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Running Title: Extracellular vesicles as a therapeutic tool in various renal diseases

Keywords: Extracellular vesicles, exosome, microvesicles, kidney, renal disease, drug delivery

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ABSTRACT

Extracellular vesicles (EVs) released from different types of kidney cells under physiologic conditions contribute to homeostasis maintenance, immune-modulation, and cell-to-cell communications. EVs can also negatively affect the progression of renal diseases through their pro-inflammatory, pro-fibrotic, and tumorigenic potential. Inhibiting EVs by blocking their production, release, and uptake has been suggested as a potential therapeutic mechanism based on the significant implication of exosomes in various renal diseases. On the other hand, stem cell-derived EVs can ameliorate tissue injury and mediate tissue repair by ameliorating apoptosis, inflammation, and fibrosis while promoting angiogenesis and tubular cell proliferation. Recent advancement in biomedical engineering technique has made it feasible to modulate the composition of exosomes with diverse biologic functions, making EV one of the most popular drug delivery tools. The objective of this review was to provide updates of recent clinical and experimental findings on the therapeutic potential of EVs in renal diseases and discuss the clinical applicability of EVs in various renal diseases.

INTRODUCTION

Extracellular vesicles (EVs) are endogenously produced, membrane-bound vesicles that are released from cells into the extracellular space (1). Exosomes and microvesicles (MVs) are major subtypes of EVs. They are known to serve important roles in homeostasis, immune modulation, and tissue regeneration under physiologic conditions (2, 3). EVs can also mediate inflammation, thrombosis, fibrosis, and tumorigenesis in pathologic conditions (4, 5). EVs contain various biologic materials including mRNA, microRNA (miRNA), proteins, and lipids. Their contents are determined by the type of host cells and microenvironments of host cells (6). The biologic function of EVs depends on their compositions and downstream responses of recipient cells.

EVs serve several pivotal roles in renal physiology including immune modulation, tissue proliferation/regeneration, antimicrobial effect, and electrolyte/water balance, which contributes to maintenance of renal homeostasis (3). In pathologic conditions, however, EVs can contribute to propagation of disease courses by enhancing inflammation, fibrosis, coagulation, and tumorigenesis in various renal disease conditions (3). The role of EVs as a novel biomarker gained a lot of attention and comprised a major proportion of EV studies in kidneys. This topic is well summarized in the review article by Karpman *et al*. (1) and this will not be addressed further in this review as it is beyond the scope of our topic.

Based on the recent advancement of stem cell research and biomedical engineering technique on EV loading and modification (7), EVs have received a lot of medical attention for treatment of various kidney diseases including AKI, CKD, and transplant graft rejection even though further verification through human studies is limited. EVs serve a crucial role in intercellular communication by delivering biological cargo to recipient cells. The potential use of EVs as biocarriers has been exploited for the delivery of endogenous or exogenous

therapeutic materials (3). Diverse cargos including miRNAs, proteins, and drugs, can be delivered to target cells by modulating EV production and cargo sorting. In this review, we will focus on the potential of EVs as intrinsic therapeutics, therapeutic targets, and drug carriers for various renal diseases.

EVs for treating various kidney diseases

More recently, both *in vivo* and *in vitro* studies have shown explosive advancements regarding the protective role of EVs in various types of renal diseases. Most EVs used in those studies originated from mesenchymal stem cells (MSCs). They can alleviate renal damage mainly through their paracrine effects rather than their differentiation potential (8). MSC-derived EVs can exert therapeutic effects by modulating various biological processes including tubular proliferation, angiogenesis, apoptosis, and fibrosis (**Figure 1**). Below, we will review the therapeutic potential of EVs in renal disease conditions in more detail which is also summarized in **Table 1**.

Acute kidney injury (AKI)

AKI is a clinical syndrome originating from acute loss of renal excretory function and typically results in accumulation of renal toxins or reduction in urine output. AKI can originate from various etiologies including dehydration, toxins, hemodynamic instability, or obstruction. AKI is associated with increased mortality and healthcare-related costs. Therefore, many studies have focused on finding novel therapeutics using EVs to prevent or improve AKI outcome which will be addressed more in detail below.

