Stem cells: potential and challenges for kidney repair

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Submitted 26 April 2013; accepted in final form 5 November 2013

KIDNEY DISEASE is a significant determinant of morbidity and mortality, and its prevalence continues to grow in association with incremental rates of obesity, diabetes, and cardiovascular disease, making it a serious global health problem (9, 78, 80, 100). Acute renal injury is significantly associated with the development of advanced-stage chronic kidney disease (CKD) (20, 52, 98). CKD is characterized by renal fibrosis and the progressive loss of functional nephrons, leading to the deterioration of renal function and end-stage renal disease (49, 134). Given the limited capacity of the kidney for regeneration, the survival of patients with irreversible kidney injury relies solely on dialysis or kidney transplantation. Dialysis leads to severe morbidity, whereas kidney transplantation is significantly limited by organ availability and the necessity for lifelong immunosuppressive treatment. The development of new therapeutic modalities is thus greatly needed. Recent advancements in the field of stem cell research have raised hopes for stem cell-based regenerative approaches to treat acute kidney disease and CKD. In this review, we summarize the current understanding of the cellular processes involved in kidney repair and the latest advances in stem cell-based kidney regeneration as well as the challenges that remain to be faced before these strategies can be implemented in humans.

Kidney Regeneration

In contrast to lower vertebrates such as the skate (37, 135) and zebrafish (31, 134), the mammalian kidney shows a limited capacity for regeneration. While partial nephrectomy can induce the formation of new nephrons (neonephrogenesis) during development, this capacity seems to be lost shortly after birth (33, 62, 127). As a result, chronic injury in the adult kidney induces irreversible loss of glomerular and tubular structures leading to fibrosis, scarring, functional loss, and organ failure. Still, in response to acute injury, the adult kidney shows some level of cellular repair characterized by enhanced proliferation and tissue remodeling (23, 35, 121). Although the exact cellular processes involved in this response remain elusive, several studies have proposed that it potentially involves epithelial cell dedifferentiation (10, 11), interstitial cell transdifferentiation (20, 58), and/or activation of stem cells (49, 80, 100) (Table 1).

In Table 2, we provide a concise overview of these processes and discuss the different cell types involved. The role of cells of the immune system, although important, has been covered elsewhere (37, 52, 98) and will not be addressed here. Tubular cells. After acute ischemic or toxic renal injury, renal epithelial tubular cells show increased proliferation and migration to the sites of damage. Indeed, within 2 days of ischemia, the expression of proliferating cell nuclear antigen (a cell cycle marker denoting the transition from G0 to G1) is maximal in the S3 segment of the proximal tubule (31, 134), a major target of injury during acute renal failure. The injured tubular basement membrane is then progressively covered by cells with mesenchymal characteristics, including an absence of Kid-1 expression (33, 135) and expression of vimentin (23, 35, 121, 134). These regenerating cells also express renal developmental markers, such as paired box 2 (Pax2) (10, 11, 62, 127), and eventually transition to an epithelial phenotype and reconstitute tubular structures.

Initial studies (128–130) have indicated that the source of this regeneration might be the mature cells of the tubular epithelium through a process of dedifferentiation. This hypothesis has been further supported by lineage tracing studies (34, 55, 71). First, using chimeric mice expressing green fluorescent protein (GFP), Duffield et al. (34) showed that tubular regeneration after injury does not involve marrow cells. Similar studies (34, 55, 71) using alternative methods to trace marrow
cells, such as β-galactosidase expression or the presence of the male Y chromosome, have validated these results, supporting the notion that cell repair in the kidney occurs via an intrinsic cell population. To investigate whether renal epithelial cells directly contribute to repair after renal injury, Lin et al. (71) crossed Z/EG double-reporter (43) mice with Cre<sup>esp</sup> transgenic mice that express Cre recombinase under the control of the nephron-specific KSP-cadherin promoter (ksp). In the kidney, Cre<sup>esp</sup> mice express Cre exclusively in tubular epithelial cells (113). As a result, Cre<sup>esp</sup> Z/EG mice have their renal tubular epithelial cells and progeny marked with enhanced GFP. When these mice were subjected to renal ischemic injury, enhanced GFP<sup>+</sup> tubular cells showed enhanced proliferation as well as the reexpression of vimentin (a mesenchymal cell marker) and Pax2 (an embryonic kidney marker), suggesting that dedifferentiation of the tubular epithelium to a less mature phenotype is involved in renal regeneration after injury (71). Using a similar approach, Humphreys and colleagues (55) used the FoxD1 or sine oculis-related homeobox (Six)2 promoter to label interstitial and tubular epithelial cells with β-galactosidase (lacZ) or red fluorescent protein, respectively. Within 2 days after renal injury, 50% of tubular epithelial cells coexpressed the proliferation marker Ki-67 and red fluorescent protein, indicating that the proliferating cells were derived from Six2<sup>+</sup> tubular epithelia. In contrast, FoxD1-lacZ-labeled cells were not detected. Overall, these data indicate that dedifferentiation of tubular epithelial cells plays an important role in proximal tubule recovery after acute renal injury.

