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Mesenchymal stem/stromal cell-derived extracellular vesicles for chronic kidney disease: Are we there yet?

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Abstract

Mesenchymal stem/stromal cells (MSCs) are the most utilized cell type for cellular therapy, partly due to their important proliferative potential and ability to differentiate into various cell types. MSCs produce large amounts of extracellular vesicles (EVs), which carry genetic and protein cargo to mediate MSC paracrine function. Recently, MSC-derived EVs have been successfully employed in several preclinical models of chronic kidney disease. However, uncertainty remains regarding EV fate, safety, and long-term effects, which might impose important limitations on their path to clinical translation. This review discusses the therapeutic application of MSC-derived EV therapy for renal disease, with particular emphasis on potential mechanisms of kidney repair and major translational barriers. Emerging evidence indicates that the cargo of MSC-derived EVs is capable of modulating several pathways responsible for renal injury, including inflammation, oxidative stress, apoptosis, fibrosis, and microvascular remodeling. EV-induced modulation of these pathways has been associated with important renoprotective effects in experimental studies. However, scarce clinical data are available, and several challenges need to be addressed as we move towards clinical translation, including standardization of methods for EV isolation and characterization, EV fate, duration of EV effects, and effects of cardiovascular risk factors. MSC-derived EVs have potential to preserve renal structure and function, but further experimental and clinical evidence is needed to confirm their protective effects in patients with chronic kidney disease.

Keywords

mesenchymal stem cells; extracellular vesicles; microvesicles; exosomes; kidney

Introduction

Chronic kidney disease (CKD) remains a growing health concern that leads to progressive and irreversible deterioration of renal function and end-stage renal disease (ESRD), requiring dialysis or kidney transplantation. The prevalence of hypertension is significantly higher in patients with CKD compared to the general population[1, 2]. Hypertension is one of

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the leading causes for CKD and they possess a mutually perpetuation potential. CKD affects almost 15% of the United States population[3], but its prevalence is projected to increase to 17% by 2030[4], underscoring the need of novel treatment approaches to retard its progression of ESRD. Similarly, hypertension affects approximately one-third of the United States population and contributes to morbidity and mortality[5]. In this context, the advent of regenerative therapies that support repair, regeneration, and restoration of damaged tissues emerged as promising approaches to preserve the structure and function of the kidney.

Mesenchymal stem/stromal cells (MSCs) are the most utilized cell type for cellular therapy. These multipotent stem cells are found in multiple tissues, including bone marrow, umbilical cord, and adipose tissue, display broad pro-angiogenic and immunomodulatory properties, and can differentiate into several cell types. Minimal criteria to define human MSCs include plastic-adherence in standard culture conditions, expression of CD105, CD73 and CD90, lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules, and transdifferentiation into osteoblasts, adipocytes, and chondroblasts in vitro[6].

Currently, preclinical evidence suggests that MSCs can foster renal regeneration and repair in several forms of CKD[7–9]. Hundreds of clinical trials involving MSCs are listed in www.clinicaltrials.gov. Clinical trials in patients with hypertension and diabetic nephropathy, entities responsible for up to two-thirds of CKD cases, underscore the safety and efficacy of MSCs to ameliorate kidney injury and dysfunction[10, 11], positioning MSCs as propitious candidates for cell-based therapy in patients with CKD.

It is now well-accepted that MSCs exert their paracrine activity partly by releasing extracellular vesicles (EVs), lipid bilayer-delimited particles. These include microvesicles (0.1–1μm diameter, shed by outward blebbing of the plasma membrane) and exosomes (30–100nm released by the fusion of multi-vesicular bodies with the plasma membrane) (Figure 1). EVs carry messages in the form of genes, proteins, and micro-RNAs (miRNAs) capable of modulating several pathways responsible for cellular injury in recipient cells, including inflammation, oxidative stress, apoptosis, angiogenesis, and fibrosis, all of which contribute to the development and progression of CKD[12]. Indeed, experimental studies in several models of CKD uncovered the feasibility and efficacy of MSC-derived EVs to preserve renal structure and function[13], forging a paradigm shift towards cell-free therapeutic interventions for renal repair.

