Regenerative Role of Stem Cell-Derived Extracellular Vesicles in Acute Kidney Injury

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Abstract

Acute kidney injury (AKI) is a frequent complication of hospital admission and worsens short- and long-term patients' prognosis. Currently, AKI treatment remains supportive and no therapy has proven significant benefit in clinical trials. Stem cells (SCs) are a promising therapeutic option, but their translation to the clinical setting is limited by the risk of rejection or aberrant differentiation. Numerous studies have shown how SC effects are mediated by paracrine factors such as extracellular vesicles (EVs). In this review, we describe the preclinical evidence about EV efficacy in acute tubular and glomerular injury and the recently generated clinical data.

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Stem Cell Therapy for AKI: The Role of Paracrine Factors and Extracellular Vesicles

Acute kidney injury (AKI) complicates up to 20% of hospital admissions [1] and considerably impairs patients' outcome. In particular, 30–50% of patients requiring renal replacement therapy do not survive and >30% of the remaining subjects develop chronic or end-stage kidney disease [1, 2]. Moreover, even when renal function completely recovers, an increased incidence of cardiovascular events, sepsis, and cancer has been observed [3].

Despite a conspicuous growth of AKI scientific literature, no specific therapies have been approved and patient management is either experimental or supportive. Renal replacement therapies allow to manage volume overload, acid-base, and electrolyte abnormalities without improving outcome otherwise [4–6]. These data warrant the research of alternative therapeutic strategies in order to limit the social and economic burden of AKI and the subsequent progression to advanced CKD.

Several studies have shown how stem cell (SC) therapies could prevent or at least limit ischemic, immune, and toxic AKI [7–10]. The largest preclinical evidence has been generated in studies focused on hematopoietic stem cells and mesenchymal stromal cells (MSCs). Multiple types of MSCs (e.g., derived from bone marrow, umbilical

cord, adipose tissue, etc.) were successfully studied in pre-clinical AKI models [11]. MSCs have been shown to localize within injured kidneys through different mechanisms including the expression of CD44, a receptor for hyaluronic acid [12]. Kidney resident CD133+ SC have a mesenchymal origin and were identified in glomerular or tubular compartments; both types promote renal regeneration after AKI [13]. Other authors demonstrated how embryonic stem cells and induced pluripotent stem cells may facilitate kidney regeneration [11]. Endothelial progenitor cells (EPCs) are a committed population known for their extraordinary vascular regenerative potential. After endothelial damage, EPCs are mobilized from the bone marrow and home to injured vessels through the surface expression of L-selectin, an adhesion receptor that regulates leukocyte trafficking in injured tissues [14].

Despite the excellent results observed in preclinical studies, clinical trials aimed to evaluate the protective role of SC in AKI have shown conflicting results, with possible benefits in immune-mediated disorders and limited data on ischemic and toxic AKI [15]. In a Phase II, multicenter study, 156 patients with early evidence of AKI after cardiac surgery were randomized to standard treatment or intra-aortic administration of allogeneic MSCs. In contrast with experimental data, MSCs did not decrease the time to recovery of kidney function [16]. In kidney transplantation trials, MSC infusion led to minimization of immunosuppressive therapy and to a slightly improved renal function after 12 months [15, 17]. Nevertheless, cell therapies pose conspicuous challenges, including the risk of rejection, aberrant differentiation (e.g., fibrogenesis), tumorigenesis, and embolization.

Different studies demonstrated that SC do not differentiate into mature renal elements but induce renal recovery by contact-independent (endocrine/paracrine) mechanisms that promote resident cell proliferation and downregulate the inflammatory response [18]. Extracellular vesicles (EVs) are cell-derived membranous structures involved in cell-to-cell communication and able to shuttle proteins, mRNAs, microRNAs (miRNAs), lipids, metabolites, and organelles from origin to target cells. According to the International Society for Extracellular Vesicles, EVs are divided into 3 main families: exosomes, shedding vesicles (or microvesicles), and apoptotic bodies [19]. Exosomes size from 30 to 150 nm, originate from multivesicular bodies, and are secreted by exocytosis. Microvesicles are larger in size (100–1,000 nm) and are generated by a membrane-sorting mechanism involving changes in intracellular calcium concentrations and activation of membrane enzymes such as flippase, floppase,

and scramblase. Last, apoptotic bodies are larger than exosomes and microvesicles and derive from cellular residues after apoptotic cell death [19].