Nevertheless, several studies have proposed that rare epithelial lineage-committed progenitors might exist, which can also participate in kidney regeneration. After injury, these progenitors could become activated to proliferate and eventually differentiate to new tubular epithelial cells. Indeed, initial studies in rodents have reported the existence of quiescent cells in the adult renal epithelium that show rare and infrequent proliferation under normal conditions, a characteristic usually associated with the presence of adult stem cells (77). When these cells were isolated, they showed the potential to generate tubule-like structures in vitro and in vivo (76). Another study by Langworthy et al. (67) identified nuclear factor of activated T cells (NFAT)<sub>c1</sub> as a marker of potential proximal tubule progenitors in the mouse kidney. Using lineage tracing, they showed that NFATc1<sup>+</sup> cells are rare in the adult kidney, although upon injury they show resistance to cell death, become activated, and give rise to the majority of new tubular cells generated (67). Still, more recent data from Humphreys et al. (54), using different thymidine analogs to track sequential rounds of DNA synthesis in the adult kidney after injury, suggest that the proliferating cells represent existing epithelial cells. The reason for these discrepancies is not yet well understood. It is possible that the markers used to trace the progenitor cells in the adult kidney pick up dedifferentiated epithelial cells that express stem cell markers. Alternatively, rare progenitor cells do exist, but their detection is challenging, as these cells become activated and thus detectable only after injury. Further lineage tracing experiments using inducible systems are required to elucidate these questions. For instance, it has recently been shown that leucine-rich repeat-containing G protein-coupled receptor (Lgr5) expression (a protein involved in Wnt signaling) marks a stem cell/progenitor population during development that gives rise to the thick ascending limb of Henle’s loop and distal convoluted tubule cells (7). Lgr5 expression was not detected in adult kidney; however, it is possible that Lgr5<sup>+</sup> cells are activated transiently after injury (7). These data not only bring attention to the possible importance of Lgr5 during adult kidney regeneration but also to the broader implications for the importance of Wnt signaling in these processes (7).

The presence of a potential adult renal progenitor population of intratubular origin is also supported by several studies (1, 15, 73, 75, 106, 116) that reported the existence of multipotent CD24<sup>+</sup>CD133<sup>+</sup> tubule cells in the human kidney. These mul-

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**Glossary**

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**Table 2. Human resident renal progenitor cells**

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<td>Papilla</td>
<td>Multipotent</td>
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<tr>
<td>CD133&lt;sup&gt;-&lt;/sup&gt;CD73&lt;sup&gt;-&lt;/sup&gt;CD90&lt;sup&gt;-&lt;/sup&gt;</td>
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PODXL, podocalyxin-like. Modified from Ref. 80.
tipotent CD24+CD133+ cells show characteristics typical of adult stem cells, such as clonogenicity, expression of stem cell markers, and the potential to differentiate into multiple cell types in vitro and in vivo. They are scattered throughout the proximal tubule and show specific gene expression (73). They also have distinct morphology characterized by less cytoplasm, fewer mitochondria, lack of basolateral invaginations, and a mature brush border, which is consistent with a less differentiated proximal tubule phenotype (116). Importantly, these cells proliferate after injury in the kidneys of patients with acute or chronic tubular damage and comprise the majority of regenerated tubules (1, 75, 116). Moreover, Angelotti et al. (1) have recently showed that a subpopulation of CD24+CD133+ renal progenitors that lack the expression of vascular adhesion molecule 1 (CD106) have a high propensity to differentiate toward tubular cells in vitro. In vivo, CD24+CD133+CD106− cells were specifically localized to the proximal tubule and distinct areas of the distal convoluted tubule but were never found in the collecting duct. Although these renal progenitors have been found in pig and primate kidneys (3), they have not been detected in rat or mouse kidneys, suggesting that either the cell markers that define the progenitor cell population are different among species or that other processes (i.e., dedifferentiation) are involved in rodent tubular regeneration (102).

