

Adult stem cells as a tool for kidney regeneration

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Abstract

Kidney regeneration is a challenging but promising strategy aimed at reducing the progression to end-stage renal disease (ESRD) and improving the quality of life

of patients with ESRD. Adult stem cells are multipotent stem cells that reside in various tissues, such as bone marrow and adipose tissue. Although intensive studies to isolate kidney stem/progenitor cells from the adult kidney have been performed, it remains controversial whether stem/progenitor cells actually exist in the mammalian adult kidney. The efficacy of mesenchymal stem cells (MSCs) in the recovery of kidney function has been demonstrated in animal nephropathy models, such as acute tubular injury, glomerulonephritis, renal artery stenosis, and remnant kidney. However, their beneficial effects seem to be mediated largely *via* their paracrine effects rather than their direct differentiation into renal parenchymal cells. MSCs not only secrete bioactive molecules directly into the circulation, but they also release various molecules, such as proteins, mRNA, and microRNA, in membrane-covered vesicles. A detailed analysis of these molecules and an exploration of the optimal combination of these molecules will enable the treatment of patients with kidney disease without using stem cells. Another option for the treatment of patients with kidney disease using adult somatic cells is a direct/indirect reprogramming of adult somatic cells into kidney stem/progenitor cells. Although many hurdles still need to be overcome, this strategy will enable *bona fide* kidney regeneration rather than kidney repair using remnant renal parenchymal cells.

Key words: Adult stem cells; Direct reprogramming; Extracellular vesicles; Mesenchymal stem cells; Paracrine factors; Indirect reprogramming

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Core tip: Although intensive studies have been performed to isolate kidney stem/progenitor cells from the mammalian adult kidney, whether stem/progenitor cells actually exist in the adult kidney is still debated. Mesenchymal stem cells seem to exert beneficial effects *via* paracrine effects rather than by direct differentiation into renal parenchymal cells. In this review, we also introduce potential roles of extracellular vesicles released

from stem cells and direct/indirect reprogramming of adult somatic cells by which kidney stem/progenitor cells will be formed in the future.

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INTRODUCTION

The kidney is a vital organ that plays various roles, such as the excretion of waste products; regulation of systemic fluid volume, electrolytes, and pH; maintenance of systemic blood pressure; and erythropoietin production. These functions are performed by the nephrons, the functional units of the kidney. If the structure and/or function of the nephrons are damaged because of diseases, such as diabetes, hypertension, and glomerulonephritis, and if such damages continue to progress, renal function gradually deteriorates. The kidney finally becomes unable to perform its critical roles, resulting in renal failure.

There are two therapeutic options for the treatment of end-stage renal disease (ESRD). One is dialysis therapy, which compromises patients' quality of life and cannot substitute for all kidney functions. Another is renal transplantation, which is limited because of the lack of sufficient donors. To explore a better treatment for ESRD, it is necessary to find strategies to regenerate the kidney. In this respect, stem cell therapy for the kidney has been intensively studied recently.

Stem cells are defined as cells that are capable of self-renewal and can differentiate into a variety of phenotypes^[1]. Adult stem cells (ASCs) are multipotent stem cells that reside in various tissues, such as the bone marrow, adipose tissue, and skeletal muscle^[2,3]. In this article, we review the possibility of kidney regeneration using ASCs.

Stem cells in the embryonic kidney

Although stem/progenitor cells in the embryonic kidney are beyond the scope of this review, we briefly describe the process of kidney organogenesis, because genetic programs that are activated during kidney organogenesis are reactivated in disease states, such as acute tubular injuries. Kidney organogenesis initiates with the interaction of the ureteric bud (UB) derived from the Wolffian duct with the metanephric mesenchyme (MM). A proportion of the MM is located adjacent to the UB, called the cap mesenchyme (CM). The CM then aggregates at the tip of the UB and differentiates into all epithelial cells of nephrons, except the collecting tubules. It is now well established that the CM contains stem/progenitor cells for kidney organogenesis^[4]. The

CM expresses unique transcription factors, such as *Pax2*, *Six2*, and *Sal1*^[5]. Although stem cells in the embryonic kidney are a promising source for kidney regeneration, their clinical use is strictly limited mainly because of the ethical concerns and small number of stem cells. Therefore, the search for stem/progenitor cells in the adult kidney has been intensively performed.