MSC-derived EVs have shown a profound protective effect on AKI through their anti-apoptotic, antioxidant, anti-inflammatory, and angiogenic activities. In a study by Chen *et al.* (9), MVs derived from human Wharton's Jelly mesenchymal stromal cells (hWJMSCs) could ameliorate renal ischemia reperfusion injury (IRI) by enhancing the regeneration and decreasing the apoptosis of renal cells while mitigating IR-induced renal fibrosis. This protection was induced by G2/M cell cycle arrest via Erk1/2 signaling. Another study has shown that hWJMSC-derived MVs possess antioxidant effects in a rat ischemic AKI model

(10). A single administration of hWJMSC-derived MVs into IR-injured kidneys decreased the expression of reactive oxygen species, reduced apoptosis, and enhanced proliferation of tubular cells *in vivo*. Renal fibrosis in the late stage of IRI was also significantly abrogated by MV delivery, leading to similar biochemical improvement (10).

As identification and characterization of noncoding RNAs become more widely available, several studies have shown therapeutic potentials of exosomal miRNAs in ischemic AKI by post-transcriptional regulation. Li *et al*. (11) have found that both human urine-derived stem cells (USC) and their exosomes could protect ischemic AKI in mice. Further sequencing and bioinformatics analysis showed that miR-146a-5p was the most abundant miRNA in USC-derived exosomes. They found that miR-146a-5p targeted interleukin (IL)-1 receptor-associated kinase 1 mRNA, subsequently inhibiting the activation of NF-κB signaling *in vitro* (11). Zhu *et al*. (12) have demonstrated that exosomes from human-bone-marrow-derived MSCs can induce anti-apoptotic effect in ischemic AKI by transferring miR-199a-3p to renal cells. This protective effect was mediated via modulation of protein kinase B and extracellular-signal-regulated kinase pathways (12). However, neither of these studies traced or targeted exosomes *in vivo*. Thus, direct visualization of exosome transfer into target renal cells could not be provided by these studies.

Several studies have shown therapeutic effects of EVs derived from various sources other than MSCs (13, 14). Pan *et al.* (13) have found that the protective effect of limb remote ischemic preconditioning (rIPC) is mediated by miR-21 transportation from preischemic limbs to remote organs via serum exosomes. Serum-derived exosomes from mice with limb rIPC or enhanced exosomal miR-21 from cultured myotubes with hypoxia and reoxygenation preconditioning integrated into renal tubular epithelial cells and targeted downstream PDCD4/NF-κB and PTEN/AKT pathways. The delivery of those exosomes attenuated sepsis-

induced AKI through their anti-inflammatory and anti-apoptotic effects. In a glycerol-induced AKI model, the delivery of urine-derived EVs (uEVs) from healthy volunteers alleviated biochemical and histological renal injury, improved tubular cell proliferation, and reduced tubular cell apoptosis (14). Biodistribution analysis confirmed the preferential localization of uEVs in damaged kidneys. Treatment with human uEVs could restore Klotho to normal levels in injured kidneys which is known to serve reno-protective role in AKI by inhibiting apoptosis, fibrosis and upregulating autophagy (15). However, uEVs from Klotho null mice did not show any reno-protective effect. On the other hand, Klotho engineered uEVs from Klotho null mice restored regenerative properties, suggesting the indispensable role of Klotho in the protective mechanism of uEVs. Dominguez *et al*. (16) have shown that exosomes from human renal tubules could reverse renal IRI in nude rats through maintenance of renal vascular and epithelial networks, protection from oxidative stress and apoptosis, and suppression of pro-inflammatory and pro-fibrotic pathways. Further comprehensive proteomic analysis on IR-injured kidneys showed that renal IRI induced significant and extensive changes in protein expression. However, treatment with human renal tubular exosomes could prevent most of these protein expression alterations (16).

Chronic kidney disease (CKD)

Renal fibrosis is a major contributor to CKD pathophysiology and can cause irreversible deterioration of renal function. The severity of renal fibrosis is significantly correlated with progression of CKD. Pathways, diagnostic potential, and therapeutic potential of EV-regulated renal fibrosis in CKD are well described in a review article by Brigstock (17). Here, we will review some representative *in vivo* studies regarding therapeutic actions of EVs in various experimental models of CKD.