Taken together, these findings support the hypothesis of a tubular stem cell. However, these cells seem to be rare, and more studies are required to define the specific markers in different species. As mentioned above, lineage fate tracking to further analyze the origin and contribution of these cells to the regenerating epithelium are also necessary.

Podocytes. Podocyte loss is a hallmark of end-stage kidney diseases (133). The kidney can partially compensate for low levels of podocyte loss by decreasing the effective area of glomerular filtration and hypertrophy of the remaining cells (132). Podocytes have a limited capacity for regeneration. However, several studies have reported that podocytes possess proliferative potential (5, 6, 85, 96, 115, 141) and/or that endogenous renal progenitors that can differentiate to podocytes do exist (1, 68, 101, 103), opening the possibility that these processes might participate in mature podocyte replacement during homeostasis and/or disease states.

Mature podocytes are usually quiescent and show a limited ability for cell division (84, 93, 112). This inability is regulated by cyclin-dependent kinase inhibitors and is considered a prerequisite for terminal differentiation (85, 111). Nevertheless, podocyte cell division has been reported in certain cases of nephropathy, such as focal segmental glomerulosclerosis and collapsing glomerulopathies (5, 6, 56). As an example, Barisoni and colleagues (4, 6) have shown that during collapsing glomerulopathies, podocytes show the expression of cell cycle genes such as Ki-67, p27, p57, cyclin A, and cyclin D1 accompanied by dedifferentiation and an increase in podocyte numbers. Similarly, other groups (24, 56, 57) have shown that human immunodeficiency virus infection increases podocyte dedifferentiation and proliferation via Tat and Nef-1 genes. More recently, podocyte-specific deletion of Dicer or Drosha, two enzymes that are responsible for the generation of mature microRNAs, led to collapsing glomerulopathy, suggesting that microRNAs play an important role in podocyte differentiation (114, 141). Podocytes in these mutant mice showed reduced levels and/or disorganized patterns of expression for specific podocyte proteins, such as synaptopodin, Wilms tumor-1, nephrin, and podocin (114, 141). Importantly, Ki-67+nestin+ double-positive cells were detected in areas of glomeruli damage, indicating the presence of proliferating podocytes (114, 141). These data highlight the importance of microRNAs in maintaining podocytes in a nonproliferative differentiated state. In addition, new evidence has been recently provided implicating the Wnt pathway as a crucial regulator of podocyte proliferation. Using a transgenic mouse overexpressing the telomerase reverse transcriptase (mTert) gene as a model for glomerular collapse, Shkrelli et al. (115) have shown that mTert-dependent activation of the canonical Wnt pathway induced podocyte proliferation, indicating that the pathway is involved in podocyte cell cycle regulation (115). Further studies to identify the particular microRNAs or cell signaling pathways involved in podocyte proliferation will be of considerable interest.

Still, as in the case of tubular regeneration, controversies exist as to whether the proliferating cells derive from dedifferentiated podocytes or represent activated adult progenitor cells. Indeed, in the adult human kidney, parietal epithelial cells that display the capacity to differentiate into glomerular as well as tubular epithelial cells have been observed at the urinary pole in Bowman’s capsule (1, 101, 103). These cells appear to generate transitional podocyte-committed progenitors, which display characteristics of both stem cells and podocytes and localize close to the vascular pole. These transitional podocyte-committed progenitors can only differentiate into a podocyte progenitor (1, 103). Both the subpopulations at the urinary pole and vascular pole express the renal stem/progenitor cell markers CD133 and CD24, with the former being CD133+CD24+ podocalyxin-like (PODXL)− or CD133+CD24+CD106− and the latter being CD133+CD24+PODXL+ (1, 103). Interestingly, similar progenitors have been identified in rodents. Zhang et al. (139, 140) have described different models of rat nephropathy during which parietal epithelial cells in Bowman’s capsule become activated and acquire the expression of typical podocyte markers such as Pax2 and Wilms tumor-1. Importantly, lineage tracing studies using transgenic mice to track parietal epithelial cells in the adult kidney have demonstrated that these cells are recruited onto the glomerular tuft and differentiate into podocytes. During these processes, transitional cells with morphological and immunohistochemical features of both parietal epithelial cells and podocytes could be detected at the glomerular vascular stalk (2). Interestingly, in mice lacking expression of β-catenin in the renal epithelium, podocytes can be found mislocalized in areas of the parietal epithelium, highlighting the importance of the β-catenin pathway in lineage differentiation of parietal epithelial cells toward a podocyte-like cell fate (42). Whether these parietal epithelial cells represent the murine equivalent of CD133+CD24+PODXL− cells identified in humans (103) remains to be tested.

