Chapter

Extracellular Vesicles in Kidney Disease

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Abstract

The kidney is the mainly apparatus in the human body, with a complex organizational structure and diverse pathological changes closely related to other organs. Extracellular vesicles are vesicles with diameters ranging from tens of nanometers to several micrometers, originating from multiple intracellular vesicles or local cell membranes. They carry various information from the source cells and operate between various cells in the kidney and extrarenal organs, conveying substances between cells. They play a large part in signal transmission within the kidney and between the kidney and other organs. Detecting changes in extracellular vesicles and their cargo can monitor both renal neoplastic and nonneoplastic diseases. Extracellular vesicles derived from various stem cells, loaded with bioactive substances, can be applied to some extent to treat kidney diseases. Bioengineering drugs using extracellular vesicles as carriers are also playing an increasingly big role in treating kidney diseases. Research on extracellular vesicles has achieved certain results and has some preclinical applications, but there is still a process for large-scale and widespread application.

Keywords: kidney disease, extracellular vesicles, cell-to-cell communication, biomarker, therapeutic potential

1. Introduction

Extracellular vesicles (EVs) are a heterogeneous population of bilayered lipid vesicles [1–3] regarded as a vital interactive courier between cells and secreted by most types of cells. EVs can be differentiated into three main categories according to their origin and size: exosomes, local microdomains assembled in endocytic membranes with the size of 30–100 nm; microvesicles, released from the plasma membrane with a size of 100 nm-1 μm; apoptotic bodies, released by cells undergoing apoptosis with a size of 1 μm–5 μm [4, 5]. The giant tumor-derived vesicles (oncosomes) and mitochondrial-originating vesicles (mitovesicles) are also considered as EVs [6, 7].

Despite the heterogeneous and diverse nature of EVs, they are released by normal cells and move in endless cycles in humor, with a major role in numerous physiological and pathological conditions. EVs are abundant in body fluids and are easy to separate and enrich, with complicated cargo reflecting the physiological and pathological condition of the source cells. EVs can be efficient transport through the cell membrane

of target cells, thus enabling communication between cells and modulating the gene expression and function of target cells [8, 9].

The kidney is one of the important organs to remain steady in the internal milieu. EVs feature in renal physiology and are widely involved in the occurrence and progression of various kidney diseases. It can be used for the diagnosis of kidney diseases and be instrumental in the treatment of renal diseases [10, 11].

2. Role of extracellular vesicles in cell-cell communication in kidney

As the characteristics mentioned above, EVs act major affection in nephron intercellular communication [12]. Proteomics results show that the exosomes in nephron mainly come from glomerular podocyte and tubular cells located in the proximal tubules, the thick segments of ascending medullary branches, the distal tubules, and collecting ducts. Some studies have confirmed that EVs transmit information in the nephron between the glomerulus and renal tubules. The upper tubule cells can release EVs, which are internalized by the cells in the lower segment of the tubule, transmitting activated cell molecules. The earliest research found that EVs could be expelled and internalized by murine renal collecting duct cells in cultivation and transfer the functional aquaporin 2 [13]. Further research has found that the EVs from proximal renal tubules can be absorbed by the distal tubules and collecting ducts [14]. A study on podocyte RNA labeling found that this RNA was subsequently detected in renal tubular cells, thus confirming the material transfer between glomerulus and renal tubular cells [15].

Not only that, the maintenance of electrolyte and acid-base equipoise is one of the crux functions of the kidney, which is fulfilled by tubular transport. A variety of proteins and transporters rich in urinary exosomes come from cells in the nephron, for example, Na+-Cl symporter (distal tubule), aquaporin (AQP)-2 protein (concentrated pipe), Na+-K+-2Cl symporter (thick ascending limb), AQP-1 (proximal tubule), podoglycocalyx protein (podocyte). The urinary extracellular vesicles (uEVs) produced at the top membrane of kidney tubular cells, cargo water, electrolyte, and acid-base transporters. When the proximal tubular cell line is affected by inflammation, they excrete more EVs from apical and basal membrane, both of which have different molecular and functional characteristics [16]. It has proved that miRNAs acted as a major role in EV-mediated intercellular communication. Jella et al. recorded by unipath patch clamp that the probability of ENaC opening Xenopus cells and isolated splitopen tubules could be reduced by proximal EVs, and this could be weakened by EVs transfected with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inhibitors [17]. Another research confirmed that ENaC activity could be regulated by EVs through purinergic signaling [18].

The distant tissues can release EVs into circulation and mediate inter-organ crosstalk. EVs released from the placenta of preeclampsia can carry anti-angiogenic factors and lead to maternal glomerular endothelial dysfunction and proteinuria [19]. EVs released from autoimmune diseases, such as antiphospholipid syndrome, thrombotic microangiopathy, systemic lupus erythematosus, and ANCA-vasculitis, can promote coagulation, thrombosis, and immune-mediated renal pathology [20].

Increasing evidence shows that circulating cells (monocytes, neutrophils, and red blood cells) and platelets attacked by toxins release the EVs, and the latter are the major elements of pathogenesis in hemolytic uremia syndrome [21]. The ability of EVs derived from mesenchymal stem cells (MSC) to alleviate myocardial

injury in experimental metabolic renovascular hypertension is partly mediated by IL-10-containing EVs [22]. The bioactive cargo of EVs in kidney transplantation (KT) includes implant antigens, cytokines, growth factors, costimulation/inhibitory molecules, and functional microRNAs (miRNAs) that may regulate gene expression in receptor cells, which act as the immune modulators and play a crucial role in maintaining complex crosstalk between graft tissue and innate/adaptive immune system. EVs are of great importance in allogeneic recognition, ischemia–reperfusion injury (IRI), autoimmunity, and allogeneic immunity and are expected to become biomarkers and therapeutic tools for KT [23].

The kidneys can also release EVs, affecting the function of other tissues and organs. A study on the proteomics of EVs in murine hearts found that some proteins come from other organs, including the kidneys [24]. Cardiovascular dysfunction is caused by a high level of circulating endothelial cell-derived particles in chronic kidney disease (CKD).