To date, few published and ongoing clinical trials have tested the safety and efficacy of MSC-derived EVs. This includes studies using allogeneic cord tissue MSC-derived EVs on B-cell mass in type-1 diabetes (NCT02138331), macular degeneration (NCT03437759), CKD[14], graft-versus-host disease[15], as well as bone marrow MSC-derived EVs containing miR-124 for ischemic stroke (NCT03384433).

Although therapeutic MSC-derived EVs are of great potential interest, challenges regarding standardization of methods for EV isolation and characterization, EV fate, and duration of EV effects, among others, represent important hurdles that need to be overcome before widespread adoption of this therapy in the clinical arena. This review discusses the

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therapeutic application of MSC-derived EV therapy for renal disease, with particular emphasis on potential mechanisms of kidney repair and major translational barriers.

MSC-derived EVs in CKD models

In recent years, the renoprotective effects of MSC-derived EVs have been investigated in several in-vivo models of CKD, including diabetic and hypertensive nephropathy, ischemia-reperfusion injury (IRI), unilateral ureteral obstruction (UUO), environmental exposure to heavy metals, and subtotal nephrectomy (STN). For example, Grange and colleagues investigated the potential therapeutic effect of EVs, isolated from human bone marrow MSCs, on the progression and reversal of fibrosis in a murine model of diabetic nephropathy[16]. Twenty-eight days after the onset of streptozotocin-induced diabetes, EVs (1×10^10 EVs/injection) were administered intravenously weekly to mice for four consecutive weeks, resulting in a 0.25mg/dl improvement in plasma creatinine and >20% reductions in tubular injury and glomerular fibrosis. The anti-fibrotic effects of EVs were supported by their enrichment with miRNAs capable of targeting biological pathways involved in the development of fibrosis. Likewise, after establishment of streptozotocin-induced diabetes in rats, weekly intravenous injection of EVs (100μg in PBS) isolated from human urinary MSCs prevented kidney complications by inhibiting by 70% podocyte apoptosis and promoting vascular regeneration and cell survival (increase CD31+/Ki-67+ endothelial area)[17]. Analysis of the cargo of EVs revealed that they contained important pro-angiogenic and pro-survival factors, such as vascular endothelial growth factor (VEGF) and bone morphogenetic protein (BMP)-7, which might have partly accounted for their reno-protective effects. Similarly, renal subcapsular administration of bone-marrow MSC-derived EVs (5.3×10^7 in 200μl PBS) 28 days after the onset of diabetes decreased urinary albumin-creatinine ratio and exerted important anti-fibrotic (almost 80% reduction in glomerular score), anti-apoptotic, and anti-inflammatory effects in the kidneys of high-fat diet-induced type-2 diabetic mice 2 weeks later[18], suggesting that MSC-derived EV therapy might be a promising tool to ameliorate the renal damage of diabetes.

The efficacy of MSC-derived EVs has been also tested in models of renovascular hypertension. In 2-kidneys, 1-clip (2K-1C) rats, two intravenous injections of EVs (100μg on 3rd and 5th weeks after clamping) harvested from adipose-tissue MSCs decreased systolic blood pressure by 40mmHg, proteinuria by 30mg/24h, and renal expression of pro-fibrotic factors, including transforming growth factor (TGF)-β and collagen-I, but increased expression of the anti-inflammatory cytokine interleukin (IL)-10[19] 6 weeks later. Our group has also shown in a swine model of endovascularly-induced renal artery stenosis (RAS) that a single intra-renal infusion of autologous MSC-derived EVs (10×10^7) preserved the structure and function of the post-ischemic kidney four weeks later[20]. Delivery of EVs decreased renal vein levels of several pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α and IL-6, but increased levels of IL-10 and the number of reparative M2 macrophages in the renal parenchyma. Importantly, the immunomodulatory effects of EVs were associated with increased post-stenotic kidney expression of pro-angiogenic factors, restoration of the intra-renal microcirculation, improvements in medullary oxygenation and fibrosis by 12%, and in turn in stenotic-kidney GFR (+28.4ml/min)[21]. However, their salutary effects were attenuated in animals treated with IL-10 knock-down EVs, implicating

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this anti-inflammatory cytokine in the protective effects of EVs. Although MSC-derived EVs were also enriched with anti-fibrotic factors[22], modulation of renal inflammation seems to be upstream of many other benefits (e.g. reduction of fibrosis). Importantly, delivery of IL-10 enriched MSC-derived EVs might be safer and more effective than delivery of IL-10, which may induce untoward immunological changes[23, 24].