The most common etiology of CKD is diabetic nephropathy, a microvascular complication from hyperglycemia-induced oxidative injury and inflammation that can ultimately lead to renal fibrosis. MSC-derived exosomes have shown renal-protective effects on diabetic nephropathy (18, 19), although the exact mechanism has not been completely understood. Using a rat model of streptozotocin-induced diabetes mellitus model, Ebrahim *et al*. (18) have reproduced improved biochemical and histological renal outcomes in a group treated with MSC-treated exosomes compared to a control group. Treatment with MSC-derived exosomes induced significant upregulation of autophagy markers, Beclin-1, and light chain-3, and downregulated mechanical target of rapamycin (mTOR) and fibrotic marker expression in renal tissues (18). The protective effect of MSC-derived exosomes was partially reversed by administration of autophagy inhibitors, suggesting that autophagy induction by exosomes could attenuate diabetic nephropathy (18). MSC-derived exosomes could also exert anti-apoptotic, anti-fibrotic, and anti-degenerative effects in tubular epithelial cells while protecting tight junction structure in streptozotocin-induced diabetic nephropathy model of rats (19).

Hypertension is the second leading etiology of CKD, causing damage to blood vessels and filtering function of the kidney. In a deoxycorticosterone acetate-salt hypertensive model, EVs from adipose-derived MSCs could ameliorate pro-inflammatory response and recruitment of immune cells into the kidney (20). Moreover, administration of these EVs could prevent cardiac tissue fibrosis and induce better blood pressure control. Further miRNA microarray profile suggested that EV administration could affect signaling pathway of epithelial-mesenchymal transition and prevent inflammation as well as fibrosis in the kidney. In an angiotensin II-induced hypertensive model, exosomes from cardiosphere-derived cells improved renal function and cardiac hypertrophy while diminishing inflammation and fibrosis in both kidney and heart in association with altered levels of IL-10 expression (21).

A study by Cantaluppi *et al*. showed that EVs from endothelial progenitor cells can decrease antibody- and complement-mediated injury in Thy1.1-treated glomerulonephritis model (22). This protective effect was significantly reduced by pre-treatment with a high dose RNase, suggesting a crucial role of RNA content in EVs. In a pig model of metabolic syndrome and renal artery stenosis, exosomes from autologous MSCs could preserve renal function by alleviating renal inflammation, tissue hypoxia, and fibrosis (23). This reno-protective capacity appeared to be mediated by exosomal IL-10, an anti-inflammatory cytokine. Song *et al*. (24) have shown that MSC-derived EVs from lean pigs are more effective in improving renal function and decreasing tubular injury and fibrosis than EVs from metabolic syndrome pigs. These beneficial effects were associated with enhanced anti-inflammatory transforming growth factor (TGF)-β signaling leading to regulatory T cell induction (24).

Graft dysfunction after renal transplantation

Renal transplant has become the treatment of choice for most of the advanced kidney disease by placing a healthy kidney from a donor into a recipient's body. Transplant procedure itself induces some degree of ischemic-reperfusion damage as well as tissue damage which has significant impact on early graft function (25). Long-term immunosuppressive treatment is also crucial to prevent graft rejection and to prolong the graft survival as well as its function maintenance. EVs are known to serve a various role in transplanted kidney through their modulatory functions in innate immunity, complement system, and coagulation system, either by activating or inhibiting them depending on the microenvironment and EV content (25). EVs are also involved in allorecognition, IRI, and the autoimmune component of antibody-mediated rejections, affecting on the graft function and survival (25). Kidney endothelial- and tubular-derived EVs can trigger graft rejection by inducing alloimmune and autoimmune responses,

while MSC-derived EVs have been investigated for their therapeutic potential in experimental transplant models (26-28). The role of EVs in the crosstalk between the renal graft and immune systems as well as the diagnostic and therapeutic role of EVs in renal transplantation are well summarized in the review article by Quaglia *et al.* (25).