In summary, although podocytes have the capacity to proliferate, true cell division is rare or occurs under specific disease states. Similarly, Bowman’s capsule seems to contain rare adult progenitor cells that hold the capacity to differentiate toward podocytes. Future studies to understand the characteristics of those progenitor cells as well as the mechanisms of podocyte proliferation and differentiation would provide useful developments in achieving podocyte regeneration.
Mesangial cells. Mesangial cell proliferation and migration are observed in several types of glomerular injuries. Hugo et al. (53), using an antibody against the Thy1 mesangial marker, were able to eliminate almost 95% of mesangial cells in a rat model and identified a mesangial “reserve population” that could repopulate the entire glomerulus (53). Although proper characterization of these repopulating cells is still lacking, Daniel and colleagues (29) have recently shown that they express nestin, a protein also found in podocytes as well as stem cells. Further studies to determine whether this reserve population represents mature dedifferentiating or true progenitor cells are required.

Renal papilla. A previous study (89) based on label retaining approaches after long-term bromodeoxyuridine (BrdU) injection have indicated that the renal papilla might act as a stem cell niche harboring a putative stem cell population. Studies (87–89, 131) in both rodents and humans have indicated that CD133+ “nestin+” cells exist in the papilla and show stem cell-like characteristics. On the basis of these observations, the renal papilla was hypothesized to be a source for tubular regeneration, and cells generated from the papilla region were proposed to migrate upward into damaged nephrons throughout the medulla and cortex (87, 89). However, the origin and characterization of these cells as “true” stem cells is still controversial (126), and recent cell lineage experiments, although revealing a subpopulation of papillary BrdU label-retaining cells that are also positive for the stem cell marker mTert (117), have shown that these cells do not seem to contribute to kidney repair after injury (117).

Interstitial/other. In addition to the sites mentioned above, several studies have identified nontubular mesenchymal populations in the renal interstitium or from whole kidney isolations with limited or no epithelial potential. Adult mesenchymal cells are quiescent under physiological conditions, but they are reactivated after acute or chronic injury. One typical example is the differentiation of mesenchymal-derived cells to myofibroblasts after injury (41, 58). Whether these adult tissue resident mesenchymal cells contain a population of multipotent mesenchymal stem/stromal cells (MSCs) is under debate.

Adult MSCs represent an “heterogeneous” population that expresses a combination of cell surface markers, none of which is exclusively found in MSCs. Moreover, there is growing evidence showing that MSC-like cells frequently exist in the perivascular space and can express pericyte markers (25), posing the question of whether there is an overlap between pericyte and MSC populations. The situation is complicated further by the fact that the in vivo characteristics of MSCs and pericytes, as well as their developmental relationships, are relatively unknown. Currently, the minimal criteria for defining MSCs are 1) plastic adherence in standard tissue culture conditions; 2) expression of CD73, CD90, and CD105; 3) lack of expression for CD11b, CD14, CD34, CD45, CD79α/CD19, and human leukocyte antigen-DR; and 4) differentiation in vitro to osteoblasts, adipocytes, and chondroblasts (32). In adult mouse tissue, MSC-like cells appear to be stem cell antigen (Sca)1+CD90+CD45− (81).