Stem/progenitor cells in the adult kidney

Four different methods have been used in an attempt to isolate stem/progenitor cells from the adult kidney (Table 1).

Label-retaining cells (LRCs): To conserve the proliferation capacity for a lifetime and to prevent genetic injuries during mitosis, stem cells cycle very slowly. Stem/progenitor cells in the kidney were isolated using this property. Cells were pulse-labeled with a dye, such as 5-bromo-2-deoxyuridine. Then, slow-cycling LRCs were detected following a chase period. Maeshima *et al*^[6] detected LRCs predominantly in the renal tubular cells of the adult rat kidney. LRCs proliferated in response to ischemia/reperfusion injury and contributed to the repair of renal tubules. In another study, Maeshima *et al*^[7] also demonstrated that LRCs were integrated into epithelial components of the nephron when transplanted into the metanephric kidney, suggesting that LRCs were multipotent stem cells. Oliver *et al*^[8] detected LRCs in the kidney papilla of adult rats. LRCs proliferated after the induction of ischemia in the kidney and migrated toward the medulla. They also injected renal papillary cells into the subcapsular area of the kidney and found that some cells were incorporated into renal tubules.

Side population cells: Because stem cells extrude dyes, such as Rhodamine 123 and Hoechst 33342, *via* the ATP-binding cassette protein^[9], they are located in a unique position on the fluorescent-assisted cell sorting scattered plot and are called side population (SP) cells. Iwatani *et al*^[10] isolated SP cells from the adult rat kidney. However, the cells did not participate in the kidney repair following experimental glomerulonephritis or gentamicin-induced nephropathy. Hishikawa *et al*^[11] isolated SP cells from the adult murine kidney. These cells expressed musclin/MyoR and improved renal function when injected systemically into mice with the induction of acute tubular injury by cisplatin administration. Furthermore, SP cells expressed renoprotective factors, such as hepatocyte growth factor (HGF), vascular endothelial growth factor, and bone morphogenetic protein 7 in a cisplatin-induced acute kidney injury (AKI) model. Challen *et al*^[12] also isolated SP cells from the adult murine kidney. These cells were located predominantly in the proximal tubules and integrated into the MM- and UB-derived structures when injected into the embryonic kidney, suggesting that they were multipotent stem cells. However, these cells were barely incorporated into the renal tissues when

Table 1 A summary of the results of isolating stem cells from the adult kidney

Species	Isolation method	Stem cell markers	Location	Incorporation into kidney tubules	Ref.
Rat	Label retaining		Proximal tubule	Yes	Maeshima <i>et al</i> ^[6]
Rat	Label retaining		Papilla	Yes	Oliver <i>et al</i> ^[8]
Rat	Side population	Sca-1, CD45	Some were derived from bone marrow	No	Iwatani <i>et al</i> ^[10]
Mouse	Side population	Sca-1	Interstitial	NE	Hishikawa <i>et al</i> ^[11]
Mouse	Side population	Sca-1	Proximal tubule	Yes (rare)	Challen <i>et al</i> ^[12]
Human	Marker	CD133	Tubules	Yes	Bussolati <i>et al</i> ^[15]
Human	Marker	CD133, CD24	Parietal epithelium in the Bowman's capsule	Yes	Sagrinati <i>et al</i> ^[16]
Mouse	Marker	Sca-1	Papilla	Yes	Dekel <i>et al</i> ^[17]
Rat	Culture	Pax2, Oct4	Proximal tubule	Yes	Gupta <i>et al</i> ^[18]

NE: Not examined.

administered to an adriamycin-induced kidney injury model, although renal function was recovered, probably because of their paracrine effect.

