as a real-time, noninvasive and tumor-specific technique can reliably monitor the progress and relapse of cancer and respond to the therapeutic effect.[58] Importantly, liquid biopsy reflects the more comprehensive tumor genetic profile than tissue biopsy, which may facilitate clinical judgment and decision-making.[59] Nonetheless, the sensitivity and specificity of liquid biopsy remain to be elevated so as to be applied to clinical detection.

As an important biological component of liquid biopsy, exosomes have several advantages compared to TEPs, CTCs and ctDNA. The RNA content of TEPs can likely be affected by therapeutic factors, pathological conditions and activated immune system.[60] CTCs and ctDNA are unstable, fragile and have short half-lives, leading to the requirement of quick processing after sample collection.[9] In addition, CTCs are phenotypically heterogeneous, with markers varying during different stages, such as the epithelial-mesenchymal transition (EMT) process. Cell-free DNA (cfDNA) mainly originated from noncancerous cells, while ctDNA only accounts for a small fraction of cfDNA, making the detection even more difficult.[9] The ctDNA of EGFR mutation in NSCLC patients with intrathoracic metastasis was at a low level, while exosomal RNA of EGFR mutation showed superior sensitivity.[61] The mechanism is that RNA or protein cargo can be protected being degraded in circulation by the surrounded lipid bilayer membrane.[9] Furthermore, exosomes can reflect stromal cell response rather than being only limited to cancer cells.[62] The enrichment of exosomes is relatively less expensive and laborious. Exosomes contained short-length (≤200 bp) tumor-derived DNA or RNA is more detectable than long-length or full-length exosomal DNA or cfDNA, which highlights the high sensitivity of short-length exosomal DNA or RNA in liquid biopsy.[63] Moreover, exosomal protein has a higher sensitivity and specificity over secretory proteins.[64] A meta-analysis confirmed the superiority of liquid biopsy in diagnosing pancreatic cancer, among which exosomes exhibited the highest sensitivity and specificity.[65] In another meta-analysis, CTCs showed the best performance in colorectal cancer (CRC) diagnosis compared to cfDNA and exosomes. But the number of studies about CTCs (5) and exosomes (6) is significantly less than that of cfDNA (51), which might introduce bias and lead to inaccurate outcomes.[66] For getting more accurate predictive outcomes, Vafaei et al recommended the combination of CTCs and blood exosomes in CRC diagnosis and prognosis.[67]

## 4 | DIFFERENT BIOMARKERS IN EXOSOMES IN CANCER DIAGNOSIS AND PROGNOSIS

Exosomes have an impact on tumor initiation and progression as well as resistance to therapy via the transfer of their contents, which indicates that exosomes with the contents can be used as

