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REVIEW

What can urinary exosomes tell us?

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Abstract

Exosomes are involved in a wide variety of biochemical processes in human body homeostasis. Exosomes also provide important information regarding communications among several organ systems. Additionally, they can serve as molecular vehicles to deliver drugs. Therefore, exosomes have received wide attention in current biomedical research for unraveling pathogenic mechanisms of diseases, searching for novel biomarkers, and discovering new drugs. This paper reviews and discusses the significance of urinary exosomes for a better understanding of human disease pathophysiology and their potential use as therapeutic targets. Isolation methods of exosomes and the latest technological advances are also discussed. Furthermore, novel urinary exosomal biomarkers are highlighted with special emphasis on their clinical applicability (particularly sensitivity, specificity, reliability, and other aspects). Finally, future trends for this field are analyzed and our perspectives are provided.

Keywords Exosomes · Drug targets · Biomarker · Urine

Introduction

All human cells can release exosomes, which are derived from plasma membrane–multivesicular body fusions. Exosomes are found in body fluids and tissues and are involved in metabolic processes, cell waste disposal, protein and nucleic acid exchange, coagulation, and immune function [1–4]. Exosomes secreted by cells are granular substances with a diameter of 50–150 nm. Their surface contains lipids and proteins derived from cell membranes, while their inside contains intracellular substances, such as nucleic acids (e.g., microRNA, messenger RNA, DNA) and proteins. Exosomes are considered to be a type of extracellular vesicle. Extracellular vesicles have microvesicles and apoptotic bodies in addition to exosomes, which have different production mechanisms and sizes.

Exosomes are generated from multivesicular bodies (or endosomes), which are endosomal organelles taken up by cells through endocytosis (a mechanism by which cells take up extracellular substances), followed by fusion with the cellular plasma membrane and secretion into the extracellular space. It is believed that the surface of exosomes contains cell membrane components and that the inside contains intracellular substances, all of which reflect the characteristics of the original cells that secreted the exosomes [1–4]. Exosomes secreted by cells exist not only in the extracellular space but also in body fluids (e.g., blood, spinal fluid, urine) in which they circulate throughout the body.

Exosome research is an emerging field that explores the role of exosome DNA, RNA, and proteins in cellular pathways and the communication and/or signaling events between human body systems. Information on exosome DNA, RNA, and proteins may yield insights into underlying disease states [5–10].

Several proteins are commonly found in exosomes. Membrane proteins such as tetraspanins (e.g., CD9, CD63, CD81), integrins, major histocompatibility complex (MHC) molecules located at the surface, and proteins related to multispore formation (Tsg101, Alix) often occur in exosomes

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[11]. Heat shock proteins (HSPs) are another protein family that is frequently observed in exosomes. Attempts are being made to collect exosomes using proteins on the surface, such as CD9.

An important function of exosomes is their ability to transmit information between cells. It has been reported that secreted cell nucleic acids (microRNA, messenger RNA) are transmitted to recipient cells via exosomes and that they are functional in the recipient cells [12]. Secreted exosomes act on receptors on the surface of the recipient cells to cause signal transduction, and so the contents of exosomes previously taken up are thought to have a functional impact in the recipient cell.

Furthermore, it was verified that exosomes profile is altered in several disease situations. Recently, an interesting report demonstrated that exosomes released from highly malignant cancer cells can reprogram other cells in the neighborhood making them equally malignant [13]. In relation to "cancer," exosomes they are involved in such processes as cancer cell survival, malignant transformation, and metastasis and thus function to favor cancer cells. Exosomes secreted by cancer cells modulate the extracellular matrix increasing the survival of tumor cells, namely by suppressing the action of the immune system and acting as proangiogenic [14]. Therefore, exosome research is crucial for understanding metastasis in different cancer cells [15].

Metastasis suggests that cancer spreads from where it originated to a certain area of the body. It is also known as Metastasis, advanced, or stage 4 cancer. Large cancer even when not spread to other areas of the body may also be called advanced cancer. When cancerous cells break free from the main tumor entering the blood circulation and lymphatic system, metastasis occurs. Fluids are transported across the body and as they settle and expand in distant areas of the body, the cancer cells will migrate far from the initial tumor and develop new tumors. Cancer cells coming from the main tumor may result in metastases as well (for example in abdomen cavity cancer in one organ can transmit this way to the adjacent organ—liver, lungs, etc.) [16].

In addition, a relationship between exosomes and diseases other than cancer, such as neurodegenerative diseases (e.g., Alzheimer's disease), has been reported [17].

As already mentioned, given that exosomes reflect the characteristics of the cells that secrete them, they can be particularly useful for diagnostic purposes. Its detection and characterization in body fluids as diagnostic markers is particularly promising [5–10].

Exosomes have therapeutic potential owing to their roles in the pathogenic mechanisms of diseases. The biogenesis pathways of exosomes provide clues for selectively blocking these pathways and for inhibiting the production, release, and uptake of exosomes to control disease progression [18]. On the other hand, exosomes may be utilized as transport agents or carriers to deliver nucleic acids and/or drugs instead of the traditional polymer-based nanocarriers, which suffer from limitations such as cytotoxicity, multidrug resistance of cancer cells [19], and unintended preferential drug accumulation in the spleen and the liver [20]. Exosomes carry significant advantages in terms of lower toxicity, reduced drug resistance, and the capability to deliver drugs to the brain across the blood–brain barrier (BBB) [21, 22].

Significance of urinary exosomes

Urinary exosomes in disease research

Researchers are more and more interested in urinary exosomes and their relationship with kidney physiology and diseases. Exosomes have the ability to transport their cargo between kidney cells and change the function of the proteome and recipient cells. They can represent the intercellular signaling mechanism along the nephron. In animal and human biomarker discovery studies, this proteome changes to reflect the underlying pathophysiology of certain kidney diseases. However, there are still major challenges, especially the optimization of current methods. Using urinary exosomes as diagnostic biomarkers is a non-invasive alternative to tissue biopsy [23]. Urinary exosomes, which reflect the average changes in many organ systems, is a particularly interesting because of its availability, ease of sample collection, and non-invasive nature of the collection procedure. The non-invasive nature when combined with the diagnostic and prognostic sensitivity of exosomes, offers a cost-effective opportunity for discovering disease mechanisms and therapeutic targets.

Non-invasive biomarkers have several advantages over invasive ones as they have a greater probability of being adopted by clinicians and patients. Urinary markers are cost-effective and easily distributable and, hence, accessible for clinical use. They provide potential evidence-based targets in the early diagnosis of at-risk groups and those who have clinically significant symptoms [24]. The availability of urine proteins and peptides for laboratory exploration of proximal, distant, and systemic diseases [25] are some other important aspects as well [26].

