Extracellular Vesicles in Diagnosis and Therapy of Kidney Diseases

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Running Title: Extracellular vesicles in kidney diseases

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Abstract: Extracellular vesicles (EV) are endogenously produced, membrane-bound vesicles that contain various molecules. Depending on their size and origins, EVs are classified into apoptotic bodies, microvesicles, and exosomes. A fundamental function of EVs is to mediate intercellular communication. In kidneys, recent research has begun to suggest a role of EVs, especially exosomes, in cell-cell communication by transferring proteins, mRNAs, and microRNAs to recipient cells as nanovectors. EVs may mediate the cross-talk between various cell types within kidneys for the maintenance of tissue homeostasis. They may also mediate the cross-talk between kidneys and other organs under physiological and pathological conditions. EVs have been implicated in the pathogenesis of both acute kidney injury and chronic kidney diseases, including renal fibrosis, end-stage renal disease, glomerular diseases, and diabetic nephropathy. The release of EVs with specific molecular contents into urine and plasma may be useful biomarkers for kidney disease. In addition, EVs produced by cultured cells may have therapeutic effects for these diseases. However, the role of EVs in kidney diseases is largely unclear and the mechanism underlying EV production and secretion remains elusive. In this review, we introduce the basics of EVs and then analyze the current information about the involvement, diagnostic value, and therapeutic potential of EVs in major kidney diseases.

Keywords: exosome, biomarker, therapy, acute kidney injury, chronic kidney disease
I. Introduction

Extracellular vesicles (EVs) are membrane-bound vesicles produced and released by a cell that contain various molecules, such as proteins, lipids, DNA, mRNA, and microRNAs. Recent research has demonstrated an emerging role of EVs in mediating cell-cell or intercellular communication (13, 40). By this function, EVs acts as a highly conserved mechanism for signal transmission between cells in diverse biological and physiological processes. As such changes and dysfunction in EVs may be associated with the development of diseases, and the molecules or molecular signature of EVs may serve as non-invasive diagnostic biomarkers of diseases. Moreover, the unique biological activity of EVs has displayed potential benefit for the correction of cellular dysfunction and in turn, the therapy of diseases (21). EVs have also been considered to be ideal nanovectors for bio-delivery, specifically for drug delivery in clinical application (22, 60). In kidneys, renal EVs are produced and secreted by kidney cells and have been implicated in renal function and diseases (40). In this review, we aim to provide an overview of the basic science of EVs and analysis of the available information regarding the involvement of EVs in major kidney diseases.

II. Extracellular vesicles

Three main types of EVs have been described, including apoptotic bodies, microvesicles or microparticles, and exosomes (62, 66). They are distinguished from each other by their origin, size, and content (Table 1). In general, the sizes of apoptotic bodies, microvesicles, and exosome are >1000, 100–1000, and 40–150 nm in diameters, respectively. In terms of origin, microvesicles and apoptotic bodies are derived directly
from the plasma membrane, whereas exosomes originate from the endosome. Endosomes form the multivesicular body (MVB) and subsequently fuse with the plasma membrane to release exosomes (58). EVs contain proteins, lipids, and RNAs, while apoptotic bodies may also contain DNA fragments. Some common proteins identified in EVs include TSG101, tetraspanins (CD9, CD63), heat shock proteins, annexins, flotillin, Alix from MVB, and membrane fusion protein GTPases. EVs also contain lipids, such as cholesterol and sphingomyelin, and RNAs, such as mRNA and microRNA (33). EV release is either spontaneous or induced, depending on cell type and functional status. It has been reported that EV production and release depends on the activation of specific cell surface receptors and a number of enzymes (18, 32). For exosomes, the endosomal sorting complex required for transport (ESCRT) involved in MVB formation is the main regulatory system, while the other regulatory molecules, such as Rab GTPase, contribute as well (27, 34).