**TABLE 2** Different biomarkers in exosomes in cancer diagnosis and prognosis prediction

| Exosomal biomarker | Cancer type | Biofluid | Indication | Clinical sample size | Ref. |
|---|---|---|---|---|---|
| TTF-1 and miR-21 | NSCLC | Serum | Diagnosis | NA | 60 |
| miR-222-3p | NSCLC | Serum | Prognosis | TP N = 50 | 62 |
| miR-193a-3p, miR-210-3p and miR-5100 | Lung cancer | Plasma | Diagnosis and prognosis | TP N = 41, HC N = 30 | 64 |
| circSATB2 | NSCLC | Serum | Diagnosis | TP N = 83, HC N = 95 | 65 |
| miR-375 | $ER^+$ BC | Serum | Diagnosis | TP N = 17, HC N = 12 | 35 |
| CPC1 | BC | Serum | Diagnosis | TP N = 12, BTP N = 5, HC N = 5 | 45 |
| miR-1246 | BC | Plasma | Diagnosis | TP N = 46, HC N = 28 | 66 |
| miR-21, miR-105 and miR-222 | BC | Serum | Diagnosis | TP N = 53, HC N = 8 | 67 |
| CD82 | BC | Serum and plasma | Diagnosis | TP N = 80, BTP N = 80, HC N = 80 | 68 |
| circSHKBP1 | GC | Serum | Diagnosis | TP N = 20, HC N = 20 | 70 |
| lncUEGC1 | Early-stage GC | Plasma | Diagnosis | TP N = 10, HC N = 5 | 71 |
| HOTTIP | GC | Serum | Diagnosis and prognosis | TP N = 126, HC N = 120 | 72 |
| miR-30d-5p, miR-181a-5p and miR-486-5p | Rectal cancer | Plasma | Diagnosis and prognosis | TP N = 24, HC N = 5 | 73 |
| tRNA-ValTAC-3, tRNAGlyTCC-5, tRNA-ValAAC-5 and tRNA-GluCTC-5 | HCC | Plasma | Diagnosis | NA | 74 |
| circUHRF1 | HCC | Plasma | Diagnosis | TP N = 240, HC N = 20 | 75 |
| miR-21 and miR-10b | Early-stage HCC | Serum | Prognosis | TP N = 124 | 76 |
| KRAS | Pancreatic cancer | Plasma | Diagnosis and prognosis | TP N = 127, HC N = 136 | 78 |
| CPC1 | Pancreatic cancer | Serum | Diagnosis and prognosis | TP N = 190, HC N = 100 | 79 |
| CKAP4 | Pancreatic cancer | Serum | Diagnosis | TP N = 47, HC N = 18 | 80 |
| miR-21 | Pancreatic cancer | Plasma | Prognosis | TP N = 5, TP with surgery N = 5, HC N = 5 | 39 |
| AR-V7 | Prostate cancer | Plasma | Prognosis | TP N = 36 | 82 |
| miR-196a-5p and miR-501-3p | Prostate cancer | Urine | Diagnosis | TP N = 48, HC N = 28 | 86 |
| PTENP1 | Bladder cancer | Plasma | Diagnosis | TP N = 50, HC N = 60 | 83 |
| lncLNMAT2 | Bladder cancer | Serum and urine | Diagnosis and prognosis | TP N = 206, HC N = 120 | 85 |
| SPRY4-IT1, MALAT1 and PCAT-1 | Bladder cancer | Urine | Diagnosis and prognosis | TP N = 184, HC N = 184 | 87 |
| E-cadherin | Ovarian cancer | Ascites | Diagnosis and prognosis | TP N = 35, HC N = 6 | 89 |
| miR-200b and miR-200c | Ovarian cancer | Serum | Diagnosis and prognosis | TP N = 163, BTP N = 20, HC N = 32 | 90 |
| let-7d-3p and miR-30d-5p | Cervical cancer | Plasma | Diagnosis | NA | 91 |
| Ig-BCR | MM | Serum | Diagnosis | NA | 92 |
| let-7b and miR-18a | MM | Serum | Prognosis | TP N = 156, HC N = 5 | 93 |
| PMSA3 and lncPMSA3-AS1 | MM | Serum | Prognosis | Bortezomib resistance N = 12, bortezomib sensitivity N = 45 | 94 |
| $BRAF^{V600E}$ | Melanoma | LD and plasma | Prognosis | LD N = 51, plasma N = 31. | 95 |
| PD-L1 | Melanoma | Plasma | Diagnosis and prognosis | TP N = 44, HC N = 11 | 96 |

Abbreviations: BTP, benign tumor patient; BC, breast cancer; ER, estrogen receptor; GC, gastric cancer; HC, healthy control; HCC, hepatocellular carcinoma; LD, lymphatic drainage; MM, multiple myeloma; NSCLC, non-small cell lung cancer; TP, tumor patient.

diagnostic and prognostic markers.[5] However, not only tumor cells release exosomal RNA to affect biological functions but also many normal cells will secrete the same exosomal RNA physiologically.[68] Thus, it is important to precisely capture and separate tumor-derived exosomes from exosomes derived from normal cells. Additionally, the roles of exosomes are probably dynamic and specific to cancer type, stage and genetics in cancer progression.[69] Herein, we summarize the different diagnostic or prognostic biomarkers in tumor-derived exosomes, which are as follows (Table 2).