Cell surface markers: Cell surface markers, such as CD133, were used to isolate stem/progenitor cells from the adult kidney. Although CD133 is not a specific marker for kidney stem cells, it is a universal marker for stem cells in other tissues, such as hematopoietic stem cells, vascular endothelial progenitor cells (EPCs), and cancer stem cells^[13,14]. Bussolati *et al*^[15] isolated CD133+ cells from the adult human kidney. These cells expressed *Pax2*, but not CD34 or CD45, markers for hematopoietic stem cells. They could also be induced to differentiate into tubular epithelial cells and endothelial cells *in vitro*. When these cells were administered intravenously in a glycerol-induced AKI model of severe combined immune deficiency (SCID) mice, they were incorporated predominantly into the proximal and distal tubules. Sagrinati *et al*^[16] isolated CD133+ and CD24+ cells from human parietal epithelial cells in the Bowman's capsule after culturing glomeruli. When cultured in appropriate conditions, these cells were differentiated into tubular epithelial cells, osteogenic cells, adipocytes, and neuronal cells. These cells were integrated predominantly into renal tubules when they were injected intravenously in SCID mice that were treated with glycerol to induce acute tubular injuries. Dekel *et al*^[17] isolated stem cell antigen-1 (Sca1)-positive cells from the adult mouse kidney. The Sca1+ cells were located mainly in the papilla. When these cells were administered to an ischemia-induced AKI model, some of these cells were integrated into renal tubules.

Cell culture: A unique cell population was isolated during the culture of dispersed cells derived from the adult kidney. Gupta *et al*^[18] isolated progenitor-like cells from the adult rat kidney that express vimentin, CD90, *Pax2*, and *Oct4*, a marker for embryonic stem cells. These cells were incorporated into renal tubules when injected under the capsule of the kidney or intra-arterially, following ischemia-reperfusion injury of the

kidney.

Arguments against the presence of stem/progenitor cells in the mammalian adult kidney

Although aforementioned results suggest that renal stem/progenitor cells exist in the adult kidney, some reports demonstrated that differentiated renal tubular cells, but not renal stem/progenitor cells, can completely regenerate renal tubules after injury. Humphreys *et al*^[19] used genetic fate-mapping techniques in which renal epithelial cells derived from the CM (from the Bowman's capsule to the junction of the connecting segment and collecting duct) were labeled with either β -galactosidase or red fluorescent protein (RFP). After ischemia-reperfusion injury, approximately 50% of the proximal tubular cells coexpressed both RFP and Ki67, a cell proliferation marker that is expressed during the S-M phases of the cell cycle. These findings suggested that intrinsic renal tubular cells proliferate in response to injury. Furthermore, approximately 95% of tubular epithelial cells expressed RFP prior to injury, after one cycle of injury, and after two cycles of injury, indicating that no dilution of the RFP+ tubular epithelial cells occurred. These results suggested that differentiated renal epithelial cells proliferate well in response to the injury and that stem and/or progenitor cells residing in the interstitium did not participate in the regeneration of the tubules. Kusaba *et al*^[20] used a genetically modified mouse in which the tdTomato protein, which fluoresces in a red color, expressed only in differentiated proximal renal tubules. No dilution of tdTomato+ cells was observed after ischemia-reperfusion injury, suggesting that stem/progenitor cells in renal tubules did not participate in the regeneration of renal tubules. Furthermore, they observed that tdTomato+ proximal tubules expressed CD24 and CD133, markers for stem/progenitor cells. These findings suggested that renal tubules were dedifferentiated and expressed stem cell markers during their proliferation and participation in the repair of renal tubules. These results did not support that stem/progenitor cells in renal tubules and in the interstitium participated in the regeneration of renal