### III. Isolation of extracellular vesicles

EVs are released by most cell types and are present in various body fluids, including urine, serum, and saliva (43). The isolation method is a key to EV research and clinical application. Currently, several EV isolation methods have been described and commonly used. Traditionally, EVs are isolated and purified by differential centrifugation (64); the differences in isolation of main types of EVs by centrifugation are summarized in Table 1. In the last few years, commercial kits for EV isolation became available, which are mainly based on sucrose or iodixanol density gradient to obtain EVs at relatively higher yields than differential centrifugation alone (61). More recently, a polyethylene glycol-
based method for enrichment of EVs, called Extra PEG, has been described (54).

Apparently, Extra PEG may provide a more convenient method as it reduces the requirement of ultracentrifugation, but the merits and pitfalls of this method remain to be verified. Very few studies have isolated EVs from kidney tissues. Nonetheless, Borges et al. reported the increase of exosomes from kidney tissues following unilateral urinary obstruction (7). In this study, exosomes were isolated from kidney cortex by mechanical sectioning and enzymatic digestion with collagenase and trypsin, followed by ultracentrifugation of the supernatant (7).

IV. Extracellular vesicles in kidneys

Recent research has implicated EVs in renal physiology and the pathogenesis of major kidney diseases. Almost all cell types in kidneys produce and secret EVs. As summarized in Fig 1, exosomal proteins may originate from all segments of the nephron, including glomerular podocytes, proximal tubules, thick ascending limb of Henle, distal tubule, and the collecting duct (50). Further studies (25, 50) demonstrated that due to the glomerular structure of mechanical and charge barriers, circulating EVs from serum cannot cross the nephron at least under physiological conditions, suggesting that urinary EVs (uEVs) are mainly derived from renal cells.

EVs in kidneys also function as an important vector for intercellular communication. Early work by Brown et al. showed that the proteins of proximal tubule cells may be transported to downstream collecting duct cells (8). This concept has been verified by recent studies. For example, Street and colleagues suggested that collecting duct epithelial cells may release exosomes that contain Aquaporin-2 (AQP2) and this
release is physiologically regulated. Exosomes can transfer functional AQP2 between cells, representing a novel mechanism for cell-to-cell communication within the kidney(57). Indeed, a number of proteins from various segments of the nephron may be released into urine or transported via EVs to other cells in kidneys (Figure 1).

V. EVs as diagnostic biomarkers for kidney diseases

AKI:

As indicated above, proteomics analysis of urinary exosomes has confirmed that exosomal proteins may be originated from all segments of the nephron. Importantly, the content of exosomes may change in response to various patho-physiological conditions. As such, exosomal proteins may be biomarkers for specific diseases. In this regard, Fetuin-A and Aquaporin-1 (AQP1) protein level in urinary exosomes have been identified as potential biomarkers for AKI. During renal ischemia/reperfusion (I/R) in rats, AQP1 protein in urine exosomes decreased significantly. Remarkably, similar decreases were detected in patients after renal allograft transplantation, suggesting that urinary exosomal AQP1 may be a biomarker for AKI induced by renal I/R (56). For fetuin-A, a marked increase was detected in urinary exosomes in both animal models and human patients of AKI; moreover, the increase of fetuin-A occurred prior to the increase of serum creatinine (69). In addition, Zhou et. al. detected an increased level of activating transcription factor 3 (ATF3) in urinary exosomes (but not in whole urine) in both cisplatin and I/R -induced AKI in mice (67). Again, this increase started prior to serum creatinine, indicating that ATF3 may be a biomarker for early diagnosis of AKI. In support of this possibility, a clinical study showed that urinary exosomes containing ATF3 mRNA were 60-fold
higher in AKI patients than that of normal controls (15).

Urinary Neutrophil gelatinase-associated lipocalin (NGAL) is a recently identified molecular biomarker of AKI. Interestingly, recent work demonstrated that NGAL in urinary exosomes correlated with delayed graft function after kidney transplantation, suggesting that urinary exosomal NGAL may be an early biomarker for prognosis in post-renal transplantation condition (2). Thus, urinary exosomes may be the sites of concentration of biomarkers of renal diseases, making it a potential source for further identification of sensitive biomarkers for AKI.