### 4.1 | Lung cancer

Yang et al used the immuno-biochip to measure the expression of miR-21 and transcription termination factor 1 (TTF-1) mRNA in serum exosomes, which achieved absolute sensitivity and specificity in discriminating healthy people from whether early-stage or late-stage NSCLC patients.[68] The upregulation of exosomal miR-222-3p was clinically relevant to the poor prognosis in NSCLC patients due to promoting the metastasis and decreasing the sensitivity to gemcitabine.[70] Jin et al confirmed the diagnostic value of tumor-derived exosomal miRNA in NSCLC by NGS. MiR-30a-3p, miR-30e-3p, miR-181-5p and miR-361-5p were adenocarcinoma specific while miR-10b-5p, miR-15b-5p and miR-320b were squamous cell carcinoma specific. Moreover, the diagnostic accuracy had been verified, indicating these miRNAs would be promising biomarkers in early NSCLC detection.[71] In addition, miR-193a-3p, miR-210-3p and miR-5100 in exosomes derived from hypoxic bone marrow-derived mesenchymal stem cells were identified as novel biomarkers for lung cancer progression. Mechanically, these three exosomal microRNAs could induce the STAT3-mediated EMT. Combining a panel of three exosomal biomarkers will increase the performance of diagnosis compared to using any individual microRNA..[72] Exosomal circRNA is also associated with the status of lung cancer. For example, circRNA circSATB2 was reported to be highly expressed in serum exosomes from lung cancer patients and related to the metastasis status. The AUC value of the ROC curve is 0.660 and 0.797 in differentiating lung cancer patients from healthy volunteers and metastatic lung cancer patients from nonmetastatic ones, respectively.[73]

### 4.2 | Breast cancer

Zhai et al detected a breast cancer-specific marker, exosomal miR-1246, by a gold nanoflare probe functionalized with the nucleic acid. This simple and cost-effective liquid biopsy had 100% sensitivity and 92.9% specificity in differentiating 46 BC patients from 28 healthy individuals, which had the potential to be exploited as a clinically diagnostic assay.[74] Besides, exosomal miR-21, miR-105 and miR-222 can serve as complementary markers in diagnosing BC and predicting therapy response to neoadjuvant chemotherapy.[75] High expression of exosomal protein CD82 acted as a diagnostic biomarker in BC. Intriguingly, the expression of CD82 in BC tissues is significantly lower than that in noncancerous tissues, which is inversely correlated with exosomal CD82. The phenomenon indicated that CD82 expression was redistributed from tissues to the blood to facilitate tumor metastasis.[76] Protein phosphorylation is the most widespread and crucial mechanism in regulating molecules, which can provide clues regarding the status of a specific disease. Thus, Chen et al used phosphoproteins in exosomes and microvesicles to detect breast cancer. It was shown that the expression of 144 kinds of phosphoproteins in exosomes and microvesicles was higher in breast cancer patients compared to the control group. The result highlighted phosphoproteins-contained exosomes and microvesicles as candidate diagnostic markers in predicting early breast cancer.[77]

### 4.3 | Digestive tract cancer

In GC, circRNA circSHKBP1 was unregulated and could be effectively packed into exosomes, which was correlated with advanced TNM stage and poor prognosis. Importantly, the expression of circSHKBP1 was sharply reduced after gastrectomy. Mechanically, circSHKBP1 promoted GC progression by regulating the miR-582-3p/HUR/VEGF axis and repressing HSP90 degradation.[78] In early GC, exosomal lncRNA lncUEGC1 was found to be expressed at a significantly higher level than that in the control group. The AUC value was 0.8760 and 0.8406 in distinguishing early GC patients from the healthy group and premalignant chronic atrophic gastritis patients, respectively, which had the better performance of using CEA.[79] Zhao et al found that exosomal lncRNA HOTTIP had a higher diagnostic capability than CA72-4, CEA and CA 19-9 with the AUC of 0.827 vs 0.639, 0.653 and 0.685, respectively. In addition, univariate and multivariate COX analysis demonstrated that the overexpression of exosomal HOTTIP could be an independent prognostic factor in GC patients.[80] The hypoxic environment usually leads to the poor therapeutic outcome of locally advanced rectal cancer, in which hypoxia-related exosomes are responsible for the poor prognosis. It was found that exosomal miR-30d-5p, miR-181a-5p and miR-486-5p were all associated with the tumor progression like organ invasion and lymph node metastases.[81]

