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Review Article

Urinary exosomes as promising biomarkers for early kidney disease detection

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Abstract: Kidney injury and disease pose a significant global health burden. Despite existing diagnostic methods, early detection remains challenging due to the lack of specific molecular markers to identify and stage various kidney lesions. Urinary exosomes, extracellular vesicles secreted by kidney cells, offer a promising solution. These vesicles contain a variety of biomolecules, such as proteins, RNA, and DNA. These biomolecules can reflect the unique physiological and pathological states of the kidney. This review explores the potential of urinary exosomes as biomarkers for a range of kidney diseases, including renal failure, diabetic nephropathy, and renal tumors. By analyzing specific protein alterations within these exosomes, we aim to develop more precise and tailored diagnostic tools to detect kidney diseases at an early stage and improve patient outcomes. While challenges persist in isolating, characterizing, and extracting reliable information from urinary exosomes, overcoming these hurdles is crucial for advancing their clinical application. The successful implementation of urinary exosome-based diagnostics could revolutionize early kidney disease detection, enabling more targeted treatment and improved patient outcomes.

Keywords: Urine exosomes, biomarkers, kidney injury, kidney disease, early diagnosis

Introduction

The kidney is one of the most important organs of the human body because it regulates the removal of metabolic waste and the balance of water and electrolytes to maintain normal physiological functions [1]. However, the Global Burden of Disease Study revealed that approximately 10,000 people die of acute kidney injury (AKI) every year [2, 3]. In addition, 20,000 people die of chronic kidney disease (CKD) every year. Moreover, a study revealed that the rate of death caused by CKD is gradually increasing, and as of 2017, the number of deaths caused by CKD had increased by 41.5% compared to that in 1990 [3]. As a result, kidney injury and disease have become global public health concerns. There are many causes of kidney damage, including infection, obesity, diabetes, tumors and radiation [4-7]. In addition to common causes such as infection, poisoning may be an independent risk factor for kidney disease [8, 9]. Another study showed that there were approximately 431,000 new cases of kidney cancer worldwide in 2020, resulting in 179,000 deaths, and its incidence increased exponentially with age [10].

However, it is disappointing that at present, the clinical treatment measures for a series of kidney injuries and diseases are minimal, mainly owing to not timely diagnosis. The routine management of AKI is to control the patient's hemodynamics, and some vasoactive drugs increase renal perfusion [11], so the common measure is to restore the patient's fluid balance to a normal level [12]. Nevertheless, it still can't avoid the occurrence of AKI. Relevant studies have shown that 43% of AKI patients are in the late stage or undetected, and more than 50% of AKI

patients have unsatisfactory treatment outcomes [13]. Therefore, early diagnosis of kidney injury and disease is necessary. CKD is a common kidney disease affecting overall health [14].

According to the diagnostic criteria proposed by the Kidney Disease Improving Global Outcomes (KDIGO), only patients with abnormal kidney structure or function for more than 3 months can be diagnosed with CKD [15]. The main reason is that a single albumin index leads to a high rate of false-negative diagnoses [16]. Moreover, a three-month diagnosis cycle delays treatment time, as many people do not have obvious clinical symptoms of kidney disease in the early stages, especially those with the Chronic kidney disease of unknown aetiology (CKDu), which is difficult to detect due to a lack of significant symptoms. As a result, kidney disease may progress undetected until it causes serious, irreversible damage. Currently, there is no specific treatment for CKD, and treatment aims to slow down the progression of the disease and reduce further deterioration of kidney function, mainly through strict blood pressure control, reducing proteinuria, and avoiding further kidney damage [17]. Studies also suggest that early intervention for CKD is recommended in many countries, rather than just providing late-stage treatment [18]. Therefore, efficient early diagnosis is particularly important for reducing the complications of CKD and improving the therapeutic outcomes for end-stage renal disease (ESRD) [19].

In addition, early diagnosis is closely associated with an increased survival rate in patients with kidney cancer. For instance, it has been reported that the 5-year survival rates of kidney cancer patients with stage I and stage IV disease are 83% and 6%, respectively [20]. The low sensitivity of healthy kidneys to serum creatinine levels is the main factor limiting the early diagnosis of AKI and CKD [21]. In recent years, many studies have focused on the exploration of diagnostic molecular markers because different biomarkers are related to various pathophysiological processes that mediate AKI and CKD [22].

At present, the identified biomarkers can be roughly divided into functional molecular markers and damage-related molecular markers. Among them, biomarkers such as interleukin (IL)-18 or kidney injury molecule 1 (KIM-1) show good predictive potential but are accompanied by poor specificity and sensitivity [23-25]. Diagnosing kidney cancer at an early stage is difficult because the underlying molecular mechanisms of kidney cancer development remain elusive [26]. With the advancements in research, many biomarkers have been discovered [27], but their clinical value needs further evaluation. Overall, the requirements for new biomarkers are accuracy, specificity, and applicability in the diagnosis of diseases.