There is increasing evidence that exosomes play a role in cardiovascular and renal physiology. Mineralocorticoid hypertension can benefit from the discovery of effective biomarkers. Exosomes mainly transport RNA and proteins, which may reflect biological events in the kidney. The information transmitted by exosomes may help diagnose different subtypes of arterial hypertension, and allow more appropriate treatments and improve the patient's quality of life. Further research is needed to determine the potential benefits of exosomes in hypertension.

Urinary exosomes can also directly reflect the pathogenic events of the kidney and of other urinary system structures [27, 28]. Therefore, research on urinary exosomes has gained wide attention over the past decade. Compared with conventional urinary and circulating biomarkers, exosomes carry and are rich in specific biomarker molecules, especially receptors, proteins, genetic material (such as DNA, mRNA, and miRNA), and lipids. Urine and blood circulation are much more abundant. Therefore, exosome markers provide advantages for the discovery of biomarkers in specific diseases, which involve abnormalities of such molecules carried by exosomes. Because these biomarker molecules are transported inside the exosome cargo, they are more stable in biological fluids than other free-flowing molecules. Moreover, being an essential source of non-invasive biomarkers, exosomes also have therapeutic potential [29–31].

Urinary exosomes have been utilized in the discovery of biomarkers of genitourinary and renal origin. Urinary exosome proteins have been widely explored for diseases of the urinary tract, acute kidney injury, chronic kidney disease, diabetic nephropathy (DN), renal cell carcinoma (RCC), prostate cancer, and bladder cancer [32]. Moreover, urinary exosomal RNAs have significant diagnostic capabilities in many kidney diseases, especially renal fibrosis [33]. A critical analysis of these insights is presented in the sections below.

Proximity disease research with urinary exosomes

Urinary exosomes play significant roles in the pathogenesis of diseases and serve as potential pharmaceutical targets. This section discusses key aspects of exosome-mediated biochemical cascades in the development of proximity diseases.

Exosomes have the ability to cross the blood–brain barrier. The liquid biopsy required to analyze biomarkers in blood or urine is minimally invasive. Exosomes can be used as a delivery system for disease biomarkers and therapy [34]. Cancer has always been the subject of exosome research. Exosomes, their content and surface proteins can allow early detection of cancer, which can improve prognosis and survival. According to the research of Chen et al. it is not necessary to enrich the protein to become a useful marker [35]. Compared with healthy controls, the number of HSP90, VTN. and MAPK1 in colorectal cancer patients decreased. It has been suggested that the presence of CD24, EDIL3 and fibronectin in circulating exosomes is a case of early breast cancer [36].

Urinary exosomes have a significant role in the early detection of proximity diseases of renal origin. For instance, exosomal miRNAs (miR-21, miR-29c, and miR-150) serve as potential biomarkers for predicting disease progression in lupus nephritis (LN) [37]. Urine-derived exosomes have also been shown to indicate several associations in the pathogenic mechanisms of genitourinary diseases. A study by Zaporozhchenko et al. (2018) indicated that urinary microvesicles and proteins in prostatic cancer patients participate in signaling associated with disease development [38].

Few studies have evaluated the role of urine exosomes in monitoring the effects of drug treatment. In patients with predominantly hypertension, the decrease in blood pressure induced by hydrochlorothiazide is related to the content of Na-Cl cotransporter in urine exosomes. Further research is needed to study its effect. Urinary exosomes are used as a monitoring tool for drug therapy [39, 40].

Urinary exosomes can be used as drug delivery system as well. Compared with other nanoparticle-based drug delivery systems (such as liposomes and polymer nanoparticles), exosomes have important advantages. Different cell sources of exosomes have been studied because the parental cells have been shown to affect their biological activity and subsequent therapeutic effects [40]. Zhuang et al. reported that exosomes effectively transport curcumin to the brain to treat diseases related to neuro-inflammation without side effects [41]. Endogenous loading technology uses biological cell devices to classify and package molecules into exosomes during biogenesis [42].

Although most researchers realize that the role of urinary exosomes is multifaceted, with their ability to contribute to diagnostic, prognostic, and therapeutic insights into disease pathogenesis, there is less evidence available on the validity of in vitro findings. In the future, more rigorous scientific studies are required to explore the heterogeneity in exosomes and to discover their roles in the mechanisms underlying the development of renal diseases [43].

Methods for the isolation of exosomes

Exosomes are secreted by cells into the extracellular space after the cell membrane fuses with multivesicular bodies (MVBs). They belong to the class of extracellular vesicles (EVs), measuring 30–140 nm. However, they are found in the extracellular space mixed with ectosomes (measuring 30–100 nm) and apoptotic bodies (measuring 50–500 nm). EVs differ in terms of biogenesis, and therefore, their functions and cargo (proteomic and genetic material) are different. This makes the content of exosomes specific to the cell from which they originated, and exosomes enable signal transmission among cells with or without direct contact [2, 21, 44–46]. Biological fluids contain chylomicrons, lipoproteins, and microvesicles that have similar size ranges to those of exosomes [47]. Commonly used methods for exosomal isolation include conventional techniques (ultracentrifugation, ultrafiltration, size-exclusion chromatography, polymer-based precipitation, and immunoaffinity)

and recently developed microfluidics-based methods [21] (see also Table 1).

Conventional methods purify exosomes based on their density, function, or size. Density-based isolation is typically performed by ultracentrifugation, which takes advantage of density differences between the medium and its constituent bioparticles or among bioparticles. On the other hand, ultrafiltration and size-exclusion chromatography take advantage of size differences among bioparticles to isolate exosomes. Polymer precipitation and immunoaffinity are based primarily on the chemical and surface properties of exosomes [21]. Immunologically based separation exploits exosomal function by using surface proteins that can interact with antibodies. In the precipitation method, volume-excluding polymers separate the molecular constituents of biological fluids. Although effective, conventional methods have some limitations in terms of unsatisfactory separation, low efficiency, low recovery yield, and the absence of high-resolution visualization techniques [21].