EV content is not only influenced by the cell source but also by a variety of physiological and pathological processes (e.g., baseline vs. inflammatory conditions); moreover, EV trafficking is always multidirectional and damaged tissues can alarm and reprogram regenerative cells. Additionally, EVs do not carry genomic DNA and ex vivo engineering can be performed without risk of transferring oncogenic sequences [20].

Major advances in EV isolation and characterization protocols have been achieved in the last few years [19], thus allowing the transition of the preclinical evidence to human studies. The International Society for Extracellular Vesicles recently released guidelines based on the evolution of collective knowledge in this field, reporting the minimal information required for EV studies and several clinical trials are currently ongoing [19]. We herein summarized the most relevant results on SC-derived EVs in experimental models of acute tubular and glomerular injury and the potential mechanisms of EV-induced renal tissue repair (Fig. 1).

SC-Derived EVs Protect from Acute Tubular Injury

Ischemia-reperfusion injury is the most studied experimental in vivo AKI model. EVs from MSCs prevent AKI and subsequent CKD progression in murine IRI by inhibiting apoptosis and by promoting tubular epithelial cell proliferation [21]. Similar results have been obtained using EVs from umbilical cord Wharton's jelly and renal glomerular progenitor CD133+ cells; of note, both these cell types have a mesenchymal origin [22, 23]. The beneficial effects of MSC-EVs have been observed also in a lethal cisplatin-AKI model; almost all treated mice survived the injection as a consequence of the limitation of tubular apoptotic cell death [24]. In these studies, EV injection was always performed pre- or immediately post-AKI with consistent timing; a scenario not always applicable to clinical AKI. After intra-muscle glycerol injection and the subsequent myoglobin release, human liver mesenchymal stem cell-derived EVs improved renal function and morphology by decreasing tubular necrosis; additionally, in vitro, human liver mesenchymal stem cell-EVs promoted tubular cell proliferation [10].

In different experimental acute tubular injury models, SC-derived EVs localized in peritubular endothelial cells and in tubular epithelial cells; EV internalization was or-

Fig. 1. Content of SC-EVs: SCs continuously release EVs that shuttle miRNA, mRNA, proteins, lipids, and organelles. SC-EVs deliver their cargo to renal cells by membrane fusion and promote tissue regeneration. SC, stem cells; EVs, extracellular vesicles; miRNA, microRNA; mRNA, messenger RNA.

chestrated by proteins expressed on their surface able to interact with adhesion molecules upregulated by injured cells [25]. EV-induced tissue regeneration seems to be mainly ascribed to horizontal mRNA and miRNA transfer. Our group demonstrated that blocking miRNA biogenesis through Droscha or Dicer knockout completely abolishes EV effects [26–28]. The transcription of miRNA-originating sequences generates a 100–1,000 base pair filament called pri-miRNA; in the nucleus, Droscha cleaves the pri-miRNA into the precursor miRNA (pre-miRNA), a hairpin structure of about 70 nucleotides. The pre-miRNA is then transferred to the cytoplasm and cleaved by Dicer into a mature miRNA [28]. EVs from Droscha knocked-down MSCs did not differ from those generated by wild-type MSCs in terms of concentration, size, surface molecule expression, and internalization within target tissue. However, when injected in experimental AKI models, Droscha-knockdown EVs did not improve renal function nor histology [28]. Gene ontology analysis revealed that AKI downregulated genes involved in fatty acid metabolism and upregulated genes involved in inflammation, matrix-receptor interaction, and adhesion molecules. All these alterations were limited by wild-type MSC-derived EVs, but not by those released by Droscha knocked-down cells.

Alix is an endosomal protein involved in EV generation and able to bind the miRNA carrier Argonaute 2. For

this reason, Alix is probably a key mediator of miRNA enrichment within EVs. Experimental Alix knockdown did not vary the number of EVs released by SC, whereas it significantly decreased miRNA expression levels [29]. Consistently, cells targeted by Alix-KO EVs had lower cytosolic miRNA concentration and did not display relevant phenotype changes.

EPC-derived EVs target mainly vascular tissues; these vesicles promote angiogenesis, prevent leukocyte infiltration, inhibit complement cascade, and ultimately prevent post-AKI capillary rarefaction and CKD progression [7]. Consistently with MSC data, EPC-EV activity was mainly due to the horizontal transfer of mRNAs and miRNAs [7]. Indeed, the protective effect of EPC-derived EVs is blunted by pretreatment with RNase or by Dicer knock-out.