These particles may mediate inflammation, vascular wall damage, and remodeling and act as an incremental risk of Vascular calcification (VC) [25–30]. VC is a pathological manifestation with high mortality, mainly manifested as abnormal calcium deposition in the vascular wall. During the process of VC, vascular smooth muscle cells (VSMCs) suffer osteogenic transformation and secrete EVs with various sources and compositions. The secreted EVs may obtain calcium-promoting properties, thus acting as the nucleation focus of hydroxyapatite crystallization and calcium transmission [31]. The serum calpain particles (CPPs) and EVs in uremia are meaningful participants in the extensive calcification mechanism of CKD, and cGRP (a protein rich in Gla) plays an inhibitory role in preventing calcification at the system and tissue levels [32].

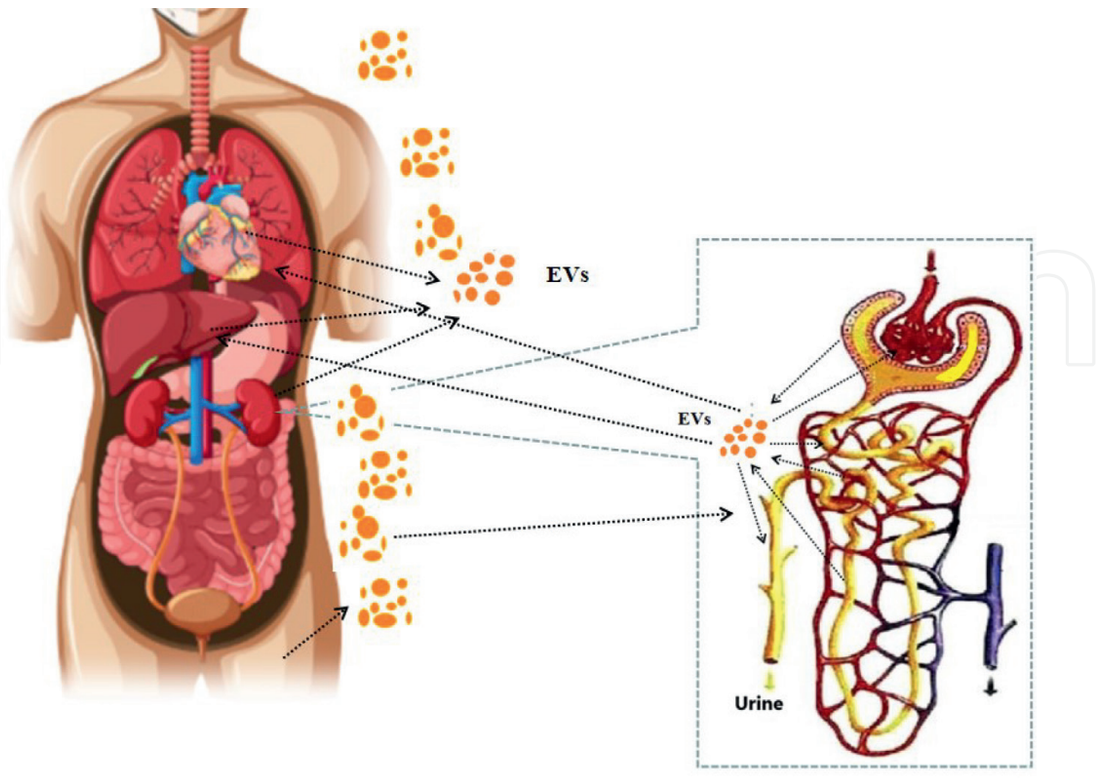

Figure 1.
Extracellular vesicles as delivery vans in cell-cell communication between renal cells and extrarenal organs (cells).

The deterioration of renal damage during liver or heart disease may be caused by EVs, while the advancement of hepatorenal or cardiorenal syndrome may involve EVs [33, 34]. Nonvalvular atrial fibrillation is associated with kidney disease because the increase in thromboembolism is mediated by a higher level of EVs from prethrombotic endothelial platelets rather than by thrombotic status and other markers of cell activation, even in anticoagulant patients [35]. In patients with hypertension, the presence of EVs indicating podocyte injury and peritubular capillary injury is detected [36]. The endothelial cells derived EVs released from peritubular capillaries were checked in the primary and renovascular hypertension patients' urine, with the density of EVs directly associated with clinical parameters and the scarcity of capillaries, but was inversely proportional to renal perfusion [37]. Therefore, the change of urinary EVs in hypertensive patients can be regarded as an early marker of renal injury caused by peritubular capillary injury, and it will change with the improvement of renal function after drug treatment in patients with primary and renovascular hypertension (**Figure 1**).

3. Extracellular vesicles as biomarkers of renal diseases

EVs are a type of disc-shape vesicles with single concave, secreted by active cell and enveloped by phospholipid bilayers [38], with sizes ranging from nanometers to micrometers [39–42]. EVs grow out from the straight sprout of small vesicles wrapped in membranes or through blend on the superficial multivesicular bodies, engage in the intercellular commutation of materials and message, with cargo proteins, lipids, and nucleic acids (microRNA, messenger RNA, circ RNA, and lncRNA, etc.) [43–47]. With the pivotal roles in an exchange between renal cells and target cells, EVs are expected to serve as new molecular markers for the detection of kidney diseases, carrying specific molecular substances in source renal cells with the lipid structure to protect the contents from being degraded [48, 49]. At present, the study of EVs, especially exosomes, has been involved in the field of liquid biopsy [50, 51].

3.1 Extracellular vesicles as liquid biopsy markers for renal cell carcinoma

Renal cell carcinoma (RCC) is a kind of malignant tumor in the urinary system originating from the renal tubular epithelium, accounting for about 3% of adult malignant tumors, ranking second in the urinary system. The partial or total nephrectomy are the current therapeutic strategy, following or not following with immune-checkpoint-inhibitors-based targeted therapies to which patients are often drug-resistant. It lacks specific symptoms at the early stage [52]. Diagnosis mostly relys on ultrasound and other examinations. At the initial diagnosis, about 17% of RCCs have distant metastasis, with a 5-year survival rate close to 12% [53]. Therefore, early diagnosis and prediction of metastasis risk are crucial to the treatment and prognosis of patients. Data has shown that EVs are the proficiency biomarkers for tumor liquid biopsy due to their specific content, species conservation, stability, and high abundance under different sources and physiological and pathological conditions [54].