Overview of exosomes

Characteristics of exosomes

Exosomes are extracellular vesicles (EVs) with a nanoscale bilayer lipid membrane structure than ranges from 30 nm to 100 nm in size [28]. Exosomes originate from the invagination of the plasma membrane. They are sorted in the endoplasmic reticulum and fuse in the Golgi complex to form intracellular multivesicular bodies (MVBs, also known as late endosomes) containing intraluminal vesicles (ILVs). With the transmission of the plasma membrane, ILVs are transported to the plasma membrane and fused, and exosomes are released into the extracellular space [29] (**Figure 1**). Exosomes carry a variety of bioactive substances, such as proteins, DNA, mRNA, and noncoding RNA (microRNA, circRNA, and lncRNA) [30]. These "cargoes" can be transported to target cells or tissues by exosomes. Exosomes contain a large number of proteins (such as CD9, CD63, CD81, CD82, HSP70, and HSP90) and lipid [31]. It is a highly heterogeneous group, whose heterogeneity is described by the size of exosomes, the substances they carry (cargoes), and the cell type that produces them (source). The combination of these different attributes creates the complexity of exosomes [32, 33]. They not only mediate signal transduction and information exchange between cells but are also involved in the pathophysiological processes of various diseases in the human body [28, 33]. Therefore, In a range of diseases, exosomes provide insight into modified cellular or tissue conditions, and their detection in biological fluids may become an effective means of diagnosis.

Exosomes in intercellular communication

Information transmission between exosomes and target cells is mainly achieved through four

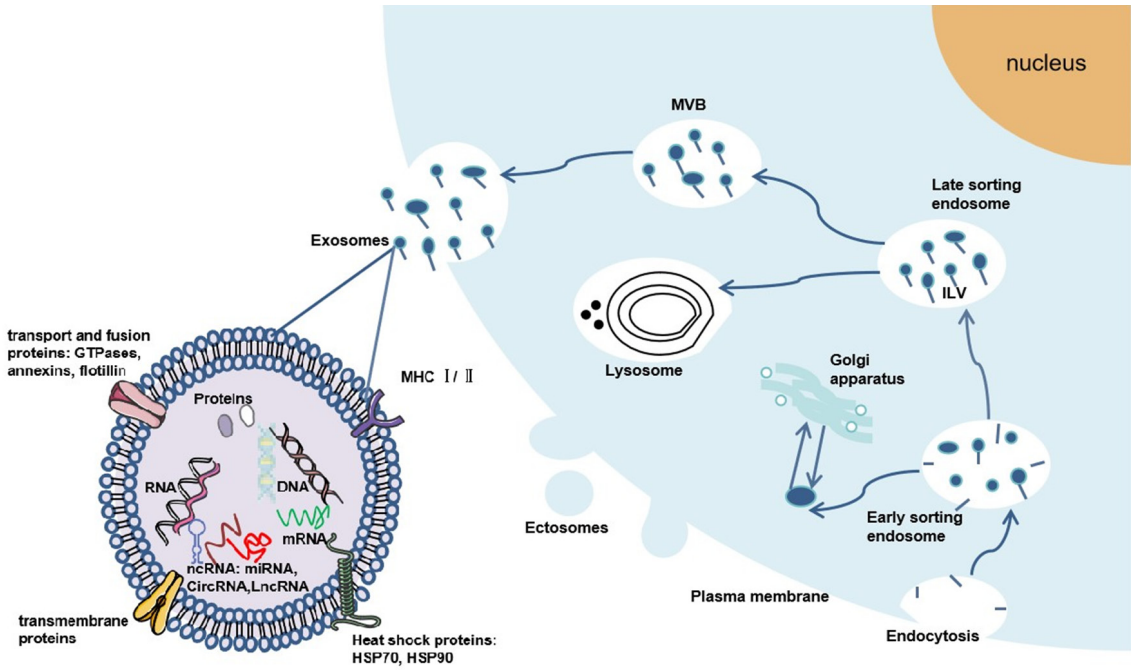

Figure 1. Schematic of the exosomes machinery. Exosomes originate from the endosomal pathway by the formation of the Early sorting endosome, Late sorting endosome, and ultimately MVB, which contain ILV. When MVB fuse with the plasma membrane, exosomes are released (size range ~30 to 100 nm). MVBs can also directly fuse with lysosomes for degradation. Exosome surface proteins include tetraspanins, Heat shock proteins, transport and fusion proteins, MHC I/II, and more. Exosomes can contain proteins, RNA, DNA, and metabolites. Exosomes can be a highly heterogeneous population and have distinct abilities to induce a complex biological response.

pathways: receptor-ligand interactions, endocytosis, macropinocytosis and membrane fusion [29]. All cells in the organism can secrete exosomes, and these exosomes can be directly absorbed by surrounding cells or indirectly absorbed by distant cells through body fluid circulation [34]. Currently, exosomes have been found in multiple body fluids, such as blood, semen, cerebrospinal fluid, breast milk, saliva, pleural effusion and urine [35]. More importantly, numerous proteins and lipids are embedded on the surface of exosomes during the assembly process, which endows exosomes with a certain degree of specificity for recipient cells and tissues [31]. Also, exosomes can cross the blood-brain barrier and enter the central nervous system through endocytosis [36]. In a nutshell, exosome-mediated signal transmission is not only ubiquitous in various cells of the body but also a key part of diverse intercellular crosstalk and communication systems.

Subsequent studies have continuously confirmed the function of exosomes in mediating information transmission. For example, research by Fuchs et al. has shown that in obese patients, exosomes derived from plasma and adipose tissue lead to the decrease of insulin signal transduction in myotubes and hepatocytes [37]. Additionally, studies have proposed that plasma exosomes derived from lean mice can regulate insulin signal transduction in adipocytes, myocytes, and primary hepatocytes in vitro, After injection into obese mice, these exosomes can improve glucose tolerance and enhance insulin sensitivity [38]. It is worth mentioning that the mRNA carried by exosomes can also be expressed in recipient cells. In some studies, new mouse proteins have been found in the recipient cells after the transfer of the exosomes derived from mice to human mast cells, indicating that the transferred exosome mRNA was translated after entering the cells [39]. All in all, the properties of exosomes determine that they are efficient and specific signal transmission mediums and of great importance in maintaining the exchange of information between cells.