Renal fibrosis and end-stage renal disease:

In a clinical study, it was observed that the mRNA level of CD2AP, a podocyte marker, was reduced in uEVs from patients with CKD. Importantly, this down-regulation was correlated with renal function, level of proteinuria, and the severity of renal fibrosis, suggesting its biomarker potential (45). Further studies analyzed microRNAs in uEVs of CKD patients. It was shown that exosomal miR-29 and miR-200 were significantly reduced in CKD patients as compared with controls, and notably the reduction correlated with renal function decline and the degree of tubular-interstitial fibrosis (44). In addition, osteoprotegerin, an inflammatory marker, was shown to be increased in uEVs of CKD patients (6). These data suggest that EVs may reflect renal fibrosis as well as the inflammatory state within the renal microenvironment of CKD patients.

In obstructive nephropathy, uEVs may be useful for evaluating the risk of developing renal dysfunction (59). uEVs in the patients affected by posterior urethral valves contained much higher levels of TGF-β1 and cell adhesion molecules than the
control group. In addition, the level of the pro-fibrotic factor TGF-β1 in uEVs showed a correlation with the glomerular filtration rate.

In ESRD, circulating EVs (endothelial microparticles, EMPs) was reported to impair endothelial-dependent vasorelaxation, which may be associated with the decrease in endothelial nitric oxide release and endothelial function (3). A prospective study involving 81 patients on hemodialysis suggested that EMPs may be able to predict all-cause and cardiovascular mortality for ESRD hemodialysis patients (47). If verified, this would help identifying patients who need more aggressive or intensive treatment. Other studies have reported that the level of circulating EVs derived from endothelial cells may correlate with arterial stiffness of hemodialysis patients (20, 23). Moreover, it was demonstrated that the increased serum EMPs may be a reliable independent predictor of outcome in patients with (ESRD)(4).

Glomerular diseases:

EVs derived from glomeruli are constantly released into urine in physiological state. As a result, changes of uEVs are considered to be a direct sign of glomerular diseases, including podocyte injury. In this aspect, Wilms' tumor 1 transcription factor (WT-1, a podocyte protein) in uEVs has been reported to correlate well with podocyte injury in both animal models and patients with chronic glomerular diseases(38). In animal models, WT-1 in uEVs is detected prior to obvious glomerular sclerosis. Clinical data further showed that WT-1 in urinary exosomes was presented in 9 out of 10 FSGS patients, but not in any of the 8 controls examined (67). Zhou et al. also reported that urinary exosomal WT-1 was significantly increased in FSGS patients as compared with healthy
controls or steroid-sensitive nephrotic syndrome (SSNS) patients (68). In addition, urinary exosomal WT-1 was significantly decreased in patients in remission for either FSGS or SSNS or following steroid treatment of SSNS patients (68). These studies suggest that exosomal WT-1 may be a promising noninvasive biomarker that can detect early progression and treatment-induced regression of podocyte injury in FSGS or SSNS. However, the potential of exosomal WT-1 as a glomerulopathy biomarker has not been verified in pediatric patients with nephrotic syndrome. WT-1 levels in uEVs did not vary according to the responsiveness to the steroid therapy in pediatric patients (42). Moreover, exosomal WT-1 was detected only in 60% of 40 children with FSGS and SSNS, indicating that WT-1 in uEVs may not be a sensitive biomarker for pediatric FSGS and SSNS (42).

uEVs may be a useful diagnostic tool to help us differentiate early IgA nephropathy and thin basement membrane nephropathy in pediatric and adult patients with microscopic hematuria. In this regard, Moon et al. identified four different biomarkers differently expressed in uEVs of these patients: levels of aminopeptidase N and vasorin precursor were higher in the thin basement membrane nephropathy group compared with the IgA nephropathy group, whereas α-1-antitrypsin and ceruloplasmin levels increased in the IgA group (49).

Proteinuria is still regarded as an easily accessible and valid marker in the evaluation of glomerular diseases, but it cannot discriminate the type of underlying pathogenesis (19). uEVs contain mRNA, microRNA, proteins as well as surface receptors of specific cell types, providing the information about the types of glomerular impairment (49, 71). For example, Rood et al. reported that lysosome membrane protein-2
(LIMP-2) was up-regulated in uEVs obtained from patients with idiopathic membranous nephropathy (iMN) as compared to normal controls as well as uEVs of patients with idiopathic focal segmental glomerulosclerosis (iFSGS)(55).