3.1.1 EVs as biomarkers for the diagnosis and staging of RCC

Some researchers conducted multi-omics analysis on tumor-related proteins and mRNA in EVs from different RCC cell lines, identified multiple candidate molecular markers, and confirmed that EVs could not only distinguish between RCC and benign

lesions, but also assisted in determining the subtypes of RCC, with great potential for clinical transformation [55]. Research has found that compared to the control HK-2 cell line, the expression level of miR-150 in EVs from the 786-O RCC cell line was upregulated by 5.2 times, while miR-205 was downregulated by 10,000 times, indicating that miR-205 and miR-150 in EVs were new prospective biomarkers for the diagnosis of RCC [56].

However, *in vitro* cell models cannot fully and in real-time reflect tumor conditions, and it is still essential to screen and validate in clinical specimens. There are reports that compared to healthy control, the expression of miR-149-3p and miR-424-3p in plasma EVs of RCC patients are upregulated, while miR-92a-1-5p is obviously downregulated, which is helpful for the diagnosis of RCC [57]. Zhang et al. observed that the levels of miR-210 and miR-1233 in serum EVs were markedly upregulated in patients with clear cell renal cell carcinoma (ccRCC) than those in the control group, and their expression were dramatically reduced after tumor resection, they has worthwhile value for RCC diagnosis and surgical effect monitoring [58]. Additionally, the expression of miR-210 is also related to their clinical prognosis in serum EVs of RCC patients, and the diagnostic performance for early patients is better than that of serum miR-155 [59]. Similarly, serum EVs miR-4525 can serve as a potential diagnostic marker for advanced RCC patients [60]. EVs from other sources of bodily fluids, besides blood also have the diagnostic potential for RCC. Urinary exo-miR-30c-5p may regulate the expression of a protein related to the progression of ccRCC (heat shock 70 kDa protein 5) and is regarded as a latent diagnostic biomarker for early ccRCC [61]. Some researchers have found that a combination detection of urine EVs miR-126-3p and miR-449a or miR-34b-5p can prominently differentiate between ccRCC and healthy individuals [62].

Recently, although most research on EVs as molecular markers of RCC has focused on miRNAs, it is also important to recognize that other specific molecules (proteins, mRNA, DNA, etc.) in EVs can also be used for auxiliary diagnosis of RCC [63]. Palma et al. found the obvious diverse in urinary exosomal shuttle RNA (esRNA) type of ccRCC patients compared to healthy population and non-ccRCC, and observed that this specific pattern increased to reach the normal level one month after partial or radical nephrectomy. They identified that the urinary esRNA levels of CEBPA, GSTA1, and PCBD1 were downregulated in patients than the healthy subjects, then suggested the RNA content in urinary EVs could be the prospective diagnostic applications for the ccRCC [64]. Some scholars describe CA9, CD147, and CD70 in EVs as tumor markers that are specific to ccRCC [65].

Some scholars characterized CA9, CD147, and CD70 as tumor-specific markers on EVs in CCRCC [65]. Vergori observed that CA9 in circulating large EVs plays a part in the diagnosis and prognosis of ccRCC [66]. Researchers regarded Polymerase I and transcript release factor (PTRF)/CAVIN1 in urinary exosomes as prospective biomarkers of ccRCC [67]. Li et al. believed that exosomal circRNAs may be the potential cancer diagnosis marker [68]. The above studies have confirmed that EVs and their contents are expected to become diagnostic and staging markers for RCC.

3.1.2 EVs as markers for RCC metastasis monitoring

The messages in EVs actively loading by cancer cells can support tumor spread; the double layer of EV protects the peculiarity. Thus EVs play a major role in metastasis by improving the content's half-life and stability, and then EVs act as markers for metastasis monitoring. Many scholars have confirmed this by animal and cell experiments.

EVs released from RCC cell line (786-O) can transfer long-chain noncoding RNA (LncRNA)_human lung adenocarcinoma of the metastasis related transcription factor 1 (MALAT1) to adjacent nonmetastatic RCC cells and enhance their growth, invasion, and metastasis abilities [69]. Meanwhile, CD103+ EVs separated from cancer stem cell (CSC) of ccRCC patients targeted regulate the protein level of phosphatase and tensin homolog deleted on chromosome ten (PTEN), which is tightly relevant to cell migration, by transporting miR-19b-3p to ccRCC cells; Cancer stem cell EVs also can be guided by CD103 to target cancer cells and organs, making ccRCC have higher lung metastasis capacity [70].

In addition, the expression of miR-1293 decreased, and the expression of miR-301a-3p increased in plasma EVs of RCC patients with metastasis, which are regarded as presumable biomarkers for the metastasis of RCC [71]. The downregulated miR-483-5p was observed in ccRCC, and Wang et al. believed that it contributed to cell proliferation, metastasis, and inflammation [72]. Evs-derived proteins also can be the marker of RCC metastasis monitoring. Some investigators observed that the serum EVs of prostate-specific membrane antigen (PMSA) levels in metastatic patients of RCC were sharply increased compared with nonmetastatic patients by establishing a sandwich ELISA method, and also believed that PMSA can reflect the angiogenesis of the primary tumor and metastasis, then monitoring the metastatic RCC in real time [73]. Furthermore, the surface or content of EVs can provide targeted recognition sites for tumor metastasis, providing a theoretical basis for developing the predicting RCC metastasis markers and targeted therapy strategies.