In a rat model of kidney transplantation, exosomes derived from regulatory T cells could delay allograft rejection, prolong the survival time of transplanted kidney, and inhibit T cell proliferation (26). This protective effect was more prominent by using the exosomes collected from donors compared to those from recipients. Pang *et al*. (27) have shown that immature dendritic cells-derived exosomes could significantly improve graft survival by alleviating inflammatory response and regulating T cell differentiation in renal grafts. They also found that immature dendritic cells-derived exosomes highly expressed miR-682 and that their protective effects are mediated through miR-682 to regulate T cell differentiation by negatively affecting Rho-associated kinase 2 expression. In a rat model of renal transplantation from cardiac death donors, when hWJMSCs-derived MVs were injected immediately after renal transplantation, they improved graft survival and renal function both in acute and chronic stages by mitigating renal cell apoptosis, inflammation, and renal fibrosis (28).

EVs as potential therapeutic targets

EVs exhibit potent effects in processes of thrombosis, inflammation, and apoptosis and are involved in propagation of various renal diseases. Therefore, blocking the release and uptake of exosomes can potentially carry beneficial effects during the disease course, even if the blocking is temporary. Various pharmacological agents can block release and uptake of EVs, including antiplatelet agents, statins, calcium channel blockers, and abciximab (29-31). However, whether modifying the release and uptake of exosomes can affect outcomes of renal

diseases has not been fully investigated yet. Mossberg *et al*. (32) have shown that patients with acute vasculitis have markedly higher levels of kinin B1-receptor-positive endothelial MVs known to carry significant neutrophil chemotactic effects. Coincubation of plasma with C1-inhibitor, the main inhibitor of the kinin system, could significantly lower the release of kinin B1-receptor-positive endothelial MVs *in vitro*, indicating a therapeutic potential of C1-inhibitor in the treatment of inflammatory diseases such as vasculitis (32). Liu *et al*. (33) have shown that inhibition of exosome release could prevent kidney fibrosis in murine unilateral IRI and unilateral ureteral obstruction (UUO) models *in vivo*. Through pharmacologic inhibition of exosome secretion from renal proximal tubular cells using dimethyl amiloride (DMA), TGFβ1-treated renal tubular cells lost their ability to activate renal interstitial fibroblast *in vitro* (33). This study also showed that blocking exosome secretion using DMA or knockdown of Rab27a, an essential protein for exosome formation, could preserve kidney function and attenuate renal fibrosis in unilateral IRI and UUO models. Of note, neither DMA treatment nor Rab27a knockout caused any noticeable abnormality in normal kidney or affected the integrity of contralateral kidneys after unilateral IRI and UUO. This study suggests a novel avenue for developing therapeutic strategies against CKD by targeting biogenesis and secretion of exosomes.

EVs as biocarriers of therapeutic materials

Better understanding of biological mechanisms of EVs and simultaneous advancement of bio-engineering technology to modulate EV production and cargo sorting have made EV one of the most preferred drug delivery systems. There are multiple biological benefits of EVs as vectors over other methods, including their stability, reduced toxicity, biostability, and low immunogenicity (34). Specific cell surface molecules on EVs enable targeted delivery of

198 therapeutics into subcellular structures including mitochondria and nucleus, while minimizing
199 off-target effects (35). Exosomal delivery of biologic materials can modulate disease processes
200 by altering genetic profiles and biological responses of recipient cells (36).

201 Several studies have investigated the therapeutic potential of EVs as a vector for drug
202 delivery in various kidney diseases. Tang *et al*. (37) have successfully produced macrophage-
203 derived dexamethasone containing MVs through co-incubation of macrophages with
204 dexamethasone. Delivery of these MVs into inflamed kidneys showed significant suppression
205 of renal inflammation and fibrosis in lipopolysaccharide- or Adriamycin-induced murine model
206 most likely through inhibition of NF-κB activity. In contrast with the traditional systemic
207 glucocorticoid treatment which can cause hyperglycemia, infection, osteoporosis, and
208 suppression of the hypothalamic-pituitary-adrenal (HPA) axis, chronic glucocorticoid
209 treatment through these MVs showed no significant effect on serum glucose, HPA axis, bone
210 metabolism, or immune response.

211 More recently, Yim *et al*. (38) have shown significant advancements in exosome
212 research regarding its production efficiency and biological compatibility using “exosomes for
213 protein loading via optically reversible protein-protein interactions” (EXPLOR), a novel
214 optogenetically engineered exosome technology. By using this EXPLOR technology, Choi *et*
215 *al*. (39) have successfully delivered super-repressor IκB-containing exosomes and shown their
216 protective effects in a murine septic AKI model by inhibiting nuclear translocation of NF-κB.
217 Recently, our group also reproduced the protective effect of exosomal super-repressor IκB
218 delivery treatment in an ischemic AKI model through its modulatory effect on inflammation
219 and apoptosis (40).