MSC-derived EVs are also effective in ameliorating chronic kidney damage consequent to IRI. Intravenous injection of EVs (30μg) isolated from human bone-marrow MSCs in rats immediately after a 45 min IRI and unilateral nephrectomy decreased creatinine levels (−1.3mg/dl) and glomerular fibrosis (−12%) 6 months later[25]. Acute effects of EVs included inhibition of apoptosis and stimulation of tubular epithelial cell proliferation, which reduced acute kidney injury (AKI), and in turn protected these kidneys from later CKD. However, inactivation of their mRNA cargo abrogated these protective effects, implying that the efficacy of EVs relies in part on the transfer of genes to target damaged cells. In a similar model in rats, a single administration of MSC-derived EVs (100μg) immediately after IRI ameliorated by 2 days later renal injury (decreased >2 points in histopathological scoring) in both the acute and chronic stages by reducing macrophage accumulation and fibrosis (decreased fibrosis score by 44%) through suppression of the pro-inflammatory and pro-fibrotic protein fractalkine (CX3CL1)[26].

Beneficial effects of MSC-derived EVs were also reported in experimental UUO models. Intravenous administration of EVs (100μg) obtained from MSC supernatants reduced tubular injury and fibrosis (−20%) and improved kidney function 14 days after UUO[27]. An important mechanism of action of EVs could have been the transfer miRNAs implicated in modulating of fibrosis and epithelial-to-mesenchymal transition (EMT). Congruently, a single intravenous delivery of MSC-derived EVs (2×10^7) in mice with UUO ameliorated peritubular capillary rarefaction via inhibition of EMT and protected against progression of renal damage by decreasing tubulointerstitial fibrosis (−70%) 7 days after UUO[28]. These observations highlight important anti-fibrotic renoprotective properties of EVs in experimental UUO, and may provide a gateway for new therapeutics in this condition.

More recently, renoprotective effects of MSC-derived EVs were studied in a model that mimics CKD secondary to long-term environmental exposure to heavy metals. Medakas fish were exposed to high cadmium for 4 days and 4×10^7 EVs isolated from human bone-marrow intravenously injected 1 day later[29]. EVs repaired the damage to apical and basolateral membranes and mitochondria of kidney proximal tubules, and improved renal function and survival by 20%. Additional studies are needed to test whether EV therapy might be useful to other forms of toxic-induced CKD. MSC-derived EVs have also shown promise in murine models of STN. In a mouse remnant kidney model, intravenous delivery of EVs (30μg) 2 days after STN ameliorated interstitial lymphocyte infiltration, prevented tubular atrophy (−1.46 points in histological score) and fibrosis, and decreased proteinuria and serum creatinine levels 7 days later[30], underscoring the potential of EV therapy in preserving the kidney in these models of CKD.

However, despite robust preclinical data proclaiming that MSC-derived EVs promote tissue repair and preserve renal function, their safety and efficacy in patients with CKD remain to

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be determined. A pilot study in CKD patients suggested that administration of cell-free cord-blood MSC-derived EVs ameliorated inflammatory immune reaction and improved overall kidney function[14]. Forty patients with estimated-GFR (eGFR) between 15–60ml/min were randomized to receive either placebo or two doses of EVs. One year after EV administration, eGFR, serum creatinine, blood urea nitrogen levels, and urinary albumin/creatinine ratio improved, circulating levels of TNF-α decreased, but levels of IL-10 increased. Cell regeneration and differentiation markers improved 3-months after therapy and no significant adverse events occurred during EV infusion or throughout the follow-up period. Although these observations suggest that MSC-derived EV therapy might safely and effectively attenuate renal inflammation and dysfunction in CKD, additional long-term follow-up trials are needed to confirm these findings.

Are EVs superior to MSCs?

Based on the available experimental data, it is reasonable to ponder whether delivery of EVs would confer more efficient reno-protection compared to their parent MSCs. Evidently, EVs possess several advantages over MSCs[31] (Table 1). Their phospholipid bilayer makes them more stable after freezing and thawing, protecting their genetic and protein cargo. EVs can be more easily prepared in a standard manner and stored for a long time compared to MSCs, allowing their use as “off-the-shelf” products. Furthermore, they can be modified to function as delivery carriers to target specific molecules, proteins, or signaling pathways in recipient cells.