Microfluidics is a novel isolation method that is considerably superior to conventional approaches by its higher sensitivity, enhanced convenience, higher speed, and lower sample requirement. It utilizes micron-sized channels to process microliter to picoliter volumes of fluids. Microfluidic platforms can isolate extremely pure exosomes with a high level of sensitivity at a low cost. They also require less time and use a modest quantity of reagents [21]. Based on the basic principles of microfluidics, many related methods have been developed to purify exosomes in recent years. A combination of centrifugal nanoparticle extraction and microfluidics can be used to generate the Coriolis force and centrifugal effect, hydrodynamic drag, and buoyancy in microchannels. The other option is to apply acoustics for isolation using either surface acoustic wave (SAW) or bulk acoustic wave (BAW) technology. However, making precise alignments involves a long fabrication process. Filtration combined with microfluidics is characterized by the use of nanoporous membranes, nanoarrays, and nanofibers for particle separation. Alternatively, inertial lift may be utilized to move particles laterally within microchannels by taking advantage of the differences in velocity and flow rate between the particles and the fluid. Viscoelastic microfluidics is a method that exerts elastic lift forces through a viscoelastic medium to separate biofluid particles [21].

After isolation, the characterization of exosomes in a heterogeneous isolate can be accomplished by imaging using electron microscopy (based on exosomal morphology) or fluorescence microscopy (by labeling the exosomal markers); nanoparticle tracking analysis (NTA) that determines particle size; and/or molecular profiling using genomics (RT-qPCR), proteomics (2DGE, LC–MS/MS), and lipidomics (MS, GC–MS) techniques [21].

Cheng et al. (2019) discussed rinsing separation as a potential exosomal isolation method and compared it with existing methods, demonstrating marked advantages over formerly used techniques [48]. They indicated that rinsing separation has superior performance in the context of protein analysis as the process they executed achieved lower contamination from non-exosome proteins. It also achieved higher efficiency than ultracentrifugation in terms of costs and time. Western blot analysis revealed that certain types of exosomal markers (CD63, TSG101, and CD9) were more enriched when rinsing separation was applied. Rinsing helps exosomes form independent units and also preserves cell morphology and tissue structure through the 2.5% glutaraldehyde pre-fixation. This was a clear advantage over ultracentrifugation as it eliminated the additional centrifugation steps that lead to exosomal damage and loss. However, researchers have recommended more in-depth studies to evaluate its suitability in various cell types and efficacy using various parameters [49].

To address the problem of tracing the exact origin of exosomes for biomarker screening, researchers have suggested a modified version of ultracentrifugation to isolate exosomes based on their densities. Exosomes were isolated from urinary samples and verified by Western blotting and transmission electron microscopy (TEM), capillary zone electrophoresis (CZE), and two-dimensional electrophoresis (2DE). With these techniques, several types of differences among exosomes in terms of their morphological characteristics, retention times, proteomic profiles, particle weights, and electrification properties could be demonstrated [50].

Last, nanotechnology is a recent technique for the analysis of exosomes and has high specificity and sensitivity. It is relatively faster, requires a smaller sample amount, and costs. The electric field-induced release and measurement (EFIRM) technique is also worth being mentioned in this context, as it is capable of detecting minute quantities of RNA and ctDNA [49].

Ultracentrifugation has been the gold standard for exosome isolation from urine samples. It is accepted as the primary method of exosome isolation. Differential ultracentrifugation is the most widely employed process even though it has some drawbacks, including high processing time, poor yield due to low exosome integrity, and chances of contaminant proteins. However, the recent developments in ultracentrifugation strategies offer an efficient and reproducible approach to separate exosomes for different starting materials [51]. Depending on the source of the biological samples, each protocol needs to be optimized to achieve a high yield of exosomes with minimal impurities. Downstream analysis of exosomes typically involves some combination of size characterization, surface marker and protein analysis, and characterization of nucleic acid content. Transmission electron microscopy (TEM), scanning electron

Table 1 Brief comparison of the most commonly used methods for urinary exosome isolation

	Method	Advantages		Disadvantages	
		Proteomic analysis	Transcriptomic analysis	Proteomic analysis	Transcriptomic analysis
1	UltraCentrifugation	Commonly used approach; provides a good and relatively pure yield	Usually no interference or contamination observed in RNA profiling after isolation procedures	Time consuming and costly; High content of Total soluble proteins from renal dysfunction patients can interfere with exosomal protein biomarker isolation; may require extra steps such as addition of reducing agents, SEC, sucrose gradient and DNAase treatment	Higher time consumption and expensive
2	Ultracentrifugation + Sucrose cushion	Can be used to remove interference from non exosomal proteins in the case of renal dysfunction patients	Particularly good yields for miRNA analysis	Time consuming, costly; Due to the density specific nature of the method, it may be disadvantageous for exosomes obtained from different pathways with unknown densities, as they would be hard to characterize	Time consuming and expensive
3	Ultrafiltration	Good total protein yield; faster and simpler procedure	Yield comparable to Ultracentrifugation	Urinary exosomes with Aquaporins and TSG101 require extra steps for isolation; Higher total protein content but very low exosomal protein purity; may not suitable for renal dysfunction patient samples	Comparably lower RNA purity
4	Polymer based Precipitation (Standard ExoQuick™)	Faster, simpler, relatively inexpensive	Faster, simpler, relatively inexpensive	Low purity and yield	Low purity and yield
6	Polymer based Precipitation (ExoQuick™ + Ultracentrifugation + DTT)	Good yield	Best yield and purity among all other methods	Purity can be improved with additional steps such as SEC	N/A

microscopy (SEM), and atomic force microscopy (AFM) have been widely used to directly observe the morphology of individual exosomes, but it is difficult to quantify exosomal size distributions and concentrations using these techniques [52, 53].

Exosomal biomarker identification for clinical applications

Exosomal biomarkers have been identified for several acute, chronic, and systemic diseases, as discussed in the sections below (Table 2).

Biomarkers from urinary exosomes

Acute kidney injury (AKI)

The major causes of AKI in decompensated liver cirrhosis are hemodynamic derangement (70%) and acute tubular necrosis (30%) [32]. To address the adverse effects of AKI in decompensated liver cirrhosis, Awdishu et al. (2019) [54] tried to find a better biomarker than serum creatine to diagnose kidney injury. Since serum creatinine does not serve as a viable clinical marker because of muscle wasting, decreased liver production, increased volume distribution, and protein-calorie malnutrition, its increase is inadequate for making a timely and accurate diagnosis. In this context, kidney injury molecule-1 (KIM-1), tissue inhibitor of metalloproteinase-2 (TIMP-2), and neutrophil gelatinase-associated lipocalin (NGAL) are AKI-related markers that have been discovered previously [54].

To find additional or better biomarkers for the diagnosis of AKI, one study has attempted to employ proteomic analysis of urinary exosomes using SDS-PAGE followed by LC/MS–MS [48]. In silico analysis of the 1572 proteins discovered using the SEQUEST search engine and statistical analysis to calculate abundance scores revealed that maltase-glucoamylase (MGAM) was the dominant marker in this study. Although this was a significant descriptive study, the sample size was small, and the data lacked robustness. Moreover, exosomal protein trypsinization would have yielded more proteins from the process. The significance of this biomarker is its ability to support the diagnosis of proximal tubular injury. However, its clinical applicability requires validation in larger sample sizes and confirmation through further research trials [55].