**Diabetic kidney disease:**

uEVs, especially urinary exosomes, have recently become the source for searching for novel and earlier biomarkers of diabetic kidney disease (DKD) or diabetic nephropathy (DN). In an animal model of DKD, two exosomal proteins (Xaa-Pro dipeptidase and Major Urinary Protein 1) were shown to be up- and down-regulated, respectively (51). Zubiri et al. further detected a group of altered proteins, including MLL3, AMBP, and VDAC1, in urinary exosomes of DKD patients as compared to healthy controls (71).

Kalani et al. recently demonstrated the presence of WT-1 in urinary exosomes of diabetic patients and notably, exosomal WT1 increased when renal function worsened(38). In addition, type 1 diabetes mellitus patients with proteinuria showed significantly higher WT-1 in urinary exosomes than those without proteinuria(38). These data suggest that the increase of WT-1 in urinary exosomes may serve as a biomarker of podocyte injury or malfunction in DKD. In further support, exosomal WT-1 was detected in all diabetic patients with proteinuria, but only in a half of those without proteinuria and only 1 of 25 healthy controls. Expression of WT-1 in uEVs also showed a significant correlation with the decrease of renal function (68). Apart from WT-1, other molecular markers of podocytes, such as podocalyxin and podoplanin, have been also detected in uEVs in DKD. For example, Burger et al. detected increased levels of podocalyxin and podoplanin in microparticles from diabetic mice prior to albuminuria(11).
In addition to proteins, differential miRNA profiling in uEVs can be used to identify miRNAs that may differentiate type 1 diabetes patients with or without DKD. Barutta et al. recently detected 22 microRNAs with altered level in patients with microalbuminuria compared to patients without albuminuria. Among these microRNAs, miR-130a and miR-145 were significantly up-regulated in patients with microalbuminuria whereas miR-155 and miR-424 were markedly decreased(5). Consistently, diabetic animals showed a significant increase in urine exosomal miR-145. In cultured mesangial cells, high glucose treatment led to the release of miR-145-containing exosomes(5). Together, these data suggest that urinary exosomal miR-145 has a good potential to be a biomarker for DKD.

**Other renal diseases:**

In exosomes isolated from healthy volunteers’ urine, Miranda and colleagues successfully detected over 1000 proteins with origins of different segments of the nephron(48). Notably, 34 of these proteins are known to be associated with kidney diseases. For example, Na-K-2Cl symport is associated with antenatal Bartter syndrome type 1, polycystin-1 (PC1) with autosomal dominant polycystic kidney disease type 1, thiazide-sensitive Na-Cl cotransporter with Gitelman’s syndrome, aquaporin-2 with autosomal dominant and recessive nephrogenic diabetes. It remains elusive how these proteins are incorporated into urinary exosomes, but their presence provides a clue for detection of some congenital or hereditary diseases. For example, Na-K-Cl cotransporter, which were normally detected in urinary exosomes of healthy subjects, were founded to be absent in patients with Bartter syndrome type1 patient, a genetic disorder caused by a
mutation of the gene encoding Na-K-Cl cotransporter (26). Likewise, Na-Cl cotransporter was not detectable in uEV from patients with Gittelman’s syndrome(37).

uEVs of patients with polycystic kidney disease (PKD) also have notable characteristics. It has been reported that Cystin and ADP ribosylation factor–like 6 are abnormally expressed in uEVs of PKD patients (30). Recently, Hogan et al. further observed a significantly higher level of transmembrane protein-2 (TMEM2) in urinary exosomes from PKD1 patients as compared with healthy controls. Remarkably, the PC to TMEM2 ratio was inversely correlated with kidney volume, suggesting that PC to TMEM2 ratio may provide a novel technique to assess kidney volume as well as disease progression in PKD patients (29).