220 As noncoding RNAs have gained more recognition for their important roles in various
221 biological processes, delivery of noncoding RNAs as novel therapeutics for regulating renal

disease progression using exosomes is in the limelight. Wang *et al*. (41) have shown that exosomal delivery of exogenous miRNA-let7c through engineered MSCs could alleviate renal fibrosis in a murine UUO model by suppressing TGF-β signaling pathway. Their group also showed that exosomal delivery of miR-26a and miR-29 could attenuate renal fibrosis and muscle wasting in a murine UUO model (42, 43). These studies showed that exosome-carried miR-26a could limit renal fibrosis by directly suppressing connective tissue growth factor, while delivery of exosomal miR-29 could down-regulate pro-fibrotic proteins in TGF-β pathway, attenuating the progression of renal fibrosis in UUO kidneys.

Conclusions

EVs carry high potentials as a novel therapeutic tool for modulation of disease courses and for drug delivery. However, application of EVs to renal diseases is still in its infancy stage despite the explosive advancement in EV research during the past decade. Clinical application of exosomes as a therapeutic tool has been mainly focused on cancer therapy and related studies in the nephrology field are relatively scarce. There are also several technical challenges to be surmounted including retaining high yields of pure exosomes, enhancing the capability of loading various cargoes, and improving targeting specificity (35). Therefore, further advancements of therapeutic application of EVs in various renal diseases need a multidisciplinary approach harnessed with better understanding of renal pathophysiology, multi-omics studies to find a novel therapeutic target, and supplementation of bioengineering technique to enhance the quality of exosomes as biocarriers. Further optimization of EV isolation techniques and scrupulous manipulation of genetic or protein compositions of EVs are mandatory to expand the therapeutic applicability of EVs. Rigorous *in vivo* and *in vitro* studies to better characterize the exact biological role of each EV treatment and evaluate potential off-target effects from EV treatment are required for individualized therapeutic application. This achievement needs to be followed by further validation through clinical trials and large-scale cohort studies before entering clinical application. These efforts will further extend the clinical applicability of exosomes as novel therapeutics of various renal diseases.

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CONFLICTS OF INTEREST

Tae-Hyun Yoo is a Scientific Advisory Board member at ILIAS Biologics Inc. The authors have no additional financial interests.

FIGURE LEGEND

Figure 1. MSC-derived EVs can ameliorate the course of AKI and CKD through modulation of various biological processes including promotion of tubular proliferation and angiogenesis, alleviation of oxidative stress, and reduction of inflammation, apoptosis, and fibrosis.

Abbreviations: MSC, mesenchymal stromal cells; EVs, extracellular vesicles; AKI, acute kidney injury; CKD, chronic kidney disease; ER, endoplasmic reticulum; MVB, multivesicular body.