Further, Sonoda et al. (2019) found that miRNAs from exosomes could serve as biomarkers for AKI progression [56]. They also hypothesized that exosomal miRNAs related to renal injury (particularly ischemic reperfusion injury) could reflect the state of the kidney. They demonstrated that the release of exosomal miRNAs into urine is dependent on their regulated sorting. Exosomal miRNAs involved in injury were linked to the renal medulla, and those that were involved in recovery had TGF-β-specific target mRNAs. The same mechanism (the release of exosomes) may be responsible for the level of miRNAs in cells. Therefore, their findings were extremely helpful in detecting AKI progression to CKD with a non-invasive method [48].

Arterial hypertension (AHT)

In AHT, a complex interplay of environmental and genetic factors is involved in altering the biochemical pathways that affect the function of the cardiovascular system [57, 58]. In addition, the role of arterial vasoconstriction and sodium/water reabsorption is significant in the disease's pathogenesis. AHT may lead to several complications, such as heart failure, end-stage renal disease (ESRD), stroke, and myocardial infarction. Primary aldosteronism coexists with AHT in 5–10% of patients, and the key hormone is a mineralocorticoid that alters sodium transport in the renal system to increase water uptake and blood volume. AHT can also induce endothelial dysfunction, oxidative stress, fibrosis, and inflammation [59–61]. In previous studies, mineralocorticoid function has been linked to aldosterone-related proteins, including GPER, RACK1, and small GTPase Rac1 [62–65]. However, there is little information on the role of exosomal GPER in mineralocorticoid-mediated AHT [4].

The sodium–water balance is maintained by several proteins of the renal system, including the sodium–hydrogen exchanger 3 (NHE3) of the renal proximal tubule, the Na–K–Cl cotransporter (NKCC2) of the ascending loop of Henle [66], the sodium chloride transporter (NCC), and the epithelial sodium channel (ENaC) of the distal nephron. Abnormalities in the function of ENaC manifest as Liddle syndrome, and the activity of WNK4-NCC presents as Gordon syndrome. Hypotension associated with Gitelman and Bartter syndromes results from alterations in the activity of NCC and NKCC2 proteins [4, 67]. Moreover, angiotensin II type I receptor (AT1R) and angiotensin II (Ang II) associated with the rennin–angiotensin–aldosterone system (RAAS) regulate blood pressure and are linked to exosomes. Ang II also plays a key role in end-stage organ damage as a result of inflammation [4]. About 45 miRNAs may be involved in AHT pathways, specifically those regulating the salt sensitivity of hypertension [68, 69].

Chronic kidney disease (CKD)

A recent study has investigated urinary exosomal biomarkers in patients with stage I, II, III, and IV CKD (classified based on glomerular filtration rate, or GFR) [63]. The study identified a total of 360 microRNAs, 116 antisense RNAs, 111 lincRNAs, 25 snoRNAs, and 4 snRNAs in urinary exosomes

Table 2 Exosome protein extraction output

Method of separating urinary exosomes	Clinical applicability	Advantages	Disadvantages	References
Exosome protein extraction and LC/MS–MS of urinary sample	Acute kidney injury	1572 proteins identified Renal brush border disaccharidase, MGAM, is identified as a robust and unique exosomal protein in cirrhotic patients with AKI Gel electrophoresis ensures that only full-length endogenous proteins present in the exosomes were compared between groups	Small sample size and low data robustness may have limited the number of proteins reaching the threshold of an acceptable score-based false discovery rate The direct exosome protein trypsinization method, instead of the 1D gel electrophoresis conducted by the authors, may have increased the number of proteins identified in the urinary sample	[137]
Urinary exosome purification, RNA isolation, RNA sequencing, and microarray analysis	Acute kidney injury progression	Proof-of-concept study: miRNA from exosomes (exo-miRs) relate to renal injury and can be considered a biomarker resource for AKI progression Non-invasively detects AKI progression to CKD	Study is carried out in rat renal ischemia–reperfusion injury (IRI) as a model of acute kidney injury (AKI), not in human samples Intercellular transfer role of exo-miRs under physiological conditions still needs to be thoroughly investigated	[138]
Urinary exosomes purification, RNA isolation, and RNA enrichment, followed by miRNA microarrays and RT-PCR for confirmation	Arterial hypertension	Technique identifies miRNAs unique to urinary exosomes	Limited exosome quantities are present in the urine. Therefore, the technique requires large pooled samples from relatively few subjects to yield enough material for array testing The effect of dietary or temporal biological variation is not explored	[139]
Independent isolation of urinary exosomal RNAs, reverse transcription into cDNA, and next-generation sequencing (NGS) analysis. To identify ncRNA, a novel bioinformatics pipeline ncRNASeqScan is implemented	Chronic kidney disease	Urinary tissue samples are used, which are more convenient than invasive methods, such as renal biopsies The method identifies dysregulated non-coding RNAs in CKD patients, such as tRNAs, tRNA fragments (tRFs), mitochondrial tRNAs, or lincRNAs The study's computational pipeline supports analysis of NGS data from multiple platforms, e.g., Illumina (Illumina, Inc.), Ion-torrent (Ion Torrent, Thermo Fisher Scientific), or 454-pyrosequencing (Roche)	Although certain tRNA fragments (tRFs) are identified, their source may be CKD or pathological stress injuries	[140]

Table 2 (continued)