### 4.4 | Hepatocellular carcinoma

tRNA-derived small RNAs (tsRNAs) is a newly identified small noncoding RNA, which can be presented in exosomes. Four exosomal tsRNAs showed significantly high levels in liver cancer patients, including tRNA-GluCTC-5, tRNA-GlyTCC-5, tRNA-ValAAC-5 and tRNA-ValTAC-3. The result not only identified the differential expression of tsRNAs in liver cancer but also provided new insight into the diagnostic potential of exosomal tsRNAs.[82] Compared to healthy control, the level of plasma exosomal circUHRF1 was higher in hepatocellular carcinoma (HCC) patients. Importantly, the level was decreased after tumor resection and increased in patients with relapse. Mechanism exploration proved that exosomal circUHRF1 could decrease the proportion and inhibit the tumor infiltration of NK cells by sponging miR-449c-5p. Furthermore, circUHRF1 may induce resistance to anti-PD1 immunotherapy.[83] Interestingly, the acidic microenvironment is usually related to the poor prognosis of HCC patients. Mechanically, it induced the upregulation of exosomal miR-10b and miR-21 to promote HCC proliferation and metastasis. Moreover, serum exosomal miR-10b and miR-21 were independent prognostic factors for disease-free survival of HCC patients in the early stage.[84]

### 4.5 | Pancreatic cancer

Pancreatic cancer is mainly driven by KRAS mutations, which are present in 80% of preneoplastic pancreatic cysts.[85] Thus, KRAS mutations are a reliable biomarker for the early diagnosis of pancreatic

cancer. Notably, KRAS mutation DNA-contained exosomes showed a superior positive rate than mutant KRAS cfDNA in detecting localized, locally advanced and metastatic PDAC patients, respectively.[86] Moreover, exosomal KRAS mutant DNA can also serve as a prognosis-related biomarker. In a prospective cohort study of pancreatic cancer patients, exosomes with KRAS mutant allele fraction ≥5% were an independent negative predictor of progression-free survival (PFS) and overall survival (OS).[85] GPC1 was found to be enriched in exosomes, which were derived from breast cancer, colorectal cancer, especially pancreatic cancer. $GPC1^+$ exosomes could carry specific KRAS mutations. Moreover, $GPC1^+$ exosomes could also serve as a better prognostic marker compared to CA19-9 and circulating GPC1.[87] Proteomic analysis of the exosome surface markers revealed that CLDN4, EPCAM, CD151, LGALS3BP, HIST2H2BE and HIST2H2BF were PDAC-specific exosome markers. Intriguingly, the positive rate of KRAS mutation in exosomes was increased from 44.1% to 73.0% after using the selected markers to capture exosomes.[86] In addition to KRAS mutations, Kimura et al identified a new dickkopf1 (DKK1) receptor, cytoskeleton-associated protein 4 (CKAP4) in exosomes as a candidate for PDAC diagnosis, which had a high expression in PDAC patient's serum.[88]