In the rodent kidney, several studies (19, 51, 60, 63) have reported the presence of a renal side population. These side population cells show multipotency in vitro and the capacity to generate embryonic kidney structures ex vivo and to engraft in the injured kidney after transplantation (19, 60). Expression analysis has shown that the majority of renal side populations express Sca1, but there is some controversy as to whether they also express the transcription factor musculin (51). Dekel and colleagues (30) have also reported the isolation of Sca1+ cells from the adult renal interstitium. These cells lack hematopoietic capacity and show characteristics typical of MSCs, such as plastic adherent and a multilineage differentiation capacity. Cell fate tracking of those cells after delivery into the kidney postinjury revealed that they hold the capacity to differentiate toward the tubular phenotype and contribute to tissue repair (30). Interestingly, a study (90) comparing Sca1+CD29+CD44+ MSCs isolated from the bone marrow, heart, and kidney showed that all three cell populations had similar morphologies and shared a common distinct gene expression signature. Still, organ-specific gene expression patterns were also observed (90). Renal MSCs showed high expression of nestin as well as enriched expression of myosin light chain kinase, myoM, desmin, endothelin-1, angiopoietin-2, and VEGF-C, suggesting a strong relationship with vascular, perivascular, or mesangial cells of the kidney (90).

In humans, similar experiments have identified a rare population of CD133+ cells localized in the cortical interstitium that could differentiate in vitro to both endothelial and epithelial cell fates (14). Cell sorting showed that these CD133+ cells were positive for the MSC markers CD73 and CD44 but not CD90. These cells also expressed the developmental renal marker Pax2 as evaluated by immunofluorescence and quantitative real-time PCR analysis.

In summary, numerous studies have supported the presence of a MSC-like population in the adult kidney. Still, the origin, characteristic, phenotype, and function of those cells are not well defined. Further studies to characterize these cells, especially in the context of their differences compared with a similar population from other tissues, are necessary for the understanding of their role in kidney regeneration.

Stem Cell Therapy for Kidney Regeneration

Despite the excitement for the discovery of endogenous renal stem/progenitor cells, so far, very little knowledge exists on how to harness their potential and how to enhance these endogenous regenerative processes to achieve kidney repair. As a result, most of the current efforts for kidney regeneration have been focused on the transplantation of stem cells from other sources, such as the bone marrow. Since the kidney is composed of at least 14 different cell types (50), the ideal strategy would be the administration of a single multipotent stem/progenitor cell from the same individual. These cells then could engraft and undergo differentiation in the diseased kidney, resulting in the replacement of the different cell types and subsequent regeneration without the risk of immune rejection-associated problems.

Below, we summarize recent studies that have investigated the potential of using transplantation of extrarenal stem cells to achieve kidney repair and regeneration under different pathological states.

Whole bone marrow stem cells. Whole bone marrow stem cell (BMSC) delivery presents an attractive approach for kidney repair and regeneration, as these cells show high plasticity and can be easily isolated, expanded, genetically manipulated in vitro, and then reintroduced into the patient in an autologous
Initial studies (44, 65, 94) using BMSCs in experimental models of kidney disease were based on the transplantation of labeled BMSCs in irradiated animals. These studies suggested that transplanted BMSCs could potentially differentiate to renal cells and regenerate the injured tissue. Still, as mentioned above, lineage tracing studies (34, 54, 61, 71) did not show differentiation of BMSCs into the tubular epithelium or mesangial cells after injury, suggesting that bone marrow transplantation has variable, if any, effects on the generation of new renal cells after injury.

Still, a previous study (138) has reported that transplanted BMSCs can engraft in the kidney and improve renal function after both acute and chronic injury. For example, Rookmaaker et al. (104), using a model of anti-Thy-1-induced renal damage, demonstrated full integration of bone marrow-derived endothelial and mesangial cells in the glomerular structure after the induction of nephritis. Moreover, Prodomidi et al. (97), using a genetic mouse model of progressive glomerular damage caused by lack of the \( \alpha_3 \) chain of collagen type IV (Alport syndrome), reported that glomerular scarring and interstitial fibrosis were significantly decreased 20 wk after the transplantation of wild-type BMSCs. In addition, serum urea and creatinine levels were lower in recipient mice compared with those that received BMSCs derived from knockout controls (97). Donor bone marrow-derived cells were detected by in situ hybridization for the \( Y \) chromosome, and experiments using fluorescence and confocal microscopy revealed that some of them showed morphology and localization typical of podocytes. Interestingly, their data suggested that the integrated cells acted by contributing to collagen type IV production in the kidney. Similar results were found by Sugimoto et al. (119), who also showed repopulation of mesangial cells in addition to podocytes and significant improvement in proteinuria after 4 and 13 wk after \( \alpha_3 \)-chains of collagen type IV knockout mice were transplanted with wild-type whole BMSCs (119). Using a mouse model of Habu snake venom-induced glomerulonephritis, Hayakawa et al. (46) found that donor-derived BMSCs can potentially contribute to glomeruli remodeling by transdifferentiation to thombomodulin \(^{-} \) endothelial-like cells. Ikasari et al. (59) showed similar findings using the more severe rat model of chronic progressive glomerulosclerosis consisting of uninephrectomy plus anti-Thy1-induced renal damage. Specifically, the authors reported the presence of GFP \(^{+} \) BMSCs in the glomerular capillaries of rats; these cells contained with the endothelial markers platelet/endothelial cell adhesion molecule 1 and RECA-1 and the mesangial marker OX7.