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Figure 1

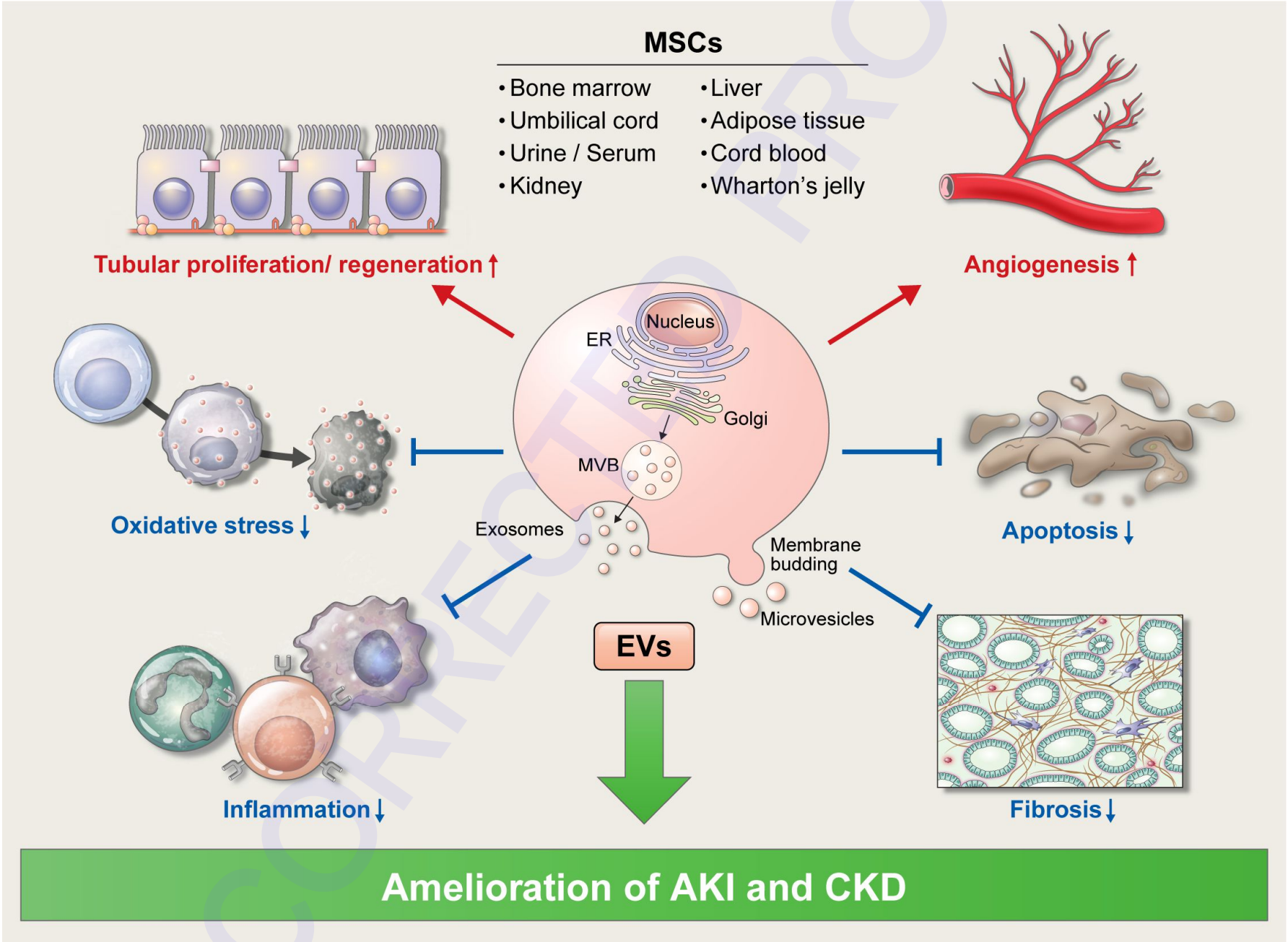

Table 1. Therapeutic application of extracellular vesicles from various origins in different kidney diseases

Disease Model	Origin	EV Type	Mechanism	Ref.
AKI	hWJMSCs	MVs	• hWJMSC-derived MVs improve renal function in ischemic AKI model by facilitating the proliferation of renal tubular cells and alleviating the apoptosis and fibrosis of renal cells *in vivo* (rats).	(9)
	hWJMSCs	Not specified	• hWJMSC-derived EVs ameliorate ischemic AKI by inhibition of mitochondrial fission through miR-30 *in vivo* (rats).	(44)
	hWJMSCs	MVs	• hWJMSC-derived MVs alleviate the oxidative stress through suppressing NOX2 expression in both *in vitro* (HUVEC) and *in vivo* (rats) IRI model. • hWJMSC-derived MVs reduce apoptosis and enhanced proliferation in renal IRI.	(10)
	hWJMSCs	MVs	• hWJMSC-derived MVs induce HGF synthesis in damaged tubular cells via RNA transfer, facilitating tubular cell dedifferentiation and regeneration in unilateral AKI model *in vivo* (rats).	(45)
	hUC-MSCs	MVs	• hUC-MSC-derived MVs mitigate epithelial cell apoptosis in low oxygen environment *in vitro* (HK-2) and ameliorated renal IRI *in vivo* (rats) via delivery of miR-21.	(46)
	hUSCs	Exosomes	• hUSC-derived exosomes ameliorate ischemic AKI *in vivo* (rats). • hUSC-derived exosomes inhibit oxidative stress after H/R injury *in vitro* (HK-2). • *miR-146a-5p* targets *interleukin-1 receptor-associated kinase 1* mRNA and subsequently inhibited the activation of NF-κB signaling *in vitro* (HK-2).	(11)
	hBM-derived MSCs	Exosomes	• Exosomes from hBM-derived MSCs play a protective role in H/R injury *in vitro* (HK-2) as well as in renal IRI *in vivo* (mice). • *miR-199a-3p* is involved in the renal protective effects of exosomes from hBM-derived MSCs by regulating Sema3A and activating the AKT and ERK pathways.	(12)
	Adipose-derived MSCs	Not specified	• Hypoxia-preconditioning increase the antiapoptotic, immune-modulatory, and anti-oxidative properties of adipose-derived MSC-EVs *in vitro*. • Adipose-derived MSC-EVs improve recovery of renal function in ischemic AKI *in vivo* (rats).	(47)