Table 2 Effects of Bone marrow mesenchymal stem cells on renal tissue repair

Origin of stem cells	Experimental model	Effects	Ref.
Mouse	Glycerol-induced AKI	Differentiation into tubular epithelial cells	Herrera <i>et al</i> ^[22]
Mouse	Cisplatin-induced AKI	Differentiation into tubular epithelial cells	Morigi <i>et al</i> ^[23]
Human	Glomerulonephropathy induced by anti-mesangial cell serum	Differentiation into mesangial cells	Wong <i>et al</i> ^[30]
Rat	I/R injury	Recovery of renal function No transdifferentiation into tubules	Lange <i>et al</i> ^[24]
Human	Cisplatin-induced AKI	Recovery of renal function Improved survival No transdifferentiation into tubules	Morigi <i>et al</i> ^[25]
Rat	Gentamicin-induced AKI	Recovery of renal function Conditioned medium and exosomes were effective	Reis <i>et al</i> ^[26]
Rat	Adriamycin-induced nephropathy	Reduced podocyte injury Increased VEGF production	Zoja <i>et al</i> ^[27]
Rat	Thy1.1 GN	Reduced mesangiolysis Increased glomerular cell proliferation Reduced proteinuria Production of VEGF and TGF- β	Kunter <i>et al</i> ^[28]
Mouse	CKD (Deficiency in collagen type IV, α -3 chain)	Reduced fibrosis Production of VEGF and BMP-7	Ninichuk <i>et al</i> ^[29]
Rat	5/6 nephrectomy	Improved renal function Reduced fibrosis Reduced expression of IL-6 and TNF- α	Semedo <i>et al</i> ^[31]
Rat	Kidney allograft	Improved renal function Reduced fibrosis Reduced expression of IL-6	Franquesa <i>et al</i> ^[32]

AKI: Acute kidney injury; I/R: Ischemia reperfusion; GN: Glomerulonephritis; CKD: Chronic kidney disease; VEGF: Vascular endothelial growth factor; TGF- β : Transforming growth factor- β ; BMP-7: Bone morphogenetic protein-7; IL-6: Interleukin-6; TNF- α : Tumor necrosis factor- α .

tubular cells in acute tubular injury. Nonetheless, a potential role of intrinsic stem/progenitor cells in kidney regeneration is not completely excluded from these results, because stem/progenitor cells may participate in the repair of other cell types, such as podocytes^[21]. It is difficult to distinguish renal stem/progenitor cells from dedifferentiated renal epithelial cells. Identification of specific markers that are exclusively expressed in stem/progenitor cells, but not in dedifferentiated renal epithelial cells, will be required to resolve this issue.

Mesenchymal stem cells

The MSCs reside in various organs, such as bone marrow, subcutaneous adipose tissue, and skeletal muscles. Bone marrow mesenchymal stem cells (BMMSCs) and adipose tissue-derived MSCs (ADSCs) are particularly interesting, because a large amount of MSCs can be collected with relatively less invasive procedures.

BMMSCs: Many studies have demonstrated the efficacy of BMMSCs in the treatment of kidney disease using animal models of AKI^[22-26], podocyte injury^[27], glomerulonephropathy^[28-30], a remnant kidney^[31], and kidney transplantation^[32] (Table 2). Although early studies indicated BMMSCs could be differentiated into renal epithelial cells^[22,23] and mesangial cells^[30], recent evidence suggests that the differentiation capacity of BMMSCs is limited. Thus, BMMSCs do not appear to differentiate into renal parenchymal cells *in vivo*^[33]. The beneficial effects of BMMSCs on renal