Method of separating urinary exosomes	Clinical applicability	Advantages	Disadvantages	References
Isolation of urinary exosomes and total RNA extraction, miRNA profiling by polymerase chain reaction (PCR)	Type 2 diabetes mellitus and diabetic kidney disease (T2DKD)	Independent validation: A global profiling approach identified altered expression of the urinary exosomal miRNAs miR-21-5p and miR-30b-5p in association with poor renal function. These changes were subsequently validated in a larger independent cohort	Classification of T2DKD was not defined by kidney biopsy, so associations with DKD could not be confirmed histologically The study is cross-sectional and therefore cannot examine causality and changes in miRNA profiles as the disease progresses Some findings could not be replicated, which was attributed to the clinical heterogeneity of the patients enrolled, specimen collection, and experimental methods, in addition to ethnicity, population structure, disease heterogeneity and classification, and study power and effect size Potential confounders regarding findings of miRNA expression in association with DKD are not ruled out	[141]
Isolation of urinary exosomes and total RNA extraction, and using small interfering RNAs (siRNAs) for Elf3	Diabetic nephropathy	The study identifies urinary exosomal Elf3 as a non-invasive diagnostic and predictive biomarker in patients with DN This method provides a novel diagnostic marker specific for the detection of podocyte injuries in DN. The current indicator for DN (microalbuminuria) has limited diagnostic accuracy since structural damages might precede albumin excretion	None stated	[141]
Enrichment of urinary microparticles by ultracentrifugation, flow cytometry, and tryptic digestion of urinary microparticle proteins, followed by one-dimensional RP–LC–MS/MS analysis	Bladder cancer	Differential isotope labeling via dimethylation, coupled with LC–CID–MS/MS and MRM–MS, substantially facilitates an MS-based workflow for biomarker discovery/verification in urinary microparticles	ELISA is used to confirm the presence of TASCD2 in urine. Other microparticle proteins are not further investigated The current workflow cannot differentiate the source of exosomes/microparticles, i.e., blood or bladder cells	[87]
Urinary ultracentrifugation, exosome protein extraction, and MALDI-TOF spectrometry	Hernia, urinary tract infection (UTI), prostate cancer, or urothelial carcinoma	Rapid examination and optimization of exosome purification efficiency Acetonitrile and fluoroacetic acid provide high protein extraction yields and are feasible for MALDI-TOF analysis	None stated	[88]
Urinary ultracentrifugation, mass spectrometry	Bladder cancer	Differential proteomics strategy. Identification of important functional proteins in bladder cancer etiology, progression, recurrence, and metastasis	Pilot study: study was carried out on a small scale The number of samples is reduced, and there is a high variability in the transcripts detected for each sample	[91]

Table 2 (continued)

Method of separating urinary exosomes	Clinical applicability	Advantages	Disadvantages	References
Urine exosome RNA isolation (Norgen), RNA sequencing	Bladder cancer	RNA-seq of urinary exosomes (UE) may allow detection of critical cancer-specific transcripts missed by previous microarray studies of UE content	Small sample size ($n = 10$ patients)	[92]

of all the samples investigated. Further, the analysis revealed different exosomal levels of ncRNAs in CKD (211 in stage I, 153 in stage II, 221 in stage III, and 117 in stage IV) as compared with healthy controls. The researchers concluded that the differential abundance of 27 ncRNAs (tRFS, mitochondrial tRNAs, and miRNAs) across CKD stages formed the basis for their use as biomarkers in CKD diagnosis [70]. The reduction in GFR in CKD is associated with increased morbidity and mortality and thus poses a significant challenge to healthcare systems [71]. Nevertheless, the contribution of miRNAs and mRNAs derived from tissue to the development of CKD and the upregulation of MiR-21 can be observed in chronic renal disease patients with urinary exosomes and after glomerular injury [27, 72].

Preventing medial calcification in people with severe kidney disease involves preserving vascular smooth health of the muscle cells. Dusso et al. 2018 [73] found that vascular smooth muscle cells increase the production and release of exosomes in order to maintain viability during CKD-induced stress, but the ultimate effect is exacerbated pathological calcification. Chen et al. [74] claimed that microvesicles from calcified smooth vascular muscle cells convey procalcifying signals to normal smooth vascular cells. Dusso et al. 2018 [73] evaluated the key regulators of microvesicle/exosome biogenesis and secretion to help devise successful strategies for disrupting the procalcifying cell-to-cell contacts.

Khurana et al. 2016 [72] developed a novel computational pipeline, called ncRNASeqScan, for the computational identification of RNA-seq data. With this pipeline, they identified 30 differentially expressed ncRNAs, obtained from urinary exosomes, as effective biomarkers for early diagnosis in CKD patients. Among these, miRNA-181a proved to be the most powerful and reliable potential biomarker, being significantly reduced in CKD patients' exosomes by around 200 times relative to healthy controls. A CKD cell culture system revealed that the urinary exosomes may indeed come from epithelial cells of the renal proximal tubule [72].

Overexpression of MiR-26a in the muscle avoided muscle wasting caused by CKD and attenuated cardiomyopathy by exosome-mediated miR-26a transfer [75]. These findings indicate potential therapeutic approaches to treating CKD complications with the help of miR-26a exosome delivery [75].

Diabetic nephropathy (DN)

The traditional method of detecting DN is measuring urinary albumin and serum creatinine. miRNA biomarkers in urinary exosomes are perceived as a potential novel way of detecting DN during its early stages. Employing a profiling approach, researchers have identified the miRNAs miR-21-5p and miR-30b-5p as biomarkers for suboptimal renal function [76]. Specifically, miR-21-5p was upregulated and

miR-30b-5p downregulated in urinary exosomes derived from patients with type 2 DN. They also showed a significant correlation of these urinary exosomal biomarkers with serum creatinine. However, more in-depth evidence is required to validate these novel exosomal markers in a larger sample size of patients with DN and in those with other types of renal diseases [77].

Sakurai et al. (2019) studied the physiological basis of podocyte loss during the onset and progression of DN [78]. They highlighted RII-Smad3 signaling and Elf3 induction in determining the kidney status (function) in diabetic patients. They also indicated that RII induction is an important aspect of DN and that Smad3 plays a significant role in podocyte injury caused by glomerular hypertrophy. Moreover, Elf3 is involved in phenotypic alterations of podocytes as a result of the activation of TGF-β signals [79], and a signature of urinary exosomal miRNAs in patients with type II diabetic nephropathy was established [80]. It has also been shown that let-7c-5p derived from urinary exosome is associated with both renal function and DN progression, indicating that this is a possible biomarker for DN [80].

Cancer

Exosomes derived from cancerous cells sustain cell proliferation through the activation of signaling pathways. Exosomes are also able to modify the microenvironment to promote cancer invasion and spread. They are known to activate fibroblasts in prostate and bladder cancer [81, 82]. Exosomes are also capable of inducing the angiogenesis process to enable the formation of vasculature for tumor proliferation [83]. In addition, they assist in metastasis at the site of distant organs and influence the immune system during disease development [21].

The recurrence rate of bladder cancer is high, and continuous surveillance of patients is a necessary protocol in clinical practice [84]. Bladder cancer can only be detected by cytoscopic examination of the bladder, an invasive technique. An alternative to this is the use of biomarkers that have wide clinical applicability [85]. However, their limitations make it impossible to implement them in practice: low specificity or sensitivity, release from benign cells leading to false-positive results, and high costs [86].

Protein markers specific to the pathogenesis of bladder cancer include tumor-associated calcium-signal transducer 2 (TACSTD2) [87], alpha 1-antitrypsin and the histone H2B1K [88], periostin [89], the cell line TCCSUP [90], the proteins GALNT1 and LASS2 [91], and lncRNA HOTAIR [92].