3.1.3 EVs as biomarkers for drug resistance and prognosis in RCC

The prognosis of RCC patients is closely related to tumor staging, grading, metastasis, and patient sensitivity to therapeutic drugs. It is deemed that partial nephrectomy is the preferred treatment strategy because some researchers observed a better ending and better postoperation renal function in the advanced stage RCC patients treated by partial nephrectomy than those that undergo radical nephrectomy [74]. The survival rate of early RCC is 90%, while that of locally advanced or metastatic cases is only 13%. Therefore, early diagnosis and treatment of RCC is extremely prominence for improving the 5-year survival rate of this disease. Liquid biopsy techniques provided for early RCC detection may bring about a superior outcome. EVs detection can guide clinical rationalization, individualization, and precision of medication by assessing the sensitivity of patients to drugs. Ras-related protein Rab-27B is one of the chief proteins involved in the secretion of EVs. Previous studies have confirmed that the high expression of this protein is related to the poor prognosis of hepatocellular carcinoma, ovarian cancer, and colorectal cancer. Tsuruda et al. identified that Rab-27B was significantly overexpressed in sunitinib-resistant renal cell carcinoma cell lines [75]. Dias et al. found that compared with patients with nonmetastatic in situ ccRCC, patients with metastatic disease had higher levels of matrix metalloproteinase inhibitor of metalloproteinase-1 (TIMP-1) mRNA in plasma EVs and lower overall survival rate. This means TIMP-1 mRNA derived from EVs is a potential prognostic marker for ccRCC [76].

Additionally, researchers also found that after 1 day of cryoablation treatment, the miR-17-5p, miR-126-3p, and miR-21-3p in mouse serum-derived EVs rapidly decreased, reflecting the number of active tumors, which can be used to evaluate tumor clearance efficacy and dynamically monitor tumor burden [77]. Du et al. applied Cox regression analysis and Kaplan Meier analysis to confirm the significant overall survival (OS) association of three EVs source miRNAs (miR-let-7i-5p, miR-615-3p, and miR-26a-1-3p. Then, these miRNAs in plasma exosomes

were used as prognostic markers for metastatic kidney cancer [78]. Nakanori et al. found that intracellular miR-224 increases in ccRCC and is obviously relevant to cancer metastasis and invasion. Then, they explored the relevance between the level of exo-miR-224 and the prognosis of 108 ccRCC patients, and they observed that progression-free survival and overall survival are shorter in the group with high exo-miR-224 expression level. Thus, they deemed that extracellular miR-224 in ccRCC patient's exosomes is a prospective prognostic marker [79]. Sunitinib is a novel multi-targeted oral drug for treating tumors and is also used for treating advanced renal cell carcinoma. The drug resistance is the main challenge of current treatment. Sun et al. detected lncRNA Activated in RCC with sunitinib resistance (lncARSR), a lncRNA related to the clinically poor response to sunitinib. LncARSR may improve the level of AXL and c-MET in RCC cells by competitively binding to miR-34/miR-449 and recovery the drug resistance of sunitinib. In addition, this lncRNA also transmits its drug resistance mechanism to sensitive cells through exosomes. Therefore, it is suggested that lncARSR may predict sunitinib resistance and a potential therapeutic target (**Table 1**) [80].

Marker	Expression disorder	Origin	Application	References
miR-150	Upregulated	RCC cell line (786-O)	Diagnosis	[56]
miR-205	Downregulated			
miR-92a-1-5p	Downregulated	Plasma	Diagnosis	[57]
miR-149-3p & miR-424-3p	Upregulated			
miR-210 & miR-1233	Upregulated	Serum	Diagnosis	[58]
miR-4525	Upregulated	Serum	Diagnosis	[59]
combination of miR-34b-5p or miR-449a & miR-126-3p		Urine and cell line mode	Diagnosis	[61]
GSTA1, CEBPA, and PCBD1	Downregulated	Urine	Diagnosis	[63]
CD147, CA9, and CD70	Upregulated	Cell lines and tissue samples	Diagnosis	[64]
CA9	Increased	Plasma	Diagnosis and prognosis	[65]
PTRF/CAVIN1	Increased	Urine	Diagnosis	[66]
MALAT1		Cell line	Metastasis monitoring	[68]
CD103		Cancer stem cell	Metastasis Monitoring	[69]
miR-1293	Increased	Plasma	Metastasis monitoring	[70]
miR-301a-3p	Decreased			
miR-483-5p	Downregulated	Plasma and tissues	Metastasis monitoring	[71]
hsa-miR-301a-3p	Increased	Plasma	Metastasis monitoring	[72]
hsa-miR-1293	Decreased			

Marker	Expression disorder	Origin	Application	References
PMSA	Increased	Serum	Metastasis monitoring	[73]
RAB27B	Increased	Cell lines	Drug resistance	[75]
TIMP-1 mRNA	Increased	Plasma	Prognosis	[76]
miR-126-3p	Decreased	Serum	Prognosis	[77]
miR-17-5p	Decreased			
miR-21-3p	Decreased			
miR-let-7i-5p	Increased	Plasma	Prognosis	[78]
miR-26a-1-3p	Increased			
miR-615-3p	Increased			
miR-224	Increased	Blood and cell lines	Prognosis	[79]
lncARSR	Increased	Cell line, plasma, and tissue	Drug resistance	[80]

Table 1.
A list of candidate dysregulated EV contents as molecular markers for RCC.

3.2 Extracellular vesicles as diagnostic markers for renal tubulointerstitial injury

Some biomolecules carried in urine EVs are closely related to the degree of renal tubulointerstitial inflammation and fibrosis; the detection of these markers in EVs can reflect the degree of renal inflammatory fibrosis progression. After genetic screening of EVs in urine of chronic kidney disease (CKD) patients, it was found that miR-29c and CD2AP mRNA were positively correlated with the degree of renal fibrosis and renal function, which may have meaningful diagnostic value for the progression of renal fibrosis [81, 82]. Based on previous findings that EVs CCL2 mRNA released by renal tubular epithelial cells (TECs) can directly transfer to macrophages and promote tubulointerstitial inflammation, some researchers found that the expression of CCL2 mRNA in urine EVs of IgA nephropathy (IgAN) patients is related to the level of eGFR and can predict the progress of renal function, which may become a new marker for IgAN prognosis monitoring [83]. Hypertension is a noteworthy cause of CKD, and the loss of peritubular capillaries (PTC) is one of the characteristics of hypertension; the level of endothelial microparticles (EMP) in the loop can report the endothelial injury systematically. Urinary EMPs levels were examined as possible markers of PTC and decreased fibrotic density. Ptc-emps were peculiar proteins that were identified as plasmalemmal vesicle-associated protein (PL-VAP) positive urinary exosomes, which were showed in PTC and Vas deferens endothelium but not in glomeruli and arteries and are supposed to be a novel biomarker of intrarenal capillary loss [84]. In obstructive kidney disease, uEVs may help assess the risk of developing renal dysfunction, and some studies have observed that the profibrotic factor TGF-β1 level in uEVs is related to glomerular filtration rate [85]. It was the upregulated of the urinary exosomal miR-181a (200-fold) in CKD patients [86] and the downregulated of the exosomal level of secreting transglutaminase-2 (a fibrosis-activating enzyme) in UUO mice [87]. CKD patients have a decrease in miR-200b of urinary exosomes, with