Cardiovascular risk factors can alter the transcriptome and proteome of swine adipose tissue-derived MSCs[32–34], which might limit their reparative function and utility as an exogenous regenerative therapy. In contrast, EVs are more resistant to the deleterious effects of an inflammatory microenvironment[35], increasing their half-life following injection (Table 1).

Despite their excellent safety profile, few reports of sarcomas[36] and teratomas[37] following exogenous MSC administration underscore their potential for transformation into tumors. Unlike MSCs, EVs are acellular and do not self-replicate, which lowers the potential for endogenous tumor-formation. Likewise, EVs have lower immunogenicity than their parent MSCs, which is partly due to their small size and lower expression of membrane histocompatibility molecules[38].

In terms of in-vivo efficacy, these small particles easily circulate and may reach distant damaged sites of the nephron. Like MSCs, injected EVs are retained in damaged kidneys and can engraft into several cell types, including proximal and distal tubular cells, endothelial cells, and macrophages[20, 39]. Yet, while MSCs primarily exert their protective effects by paracrine mechanisms, like release of EVs, growth factors, and cytokines, they may also replace damaged cells, which might extend and prolong their in-vivo efficacy compared to EVs.

Unfortunately, few experimental studies have compared the efficacy of MSCs and EVs in kidney repair. In murine IRI and STN models, delivery of MSCs and EVs exhibited similar potential to preserve renal structure and function[30, 40]. However, EVs in mice with UUO conferred reno-protective effects exceeding those of their parent MSCs[27]. In acute IRI, the

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combination of MSCs and MSC-derived EVs was more effective to either one alone in reducing proteinuria and preserving kidney function, suggesting additive effects[41]. More recently, we found that intra-renal delivery of either adipose tissue-derived MSCs or their daughter EVs improved stenotic-kidney function and decreased injury in swine RAS, albeit by slightly different mechanisms[42]. MSCs were more effective in suppressing inflammatory cytokines and preserving the intra-renal microcirculation, whereas EVs bestowed better preservation of renal cellular integrity. Yet, both strategies recovered renal structure and function, supporting the notion that EVs recapitulate the salutary effects of MSCs.

Obviously, longer-term studies would be useful for comparing MSCs and EVs for CKD. There are currently robust experimental data and several ongoing or completed clinical trials testing the safety and efficacy of MSCs for renal disease, some in common forms of CKD like diabetic nephropathy and hypertension[43]. No doubt, additional experimental research is needed to determine whether EVs confer more efficient reno-protection than MSCs.

Specific renal injury pathways modulated by MSC-derived EVs

Accumulating experimental evidence suggests that MSC-derived EVs are capable of modulating several pathways responsible for renal injury in CKD, including inflammation, oxidative stress, apoptosis, microvascular damage, and fibrosis (Figure 2). Circulating pro-inflammatory cytokines and subsequent inflammatory cell infiltration favor production of reactive oxygen species (ROS) and microvascular damage, leading to tubular injury and fibrosis[44]. In patients with CKD, increased ROS production contributes to the acceleration of GFR decline and several complications, such as hypertension, atherosclerosis, and anemia[45]. Similarly, intra-renal microvascular rarefaction and dysfunction are major determinants of the progression of CKD[46], and loss of renal tubular cells from apoptosis leads to expansion of the extracellular matrix and fibrosis, and in turn progression of CKD[47]. MSCs pass on important anti-inflammatory, anti-oxidant, anti-apoptotic, anti-fibrotic, and pro-angiogenic properties to their daughter EVs, rendering them an attractive tool to treat and improve outcomes in CKD[12, 13].