VI. EVS as therapeutic agents for kidney diseases

AKI:

Besides being potential biomarkers for AKI diagnosis and prognosis, EVs have shown promising beneficial effects on tissue repair and regeneration during AKI (Table 2). Microvesicles derived from bone marrow, mesenchymal, or endothelial progenitor stem cells could protect against various types of AKI and promote kidney repair or recovery (9, 10, 24). Exosomes from mesenchymal stem cells could also protect rats against gentamicin-induced kidney injury(53). Zhou and colleagues further demonstrated that exosomes derived from human umbilical cord mesenchymal stem cells (hucMSCs) could alleviate AKI induced by cisplatin in rats. Mechanistically, HucMSCs-derived exosomes reduced cisplatin-induced oxidative stress and apoptosis in vivo and facilitate renal epithelial cell proliferation in vitro (70). Gatti et al. have reported that administration of
microvesicles released from human adult mesenchymal stem cells, immediately after
renal I/R, could protect rats from AKI by inhibiting apoptosis and stimulating tubular
epithelial cell proliferation. Functionally, the rats treated with microvesicles had
significantly lower serum creatinine and BUN than those injected with vehicle
alone(24). Similarly, in mice with ischemic AKI model, administration of exosomes
derived from Human Endothelial Colony-Forming Cells (ECFCs) at the time of
reperfusion significantly attenuated the increases in plasma creatinine, tubular necrosis,
macrophage infiltration, oxidative stress, and apoptosis in kidney tissues in mice(12).
Beneficial effects of EVs derived from human liver stem cells have also been reported in
glycerol-induced AKI in mice(28).

Mechanistically, several studies attributed the protective effect of EVs on kidney
diseases mostly to their RNA content, especially microRNAs (16, 63). In this regard,
Collino and colleagues reported that EVs derived from the cells with silenced Drosha, a
key enzyme for microRNA biogenesis, were ineffective in promoting morphologic and
functional recovery in AKI. RNA sequencing analysis further showed that differentially
expressed kidney genes after injury were restored by treatment with wide type cell
derived EVs, but not with Drosha-knockdown cell vesicles. In gene ontology analysis,
those genes were associated with fatty acid metabolism, inflammation, matrix-receptor
interaction, and cell adhesion(17).

Of note, EVs are not always beneficial to kidneys as they can also aggravate the
progression of diseases. This notion is supported by that observation that in renal I/R,
EVs from proximal tubular cells may induce fibroblast activation and expansion to
promote interstitial fibrosis and tissue deterioration(52).
**CKD:**

As presented above, there is an intensive research on the potential of EVs as biomarkers for CKD. In contrast, very limited is known about the therapeutic effect of EVs in CKD. In this regard, Gatti et al. reported that microvesicles derived from human adult mesenchymal stem cells not only protected against ischemic AKI but also alleviated its progression to CKD (24). Of note, in this study the effect of microvesicles on AKI to CKD transition was most likely due to the amelioration of initial AKI. Mechanistically, the beneficial effect of microvesicles was diminished after pretreatment with RNases, suggesting a critical role of RNAs. As a significant extension of this observation, ischemically injured renal tubule epithelial cells were shown to release exosomes to activate fibroblasts via TGF-β mRNA, contributing to the development of renal fibrosis in post-AKI kidneys (7). However, it is unclear how the production and release of exosomes are regulated in injured tubular cells. Nonetheless, exosomes may be a brand new candidate for tubulointerstitial communication in renal disease progression.

In the anti-Thy1.1-induced model of glomerulonephritis, EVs derived from endothelial progenitor cells (EPC) showed significant beneficial effects (14). These included the alleviation of mesangial cell activation, leukocyte infiltration, and apoptosis, associated with the reduction of proteinuria. Serum complement haemolytic activity and renal function were also increased by EVs of EPC in this model. Mechanistically, the effect of EVs may be related to its content of mRNAs coding for anti-apoptotic factors and the complement inhibitors (14).

The latest work by Jiang et al. examined the effect of exosomes derived from urine-
derived stem cells on DKD. They showed that intravenous injections of exosomes reduced urine volume, microalbuminuria, and podocyte and tubular epithelial cell apoptosis in streptozotocin-induced diabetic rats. In addition, the exosomes increased the proliferation of glomerular endothelial cells (36). Thus, exosomes from urine-derived stem cells may have therapeutic effects on DKD.