Potential of exosomes as biomarkers

The production of exosomes is a process that is strictly regulated by environmental factors such

as cell state and stress conditions. A related study revealed that the "cargo" loaded in the exosomes in systemic circulation can represent the state of primitive cells [40]. Studies have shown that exosomes released by cells into the circulation and body fluids carry different amounts of proteins and RNA in healthy subjects and patients with different diseases [41]. thus, they can be used as potential diagnostic markers. For example, a variety of noncoding RNAs in tumor-derived exosomes can be used as tumor markers [42]. Multiple miRNAs inside exosomes, including miR-21, miR-26, miR-122, and miR-150, have been identified as biomarkers for noninvasive diagnosis of cholangiocarcinoma [43]. LncRNA-ATB is a new type of cancer-associated gene that is abnormally expressed in hepatocellular carcinoma, colorectal cancer, gastric cancer, and kidney cancer. Predominantly, lncRNA-ATB induces epithelial-mesenchymal transition by competitively binding to miRNAs (miR-200 family members), thereby promoting tumor occurrence and development [44].

Moreover, Bai et al. reported that exosomal circ_DLGAP4 promotes diabetic kidney disease progression by sponging miR-143 and targeting the ERBB3/NF-κB/MMP-2 axis [45]. In addition, some studies have also revealed that lncARSR transmitted by exosomes promotes sunitinib resistance in RCC by serving as a competitive endogenous RNA. Despite obesity being considered a promoting factor for type 2 diabetes (T2D), the pathogenesis of the two conditions is still different, with T2DM primarily occurring due to insulin resistance and impaired insulin secretion, while obesity, in addition to insulin resistance, is closely related to chronic inflammation, immune dysregulation, and changes in gene expression [38, 46]. Studies shows that the plasma RNA expression profile of obese and diabetic patients is also different, with miR-152 and miR-17 expression significantly higher in obese patients compared to patients with T2D, while miR-138 expression is markedly lower. Therefore, miR-152, miR-17, and miR-138 may serve as potential biomarkers for the two conditions [47]. Moreover, Hsp90 in tumor-derived exosomes is considered an important factor for maintaining intercellular communication between tumor cells and stromal cells and is closely related to the migration of cancer cells [48]. As mentioned previously, exosomes widely exist in body fluids, have certain specificity, and can sensitively reflect changes in the cell state. Therefore, signal molecules derived from exosomes are potential biomarkers for various diseases.

The role of urinary exosomes in healthy kidneys

Urinary exosomes as a source of renal-specific biomarkers

Urinary extracellular vesicles (uEVs) are nanosized membrane vesicles excreted by renal and urethral cells, mainly including exosomes, microvesicles, or apoptotic bodies [49]. Exosomes are the most widely studied subtype of uEVs [50]. Previously, it was believed that the main physiological function of urine exosomes was to process aging proteins from cells, which may be a more effective way to eliminate protein than proteasome and lysosome degradation [51]. As early as 2004, it was reported that there were a large number of outer membrane vesicles (MVs) with a diameter of 30-50 nm in the normal urine sediment of healthy individuals, and the MVs had typical MVB markers [52, 53].

Stahl et al. reported that exosomes play a key role in cell-to-cell communication in the nephron segment in kidney physiology [54]. For example, Street et al. demonstrated that exosomes secreted by renal collecting tubule cells can act as functional channels to transmit information to multiple cells [55], indicating that exosomes may be a new mechanism of intercellular communication in the kidney. In renal biopsies from patients with vasculitis, neutrophil-derived MVs expressing B1 receptors docked with glomerular endothelial cells, and B1 receptor-labeled MVs were observed in docked glomerular endothelial cells, where intercellular communication was achieved [56].

Notably, the exosomes in the urine of these healthy individuals carried a large number of proteins and transporters from various parts of the nephron, including podocalyxin and podocin, which are unique to glomerular podocytes, aquaporins, which are unique to proximal convoluted tubules, and sodium and chloride cotransporters, which are produced by distal convoluted tubules [57]. Subsequent proteomics studies showed that most of the exosomes in

Table 1. Factors or genes regulating the secretion of exosomes by kidney cells and tissues

Tissue/cell	Secretion of exosomes	Regulating factors	References
Podocytes	Yes	Hydrogen peroxide, TRPML1, Asah1, rapamycin, Amitriptyline, Sirt1, Elf3	[46, 47, 49, 53]
Renal tubular cells	Yes	Unknown	
Renal tubular epithelial cells	Yes	HNRNPA1, Epsin1, HIF-1, Oxalate	[54-56, 138]
Proximal renal tubular cells	Yes	HIF-1	[58]

the nephron mainly originate from glomerular podocytes and renal tubular cells. In addition, exosomes can be observed in proximal tubules, loops of Henle, distal tubules, and collecting tubules [58]. Collectively, the majority of exosomes in urine are derived from kidney tissues.