The primary mode by which EVs modulate renal injury pathways involves their renal engraftment and transfer of their cargo to injured renal cells. Studies described the biological signature of MSC-derived EVs and found that they are selectively packed with genes and proteins involved in immune regulation, extracellular matrix remodeling, apoptosis, angiogenesis, and redox cellular response[12]. For example, EVs derived from human umbilical cord MSCs are packed with the mitochondria-located antioxidant enzyme manganese superoxide dismutase (MnSOD)[48], and their delivery decreases renal oxidative stress[49]. Similarly, EVs released from bone marrow-derived MSCs transfer anti-apoptotic mRNAs to recipient cells and exert a pro-survival benefit on renal cells in-vitro and in-vivo[50]. We have shown that EVs harvested from swine adipose tissue contain several genes and proteins involved in angiogenesis, including the proangiogenic genes hepatocyte growth-factor, as well as VEGF and von Willebrand factor proteins[22, 51]. The genetic and protein cargo of swine MSC-derived EVs included modulators of TNF-α and TGFβ signaling[52], implying regulation of inflammatory response and extracellular matrix remodeling, as well as several anti-oxidants proteins, including MnSOD, catalase, and

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glutathione peroxidases. Importantly, delivery of these EVs into a stenotic swine kidney ameliorated inflammation and oxidative stress, and preserved its microcirculation and GFR[20, 21], underscoring the therapeutic potential of MSC-derived EVs to modulate renal injury pathways and improve kidney function in experimental CKD.

In addition to genes and proteins, MSC-derived EVs possess an important cargo of miRNAs, small non-coding RNAs which control gene expression post-transcriptionally and modulate important pathways responsible for renal injury in CKD. Among them are miR-24, an important modulator of vascular inflammation[53] implicated in EV-induced renal repair after ischemia[54], and miR29, which suppresses TNF-α production[55]. Swine MSC-derived EVs are enriched with miR148a-3p, which targets genes that regulate apoptosis and angiogenesis[51]. EVs decrease apoptosis and restore expression of angiogenic factors in the stenotic kidney[39], suggesting that their renoprotective effects might be attributed partly to their miRNA cargo.

Challenges

Experimental studies have greatly expanded our knowledge on the cargo, mechanisms of action, and reno-protective properties of MSC-derived EVs, yet clinical translation of this novel approach for patients with CKD remains associated with several challenges. In preclinical studies EVs were isolated by variable methods, including differential ultracentrifugation, filtration, and size exclusion chromatography among others[56]. Similarly, handling and storage of isolated EVs widely vary among studies. While each method has advantages and disadvantages for a given research hypothesis, the purity, concentration, morphology, size range, and even functional activity of EVs might differ with each different method or protocol for handling and storage[57, 58]. For example, preparations may contain different proportions of microvesicles and exosomes. Therefore, it is critical to define and standardize accurate, reliable, and easily implemented techniques for EV isolation before widespread use in CKD patients. The recent guidelines from International Society for Extracellular Vesicles[59] are an important step in this direction.

Despite the growing number of experimental studies testing the efficacy of MSC-derived EVs in many forms of CKD, uncertainty remains regarding their fate. Unfortunately, their small size is not conducive to tracking injected EVs. In AKI and CKD models, systemically injected EVs primarily engrafted into damaged kidneys[28, 60], with some retention in healthy kidneys[26]. EVs were taken up by several renal cells types, but tubular cells and peritubular capillaries exhibited higher retention rates compared to glomeruli[28, 60]. EVs also lodge preferentially in injured kidneys. Intra-renal administration in swine RAS was associated with higher EV retention in stenotic than contralateral kidneys four weeks after delivery, with significant uptake by tubular epithelial cells, peritubular capillary endothelial cells, and macrophages[20, 39]. Indeed, renovascular hypertensive kidneys release adhesion molecules and chemoattractants, like stromal-derived factor-1 and stem-cell factor, to promote migration and retention of progenitor cells[61]. Congruently, targeting murine MSCs to kidney injury molecule (KIM)-1 improves their therapeutic efficacy in murine chronic ischemic kidney injury[62], suggesting organ-specific recruitment strategies (e.g., injury signals) to attract MSCs and their EVs to the site of injury. The mechanisms by which EVs reach renal

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tubules may involve endocytosis in glomerular endothelial cells[63] or vascular extravasation via endothelial cell fenestrations or inter-cellular junctions, as we have shown in experimental RAS[21]. Nevertheless, studies are needed to clarify the mechanisms underlying renal cell uptake of EVs and establish the preferred route of delivery for clinical trials.