	Mouse serum	Exosomes	• Delayed remote ischemic preconditioning exerts renoprotection in septic AKI through exosomal *miR-21* derived from preischemic limbs *in vivo* (mice). • Exosomal *miR-21* attenuates septic AKI both *in vivo* (mice) and *in vitro* (mTECs) through PDCD4/NF-κB and PTEN/AKT pathways inducing anti-inflammatory and anti-apoptotic effects.	(13)
	Human urine	Not specified	• Urinary EVs alleviate AKI generated by glycerol injection and accelerate renal recovery *in vivo* (mice). • The protective role of urinary EV is mediated through repletion of Klotho in injured renal tissue.	(14)
	Human renal tubular cells	Exosomes	• Exosomes from human renal tubular cells prevent ischemic renal injury in Nude rats by preventing renal oxidant stress and apoptosis and suppressing pro-inflammatory and pro-fibrotic pathways.	(16)
Diabetic nephropathy	Rat BM-derived MSCs	Exosomes	• BM-derived exosomes improve renal function, morphology, and fibrosis in streptozotocin-induced diabetic nephropathy model *in vivo* (rats) in parallel with increased autophagy markers, LC3 and Beclin-1, and decreased mTOR and fibrotic markers expression in renal tissue.	(18)
	Rat BM-derived MSCs	Exosomes	• Exosomes from BM-derived MSCs ameliorate renal inflammation and fibrosis while protecting tight junction structure in streptozotocin-induced diabetic nephropathy *in vivo* (rats). • Exosomes from BM-derived MSCs suppress apoptosis and degeneration of tubular epithelial cells in primary renal cell culture of streptozotocin-induced diabetic rats *in vitro.*	(19)
	hUC-MSCs	Exosomes	• hUC-MSC-derived exosomes decrease the production of pro-inflammatory and pro-fibrotic cytokines in high glucose-injured renal tubular epithelial cells and renal glomerular endothelial cells *in vitro*.	(48)
	hBM-derived MSCs and HLSCs	Not specified	• EVs from hBM-derived MSCs and HLSCs alleviate renal fibrosis and proteinuria in streptozotocin-induced diabetic nephropathy model *in vivo* (mice).	(49)
Hypertensive nephropathy	Adipose-derived MSCs	Not specified	• Adipose-derived MSC-EVs improve renal function, decreased urinary protein excretion, and renal fibrosis while preventing cardiac tissue fibrosis and inducing better blood pressure control in DOCA-salt hypertensive model *in vivo* (rats).	(20)