Traditional diagnostic methods of prostate cancer either cause adverse effects (as in prostate biopsy or digital rectal exam) or have low sensitivity and specificity (as in PCA3) [93, 94]. To find a better method of disease diagnosis, researchers precipitated PC urine samples through high-speed centrifugation and observed pellets using transmission electron microscopy. Further, they isolated nucleic acids and applied statistical methods to calculate specificity and sensitivity [95].

The extracellular vesicles found in urine were protasomes, exosomes, oncosomes, microvescicles, and estosomes [95]. A significant finding was the abundance of miRNAs in the exosomes, suggesting their function as transport agents for nucleic acids and their role as possible biomarkers [95].

Several marker panels for prostate cancer have been proposed: delta-catenin, prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA), oncofetal protein 5T4, the cell invasion proteins integrin alpha 3 and integrin beta 1 [96], PCA3 lncRNA, ERG mRNA [97], the tumor-suppressive protein CDH3 [98], the alternatively spliced AGR2 gene [99], and the lipid classes of diacylglycerol (DAG) and triacylglycerol (TAG) [100–102].

Rodriguez et al. (2017) [103] also studied five microRNAs and found that miR-501-3p and miR-196a-5p were potential biomarkers for prostate cancer. The performance of candidate markers was explored using next-generation sequencing (NGS) and polymerase chain reaction (PCR). These miRNAs were found to be downregulated in the exosomes of prostate cancer patients [103].

Yazarlou et al. (2018) [104] explored the role of long non-coding RNA in the pathogenesis of bladder cancer by isolating urine exosomes from transitional cell carcinoma samples and found five different lncRNAs for diagnostic or prognostic purposes: LINC00355, UCA1-201, UCA1-203, UCA1-202, and MALATI. The researchers highlighted several findings based on their experiment and evidence from previous studies: MALATI might be a mediator for bladder cancer; UCA1-203 and UCA1-201 may have different roles in cancer and must be explored further; and exosomal expression levels of LINC00355 and MALAT1 indicate that their regulation may be influenced by cigarette smoking or opium addiction. The data from the study had high sensitivity and specificity and provided evidence of a correlation between the different candidate markers. In particular, they proved that lncRNAs from exosomes are potential biomarkers for the diagnosis of cancer [104].

Investigating Xp11.2 translocation renal cell carcinoma (Xp11 tRCC), Kurahashi et al. (2018) indicated that miR-204-5p was upregulated in urine exosomes belonging to RCC samples of mouse models. The increased levels were found in pretumorigenic and tumor stages. The researchers also confirmed that tRCCs secrete exosomes with miR-204-5p and that miR-204-5p could serve as a biomarker for early disease detection [105].

Gu et al. [106] have studied the clinical significance of urine prostate exosomal proteins in the diagnosis of prostate cancer. PSA can be elevated in non-malignant diseases

(such as prostatitis), leading to unnecessary prostate biopsies. Urine prostate exosomal protein (PSEP) is a promising biomarker of prostate inflammation. The presence of histopathological inflammation in prostate biopsies from 674 patients was assessed. Among them, 286 were diagnosed as PCa, and prostate inflammation was observed in 33.7% of the biopsies. The presence of histological inflammation was significantly associated with a lower risk of PCa ($p < 0.001$). The urine level of PSEP in PCa patients was significantly lower than that in the control group ($p = 0.003$) [106].

Understanding the molecular and cellular properties of exosomes provides advantages for liquid cancer biopsy diagnosis and its application in therapeutic drug delivery systems [107]. Studies have shown that genetic or molecular engineering of exosomes can improve target specificity and anti-cancer activity with less toxicity. Therefore, a better understanding of the biological properties of exosomes will contribute to their therapeutic potential as innovative drug delivery systems [108].

However, a successful clinical translation of exosome therapy depends not only on our understanding of the mechanisms of exosome treatment but also on our ability to isolate and design exosomes for therapeutic purposes [108, 109].

Xp11 tRCC is a rare sporadic pediatric renal cell carcinoma caused by a constitutively active TFE3 fusion protein. Tumors in Xp11 tRCC patients tend to recur and metastasize, partly because of the lack of early detection methods. Oike et al. [110] produced a transgenic Xp11 tRCC mouse model (Tg), with mice overexpressing the human PRCC-TFE3 fusion gene in renal tubular epithelial cells.

At 20 weeks of age, the kidneys of the mice had no histological abnormalities, but at 40 weeks of age, they showed the development of Xp11 tRCC and related morphological and histological changes. Compared with control mice, the 40-week-old Tg mice showing tRCC had significantly increased levels of microRNA (miR)-204-5p in urine exosomes. In the primary renal cell carcinoma cell lines established by two Tg mice, the expression of microRNA-204-5p also increased significantly [110].

All these cell lines secrete exosomes containing miR-204-5p. In particular, the researchers observed that 20 weeks before the occurrence of tRCC, the levels of miR-204-5p in the urinary exosomes of renal PRCC-TFE3 mice increased, and these levels were the same as those in the 40-week Tg mice, suggesting the occurrence of tRCC. Previously, the increase in miR-204-5p was found to follow the expression of the constitutively active TFE3 fusion protein in renal tubular epithelial cells [110]. Finally, the researchers confirmed that after overexpression of the PRCC-TFE3 fusion gene, the expression of miR-204-5p in non-cancerous human kidney cells increased significantly. These findings suggested that miR-204-5p in urinary exosomes may be a useful biomarker for the early diagnosis of Xp11 tRCC patients [110].

However, the problem is that clinical samples require more precise and standardized purification methods. Again, there are several biological activators in exosomes, and it is not easy to determine their main functional components. The basic mechanism or characterization of GC exosome biology has not been determined. Therefore, it is necessary to continue in-depth investigations [111]. With the help of cell-free urine, a model of five microRNAs was proposed by Fredsøe et al. [112]. There is an urgent need for improved biomarkers for the risk stratification of prostate cancer (PC). Fredsøe et al. [112] aimed to develop a novel model based on a minimally invasive sampling of blood and urine and a multimarker model for radical prostatectomy (RP) to predict biochemical recurrence (BCR) after radical prostatectomy. They initially measured the levels of 45 selected miRNAs by RT-qPCR in acellular urine samples rich in exosomes collected before PR of 215 PC patients [112]. They created a new logistic regression model (pCaP), which includes five urine miRNAs (miR-151a-5p, miR-204-5p, miR-222-3p, miR-23b-3p, and miR-331-3p) and serum prostate specific antigen (PSA). The model can significantly predict the BCR time in cohort 1 using univariate Cox regression analysis: the hazard ratio (HR) = 3.12 ($p < 0.001$). Then, using the same numerical dichotomy as in cohort 1, they tested and successfully verified the prognostic potential of pCaP in the other two cohorts [112]. There were 199 patients with RP (cohort 2, HR = 2.24, $p = 0.002$) and 205 patients (cohort 3, HR = 2.15, $p = 0.004$). After adjusting for pathological T staging, surgical margin status, and Gleason grading groups, PCaP is still an important predictor of RBC ($p < 0.05$ in Cox multivariate regression analysis, with HR values for cohorts 1, 2, and 3 of 2.72, 1.94, and 1.83, respectively) [112]. Further, in the three PC cohorts, the pCaP score was positively correlated with the established clinical risk stratification nomogram CAPRA. The results indicate that the minimally invasive pCaP model may be used to improve PC risk stratification and guide more personalized treatment decisions in the future [112].