Mechanism of exosome release in the kidney

Exosomes are consistently released from diverse resting cells, especially during cell growth [29, 50]. However, during cell activation or cell stress induced by changes in the extracellular microenvironment or self-states, the release of exosomes changes significantly. For example, when cells are exposed to tumor necrosis factors, factors such as bacterial toxin and virus components, or cell damage, senescence, and canceration will change the packaging and release of exosomes [29]. As mentioned above, many tissues and cells in the kidney can produce and secrete exosomes.

Li et al. suggested that exosomes secreted by podocytes are related to the initiation of glomerular inflammation during hyperhomocysteinemia (hHcy). Briefly, in hHcy podocytes stimulated by hydrogen peroxide, the activity of the mammalian mucolipin TRP channel subfamily (TRPML1) decreases significantly, promoting the release of exosomes [59]. Some studies have shown that the deletion of the Asah1 gene, which is specifically expressed by podocytes, also causes abnormalities in TRPML1 channel activity, thus stimulating the release of exosomes [60]. Additionally, other studies have revealed that the addition of amitriptyline and rapamycin during hHcy can significantly reduce the production of podocyte-derived exosomes. Amitriptyline is an inhibitor of serotonin/norepinephrine reuptake transporters [61], and rapamycin is an enhancer of lysosomal function [62]. Furthermore, numerous studies have revealed other regulatory factors, all of which are shown in **Table 1**. Zhao et al. and Lv et al. discovered that exosomes derived from renal tubular cells and renal tubular epithelial cells have biological functions [63, 64]. Liu et al. stated that HNRNPA1 can mediate the assembly of miRNA in exosomes by renal tubular epithelial cells [65].

Furthermore, epsin1 modulated the interaction between tubular cells and macrophages in diabetic nephropathy (DN) by facilitating the sorting of exosomes containing Dll4 [66]. Besides, during hypoxia, the exosomes produced by renal proximal tubular cells activated by hypoxia-inducible factor 1 (HIF-1) have protective effects on renal tubular cells [67]. The above studies have suggested that exosomes can be used as a marker of the change of metabolic state of source cells or tissues. With the deepening of research, more factors regulating the release of exosomes in kidney tissue will be reveal.

Regulatory effect of urine exosomes on kidney physiological function

Due to carrying abundant signal molecules, exosomes can be transferred from one cell type to another, may significantly affect target cell homeostasis, and have significant regulatory functions. studies have pointed out that urine exosomes participate in the control and regulation of kidney development by activating Wnt family members and their signal transduction pathways [68]. In addition, exosomes are involved in the regulation of kidney physiological functions. Studies have shown that aquaporin (AQP)-2 is highly expressed in exosomes secreted by collecting tubule cells and can be absorbed and utilized by surrounding recipient cells, thereby enhancing the water reabsorption ability of recipient cells [69].

Table 2. Specific biomarkers of kidney injury

Markers	Changing tendency	References
TGF-β1	Up-regulated	[66]
miR-500a-3p	Down-regulated	[67]
NHE3	Up-regulated	[139]
fetuin A	Up-regulated	[71]
ATF3	Up-regulated	[68]
AQP-1	Down-regulated	[140]
miR-146a-5p	Down-regulated	[73]
miR-200a, miR-200c, miR-429, miR-24, miR-16	Up-regulated	[67]
Complement C4, Complement C3, glycan-binding protein Galectin 2 and other inflammation-related proteins	Up-regulated	[74]
miR-21	Up-regulated	[75]
CD26	Down-regulated	[76]

Other studies have reported that exosomes derived from proximal renal tubular epithelial cells can be transferred to the distal glomerulus and further regulate and induce the differentiation of myofibroblasts [70]. More interestingly, the exosomes in urine may be transported to or embedded in uromodulin, and this large protein may participate in the regulation of interactions between extracellular vesicles and their target cells [71]. Consequently, urine exosomes are involved in the regulation of the physiological function of the kidney through body fluid circulation. However, the role of urine exosomes in renal physiological information transmission remains unclear, and further exploration is still needed.

Urinary exosomes as biomarkers for specific kidney diseases

AKI

AKI is a common disease that can endanger people's lives [72]. It mainly manifests as acute tubular necrosis caused by ischemia or nephrotoxicity, acute glomerulonephritis, and acute interstitial nephritis [73]. AKI is correlated with acute morbidity and mortality, and the development of CKD or the increasing risk of cardiovascular events [74]. In a multicenter study, Liano et al. reported that the most common causes of AKI were acute tubular necrosis (45%) and prerenal factors (21%) [75]. Renal tubular epithelial cell damage is the main feature of AKI in the initial stage. Generally, a decline of renal blood flow to a level insufficient to maintain adenosine triphosphate (ATP) depletion in cells will trigger the initial stage of acute tubular necrosis, resulting in acute cell damage and dysfunction [76].

Moreover, because of renal tubule damage, renal tissue hemodynamics are abnormal, which further causes kidney injury [77]. Early diagnosis of AKI is particularly important for controlling the development of the disease, and early diagnosis has been shown to be related to the prognosis and survival rate of patients with AKI [73]. At present, the main criteria for diagnosing AKI are the serum creatinine concentration and urine volume. However, these two indices are relatively insensitive, and the changes can be rather obvious only in the late stage of AKI. Therefore, it is particularly important to improve the diagnostic efficiency of AKI. The cargo carried by exosomes from different renal tissues varies significantly during renal injury. In **Table 2**, we present several factors from urinary exosomes that have potential for use in the diagnosis of AKI. Sonoda et al. reported that in vivo renal ischemia-reperfusion injury (IRI)-induced AKI model, damaged epithelial cells releaserelease miRNAs in exosomes (exo-miRs), such as miR-16, miR-24, miR-200c, miR-9a, miR-141, miR-200a, and miR-429, the release of these exo-miRs is regulated by transforming growth factor β1 (TGF-β1), which can activate the proliferation and activation of renal fibroblasts [78].