the greatest reduction in urinary exosomes originating from cells outside the proximal renal tubules [88]. The upregulation of the urinary exosomal ceruloplasmin in CKD patients and animals with passive Heyman nephritis was observed [89]. Moreover, osteoprotegerin, an inflammatory marker, was shown to be increased in the uEVs of patients with CKD [90].

The kidney is a major organ that regulates the body's water and salt metabolism. Ions and aquaporin are distributed in different segments of TECs. During the injury of TECs, these ions and water transporters in urine EVs also changed correspondingly. For example, the decrease of aquaporin (AQP) expression in urine EVs was observed in the rat model of Acute kidney injury (AKI) induced by cisplatin and ischemia/reperfusion, suggesting its potential impact on the change of renal concentration function [91]. The level of EVs Na + transporters in urine may also indicate certain pathological conditions in the kidneys. The activation of renin angiotensin aldosterone system (RAAS) after sodium-restricted diet acute aldosterone infusion in patients with hypertension increases the content of epithelial sodium channel (ENaC) in urine EVs by nearly 20 times, suggesting that it may be a novel means to monitor RAAS activation [92]. Moreover, the significant reduction of furosemide-sensitive sodium potassium chloride cotransporter (NKCC2) and sodium chloride cotransporter (NCC) in urine EVs has been used to distinguish different phenotypes of hereditary desalinization renal tubular disease [93, 94]. These studies suggest that changes in ion and water transport proteins in urine EVs may reflect renal tubular damage.

3.3 Extracellular vesicles as diagnostic markers for glomerular injury

EVs derived from glomeruli are continuously released into urine under physiological conditions, so changes in urine EVs can reflect the degree of glomerular disease, including podocyte damage. In early studies, Wilms Tumor Protein 1 (WT1) has been identified as a key regulator of podocyte expression, and the WT1 target gene is crucial to maintain the glomerular filtration barrier [95]. WT1 in uEVs was confirmed to be detectable before obvious glomerular sclerosis and urinary EVs WT1 appeared prior to proteinuria and glomerular histological damage in focal segmental glomerulosclerosis (FSGS) animal models [96]; moreover, urinary exosomal WT-1 was significantly decreased in patients in remission for either FSGS or steroid-sensitive nephrotic syndrome (SSNS) or following steroid treatment of patients with SSNS [97]. Compared with patients with minimal change nephropathy (MCN), the WT1 mRNA expression was increased significantly in urine EVs of patients with diabetes nephropathy (DN) and was associated with decreased eGFR; thus the high expression of WT1 mRNA in urine EVs distinguishes patients with DN from patients with MCN [98]. In addition, hyperglycemia can stimulate the release of WT1 from podocyte to urine EVs, so the detection of WT1 in urine EVs can indicate early damage of podocyte in diabetes patients. WT-1 may be a biomarker for early diagnosis of podocyte injury, as suggested by these data. Recently, it has been found that the expression of the upper pitt-specific transcription factor ELF3 protein in urine EVs is in patients with DN, but not in patients with MCN. Urine EVs ELF3 can predict the decline of eGFR in patients with DN in the next few years [99]. It is a practical diagnostic tool that uEVs is applied to distinguish early IgA nephropathy (IgAN) and thin basement membrane nephropathy with microscopic hematuria in children and adults. Moon et al. discovered four various biomarkers that have different expressions in the uEV of these patients and the high levels of aminopeptidase N and vasoactive precursors

in the thin basement membrane nephropathy group, while α-1-Elevated levels of antitrypsin and ceruloplasmin were higher in the IgAN group [100].

3.4 Extracellular vesicles as diagnostic markers for other kidney disease

The biological processes involved in cytoskeleton-regulating and Ca(2+)-binding proteins are closely related to the pathogenic state of renal tubular epithelial cells in autosomal dominant polycystic kidney disease (ADPKD). Some study found by iTRAQ-based quantitative proteomics that this differential expression of proteins in urine EV of ADPKD demonstrates the possibility of using urine EV to monitor patient status [101, 102]. Fabry disease, also known as Anderson-Fabry disease, is the most common lysosome accumulation disease [103]. It is an X-linked congenital defect in the pathway of glycosphingolipid metabolism, which causes the accumulation of globotriaosylceramide (Gb3) in a variety of lysosome, leading to a series of clinical manifestations. Fabry nephropathy is kidney impairment, mainly manifested as hypertension, hematuria, mild proteinuria and fatty urine, and various renal tubular dysfunction, such as concentration and dilution function [104]. Some scholars observed the increased expression of miR-29a-3p and miR-200a-3p in uEVs of Fabry nephropathy patients may reveal an attempt by this organism to inhibit the progression of renal lesions leading to end-stage renal disease (ESRD).