function seem to be largely mediated by paracrine factors produced by BMMSCs that have anti-apoptotic, proangiogenic, and/or immune modulatory effects^[34-36]. The transdifferentiation of BMMSCs observed in early reports may reflect cell fusion. If BMMSCs fuse with resident cells in the kidney, they acquire a phenotype of resident cells. Thus, it appears as if BMMSCs were differentiated into resident cells. Indeed, several reports have demonstrated that BMMSCs are capable of fusing with other cell types^[37,38]. A phase I clinical study evaluated the safety and efficacy of allogenic BMMSC administration for the prevention of AKI after open-heart surgery^[39]. This study enrolled 16 patients who required on-pump cardiac surgery and who were at a high risk of postoperative AKI due to underlying chronic kidney disease (CKD), advanced age, diabetes mellitus, and congestive heart failure. Allogenic BMMSCs were injected into the suprarenal aorta after surgery. The primary objective was the safety of BMMSC administration. The secondary objective was the efficacy of this treatment compared with well-matched historical controls. This treatment appeared to be both safe and effective as no adverse events related to the procedure were reported, and renal function was well preserved post-operatively, with no patients requiring hemodialysis after surgery, whereas 20% of the controls developed AKI. This is the only clinical trial published so far in which ASCs were used to treat kidney disease.

ADSCs: ADSCs are another type of MSCs residing

Table 3 Effects of adipose tissue-derived mesenchymal stem cells on renal tissue repair

Origin of stem cells	Experimental model	Effects	References
Rat	I/R injury	Recovery of renal function Reduction in oxidative stress	Chen <i>et al</i> ^[40]
Human	Cisplatin-induced AKI	Recovery of renal function Conditioned medium was effective	Kim <i>et al</i> ^[41]
Human	Folic acid-induced AKI	Recovery of renal function HGF and VEGF production	Katsuno <i>et al</i> ^[42]
Rat	Anti-GBM disease	Reduced renal injury and proteinuria Conversion of macrophages to immunoregulatory cells	Furuhashi <i>et al</i> ^[43]
Swine	Renal artery stenosis	Recovery of renal function Improved angiogenesis	Eirin <i>et al</i> ^[44]
Swine	Renal artery stenosis	Increased production of VEGF and bFGF Recovery of renal function Improved angiogenesis	Zhu <i>et al</i> ^[45]
Swine	Renal artery stenosis	Decreased oxidative stress Recovery of renal function Improved angiogenesis	Ebrahimi <i>et al</i> ^[46]
Mouse	Renal fibrosis (unilateral clamping of the renal pedicle)	Increased production of VEGF Recovery of renal function Reduced fibrosis Decreased expression of IL6 and TNF- α	Donizetti-Oliveira <i>et al</i> ^[47]

I/R: Ischemia reperfusion; AKI: Acute kidney injury; GBM: Glomerular basement membrane; HGF: Hepatocyte growth factor; VEGF: Vascular endothelial growth factor; bFGF: Basic fibroblast growth factor; IL-6: Interleukin-6; TNF- α : Tumor necrosis factor- α .

in subcutaneous adipose tissues. Because the subcutaneous adipose tissues are abundant and can be easily harvested using liposuction, ADSCs are promising stem cells for clinical use. The efficacy of ADSC administration in the treatment of kidney disease has been demonstrated in animal models of AKI^[40-42], glomerulonephropathy^[43], renal artery stenosis^[44-46], and progressive renal fibrosis^[47] (Table 3). ADSCs also seem to recover renal function largely *via* paracrine effects^[41,42].

EPCs: EPCs were originally isolated from human peripheral blood using CD34 as a marker for positive selection^[48]. The CD34+ mononuclear blood cells obtained the characteristics of vascular endothelial cells (VECs) when cultured on fibronectin-coated dishes. EPCs were reportedly incorporated in ischemic tissues *in vivo* and expressed markers for VECs such as CD31 when introduced into the circulation using a hindlimb ischemia model. The efficacy of EPC administration in the recovery of renal function was reported in animal models of AKI^[49] and renal artery stenosis^[50,51]. Interestingly, the function of EPCs was deteriorated in CKD patients^[52], suggesting that the autologous transplantation of EPCs may not be suitable for the treatment of CKD.