Additional research is also needed to establish the best timing window for EV administration. In rats, delivery of MSC-derived EVs prior to treatment with cisplatin prevents nephrotoxicity[64], yet their administration in mice after cisplatin-induced nephrotoxicity also ameliorates tubular injury and improves kidney function[50], suggesting that this intervention may both prevent and reverse established disease. EV efficacy also likely depends of the disease severity. Our previous studies in swine RAS suggest that MSC-derived EVs prevent rather than reverse further development of tubulo-interstitial fibrosis and glomerulosclerosis[20]. Clearly, more studies are needed to identify optimal timing for EV delivery.

Likewise, studies are needed to determine the duration, long-term effects, and optimal regimen of MSC-derived EVs. Most experimental studies using MSC-derived EVs were performed in AKI models followed ≤2 weeks after injection. However, in rats with IRI, MSC-derived EVs lowered the incidence of CKD 6 months after therapy[25]. Although these observations suggest that the beneficial effects might be sustained for at least few months, repeated doses might be necessary. In mice with AKI, multiple EVs injections decreased mortality and improved renal function compared to a single injection regimen[50]. Further studies should explore the duration of reno-protective effects of EVs and the efficacy of escalating doses to determine the adequate dose regimen for future clinical trials.

The source of MSCs may also impact the immunomodulatory or therapeutic effects of EVs. In diabetic mice, EVs derived from adipose tissue MSCs promote wound healing better than those derived from bone-marrow MSCs[65], probably thanks to different protein and miRNA cargo. Furthermore, liver-derived human MSCs produce more pro-angiogenic, anti-inflammatory, and anti-apoptotic cytokines than bone marrow-derived MSCs[66], but are comparable to adipose-tissue MSCs[67], and their EVs accelerate hepatic regeneration[68], underscoring the importance of the choice of parent MSC for EV-based therapy.

Studies are also needed to explore potential long-term detrimental effects of EVs. Immune response is not commonly observed following MSC or EV administration at standard doses. MSCs are hypoimmunogenic or ‘immune-privileged’, expressing low levels of MHC class-I and no MHC class-II or co-stimulatory molecules, enabling MSC transplantation across major histocompatibility barriers[69]. Indeed, cord-blood MSC-derived EVs ameliorate inflammatory immune reaction and improve kidney function in patients with CKD[14]. Furthermore, being acellular, EVs do not proliferate and are likely devoid of potential tumor growth, malformation, or microinfarctions attributed to MSCs[36, 70].

Emerging experimental evidence suggests that comorbidities and cardiovascular risk factors can impair the in-vitro and in-vivo functionality of autologous MSC-derived EVs. EVs released from adipose tissue-derived stem cells of obese individuals have limited pro-angiogenic potential, partly attributed to their reduced content of VEGF and miR-126[71]. We

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have shown that diet-induced metabolic syndrome (MetS) in swine alters the packaging of genes, proteins, and miRNAs of MSC-derived EVs, which contained genes involved in modulation of inflammation, but lacked mRNAs related to TGF-β signaling[72]. Additionally, target genes of miRNAs enriched in swine MetS-EVs were implicated in the development of MetS and its complications[73], whereas proteins were linked to pro-inflammatory pathways[74]. Notably, MetS-induced changes in MSC-derived EV cargo impaired their ability to support angiogenic function of endothelial cells in-vitro, and blunted their capacity to preserve the microvasculature in the post-stenotic kidney in-vivo[52, 75, 76]. Interestingly, MetS also induces release of smaller EVs packed with genes commonly associated with exosomes, whereas Lean EVs were larger (macrovesicles)[34]. Although the functions of these EV subsets might overlap, their biogenesis and molecular contents are different, warranting further studies to determine the impact of EV size distribution on their function. Overall, our observations may raise concerns about the use of autologous MSC-derived EVs in patients with cardiovascular risk factors, and need to be considered when designing clinical trials in CKD.

Summary and future perspectives: Are we there yet?