### 4.6 | Urological cancer

High expression of exosomal miR-375 or miR-1290 could predict the shorter OS of castration-resistant prostate cancer. Notably, incorporating miR-375 and miR-1290 into putative clinical prognostic factor-based models using androgen-deprivation therapy failure time and PSA level could significantly improve predictive performance with an AUC increase from 0.66 to 0.73.[89] Besides, the upregulation of plasma-derived exosomal RNA of androgen receptor splice variant 7 (AR-V7) was found to predict the poor response to hormonal therapy in prostate cancer patients with metastasis, making it a potential prognosis-relevant biomarker.[90] In bladder cancer, PTEN pseudogene 1 (PTENP1) was reported to be significantly reduced in both tissues and plasma exosomes, which could increase the expression of PTEN to repress cancer progression via serving as a ceRNA of miR-17. These results indicated exosomal PTENP1 would be a promising biomarker for the clinical detection and prognosis evaluation of bladder cancer.[91] VEGF-C was proved to play an important role in lymph node metastasis in bladder cancer. Nonetheless, there is approximately 20% of metastatic bladder cancer exhibiting low VEGF-C expression, which indicates a VEGF-C-independent mechanism exists.[92] Chen et al found an exosomal lncRNA LNMAT2 promoting lymphangiogenesis and lymphatic metastasis of bladder cancer by upregulating prospero homeobox 1 (PROX1). Notably, exosomal LNMAT2 had the AUC value of 0.769 and 0.881 in diagnosing bladder cancer and lymph node metastatic bladder cancer, respectively. Besides, the higher expression of exosomal LNMAT2 was associated with shorter OS and disease-free survival.[93] In urological cancer, urinary exosomes may be effective biomarkers for diagnosis and prognosis prediction. For example, Rodríguez et al reported that miR-196a-5p and miR-501-3p in urinary exosomes would be promising biomarkers in diagnosing prostate cancer.[94] Furthermore, for the diagnosis of bladder cancer, an approach based on detecting a panel consisting of three urinary exosome-derived lncRNAs (SPRY4-IT1, MALAT1 and PCAT-1) was conducted. The performance of the panel was significantly better than that of urine cytology with the AUC value of 0.813 vs 0.619. Moreover, exosomal PCAT-1 in urine could be regarded as an independent prognostic factor for evaluating the relapse-free survival of non-muscle-invasive bladder cancer.[95]

### 4.7 | Gynecological cancer

Yokoi et al improved a novel diagnostic model by using eight circulating serum miRNAs (miR-26a-5p, miR-130b-3p, miR-142-3p, miR-200a-3p, miR-328-3p, miR-374a-5p, miR-766-3p and let-7d-5p), which were primarily packed into exosomes. The model could successfully differentiate ovarian cancer patients from healthy individuals and early-stage ovarian cancer from patients with benign tumors with the AUC of 0.97 and 0.91, respectively.[96] Exosomal soluble E-cadherin was found to induce angiogenesis by activating the β-catenin and NF-κB signaling. in vivo and clinical data showed the relevance between high expression of exosomal soluble E-cadherin and peritoneal dissemination as well as the formation of malignant ascites in ovarian cancer. These results indicated exosomal soluble E-cadherin would be a promising marker for diagnosis and prognosis.[97] In addition, exosomal miR-200b and miR-200c were related to worse OS in ovarian cancer, which was significantly related to the levels of CA-125.[98] The most effective measure to screen cervical cancer is detecting and treating cervical intraepithelial neoplasia (CIN) early. Zheng et al used exosomal let-7d-3p and miR-30d-5p to distinguish CIN II + patients (including CIN II) from CIN I- patients (including CIN I and healthy controls). The AUC value was 0.828, which is higher than that based on the cytological test (0.766).[99]

### 4.8 | Hematological malignancies

Multiple myeloma (MM)-derived exosomes usually express the immunoglobulin B-cell receptor (Ig-BCR), which are relevant to tumor progression. Ig-BCR-expressed exosomes can be targeted by idiotype-binding peptides (Id-peptides). Therefore, Iaccino et al developed a diagnostic approach based on Id-peptides to detect MM-derived exosomes, which could predict clinical disease progression.[100] Moreover, circulating exosomal let-7b and miR-18a were identified to predict the OS and PFS in patients with newly diagnosed MM.[101] High expression of exosomal PMSA3 or lncPMSA3-AS1 in MM patients' serum was also correlated with the decreased PFS and OS. Mechanically, exosomal PMSA3 could reduce the sensitivity of proteasome inhibitors, and lncPMSA3-AS1 could enhance the stability of PMSA3 to further promote the drug resistance. Therefore, exosomal PMSA3 or lncPMSA3-AS1 could not only be prognostic predictors for drug response but also act as therapeutic targets for the

patients with proteasome inhibitors resistance.[102] In acute myeloid leukemia (AML), Lin et al identified that the upregulation of plasma exosome-derived miR-532 was related to the favorable OS, which played a role in reducing the major energy substrate for the growth of AML cells.[103] In contrast, as an independent prognostic predictor, a high level of exosomal miR-125b indicated higher risks of relapse and overall death of AML patients.[104] Yeh et al conducted miRNA profiling on plasma-derived exosomes from 15 healthy volunteers and 69 chronic lymphocytic leukemia (CLL) patients and identified a distinct miRNA signature, in which miR-150 and miR-155 were upregulated while miR-223 was downregulated.[105] However, the potential diagnostic and prognostic value of these exosomal miRNAs in CLL should be further verified.