	Cardiosphere-derived cells	Exosomes	• Administration of exosomes from cardiosphere-derived cells attenuate renal injury and cardiac hypertrophy in angiotensin II-induced hypertension model *in vivo* (mice), which appears to be associated with changes in the expression of interleukin-10.	(21)
Glomerulonephritis	hEPC	Not specified	• hEPC-derived EVs alleviate complement-mediated mesangial injury in anti-Thy1.1-induced glomerulonephritis model *in vivo* (rats) by inhibiting mesangial cell activation, leukocyte infiltration, and apoptosis. • hEPC-derived EVs inhibit complement-mediated renal mesangial cell injury and C5b-9 deposition *in vitro*.	(22)
Other CKD	Adipose-derived autologous MSCs	Not specified	• Autologous MSCs-derived EVs restore renal function through attenuation of renal inflammation, tissue hypoxia, and fibrosis in metabolic syndrome and renal artery stenosis model *in vivo* (pigs). • These protective effects are blunted in pigs treated with interleukin-10-depleted EVs.	(23)
	MSCs	Not specified	• MSC-derived EVs from lean pigs more effectively improve renal function and decrease tubular injury and fibrosis compared to those from pigs with metabolic syndrome. • The beneficial effect of MSC-derived EVs appears to be associated with up-regulated TGF-β signaling and enriched regulatory T cells.	(24)
	hCB-MSCs	Not specified	• Cell-free hCB-MSCs-EVs ameliorate the inflammatory immune reaction and transiently improve the overall kidney function in CKD patients. • Cell-free hCB-MSCs-EVs do not induce any significant adverse events throughout the study period (one year).	(50)
	hBM-derived MSCs	Exosomes	• MSCs-derived exosomal anti-let-7i-5p attenuates the pro-fibrotic response induced by TGF-β1 *in vitro* (NRK52E cells). • MSC-derived exosomal anti-let-7i-5p improves renal function and attenuates renal fibrosis in UUO-induced renal fibrosis model *in vivo* (mice).	(51)
	hWJMSCs	MV	• hWJMSC-derived MVs attenuate ischemia-induced renal fibrosis *in vivo* (rats) and promote M2 macrophage polarization *in vitro* (THP-1 macrophages) via transferring HGF.	(52)

	Human adipose-derived MSCs	Exosomes	• GDNF-modified human adipose-derived MSCs ameliorate renal fibrosis in murine UUO model. • GDNF-modified human adipose-derived MSCs exert cytoprotective effect on HUVEC in hypoxia/serum deprivation injury model by promoting angiogenesis through activation of SIRT1/eNOS signaling pathway.	(53)
Graft dysfunction after renal transplantation	Tregs	Exosomes	• Treg-derived exosomes can postpone allograft rejection and prolong the survival time of transplanted kidney *in vivo* (rats). • Treg-derived exosomes suppress T cell proliferation *in vitro*.	(26)
	Mouse immature DCs	Exosomes	• Immature DC-derived exosomes improve the survival in isograft mice by alleviating inflammatory response, reducing CD4 T cell infiltration, and increasing regulatory T cells in spleen and kidney tissues. • miR-682 is highly expressed in immature DC-derived exosomes which can promote regulatory T cell differentiation and immune tolerance in renal allograft *in vivo* (mice).	(27)
	hWJMSCs	MV	• hWJMSC-derived MVs improve survival rate and renal function after renal transplantation *in vivo* (rats). • hWJMSC-derived MVs mitigate renal cell apoptosis and inflammation and enhance proliferation in the acute stage while abrogating renal fibrosis in the late stage.	(28)

Abbreviations: EV, extracellular vesicles; AKI, acute kidney injury; hWJMSCs, human Wharton's Jelly mesenchymal stromal cells; MVs, microvesicles; miR, microRNA; HUVEC, human umbilical vein endothelial cells; IRI, ischemia-reperfusion injury; HGF, hepatocyte growth factor; hUC-MSCs, human umbilical cord mesenchymal stem cells; HK-2, human tubule epithelial cells; hUSCs, human urine-derived stem cells; H/R, hypoxia/reoxygenation; hBM, human bone marrow; MSCs, mesenchymal stem cells; AKT, protein kinase B; ERK, extracellular signal-regulated kinase; mTECs, mouse tubular epithelial cells; mTOR, mammalian target of rapamycin; HLSCs, human liver stem-like cells; CKD, chronic kidney disease; DOCA, deoxycorticosterone acetate; TGF-β1, transforming growth factor beta-1; hCB-MSCs, human cord blood mesenchymal stem cells; UUO, unilateral ureteral obstruction; GDNF, Glial cell line-derived neutrophic factor; SIRT1, Sirtuin 1; eNOS, endothelial nitric oxide synthase; hEPC, human endothelial progenitor cells; Tregs, regulatory T cells; DCs, dendritic cells