Umbilical cord blood-derived MSCs

Umbilical cord blood (UCB) contains MSCs, and the efficacy of UCB administration in the restoration of renal function has been reported in animal AKI models^[53,54]. Morigi *et al*^[53] injected human UCB-derived MSCs to immunodeficient mice with cisplatin-induced acute tubular injury. They demonstrated that these cells ameliorated tubular injury, resulting in the recovery

of renal function. They also cocultured UCB-derived MSCs with cisplatin-treated proximal tubular cells (HK-2 cells) and demonstrated that the expression of HGF was particularly induced and that of interleukin 1- β and tumor necrosis factor- α was significantly decreased in the coculture system. These findings suggested that the modulation of paracrine factors in the kidney was implicated in the UCB-induced recovery of renal function. Panepucci *et al*^[55] compared the gene expression profile of UCB-derived MSCs and BMMSCs. Although both MSCs expressed similar sets of genes, BMMSCs predominantly expressed a set of genes related to antimicrobial activity and osteogenesis, whereas UCB-derived MSCs predominantly expressed genes related to matrix remodeling and angiogenesis, suggesting that UCB-derived MSCs and BMMSCs may have distinct activities *in vivo*.

Amniotic fluid stem cells

Human amniotic fluid contains stem cells derived from embryos, and thus, is a promising source of stem cells. The efficacy of human amniotic fluid stem cells (HAFSCs) has been demonstrated in animal models of AKI^[56,57]. Houser *et al*^[56] compared the characteristics of HAFSCs with that of BMMSCs. They found that compared with BMMSCs, HAFSCs had a more potent anti-apoptotic activity against renal tubular cells but lesser stimulatory activity for the proliferation of renal tubular cells. They also demonstrated that HAFSCs and BMMSCs expressed distinct sets of paracrine factors, suggesting that HAFSCs and BMMSCs may have distinct activities *in vivo*.

Direct/indirect reprogramming of adult somatic cells

Another strategy for kidney regeneration is to create

pluripotent stem cells and progenitor cells, whose destination is limited to one or several cell lineages, or terminally differentiated renal parenchymal cells from adult somatic cells *via* direct/indirect reprogramming. Indirect reprogramming is a strategy in which adult somatic cells are induced to dedifferentiate into pluripotent stem cells and re-differentiate into specific cell types. Direct reprogramming involves a strategy in which adult somatic cells are induced to differentiate directly into another cell type. Since the discovery of induced pluripotent stem (iPS) cells^[58,59], it is not difficult to prepare iPS cells from various adult somatic cells. Indeed, several reports have demonstrated a successful preparation of nephrogenic intermediate mesoderm, from which the MM and the UB derive, using iPS cells^[60-62]. However, several hurdles remain to be overcome before iPS cells can be used practically to regenerate the kidney. First, the efficiency of iPS cell preparation from adult somatic cells is still low. Second, it is still challenging to prepare kidney stem/progenitor cells that exist in the CM from iPS cells. Third, even if kidney stem/progenitor cells are successfully created from iPS cells, it is difficult to continue to culture and expand those stem/progenitor cells while maintaining their unique properties. The reason for this limitation is that the niche for kidney stem/progenitor cells has not been clearly understood. Therefore, further studies will be required to use indirect reprogramming for kidney regeneration. Recently, several reports have demonstrated that a direct reprogramming method was useful for kidney regeneration. Hendry *et al.*^[63] introduced 6 transcription factors into a human adult renal proximal tubular cell line. These cells were localized in *Six2+* and *Wilm's tumor 1+* compartment in an *ex vivo* organoid culture assay. These findings suggest that these cells obtained properties similar to kidney stem/progenitor cells. Papadimou *et al.*^[64] incubated permeabilized human BMMSCs with the extracts of human proximal tubular epithelial cells and obtained a cell population similar to proximal renal tubular cells. These cells were integrated in tubular structures in an *ex vivo* organoid culture assay. Furthermore, these cells were engrafted in renal tubules when administered to a cisplatin-induced AKI model. Therefore, direct reprogramming seems to be a promising strategy for kidney regeneration. It has been reported that iPS cells derived from various cell types are not identical in their differentiation capacity^[65-67], probably because iPS cells maintain epigenetic memory of their parental cells. Thus, it may be better to use renal parenchymal cells for reprogramming than cells derived from tissues other than the kidney.