Pathema	Liquid biopsy marker	Level related to healthy	Type of regeneration or lesion	Detection way
Autosomal dominant polycystic kidney disease (ADPKD)	S100-A9, S100-A8, annexin A1, and annexin A2	increased	urinary EVs	iTRAQ-based quantitative proteomics [101, 102]
Fabry nephropathy	miR-200a-3p, miR-29a-3p	increased	Urine EVs	qPCR [105]
Acute kidney injury	AQP1	Decreased	Tubular cell injury	Western blot [107]
	ATF3	Increased		qRT-PCR [96]
	Fetuin A	Increased		LC-MS/MS, MALDI-TOF, western blot [108]
	miR-130a and miR-145	Increased	Glomerular	qRT-PCR [109]
	miR-155 and miR-424	Decreased	mesangial damage	
	ELF3	increased	Podocyte damage	Western blotting [99]
	WT1	Increased	Podocyte damage	Western blot [110]
	AEBP1 mRNA	increased	Not known	Bioinformatics analysis [111]
	hsa-miR-503 and hsa-miR-451a	increased	uEVs	next-generation small RNA sequencing & qRT-PCR [112]

Pathema	Liquid biopsy marker	Level related to healthy	Type of regeneration or lesion	Detection way
Hypertensive kidney injury	PL-VAP	increased	Urine	flow cytometry [84]
Focal segmental glomerulosclerosis	WT1	Increased	Podocyte damage	Western blot [97]
Chronic kidney disease	CCL2 mRNA	Increased	tubular epithelial cells	qRT-PCR [83]
	CD2AP mRNA	Decreased	Podocyte damage	qRT-PCR [82]
	miR-29	Decreased	Fibrosis	qRT-PCR [81]
	miR-200b	Increased		
	secreting transglutaminase-2	Increased	UUO	Proteomic [87]
	ceruloplasmin	increased	Urine	ELISA [89]
	miR-181a-5p and miR-451	Increased	Not known	qRT-PCR [86, 113]
IgA nephropathy	miR-378, miR-215-5p	Increased	Not known	qRT-PCR [114]
	miR-205-5p, miR-29c	Decreased		
	α-1-antitrypsin & ceruloplasmin	increased	Urine	semi-quantitative immunoblot analysis [100]
Kidney transplantation	NGAL	Increased	Tubular cell injury	Western blot [115]
	CD133	Decreased	Tubular cell regeneration	FACS, western blot [116]
Medullary Sponge Kidney Disease	Ficolin 1, Mannan-binding lectin serine protease 2, and Complement component 4-binding protein β	increased	Urine EVs	ELISA [117]
Lupus nephritis	miR-146a, miR-150, miR-21	Increased	Fibrosis	qRT-PCR [118]

Table 2.
Deregulated EVs and their contents as the molecular marker of nonneoplastic kidney disease.

Meanwhile, the expression of miR-30b-5p increased within 10 years in uEVs of patients without renal dysfunction, which may play a defensive part in podocyte trauma and may play a vital role in Fabry nephropathy [105].

In summary, the "liquid biopsy" based on EVs currently shows good application prospects in the diagnosis of kidney disease. In future research, it is necessary to further explore the standardization of urine sample separation, storage, and processing [106], strengthen the large-scale cohort study of the diagnostic, prognostic and predictive value of EVs biomarkers in different kidney diseases, and promote the technical research on the traceability of urine EVs kidney cells (**Table 2**).

4. Extracellular vesicles in the therapeutic role

Renal pathophysiology is a multivariate procedure concerned with different kidney structures. Acute kidney injury (AKI) is a group of clinical syndromes, the manifestations include oliguria, anuria, edema, loss of appetite, etc., with the character of tubular necrosis and glomerular hyperfiltration. The maladaptive repair after AKI can easily lead to chronic kidney disease, also known as CKD. CKD is a growing and irretrievable damage of kidney function, the athological manifestation being fibrosis that could lead to ESRD. Currently, kidney disease therapies based on EVs can be divided into two categories: firstly, certain specific cells, such as stem cell-derived EVs, can be directly applied as therapeutic drugs due to their carrying bioactive molecules; Secondly, EVs can serve as delivery carriers for various types of drugs for the treatment of kidney diseases [119, 120].

4.1 Application of stem cell-derived EVs in the treatment of kidney disease

Timmers et al. first confirmed that the conditioned medium of human mesenchymal stem cells had a protective effect on myocardium in 2007, they also confirmed that the main active substances involved this were at 100–220 nm by size analysis [121]. The team further isolated and identified the substance in 2010, identifying it as exosomes. Since then, more and more studies have confirmed that the EVs derived from mesenchymal stem cell (MSC-EVs) play a major part in tissue damage repair and immune regulation, and have developed their therapeutic potential in the matter of regenerative medicine. In recent years, stem cell-derived EVs have been studied in different animal kidney disease models in vivo, including AKI, diabetes nephropathy, hypertensive nephropathy, unilateral ureteral obstruction, and subtotal nephrectomy.

4.1.1 Acute kidney injury (AKI)

AKI is a general clinical condition, and there is no clear and effective treatment method. In recent years, stem cell EVs have been proven to play therapeutic roles in AKI, including anti-apoptotic, anti-inflammatory, and antioxidant stress. BRUNO et al. proved that in the glycerol-induced AKI mouse model, the bone marrow mesenchymal stem cells derived EVs accelerate the repair of damaged renal tubular cells, promote renal tubular cell proliferation, and protect cells from apoptosis [122]. Extracellular vesicles derived from bone marrow mesenchymal stem cells were also verified in the toxic AKI model induced by cisplatin and gentamicin [123, 124], which can improve renal function, reduce histological damage, and alleviate renal fibrosis. The bone marrow mesenchymal stem cells derived EVs have also achieved the same effect in renal ischemia–reperfusion (I/R) injury models [125, 126].

There are many mechanisms by which stem cell-derived EVs can improve AKI. Studies have found that the bone marrow mesenchymal stem cells derived EVs may carry specific mRNAs to activate the proliferation process of surviving renal tubular cells after injury, so that the impaired cells can re-enter the cell cycle.