Researchers have determined the importance of glycans as biomarkers for prostate cancer [113]. Because prostate cancer is a heterogeneous and multifocal disease, several biomarkers may be needed to guide clinical decision-making. Liquid-based biomarkers will be ideal, and attention is now turning to minimally invasive liquid biopsies, which can analyze tumor components in the patient's blood or urine [113]. An effective diagnosis using liquid biopsy will be required, and a recent high-level review discusses the combination of several analytes, including modifications to the transcriptome, epigenome, proteome, and tumor metabolome [113]. However, liquid biopsy analysis of genomics-based parameters may miss important aspects [114, 115].

Glycans have shown broad prospects as disease biomarkers, and data indicate that the integration of biomarkers into a multianalyte platform (including glycome modification) may improve glycoprotein stratification [114, 115]. Extensive glycan changes have been observed in prostate cancer, including changes in PSA glycosylation, increased central sialylation and fucosylation, O-GlcNacylation, the appearance of recessive and branched-chain N-glycans, and the modification of galectin and proteoglycans [113].

Amuran et al. [116] studied the relationship between urine protein and exosomal miRNA. The most significant difference in the diagnosis of British Columbia diabetic nephropathy was found by logistic regression analysis. The logistic regression model diagnosed bladder cancer (BC) with a sensitivity of 80% and a specificity of 88% (area under the curve [AUC] = 0.903) [116]. The same model distinguished low-risk (LR) patients from healthy controls (HC) with a sensitivity of 93% and a specificity of 97% (AUC = 0.976) [116]. In the early stage of LR patients, the panel was more sensitive and prompted for changes. Other studies using cultures clarified this idea. However, an expert team can prevent unnecessary cystoscopy, increase patient comfort, and reduce the financial burden of LR patients [116]. By using the same biomarkers, it is very advantageous to distinguish LR and BC patients from HC in terms of work, time, and cost [116]. Also, including miRNAs in the model that are not based on expression level but on the presence or absence of expression minimizes single errors, as this will eliminate the standardization and quantification steps. The sensitivity and specificity of the developed group are superior to those of urine cytology, ranging from 30 and 86% to 83% and 43% in various studies [116]. The sensitivity and specificity of the test panel became better than those of urine cytology when the same molecule was used to distinguish between BC and HC and LR, BC, and HC [116]. Although it is more sensitive and specific than FDA-approved urine biomarkers, the method still needs to be analyzed in a larger cohort. However, in terms of bladder cancer diagnosis, urine concentrations of exosomal miR-19b1-5p, 136-3p, and 139-5p and CRK APE1/Ref1, BLCA-4, and CRK are promising candidates [116].

Research on the human exosomal glycome is still in its early stages [117]. Comprehensive characterization of each sample is not only a methodological exercise but also requires a comprehensive characterization of the sample. Some confirmed results highlight the importance of structurally abnormal glycosylation in cancer and other diseases [118]. In a noteworthy study, it has been suggested that the position of fucosyl substitution and the position of sialic acid bonds are related to cancer cell proliferation, angiogenesis, and metastasis [119]. However, these potentially important structural changes may be the most important, and these structural changes can appear only as minor components in "bulk" glycan profiling studies [117]. When analyzing sera from different cohorts of ovarian cancer patients, some of us emphasized this observation. In these patients, 4-year-old glycans, which were previously overlooked, appear to be important [118]. An independent team of clinicians confirmed these findings. Similar considerations may apply to the detection and measurement of sulfate structures and glycan groups, which seem to be related to cancer [117]. Song et al. [119] conducted a thorough characterization of biological samples that is important for (a) covering major and minor components in complex samples and (b) promoting structural function based on structural similarities and differences in glycans (C). Once the visible glycans are determined, it may lead to simpler and more reliable analysis procedures for identifying important features for diagnosis and prediction (perhaps based on non-MS methods) [119].

Focal segmental glomerulosclerosis (FSGS)

Huang et al. (2016) studied focal segmental glomerulosclerosis (FSGS), a condition that plays a key role in the progression of end-stage renal disease (ESRD) [120]. FSGS patients do not respond to corticosteroids. In a renal biopsy, diagnosis and classification of FSGS are difficult because of sampling inadequacy and limitations in differentiating FSGS from minimal change disease (MCD) on account of limited glomerular findings. Evidence suggests the efficacy of miR-193a in urine exosomes in the adult population [121]. To explore the efficacy of this biomarker in children, researchers isolated the urine supernatant by ultracentrifugation and explored the samples using transmission electron microscopy. They quantified miRNA by quantitative real-time polymerase chain reaction (qRT-PCR) and analyzed it using statistical methods [122].

FSGS pathogenesis is related to injury to podocytes, which stabilize the structure and function of the glomerulus [123]. In their analysis, researchers found elevated levels of miR-193a in children. Although miR-193a could be a potential biomarker, limitations in their study included a small sample size, a limited age range, and the unavailability of follow-up data [122].

Kidney tissue and urinary exosomes from diabetic kidney disease patients displayed elevated ceruloplasmin (CP) levels, which can function as an effective biomarker [124].

Lupus nephritis (LN)

Sole et al. (2015) [125] used urinary exosome samples to test whether miR-29c, a microRNA, could be effective in detecting renal fibrosis during its early stages in lupus nephritis (LN) patients. They observed a 2.75-fold decrease in miR-29c in LN and non-lupus CKD patients. The marker was negatively correlated with renal chronicity but showed

no correlation with tubular atrophy, interstitial fibrosis, and fibrous crescents. It also did not correlate with clinical indicators of disease damage, including proteinuria, BUN, and creatinine levels. Further, the expression of MMP2 and Smad3 was upregulated in LN patients and correlated positively with chronicity and indirectly indicated a negative correlation with miR-29c [37]. The study was limited in terms of its small sample size and the absence of comparative evaluation through kidney biopsies to validate the results. However, miR-29c could serve as a non-invasive marker to predict "histological fibrosis" in the early stages of LN [125].