To further demonstrate the prominent vascular role of EPC-EVs, we transfected EPCs with miRNA-blocking transcripts (antagomiRs) directed to the angiogenic miR-126 and miR-296 [27]. As expected, the EVs from antagomiR-EPC did not improve AKI and did not halt the vascular rarefaction observed in AKI to CKD transition. In vitro studies on hypoxic renal endothelium confirmed the pro-angiogenic effects observed in vivo and their dependence on specific miRNAs.

Finally, EV effects are also mediated by protein trafficking. In a murine study, MSCs released IGF-1 follow-

ing cisplatin-induced renal injury thus promoting renal repair [30]. The authors demonstrated that MSC-EVs deliver IGF-1 receptor to injured tubular epithelial cells, thus enhancing their regenerative potential.

SC-Derived EVs Protect from Acute Glomerular Injury

SC-derived EVs have also been evaluated in a wide range of experimental models of glomerular injury. A recent study measured the levels of the progenitor cell marker CD133 on EVs isolated from urine of patients with acute and chronic glomerular diseases. Urinary CD133+ EVs were significantly decreased in concomitance with any glomerulopathy [31]. Moreover, CD133 levels were restored in EVs after the treatment of the underlying renal disease and ROC curve analysis indicated that CD133 urinary levels are diagnostic for glomerulopathy. In vitro studies confirmed a significant decrease of CD133 in renal progenitor cells treated by high glucose concentrations and albumin overload.

Intravenous injection of anti-Thy1.1 antibody is a validated rat model of acute mesangio-proliferative glomerulonephritis characterized by complement-mediated injury of mesangial cells and impairment of glomerular angiogenesis. In anti-Thy1.1 treated rats, human EPC-derived EVs promptly localized within injured glomeruli, inhibited leukocyte infiltration, and prevented endothelial and mesangial cell activation and apoptosis [7]. These effects resulted in significant decrease of proteinuria and in improvement of renal function. EPC-derived EVs inhibited the progression of glomerulosclerosis by decreasing tissue deposition of the terminal component of the complement cascade C5b9 (membrane attack complex) and the subsequent fibrosis characterized by the overexpression of smooth muscle actin. In addition, EPC-EVs preserved the expression of both endothelial (RECA-1) and podocyte (synaptopodin, nephrin) antigens, suggesting their role in the maintenance of glomerular integrity. These effects were mainly mediated by mRNA/miRNA transfer; targeted glomerular cells expressed enhanced levels of vascular endothelial growth factor and complement-inhibiting proteins (decay accelerating factor or CD55, CD59, and factor H). As reported in the above-mentioned studies about MSCs, the pretreatment of EPC-EVs with RNase abolished their regenerative effects. Additionally, glomerular healing was specific for EPC-derived EVs since EVs originated from other cell types

(including fibroblasts and monocytes) were ineffective or even detrimental.

Preeclampsia (PE)-associated glomerular injury is characterized by severe endothelial dysfunction and detrimental complement activation [32]. Of note, circulating EPCs are increased in preeclamptic women with a positive correlation between their number and systolic blood pressure, thus suggesting that EPCs may represent a physiological response of the bone marrow to PE-related vascular injury [33]. Our preliminary data (unpublished) suggest that EPC-derived EVs may enhance angiogenesis and limit complement-mediated endothelial cell lysis in PE.

Conclusions and Perspectives

EVs are complex endocrine factors that shuttle a wide variety of macro-molecules and are able to reprogram target cells, mainly via epigenetic mechanisms. In AKI, EV infusion is potentially safer than cell therapies and has been proven effective in multiple disease models. A recent meta-analysis pooled the results of 39 preclinical AKI studies on SC-EVs. The beneficial effects were consistent among the reports and comparable to SC infusion [34]. Different clinical trials are now recruiting patients, none has been published so far about AKI. One randomized Phase II study on CKD reported an increase of GFR after 1 year in MSC-EV-treated patients [35]; in addition, the authors observed increased circulating levels of TGF- β and IL-10 and reduced TNF- α .

However, we recognize that several limitations should be overcome before the safe use of EV-based therapies in the clinical setting. EV isolation protocols have been significantly improving over the last years, but the steps of ultracentrifugation and/or gradient separation still remain essential although not efficient and time consuming. Like SC, EVs require specialized and certified cell factories with high costs of production. Finally, most EV types have not been comprehensively characterized and the vast majority of EV-related regenerative effects are unknown.

However, despite these limitations and the need for more clinical and preclinical studies, available data make SC-derived EVs one of the most promising and innovative therapies for AKI at the horizon; in particular, EVs are thought to be as effective as SC but with an improved safety profile.

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Conflict of Interest Statement

All authors declare no conflict of interest.

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