Recently, a study showed that the lack of miR-500a-3p in exosomes from AKI urine significantly increased the cisplatin-induced expression of kidney injury molecule 2 (KIM-2) in renal

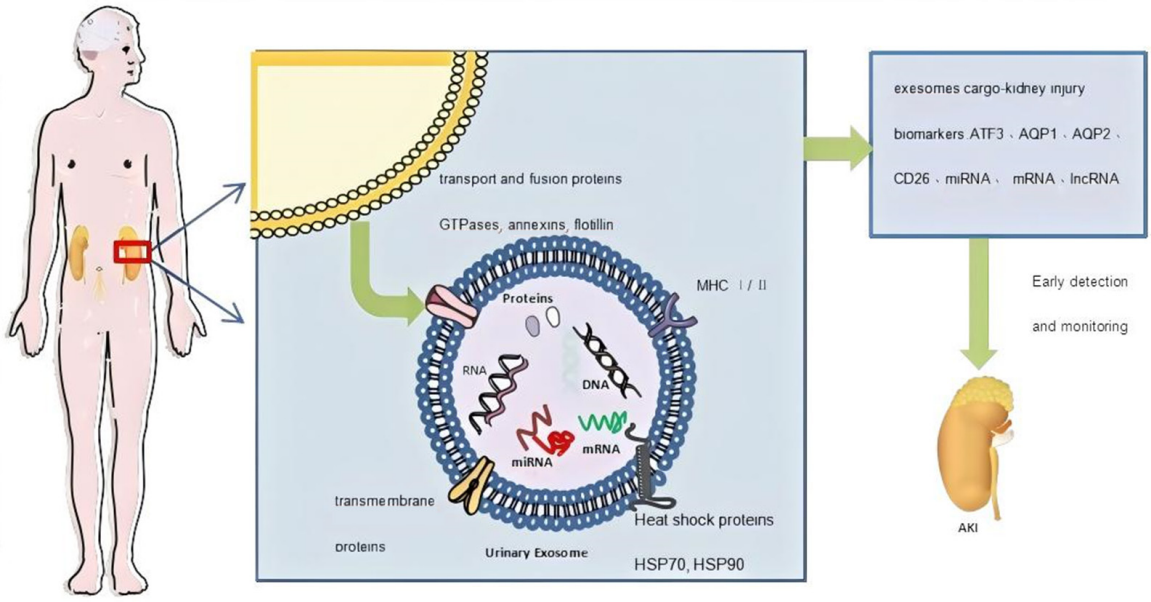

Figure 2. A schematic diagram of the cargo carried by exosomes in urine in acute kidney injury. Urinary exosomes carrying DNA, non-coding RNA, protein and other cargoes, such as ATF3, AQP1, AQP2, CD26, miRNA, mRNA, lncRNA, may be biomarkers for early diagnosis of AKI.

tubular epithelial cells located in proximal renal tubules [79]. In the process of identifying early diagnostic markers of AKI, Panich et al. reported that the expression of ATF3 increased significantly with the early development of AKI in the urine exosomes of mice with sepsis induced by cecal ligation and puncture [80]. In addition, Yun et al. reported that urinary exosomal miRNA-21 had good diagnostic ability for scrub typhus-associated acute kidney injury, and the AUC was 0.907 [81]. In summary, the unique signaling molecules in urine exosomes can act as efficient and specific biomarkers for the early diagnosis and detection of kidney injury (**Figure 2**).

CKD

In addition to kidney injury, kidney disease also has a great impact on human health worldwide. Based on the structure of the kidneys, kidney diseases can be classified into tubular diseases (renal tubular acidosis) [82], glomerular diseases, interstitial diseases (acute and chronic interstitial nephritis), renal vascular diseases (renal artery embolism and thrombosis, renal artery stenosis, renal vein thrombosis), renal stones, renal cysts, and renal tumors [83]. Moreover, glomerular diseases can be further categorized into primary, secondary, and hereditary diseases according to their etiology. Among them, the clinically common types include DN, immunoglobulin A nephropathy (IgAN), renal fibrosis, autosomal dominant polycystic kidney disease (ADPKD), and kidney tumors [18].

DN: DN is the most common underlying cause of CKD and may eventually lead to end-stage renal disease [82]. Therefore, early detection of DN is essential for improving clinical management. However, microalbuminuria cannot accurately predict DN, so new biomarkers are needed to identify early DN [83]. Researchers have extensively investigated urinary exosomal markers for the diagnosis of CKD, and numerous potential markers related to DN have been identified. For example, The content of UMOD mRNA in urine exosomes was elevated in the initial stage of DN even before the appearance of microalbuminuria [84]. Moreover, Li et al. reported that urinary exosomal let-7c-5p, miR-29c-5p and miR-15b-5p can predict the occurrence of DN, and let-7c-5p is associated with the progression of DN [85]. Notably, miR-151a-3p and miR-182-5p upregulated in T2D with DN and downregulated in T2D without DN, so they could be used as early predictors for DN [86].