It is extremely relevant to detect fibrosis early on in the treatment of LN [37]. The characterization of urinary exosomal miRNAs can be used as a possible multimarker phenotyping tool for detecting early fibrosis. In vitro studies indicate that through the effect of profibrotic molecules on SP1 and Smad3/TGFβ pathways, these miRNA combinations facilitate renal fibrosis. A multimarker urinary exosomal panel consisting of miR-21, miR-150, and miR-29c offers a non-invasive tool for detecting early renal fibrosis and predicting disease progression in LN [37]. In patients with LN, the exosome S2D:5 targets renal tubular epithelial cells that transmit inflammatory Epstein–Barr virus-encoded small RNA (eber1) [126, 127].

Several miRNAs associated with disease activity and fibrosis development have been identified in data on exosomal-derived urinary miRNAs, but prognostic studies are missing. HIF1A was described as a possible common target [128], and low protein levels in non-responder renal biopsies were observed. HIF1A inhibition decreased mesangial proliferation, as well as endothelial cell development of IL-8, CCL2, CCL3, CXCL1, and IL-6/VCAM-1. Urinary exosomal miR-135b-5p, miR-107, and miR-31 are potential novel markers for clinical outcomes that control HIF1A inhibition of LN renal recovery [128].

Summary and future perspectives

As discussed above, several lines of evidence have demonstrated the relevance of exosomes in understanding the pathophysiology of diseases and the discovery of therapeutic targets. Exosomes are relevant in these areas because of the presence of certain common heat shock proteins, tetraspanins, fusion proteins, membrane transport proteins, lipids, and proteins related to biogenesis within the exosomal cargo. They also serve as an ideal source for biomarker discovery and can assist in drug delivery owing to their ability to cross the blood–brain barrier [129]. Investigation of complex diseases, such as diabetes and cancer, is made more feasible through exosomal research [21]. Moreover, exosomes have the potential to be used in diagnostic procedures with a high level of specificity and sensitivity, assisting in realizing the goals of personalized medicine [23].

However, exosomal research has currently some limitations. For example, the presently available methods for isolation of exosomes may not offer the ideal purity and efficacy that would be expected. Different exosomes may also possess widely different properties, and delivering cargo may require in-depth studies. Moreover, mass production of exosomes is not possible as the required level of standardization in isolation has not been achieved and characterization techniques with high reliability and efficiency are not yet available [21].

Although urinary exosomal markers may have a great potential for wide applications in local, remote, or systemic diseases, it is important to understand and resolve the effects of confounders that may affect the result of the analysis. These confounders may arise at several stages, including sample collection, transportation, storage, and dilution. Additional confounding factors include specific gravity, osmolality, urinary creatinine, and conductivity [86]. Therefore, normalization methods should be investigated to strengthen data analysis and interpretation to obtain more precise results.

Besides, most markers based on urinary exosomes have not been incorporated into mainstream clinical practice owing to the inherent differences in performance, low reliability, and high costs. Other limitations exist in terms of a lack of standardization and time-consuming isolation procedures. In the future, high-throughput approaches that can account for biases and that can offer the required sensitivity, reliability, and specificity will be able to cater to the unmet needs of efficient diagnostic procedures in clinical practice [86].

The solution based on microfluidic technology may be a promising strategy to solve these issues, which combines many separation and detection functions, which can be used for the separation, detection, isolation, and analysis of exosomes. Microfluidic strategy can also give emphasis on clinical application and points of care. These new features are expected to promote basic research and pave the way for routine personalized medicine through exosome-based liquid biopsy in cancer diagnostics [130]. Thus, exosomes identified in different biological fluids can be used as potential biomarkers for early detection of cancer. However, due to the lack of reliable strategies for its isolation and detection, clinical translation of exosomes is still difficult [131]. Although a single microfluidic platform shows unique characteristics with widely varied performance, the precise exosome capture with antibodies immobilized on a solid surface illustrates the most commonly employed method for its isolation [130]. Extravesicular exosomal proteins targeted by the capture antibody are known as targeted exosomal markers. These are dependent on specific application of exosomes and

the isolation of specific subgroups [131]. Researchers have integrated many separation and sensing functions for exosome isolation, detection, and analysis, emphasizing clinical and point-of-care settings. These novel approaches are expected to promote research advancement and pave the way for personalized medicine through routine exosome-based liquid biopsy [130, 131]. Microfluidic devices were used by Contreras-Naranjo et al., 2017 specifically designed inner capture surface(s) to improve the interaction between functionalized surfaces and the target exosomes, while attaining relatively high flux and good recovery efficiency [131]. Ashcroft et al. employed a detachable microfluidic circuit on the reformed mica surface to increase the captured concentration of micro-particles. Later, they were further examined by atomic force microscopy (AFM) [132]. Another device was designed by Kanwar et al. having multiple circular interconnected (by narrow channels) capture chambers, thereby increasing the retention time of exosomes [133].

Current efforts to improve the microfluidic systems sensitivity have resulted in the implementation of nanostructured coatings [134] and nano-shearing effects [135] for improved efficiency in immune-capture of target exosomes while inhibiting non-specific capture of on-target ones. Yang et al. (2020) evaluated a new integrated microfluidic device to collect exosomes from urine samples, designed precisely for in situ detection and isolation of exosomes specific for lung cancers [136]. This device is made by combining polymethyl-methacrylate (PMMA) and nano-porous gold (Au) nanocluster membranes modified with capture antibodies. Then the second antibody-conjugated nano-rod Au probe was further loaded for the identification and quantification of these lung cancer- specific exosomes with the help of dark field microscopy [136].

These developments demonstrate how the antibody-based exosome capture strategy has great potential for the development of microfluidic platforms, which can be used for comprehensive analysis in clinical and point-of-care settings. Similar approaches may be adopted for other diseases that may help early diagnosis and treatment of life-threatening conditions.

Conclusion

In conclusion, exosome-based biomarkers for the prognosis and diagnosis of diseases have not found clinical applicability in spite of extensive research in the field. Inherent problems still exist in terms of the lack of a standardized method for translating research data into clinical insights. Further, cost factors and the reliability of the available methods are still questionable. Nevertheless, novel isolation and characterization techniques of exosome analysis are underway and may make it possible to obtain an appropriate cost and sensitivity/specificity equation in the future.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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