Possible roles of extracellular vesicles released from MSCs

MSCs not only secrete bioactive molecules directly into the circulation but also release extracellular vesicles (EVs)^[68], such as exosomes that contain proteins, mRNA, and microRNA^[69,70]. Several reports have demonstrated that EV administration restored the kidney

function in animal models of AKI^[26,71-73]. Bruno *et al.*^[71] isolated EVs from supernatants of human BMMSCs and examined their effects on the proliferation and apoptosis in renal epithelial cells. EVs were incorporated in renal epithelial cells *in vitro* and the incorporation depended on CD44 and β 1-interin. EVs stimulated the proliferation and inhibited apoptosis of renal epithelial cells. These effects were diminished when EVs were treated with RNase prior to administration. Furthermore, the authors administered EVs to immunodeficient mice with glycerol-induced acute tubular injury and demonstrated that EV administration restored renal function. Moreover, these beneficial effects were diminished when EVs were pretreated with RNase. Tomasoni *et al.*^[74] isolated EVs from supernatants of human BMMSCs and demonstrated that EVs contained mRNA for the insulin-like growth factor-1 receptor (IGF1R). When cisplatin-treated renal epithelial cells were incubated with EVs, proliferative capacity of renal epithelial cells increased significantly; however, the stimulatory effect was diminished when the expression of IGF1R mRNA in BMMSCs was suppressed using small interfering RNA to IGF1R prior to EV harvest. Zhou *et al.*^[75] administered EVs harvested from human UCB-derived MSCs to a rat model of cisplatin-induced AKI. EV administration significantly restored renal function and morphology. Therefore, EVs seem to contain various bioactive molecules that can be used for the treatment of kidney injury.

FUTURE DIRECTIONS

It seems that there are two major directions to improve the quality of stem cell therapy for kidney diseases. One is to analyze bioactive molecules released from stem cells in more details. If an ideal combination of bioactive proteins, mRNA, and/or microRNA is elucidated, stem cells *per se* will not be necessary in the future. Although BMMSCs have been used in the clinical setting to treat patients with cardiovascular disease^[76-80], concern about tumorigenesis still remains^[81]. Furthermore, BMMSCs isolated under uremic conditions have less capacity for the proliferation, survival, and secretion of paracrine factors compared with those isolated from normal controls^[82-84]. Patients who need stem cell therapy are probably not a suitable source of high-quality stem cells, indicating that autologous stem cell transplantation may not be effective in these patients. Therefore, cell-free therapy seems attractive. Another alternative is to explore strategies to directly and/or indirectly reprogram adult somatic cells into kidney stem/progenitor cells. If efficient and safe methods to induce direct/indirect reprogramming are explored, bona fide kidney regeneration rather than kidney repair using remnant renal parenchymal cells will be possible in the future.

CONCLUSION

Although intensive studies have been performed to isolate kidney stem/progenitor cells from the adult

kidney, it is debated whether kidney stem/progenitor cells actually exist in the adult kidney. There are no specific markers and/or assays to discriminate kidney stem/progenitor cells from dedifferentiated renal epithelial cells. MSCs are effective to repair the kidney in various animal models; however, their beneficial effects can be largely attributed to paracrine factors that are secreted from MSCs. In addition, MSCs release EVs that contain mRNA and microRNA as well as proteins. These EV-derived molecules may also play beneficial roles in the repair of the kidney. It will be necessary to elucidate ideal combinations of the molecules released from MSCs to establish a strategy for the maximal stimulation of kidney repair without using stem cells. It will also be necessary to explore strategies to directly and/or indirectly reprogram somatic cells to kidney stem/progenitor cells to regenerate the kidney.

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