### 4.9 | Melanoma

BRAF belongs to the RAF family of serine/threonine protein kinases, whose mutation accounts for 40% to 60% of cutaneous melanomas. $BRAF^{V600E}$ mutation could be detected in the lymphatic drainage-derived exosomes, which had a better performance in predicting the relapse risk of melanoma patients than that from plasma exosomes.[106] In addition, the expression of exosomal PD-L1 was positively related to the overall tumor burden, which indicated a poor prognosis.[107] Compared to total PD-L1, microvesicle PD-L1 and EV-excluded PD-L1, exosomal PD-L1 showed the best performance in not only distinguishing melanoma patients from healthy donors but also differentiating pembrolizumab responders from nonresponders. These results suggested exosomal PD-L1 may be as a negative predictor for anti-PD-L1 treatment in melanoma.[107]

## 5 | CONCLUSION AND PERSPECTIVE

Although tissue biopsy remains the gold standard for cancer diagnosis, its limitation has been gradually revealed in the era of precision cancer therapy. The difficulty we confront is dictating a therapeutic course of action according to only a single biopsy under the tumoral heterogeneity. In contrast, liquid biopsy as an emerging method can provide a comprehensive and dynamic genome landscape, which reflects the information from multiple tumor sites. Thereinto, exosomes show the superiority of high sensitivity, specificity and stability compared to other biological components of liquid biopsy like CTCs and ctDNA. For the past few years, a growing number of studies report that exosomal nucleic acid and protein play a pivotal role in tumorigenesis and tumor progression, which indicate that they can serve as a diagnostic or prognostic biomarker.[6] Nonetheless, the studies concerning exosomal lipids and metabolites as diagnostic or prognostic markers are insufficient. Though metabolomic or lipidomic profiling of exosomes in some cancer types including prostate cancer and pancreatic cancer has been conducted,[108-110] the performance of identified exosomal metabolites or lipids in clinical diagnosis and prognosis prediction remains to be further evaluated in a larger sample size meanwhile their roles in tumorigenesis and tumor progression should be explored.

Despite numerous advantages, the application of exosomes as cancer biomarkers is also faced with challenges. To begin with, the current techniques for isolating and enriching exosomes are low throughput and purity. There also exist discrepancies concerning the exosome isolation methods. Thus, the current priority in this field is to optimize the process of exosomes isolation and enrichment, develop more efficient characterization techniques and determine a standard exosome-based technique eventually. Besides, whether the relatively low concentration of exosomes in biofluids is sufficient to detect minute alterations remains unknown, which is often missed in clinical detection. Evaluation of global alterations like chromosomal instability may contribute to overcoming this problem.[111] Furthermore, the exhibition of the superiority of exosomes as the liquid biopsy is just based on the small cohorts of patients and lacking in a clear clinical benefit.[112] Therefore, it is urgent to identify reliable exosomal biomarkers for early-stage cancer diagnosis and prognosis prediction in large-scale samples, which can be adapted to clinical application. Additionally, we should verify the unique diagnostic and prognostic values of biomarkers within exosomes in cancer with a specific status.

With the gradual understanding of the nature of exosomes, the number of researches about their diagnostic and therapeutic application gets growing, and the corresponding techniques are also improving. Generally, the development of techniques is accompanied by novel statistical tools, which can utilize high-dimensional machine learning approaches to analyze the massive data and provide sound and timely decisions. Therefore, the future of exosomes as a liquid biopsy will be involved in multiple disciplines, such as molecular biology, machine learning and statistics.[113] Besides, novel techniques are transferring the traditional two-step process of isolation and characterization to an integrated one-step procedure, which is adapt to point-of-care testing of exosomes in the clinic. Critically, only when the clinical validity is demonstrated, can the exosomes reach the full potential in cancer diagnosis and prognosis prediction. Regardless, the perspective of exploiting exosomes for screening and prediction purposes is fascinating and probable to attract prominent interest in the future.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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