Recently, CAO and his team sequenced human umbilical cord mesenchymal stem cells derived EVs and found that they are rich in miR-125b-5p and can inhibit G2/M cell cycle arrest and apoptosis of TECs by targeting p53, thereby promoting renal tubular repair and improving ischemic AKI [127]. Li et al. found that human urine-derived stem cells EVs can defend renal function of ischemia–reperfusion rats by carrying miR-146a-5p, which can target the 3′-untranslated coding region of

IL-1 receptor-associated kinase (IRAK), thereby inhibiting NF-κ activation of the B signaling pathway and infiltration of inflammatory cells [128]. Lately, someone conducted EVs derived from human bone marrow combined with pulse-focused ultrasound therapy on a cisplatin-induced mouse model and found that the decreased expression of NLRP3 inflammasome and its downstream pro-inflammatory cytokine IL-1βand IL-18 decreased promoted renal repair after AKI [129]. These studies suggest that MSC-EVs exert the therapeutic effect of AKI through anti-inflammatory effects. The study also found that MSC-EVs can also inhibit mitochondrial damage in the IRI model through various pathways, thereby alleviating AKI. MSC-EVs derived from human placenta activate the Keap1-Nrf2 signaling pathway, stimulate mitochondrial antioxidant defense mechanisms to maintain the stability of TEC mitochondrial structure and regulate mitochondrial function, participate in TEC damage repair, and promote renal function recovery [130]. In addition, there are reports that MSCs-EVs can reduce mitochondrial damage and inflammation caused by AKI through the mitochondrial transcription factor A (TFAM) pathway [131]. EVs originating in umbilical stalk blood mesenchymal stem cells promote dedifferentiation and proliferation of renal tubular cells; extracellular vesicles derived from umbilical cord Wharton glue mesenchymal stem cells stimulate cell proliferation, reduce inflammation, and apoptosis through mitochondrial protection. 3D cultured placental mesenchymal stem cells derived EVs more effectively inhibit cell apoptosis, inflammation and improve renal function [132–134]; Human urinary mesenchymal stem cells derived EVa accelerate renal recovery, stimulate renal tubular cell proliferation, reduce the expression of inflammatory and injury markers, restore endogenous Klotho loss, and thus protect renal function [135, 136].

4.1.2 Chronic kidney disease (CKD)

It is a significant pathological manifestation in CKD progression of persistent kidney tubulointerstitial fibration. Scholars have found that hBMSC-EVs can inhibit TGF through their rich miR-294/miR-133-β1 mediates epithelial-mesenchymal transformation in CKD rats, thereby alleviating renal interstitial fibrosis (RIF) [137]. Moreover, particles derived from renal-derived MSCs can improve peritubular capillary sparsity in the kidney and delay the progression of renal injury by inhibiting tubulointerstitial fibrosis in mice UUO [138]. EVs produced by bone marrow mesenchymal stem cells derived EVs alleviate UUO renal fibrosis, partially by inhibiting the RhoA/ROCK pathway [139]. In a mouse CKD model induced by chronic cyclosporin, bone marrow derived EVs can improve the kidney function in the inflammatory microenvironment [140]. Extracellular vesicles rich in miR-196b-5p mediate crosstalk between proximal tubular epithelial cells and fibroblasts, which may be related to the STAT3/SOCO2 signaling pathway and mediate aldosterone-induced renal fibrosis with diabetes [141]. However, miR-221 in the EVs derived from podocyte reversed DN by inhibiting Wnt/β-Catenin signaling mediated proximal tubular cell damage [142]. EVs from human liver stem cell through miR29b reduce renal fibrosis by disturbing the β-Catenin pathway [143]. In a recent study, researchers synthesized a biological scaffold, which integrates MSC-EVs, extracellular matrix, poly (lactic acid Glycolic acid) copolymer, poly (Deoxyribonucleotide), etc. This biological scaffold achieved renal tissue remodeling in a partial nephrectomy mouse model by promoting cell proliferation, angiogenesis, and inhibiting fibrosis and inflammation [144]. Eirin et al. found that intrarenal injection of adipose-derived mesenchymal stem cells EVs improved pigs with metabolic syndrome and renal

artery stenosis disease by ameliorating renal inflammation and fibrosis. Further research confirmed that these renal protective effects were mediated by the carrier of anti-inflammatory cytokine IL-10 carried by MSC-EVs [145]. The secretion of EVs from mesenchymal stem cells stimulated by melatonin inhibits fibrosis in renal tissue by regulating cell apoptosis and proliferation of fibrosis related cells [146]. Stem cell-derived EVs also improve CKD related lesions. CHOI et al. found that renal mesenchymal stem cells expressing erythropoietin EVs improve renal anemia in mice with chronic kidney disease [147].

4.1.3 Diabetic nephropathy (DKD)

Diabetes nephropathy (DKD) is one of the elemental etiology of terminal-stage kidney disease [148]. For a long time, there is no specific drug for DKD, and the current treatment is limited to blood glucose control, blocking of renin-angiotensin-aldosterone system, and changes in lifestyle [149]. MSC-EVs can protect cells from high glucose-induced damage by promoting regeneration through anti-apoptosis, anti-fibrosis, and autophagy. Jin et al. confirmed that miR-486 carried by EVs derived from fat MSCs can be transferred to podocyte, which leads to increased autophagy flux and decreased apoptosis by inhibiting the Smad1/mTOR signal pathway of podocyte in DKD mice [150], thereby improving podocyte injury in DKD mice. In addition, MSC-exos can reduce the overexpression of TGF-βto improve tubulointerstitial fibrosis in DN mice and regulate the expression of ICAM-1 for inhibiting inflammatory cell infiltration, thereby reducing diabetes kidney damage [151]. Xiang et al. found that umbilical cord mesenchymal stem cells derived EVs can reduce inflammatory factors (IL-6, TNF-α) in renal tubular cell expression, reduce inflammatory cell infiltration, interstitial fibrosis, and other pathological changes of diabetes nephropathy in renal tissue [152]. Mesenchymal stem cells derived EVs can regulate autophagy through the mTOR pathway, upregulate autophagy proteins such as LC3 and Beclin-1, and observe an increase in autophagic vesicles under electron microscopy, and the treatment of the MSC-derived EVs decreased the urine protein and serum creatinine in diabetes nephropathy mice. Renal biopsy showed that the renal pathological changes of diabetes nephropathy such as mesangium expansion and fibrosis were alleviated [153]. Jin et al. observed that the adipose stem cells derived exosomes could reverse the damage of renal function caused by high glucose environment and further found that miR-486 is a critical determiner in the reverse process, which can reduce the expression of Smad1, increase cell autophagy, and reduce podocyte apoptosis [150].