Hypertension and CKD are closely associated pathophysiologic states, and warrant novel treatment approaches to retard their progression. Experimental evidence demonstrates that MSC-derived EVs might confer protective effects in kidneys exposed to different forms of CKD, whereas clinical data supporting their safety and feasibility are clearly very limited, raising a concern about potential impediments for the translational process. First, we must ensure that important hurdles are overcome before moving towards designing clinical trials. This includes standardization of methods for EV isolation and characterization, which may increase reproducibility and facilitate interpretation of preclinical data. The International Society for Extracellular Vesicles proposed minimum criteria for characterization of EVs (e.g. nanocyte tracking analysis, western blotting, electron microscopy, and flow cytometry analysis), and suggested protocols and steps to document specific EV-associated functional activities[59]. At the same time, we need to elucidate the complex mechanisms of EV-induced reno-protection. Experimental evidence suggests that following systemic or intra-renal injection EVs are taken up by renal tubular cells and peritubular capillaries. In-vitro data support the transfer of protein, mRNA, and miRNA content into recipient cells, which contribute to modulate pathways implicated in renal damage in CKD. The ability to target EVs to the kidney could increase their engraftment and minimize off target effects[77]. Therefore, experimental data suggest that delivery of MSC-derived EVs might be an attractive therapy for individuals with stage 3–4 CKD to delay or decrease the need for renal replacement therapy. However, unmet needs remain in the field regarding EV fate, duration of their effects, and impact of cardiovascular risk factors. There is definitely a need for additional experimental studies to develop sound evidence-based guidelines for EV therapy. The future of MSC-derived EV therapy for renal repair is in its infancy, and will hopefully mature to establish this promising intervention in patients suffering from CKD.

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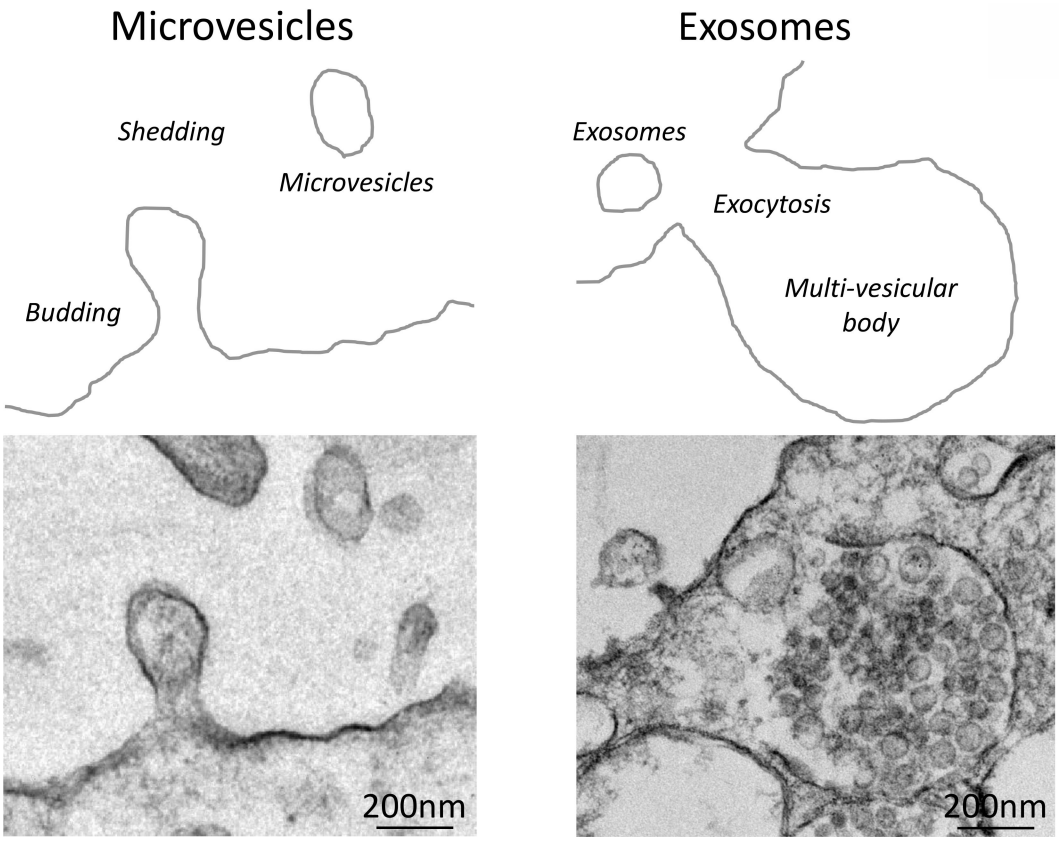

Figure 1. MSCs release extracellular vesicles.