VII. Conclusions and Perspectives

Research of EVs, especially exosomes, is currently one of the most exciting areas in cell biology. In the kidney field, most previous studies focused on the biomarker potential of EVs, particularly those detected in urine. Nonetheless, the therapeutic effect of EVs has been recognized. The most promising source of therapeutic EVs appears to be the mesenchymal stem cell of different origins (52), while exosomes from epithelial cells cultured in conditioned media also showed some effect (7). In addition, EVs may be used to deliver therapeutic molecules, such as proteins, mRNAs, siRNAs and microRNAs (1).

Despite these studies and potentials, the investigation of EVs in kidney diseases has just begun its journey. There are numerous questions to be raised and answered. EVs are roughly classified mainly according to their origins and sizes, but apparently even the exosomes derived from the same cell may not have the same contents. Also it is unclear how they are produced and released under normal physiological condition and how they are changed under pathological or disease conditions. After their release, where do they go and what are their specific recipient cells? In recipient cells, what are the main molecular contents that account for their cell biological effects? Although the biomarker potential of uEVs has been suggested, it has to be verified by large clinical studies. In
addition, the therapeutic efficacy of EVs needs to be further examined in experimental models and then hopefully translated to the bedside. As an emerging frontier in regenerative medicine, the research of EVs needs to identify the most suitable cell type(s) and the most efficient condition for the production of beneficial EVs. It is also necessary to elucidate where and how the EVs travel and work in the recipient cells in diseased organs. Addressing these and other related questions would lead to an in-depth understanding of the cell biology of EVs and their clinical potentials, including those for diagnosis and treatment of various kidney diseases.

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<th>Exosomes</th>
<th>Microvesicles</th>
<th>Apoptotic bodies</th>
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<tr>
<td><strong>Size</strong></td>
<td>40-150 nm</td>
<td>100-1000 nm</td>
<td>&gt;1000nm</td>
</tr>
<tr>
<td><strong>Origin</strong></td>
<td>Late Endosome</td>
<td>Plasma membrane</td>
<td>Apoptotic cell</td>
</tr>
<tr>
<td></td>
<td>(multivesicular body)</td>
<td></td>
<td>(Plasma membrane)</td>
</tr>
<tr>
<td><strong>Main functions</strong></td>
<td>Transmission of proteins and RNAs</td>
<td>Transmission of RNAs and DNAs</td>
<td>Not clearly defined</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>ESCRT components, adhesion molecules</td>
<td>Integrins, Flotillins, Selectins, CD40,</td>
<td>Anexin, Histones</td>
</tr>
<tr>
<td></td>
<td>(tetraspanins:CD63,CD9), membrane</td>
<td>Metalloproteinas</td>
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<td></td>
<td>transport/fusion protein(flotillins,</td>
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<tr>
<td></td>
<td>annexins), antigen presentation(MHC I),</td>
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<td></td>
<td>signal transduction, enzymes,</td>
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<tr>
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<td>cytoskeletal proteins, other cytosolic</td>
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<td></td>
<td>proteins(TSG101), Heat Shock protein(HSP</td>
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<td></td>
<td>70)</td>
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<tr>
<td><strong>Lipids</strong></td>
<td>Lysobisphosphatidic acid, cholesterol,</td>
<td>High amount of cholesterol,</td>
<td>High concentration of phosphatidylserine</td>
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<td></td>
<td>ceramide, sphingomyelin and low</td>
<td>sphingomyelin, ceramide, high</td>
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<tr>
<td></td>
<td>concentration of phosphatidylserine</td>
<td>concentration of phosphatidylserine</td>
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<tr>
<td><strong>Nucleic Acids</strong></td>
<td>mRNA and miRNA</td>
<td>mRNA and miRNA</td>
<td>mRNA, miRNA, fragments of DNA</td>
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<td><strong>Morphology</strong></td>
<td>Homogenous cup-shape</td>
<td>Heterogeneous irregular</td>
<td>Heterogeneous irregular</td>
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<tr>
<td><strong>Isolation method</strong></td>
<td>Immunoprecipitation (commercial Kit),</td>
<td>Ultracentrifugation (10,000-60,000 g)</td>
<td>No standardized protocol</td>
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<td>ultracentrifugation (100,000-200,000 g),</td>
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<td>ultracentrifugation with density gradient</td>
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<td><strong>References</strong></td>
<td>(35, 41, 46)</td>
<td>(39, 41, 65)</td>
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Table 2. Therapeutic test of EVs in kidney disease models