Recently, researchers injected two doses of MSC-EVs into patients with CKD phase III and IV in a single center, stochastic, placebo-contrast phase II/III clinical trial, and the results showed that the renal inflammation and function were effectively improved in the treatment group, providing worthwhile clinical evidence for the employ of MSC-EVs in the therapy of CKD. Unfortunately, this study did not investigate its mechanism and related renal pathological types [154]. Obviously, it has extremely clinical significance to further strengthening research.

4.2 Application of EVs based drug targeted delivery in the treatment of kidney disease

In 2010, Sun et al. wrapped curcumin with anti-inflammatory and antioxidant effects in EVs for the first time to treat sepsis in mice and achieved good therapeutic

effects [155]. Since then, targeted drug development based on EV carriers has continued to heat up. Existing research suggests that EVs can successfully load various types of drugs such as nucleic acid drugs, protein drugs, and small molecule drugs for the treatment of kidney disease.

4.2.1 EVs as nucleic acid drug carriers

Various types of nucleic acids are the main ingredients of EVs; therefore, it has aroused widespread interest in using EVs as delivery carriers for nucleic acid drugs. Previous studies have shown that EVs can load and transport nucleic acids such as mRNA, miRNA, and small interfering RNA (siRNA) for disease treatment [156]. Among them, siRNA therapy has shown great potential in the treatment of human diseases. However, due to its instability and missing target effect, the clinical application of RNA interference technology has been limited. Recently, TANG et al. successfully developed a siRNA delivery system based on red blood cell-derived EVs, which utilizes renal injury molecule-1 targeted peptide A Kim-1-binding peptide (LTH) to modify red blood cell-derived extracellular vesicles (REVs) and successfully deliver siP65 and siSNAI1 to the injured renal tubules, effectively improving renal tubulointerstitial inflammation and fibrosis induced by ischemia-reperfusion and UUO, and blocking the chronic progression of AKI [157]. Meanwhile, they also applied muscle-targeted peptide-modified EVs with loaded miR-26a, significantly improving Sarcopenia in 5/6 nephrectomized CKD mice [158]. Combining EVs with a kidney-targeting peptide, Rabies Virus Glycoprotein (RVG), and loading miR-29a into it, can not only alleviate the myopenia in mice with UUO but also improve renal fibrosis through EVs-mediated communication between skeletal muscle and renal organs [159]. Therefore, the targeted therapy of nucleic acid drugs based on EVs can not only delay the progression of kidney disease but also improve its complications. A different strategy was applied and studies have shown that EVs from engineered MSCs overexpressing miR-let7c can shift miRNA to renal cells and inhibit RIF [160]. In addition, BM-MSC-derived EVs overexpressing miR-34a embellished by lentivirus inhibited TGF-β1 begotten epithelial-mesenchymal transition (EMT) in human renal tubular cells [161].

4.2.2 EVs as protein drug carriers

Protein deficiency and dysfunction are important causes of many diseases. Therefore, it is one of the methods for treating diseases by increasing the corresponding protein levels. However, protein drugs themselves have drawbacks, such as high molecular weight and poor stability, which limit their clinical application. The loading performance and modifiability of EVs have brought dawn to this field. Recently, Researchers constructed a cytokine IL-10 delivery system (IL-10 + EVs) using macrophage-derived EVs as a vector. The loading of EVs not only improved the stability of IL-10 but also demonstrated a unique ability to target kidney damage. Further mechanism research has found that IL-10 + EVs can promote mitochondrial autophagy in TECs by suppression the sensitization of the mTOR signaling pathway, significantly improving renal injury and chronic lesions induced by ischemia-reperfusion [162]. Kim et al. utilized a novel photogenetic engineering technique to load NF-κ inhibition protein srIκB into EVs, effectively improving the inflammatory response and cell apoptosis in the kidney after ischemia [163].

4.2.3 EVs as small molecule drug carriers

Research has found that the encapsulation and delivery of EVs can improve the targeting, cell uptake efficiency of small molecule drugs, also improve drug stability, and reduce toxic side effects [119, 164]. MSCs-EVs have been considered as another prospective acellular therapy for AKI. A Supermolecule hydrogel containing Arg-Gly-Asp (RGD) peptide has been manufactured to enhance the efficiency in the therapy of AKI. Data shows that RGD-EV hydrogel has a good rescue influence on renal function at the early stage of AKI, by dwindling tubular damage and facilitating cell proliferation through the combination of RGD and integrin [165]. Recently, researchers constructed M2 macrophage-derived EVs loaded with dexamethasone (DEX) and found that it not only targets damaged kidneys but also has effective anti-inflammatory and anti-fibrotic effects. Moreover, it significantly alleviates the adverse effects of DEX on blood glucose and the hypothalamic-pituitary renal gland axis in mice impact [166]. In the AKI model, EVs derived from MSCs ameliorative to over-express the octamer binding transcriptional factor 4 (OCT4) showed decreased expression of Snail, a trigger factor for epithelial-mesenchymal transition (EMT). Therefore, the administration of EV-ameliorated MSCs has achieved better renal tissue recovery, cell proliferation improvement, cell death elimination, and the initial fibrosis process block [132].

All in all, EVs have become a worthwhile carrier for the next generation of targeted drug delivery and have received widespread attention in recent years based on the advantages of good biocompatibility, low immunogenicity, and modifiability of EVs Cell therapy. Of course, there are still issues that need to be addressed: producing EVs that are repeatable, large-scale, high-throughput, and meeting the clinical application level; suitable parental cells selecting, culture systems such as cell factories, bioreactors, and hollowing fiber tubes establishing to achieve large-scale expansion production [167]; standardized EVs separation technology suitable for large-scale production building, a high-throughput detection platform developing, the quality of the EVs production process control and quality monitoring at the single particle level achieving, thereby further achieving standardized quality control and improving drug loading efficiency.

5. Conclusion

In the past 20 years, research on EVs has made rapid progress. More and more preclinical studies have shown that biomarkers and related treatment technologies based on EVs have great prospects in detecting and treating kidney disease, laying the foundation for their clinical application. Further strengthening the basic and clinical research on the role of EVs in making a diagnosis and giving treatment of kidney diseases, developing standardized, clinical-level EVs separation, purification, and quality control, and strengthening clinical queue research will offer technical support for the clinical transformation and application of electric vehicles.

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