In addition, another study proposed evaluating the progression of DN by detecting the expression level of miR-483-5p in urinary exosomes

Table 3. Specific biomarkers of kidney diseases in urine exosomes

Diseases	Markers	Changing tendency	References
DN	miR-92a-1-5p	up-regulated	[51]
	miR-483-5p	up-regulated	[62]
	UMOD	up-regulated	[82]
	let-7c-5p, miR-29c-5p, miR-15b-5p	up-regulated	[85]
	miR-151a-3p, miR-182-5p	up-regulated	[84]
	C-Megalin	up-regulated	[86]
	AQP5, AQP2	up-regulated	[89]
	CD63	up-regulated	[102]
	Elf3	up-regulated	[141]
	circ_0008529	up-regulated	[104]
IgAN	miR-29c, miR-205-5p	Down-regulated	[89]
	miR-215-5p, miR-378i	up-regulated	
	hsa-miR-451a, hsa-let-7d-3p	up-regulated	[92]
	miR-4639, miR-210 miR-199a-3p, miR-16-5p	up-regulated	[94]
	CCL2	up-regulated	[93]
	aminopeptidase N, vasorin precursor, α-1-antitrypsin, and ceruloplasmin	up-regulated	[106]
Kidney fibrosis	CD36	up-regulated	[95]
	miR-21	up-regulated	[96, 98, 99]
	miR-142-3p, miR-155	up-regulated	[99]
	hsa_circ_0036649	up-regulated	[100]
	hsa_circ_0008925	up-regulated	[101]
ADPKD	PC1, PC2	Down-regulated	[103]
	TMEM2	up-regulated	
	plakins, complement C3 and C9	up-regulated	[104]
	miR-192-5p, miR-194-5p, miR-30a-5p,miR-30d-5p, miR-30e-5p	up-regulated	[105]
Wilms tumor	WT-1	up-regulated	[97]
RCC	GSTA, CEBPA, PCBD1	Down-regulated	[110]
	miR-210-3p	up-regulated	[111]
	miR-126-3p, miR-17-5p, miR-21-3p, miR-25-39	up-regulated	[112]
	RAB27B	up-regulated	[142]

Abbreviations: DN: diabetic nephropathy; IgAN: immunoglobulin A nephropathy; ADPKD: autosomal dominant polycystic kidney disease; RCC: renal cell carcinoma.

[65]. Researchers have found that high glucose-induced exosomes from mesangial cells can induce podocyte injury [87], and HIF-1-induced exosomes from proximal renal tubular cells can reduce the level of apoptosis of the cells themselves [67]. The content of c-megga-lin in urinary exosomes increases with the progression of DN [88]. Moreover, it has been reported that the levels of AQP5 and AQP2 in the urinary exosomes of DN patients are significantly increased [89]. Urinary exosome biomarkers associated with other kidney diseases are shown in **Table 3**. In addition to these discovered biomarkers, many potential key factors have not been identified and deserve further exploration.

IgAN: IgAN is recognized as the most prevalent form of primary glomerulonephritis globally. Due to the fact that biopsy is considered the gold standard for diagnosing IgAN, and this invasive method to some extent limits its implementation for diagnosis and treatment [90]. Therefore, it is necessary to explore biomarkers of IgAN in urine exosomes. Studies have shown that the levels of miR-215-5p and miR-378i in

the urine exosomes of IgAN patients are significantly upregulated, and the levels of miR-29c and miR-205-5p are significantly downregulated [91].

In addition, Li and colleagues evaluated the diagnostic value of hsa-miR-451a and hsa-let-7d-3p in urine exosomes by ROC curve analysis, and the results showed that hsa-miR-451a (AUC = 0.805, P = 0.001) and hsa-miR-7d-3p (AUC = 0.76, P = 0.0049) had good diagnostic value [92], indicating that miR-215-5p, miR-378i, hsa-miR-451a and hsa-let-7d-3p may be biomarkers for the diagnosis of IgAN.

Furthermore, Yoon et al. reported that microRNAs such as miR-16-5p, miR-199a-3p, and miR-335-3p were associated with the progression of IgAN [93]. Meanwhile, exosomal miR-4639 and miR-210 in plasma and urine can serve as biomarkers for IgAN, playing an important role in the diagnosis and assessment of the severity of the disease [94]. In addition to miRNA, the expression level of chemokine (C-C motif) ligand 2 (CCL2) mRNA in urine exosomes is associated with the glomerular filtration rate, tubulointerstitial inflammation and C3 deposition [95]. In summary, the exploration of IgAN-related biomarkers in urine exosomes is not limited to miRNAs, and further studies are still needed.

Renal fibrosis: Renal fibrosis is characterized by the buildup of scar tissue in the kidney's parenchyma, impairing its self-repair capabilities and potentially resulting in renal failure [96]. Fibrosis often represents a final stage for nearly all chronic and progressive kidney disorders. which may be the reason for the difficulty of anti-fibrosis treatment. The prerequisite for early treatment is early detection. Therefore, it is very important to find early biomarkers to predict and evaluate renal fibrosis. Studies have shown that podocyte-derived microparticles promote the signal transduction of proximal renal tubular fibrosis through p38 MAPK and CD36, accelerating the development of renal fibrosis [97]. Recently, Earle et al. summarized miRNAs in urine exosomes that can be used as biomarkers for CKD, including miR-21, miR-29, miR-146, and miR-200 [98]. The upregulation of miR-21 in urine exosomes is closely related to renal fibrosis [99].