Top: Schematic of the mechanisms of formation of microvesicles (left) and exosomes (right). Bottom: Representative transmission electron microscopy images of MSCs releasing microvesicles (left) and exosomes (right).

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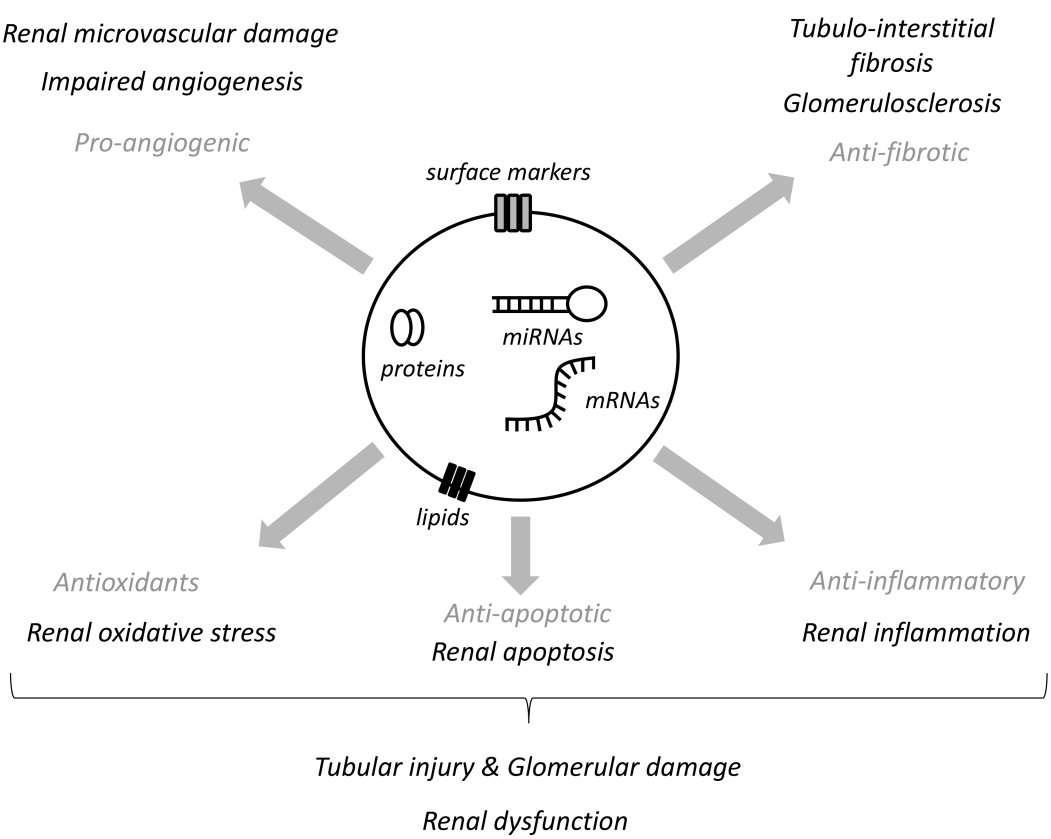

Figure 2. MSC-derived EVs modulate renal injury pathways in recipient cells.

MSC-derived EVs contain mRNAs, proteins, and micro-RNAs (miRNAs) capable of targeting pathways responsible for renal injury, including the renal microvasculature damage and defective angiogenesis, tubulo-interstitial and glomerular fibrosis, as well as renal inflammation, apoptosis, and oxidative stress.

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Table 1.

Advantages and disadvantages of EVs over MSCs for CKD.

Therapy	**MSCs**	**EVs**
In-vitro:		
Half-life *(following freezing and thawing)*	+	++
Stability *(in inflammatory microenvironment)*	−	+
Utility *(use as "off-the-shelf" products or delivery carriers)*	+/−	+
In-vivo:		
Tumorigenicity *(ability to give rise to benign or malignant tumors)*	+/−	−
Immunogenicity *(ability to induce humoral or cell immune responses)*	+	−
Efficacy *(preservation of renal structure and function in CKD)*	++	++
Efficiency *(reaching damaged sites within the neprhon)*	+	++
Targeting *(ability to ensure renal homing)*	+	+