<table>
<thead>
<tr>
<th>EV origin</th>
<th>Type</th>
<th>Disease model</th>
<th>Result</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>mesenchymal stem cells</td>
<td>exosome-like microvesicles</td>
<td>gentamicin mediated Nephrotoxicity in rats</td>
<td>Inhibited Cr increase, necrosis, apoptosis and increased cell proliferation.</td>
<td>(53)</td>
</tr>
<tr>
<td>Mesenchymal stem cell</td>
<td>microvesicles</td>
<td>glycerol-induced AKI in SCID mice</td>
<td>accelerated the morphologic and functional recovery</td>
<td>(10)</td>
</tr>
<tr>
<td>human adult mesenchymal stem cells</td>
<td>Microvesicles</td>
<td>AKI induced by ischaemia-reperfusion injury (IRI) in Rats</td>
<td>inhibited apoptosis and stimulated tubular epithelial cell proliferation</td>
<td>(24)</td>
</tr>
<tr>
<td>mesenchymal stem cells</td>
<td>Microvesicles</td>
<td>lethal cisplatin-induced AKI of SCID mice</td>
<td>ameliorated renal function and morphology, and improved survival</td>
<td>(9)</td>
</tr>
<tr>
<td>human umbilical cord mesenchymal stem cells</td>
<td>exosomes</td>
<td>cisplatin-induced AKI in Rat</td>
<td>ameliorated oxidative stress and cell apoptosis, promoted cell proliferation</td>
<td>(70)</td>
</tr>
<tr>
<td>Human endothelial colony-forming cells</td>
<td>exosomes</td>
<td>a mice model of ischemic AKI</td>
<td>attenuated Cr, tubular necrosis, macrophage infiltration, oxidative stress, and apoptosis</td>
<td>(12)</td>
</tr>
<tr>
<td>Human liver stem cells</td>
<td>extracellular vesicles</td>
<td>Mice AKI induced by intramuscle glycerol injection</td>
<td>ameliorated renal function and morphology</td>
<td>(28)</td>
</tr>
<tr>
<td>mesenchymal stromal cells vesicles</td>
<td>extracellular vesicles</td>
<td>glycerol-induced AKI in severe combined immunodeficient mice</td>
<td>induced morphologic and functional recovery in AKI</td>
<td>(17)</td>
</tr>
<tr>
<td><strong>tubular epithelial cells under hypoxia</strong></td>
<td>exosomes</td>
<td>Fibroblasts</td>
<td>Fibrosis proteins expression increased</td>
<td>(7)</td>
</tr>
<tr>
<td>------------------------------------------</td>
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</tr>
<tr>
<td><strong>Endothelial progenitor cells</strong></td>
<td>extracellular vesicles</td>
<td>experimental anti-Thy1.1 glomerulonephritis in Rats</td>
<td>inhibited antibody- and complement-mediated injury of mesangial cells</td>
<td>(14)</td>
</tr>
<tr>
<td><strong>human urine-derived stem cells</strong></td>
<td>exosomes</td>
<td>Type 1 diabetic nephropathy</td>
<td>reduced the urinary microalbumin excretion, prevented apoptosis, increased glomerular endothelial cell proliferation</td>
<td>(36)</td>
</tr>
</tbody>
</table>

**Figure Legend**

Figure 1. Proteins originated from different cells type of the nephron transported via EVs.
Proximal tubules
megalin
cubilin
aquaporin-1
type IV carbonic anhydrase
γ-glutamyltransferase
aminopeptidase N
Fetuin-A
Dipeptidyl peptidase-4
Activation transcription factor 3

Distal tubules
thiazide-sensitive Na-Cl cotransporter

Collecting duct
aquaporin-2
mucin-1
Rh type C glycoprotein

Podocyte
podocin
podocalyxin
Wilms' tumor 1

Henle
type 2 Na-K-2Cl cotransporter
Tamm-Horsfall Protein
CD9