Meanwhile, Lv et al. reported that urinary exosomal miR-29c and miR-21 had good diagnostic ability for renal fibrosis, and the AUC was 0.833 and 0.973 [100]. In addition, miR-21, miR-142-3p, and miR-155 are considered markers of renal fibrosis in renal transplant recipients [101]. Interestingly, circRNAs in urine exosomes were also identified as potential biomarkers of CKD through circRNAs microarray analysis, and hsa_circ_0036649 might potentially cause kidney fibrosis [102]. Moreover, the research indicated that the expression level of hsa_circ_0008925 in urine exosomes from patients suffering from renal fibrosis was notably increased, and correlated with the score of tubulointerstitial fibrosis (TIF) and the score of glomerular sclerosis. ROC analysis revealed that hsa_circ_0008925 can effectively diagnose renal fibrosis at a cutoff value of 0.093, achieving a sensitivity of 52.2% and specificity of 96.4% [103].

ADPKD: ADPKD is a common hereditary kidney disease characterized by multiple cysts in the kidney and other organs, leading to CKD and finally results in ESRD [104]. The main causes of ADPKD are polycystic protein-1 (PKD1) and polycystic protein-2 (PKD2) gene mutations. In patients with PKD1 gene mutations, the levels of urinary exosomal PC1 and PC2 were decreased, and the level of transmembrane protein 2 (TMEM2) was increased [105]. Furthermore, Salih et al. found through proteomic analysis of urine extracellular vesicles (uEVs) and ADPKD animal model studies that the expression of plakins and complement proteins C3 and C9 was upregulated in patients with ADPKD, the expression of C3 and C9 was significantly increased in the early stage, and the expression of plakins was increased in the late stage [106].

In addition, Magayr et al. demonstrated that the levels of miR-192-5p, miR-194-5p, miR-30a-5p, miR-30d-5p, and miR-30e-5p were significantly reduced in urine exosomes from patients, as well as in cystic kidneys of murine PKD1 models and human PKD1 cystic kidney tissues [107]. Therefore, the measurement of urinary exosome polycystin-1 (PC1)/Transmembrane protein 2 (TMEM2), polycystin-2 (PC2)/TMEM2, plakins, complement C3 and C9, and miRNA may have potential value in the diagnosis and monitoring of polycystic kidney disease.

Kidney tumors: Renal-associated cancers are dangerous to human health and cannot be

ignored [86]. Patients with Wilms tumor often have no specific symptoms in the early stage, leading to a difficult diagnosis [108]. Unfortunately, few studies have investigated diagnostic markers of Wilms tumor in urine exosomes. Zhou et al. demonstrated by western blot that the level of WT-1 in the urine exosomes of patients with Wilms tumor was significantly increased, and the level of WT-1 in the urine exosomes 1 week after injury was related to the severity of glomerular injury 3 weeks later [109]. Therefore, urine exosomes are a new target for exploring diagnostic markers of Wilms tumor. Furthermore, the prevalence of renal cell carcinoma (RCC) is also increasing annually [110]. Moreover, approximately 20%-30% of RCC patients have metastatic disease at the time of diagnosis, and another 30% of local RCC patients have metastasis during follow-up [111].

Therefore, it is necessary to identify biomarkers that can aid in early diagnosis, indicate poor prognosis and improve treatment of renal tissue-related diseases. Studies have shown that the levels of Glutathione S-transferase alpha 1 (GSTA1), CCAAT/enhancer-binding protein alpha (CEBPA) and Protein C-terminal binding protein 1 (PCBD1) exosomal shuttle RNA (esRNA) in the urine of patients with clear cell RCC (ccRCC) were significantly decreased, and the mRNA levels of these three genes were significantly increased after ccRCC was cured [112]. In addition, Petrozza et al. reported that the expression level of miR-210-3p in the urine exosomes of patients with ccRCC decreased with patient recovery [113]. Moreover, Henriett Butz et al. have found that renal cancer cell lines can secrete exosomal miR-126-3p, miR-17-5p, miR-21-3p, and miR-25-3p, and have pointed out that these exosomal miRNAs can serve as potential biomarkers for ccRCC [114].

Technical considerations and challenges

Separation and characterization of urine exosomes

Purification of urine exosomes has always been a major clinical challenge. Currently available separation methods include ultracentrifugation (UC), Size-exclusion chromatography (SEC), ultrafiltration (UF), polymer-based precipitation. ultracentrifugation is the most commonly used technique for separating and concentrating primary EVs from exosomes [115, 116]. Ultracentrifugation is divided into sucrose density gradient centrifugation and differential centrifugation. Sucrose density gradient centrifugation is more complex and can obtain fewer exosomes; while differential centrifugation is more convenient and is one of the more common methods for separating exosomes currently [117]. This method has the advantages of high standardization and repeatability, but it is time-consuming, low in purity, and susceptible to contamination by small particles of EVs [117, 118]. In the light of these problems, other separation strategies have been applied, such as SEC, UF, polymer-based precipitation, Immunoaffinity capture and so on [119]. SEC is a size-based separation technique in which an aqueous solution is passed through a column made of starch and water to separate solutes of different molecular weights [120].

The advantage of using the SEC method to collect exosomes is that it is time-saving, high-yielding, and retains the natural activity of exosomes. The disadvantage is that the equipment is expensive, and it is difficult to separate proteins of similar size to the diameter of exosomes [121]. Immunoaffinity capture isolates exosomes by binding specificity between a protein marker and its corresponding antibody, making it an ideal method for isolating subpopulations of exosomes with specific origins [122]. Currently, exosome separation products on the market include exosome separation and Analysis Kit (Abcam), Exosome Human CD63 separation reagent (Thermofisher), and exosome separation kit CD81/CD63 (Miltenyi Biotec). Although immunoaffinity capture has the obvious advantages described above, its application is limited by its high cost and low yield associated with antibody development and production, and is not suitable for large-scale production. Due to the inherent limitations of various isolation methods, it is recommended to use combined exosome isolation techniques to improve the purity and quantity of exosomes.

These methods include UC combined with UF, SEC combined with low-speed centrifugation, and a combination of iodixanol density gradient ultracentrifugation and bind-elute chromatography [115, 123].

Selection of exosome identification methods

Proper identification of exosomes is crucial to ensure their quality and efficacy. Techniques such as western blotting (WB), flow cytometry (FCM), immunofluorescence (IF), nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM) can be used to characterize the size, morphology, concentration and molecular phenotype of exosomes. WB and FCM can be used for molecular phenotyping with high sensitivity and specificity [124]. Western blotting is more accurate and requires relatively simple equipment, but WB takes a long time [125]. FCM may capture false signals and require more advanced equipment, with the advantage of being faster and more sensitive, requiring a smaller sample size, and being suitable for fluorescent labeling and specific antibody labeling of exosomes [125, 126]. NTA, dynamic light scattering, and resistive pulse sensing are used to characterize the diameter of exosomes. NTA is the most widely used among the three approaches, determining the size and concentration of exosomes in liquid suspension using the properties of Brownian motion and light scattering.

TEM is the gold standard for identifying exosomes, which depicts a cup-shaped double-membrane structure of exosomes. Before analyzing urinary exosomes by TEM, complex sample preparations are required, including fixation and dehydration. These two processes may lead to the shrinkage of urinary exosomes, thus affecting their morphology and size. In contrast, cryo-electron microscopy can avoid fixation and dehydration, and rapid freezing can better maintain the morphology and size of exosomes [126, 127]. Unfortunately, cryo-electron microscope is very expensive. In addition, enzyme-linked immunosorbent assay (ELISA) can also be used to detect proteins on the surface of exosomes, but with poor sensitivity and specificity. Because the various identification methods have certain limitations, a variety of complementary methods can be used to better characterize the characteristics of exosomes and improve their quality.

Information mining and verification of urine exosomes

Proteomics and high-throughput RNA sequencing are common methods for analyzing the biological functions of urine exosomes [128, 129]. Proteomic analysis can be used to visualize the proteins in exosomes and screen for key proteins through comparison with those in the control group. the proteomic results can be verified by western blotting [130]. There are many published raw proteomic data related to human urine exosomes in the database of urine exosomes (http://hpcwebapps.cit.nih.gov/ESBL/D-atabase/Exosome/). This website provides researchers with opportunities to observe protein information in urine exosomes. High-throughput RNA sequencing is an analysis method used to observe changes in RNA expression in urine exosomes in an experimental group compared with a control group [131]. According to the analysis results, RNA with specific expression changes can be screened, and the results of RNA sequencing can be verified by real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) [132]. The biological functions of the obtained key proteins and RNAs, such as their effects on cell proliferation and apoptosis, can be verified in vitro or in vivo [133].

Limitations and future directions

On the one hand, current reports on renal injury and disease mainly focus on studies that reveal the potential of miRNAs as biomarkers in urinary exosomes [134], but there are relatively few studies on other noncoding RNAs (such as lncRNAs and circRNAs). On the other hand, omics studies mainly focus on proteins and RNA, but metabonomics is also an important field that should be considered. This is because the concept of a “metabolic state” has been proposed, and small molecular metabolites may affect the cell state [135]. Succinate has been shown to induce the accumulation of renal lipids and ROS in DN mice [136].

In addition, the metabolic state of renal tissue in DN rats is different from that in normal rats, and oral administration of astragaloside IV can reverse metabolic disorders in rats [137]. However, the use of small molecular metabolites as biomarkers is not as convenient as the use of protein or RNA. Accurate determination of metabolite content requires targeted metabolomics analysis, certain operation experience, and the analytical ability of mass spectrometry. Despite the technical challenges at this stage,

metabonomics is essential in the process of exploring kidney diseases. Moreover, identifying the source of exosomes is also a major clinical challenge. If the source of exosomes can be accurately distinguished, the diagnostic value and accuracy of the use of urine exosomes as biomarkers could be improved.

Conclusion and prospects

The biomolecules contained within urine exosomes can reflect the state of the kidney and provide information about the location of renal injury or disease. However, there are still challenges to overcome in terms of standardizing isolation and analysis methods and validating the utility of urine exosomes in clinical settings. With further research, urine exosomes could become a valuable diagnostic tool for the early detection of renal injuries and diseases, leading to improved patient outcomes. Exosomes are functional channels for information transmission, and they are specific and highly efficient. Therefore, urine exosomes also have the potential to serve as an auxiliary tool for delivering drugs, receptors, and ligands to change cell processes and are expected to become a natural tool for precision therapy.

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Disclosure of conflict of interest

None.

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