Collectively, these data support a potential use of BMSCs for renal stem cell therapy. Still, only a small population of transplanted cells is recruited by the kidney, and, even then, the ability of these cells to differentiate to renal cells is controversial. The bone marrow includes hematopoietic stem cells (HSCs), MSCs, and endothelial progenitor cells (EPCs); thus, investigators have proposed that the beneficial effects could arise from a subpopulation of BMSCs. Moreover, these effects might be the result of paracrine factors secreted from these cells, as we describe below.

MSCs. MSCs represent an important stem cell component of the bone marrow comprising \( \sim 0.01\% \) of total bone marrow cells. Several studies using different models of renal damage have shown that the delivery of bone marrow-derived MSCs results in kidney repair and improvement of renal function. In one of the first studies, Morighi et al. (82) demonstrated that intravenous injection of MSCs, but not HSCs, of male bone marrow origin significantly reversed the augmented blood urea nitrogen in female mice subjected to a model of drug-induced acute injury. These effects were accompanied by a rapid improvement of structural damage and enhanced proliferation of renal cells in the area of injury. However, the low engraftment of MSCs ruled out the possibility that they contributed to renal repair by transdifferentiation to renal cells, suggesting that MSCs might indirectly affect renal repair by paracrine mechanisms. These effects do not seem to be limited only to bone marrow-derived MSCs but appear to be common in MSCs derived from other tissues (21, 36). As an example, it has been recently reported that adipose tissue-derived MSCs have the ability to regenerate the kidney vasculature in a swine model of renal artery stenosis (36). Importantly, the discovery of renal MSC-like cells has resulted in efforts aiming to use this cell type in transplantation studies, with the hope that it might offer a higher regenerative capacity for the kidney compared with other MSC sources. In a representative example, mouse kidney MSCs were injected into the renal parenchyma of the outer cortex and an additional bolus in the renal circulation at the time of adriamycin administration to induce progressive renal damage (19, 144). Although cell transplantation resulted in a reduced albuminuria-to-creatinine ratio, there was no significant evidence for tubular integration and regeneration, suggesting a humoral role for kidney-derived MSCs in renal repair.

Given the low engraftment and differentiation levels, the current consensus is therefore that the majority of the renal repair effects of MSC transplantation are rather due to the secretion of paracrine factors. The role of MSC-secreted humoral factors on kidney regeneration has been addressed directly by studies using conditioned media obtained from cultures of MSCs in vitro. MSC-conditioned medium reduced apoptosis and induced migration and proliferation of tubular epithelial cells, suggesting that the improvement of renal function observed with MSC treatment is likely caused by the actions of secreted humoral factors acting on the injured kidney (8). Additionally, several reports (22, 39, 69) have shown that MSCs can mitigate the progression of diabetic nephropathy and CKD models accompanied with decreased circulating cytokine levels, suggesting that MSC therapy can modulate the local inflammatory response and suppress kidney remodeling in CKD in a paracrine fashion. Indeed, MSC delivery has been shown to improve renal function by suppressing proinflammatory IL-1\( \beta \), TNF-\( \alpha \), and interferon-\( \gamma \) (26, 110, 123) and increasing immunosuppressive factors such as IL-10 (109). In addition, since MSCs secrete large amounts of VEGF, paracrine release of VEGF could constitute a mechanism of inhibiting the profibrotic activity of TNF-\( \alpha \), which is a major factor for the epithelial-mesenchymal transition that leads to kidney fibrosis (16). Similar paracrine effects have been reported for human MSCs (27, 83). Thus, intra-arterial delivery of MSCs and enhancement of local paracrine mechanisms may constitute a powerful therapeutic strategy for the treatment of chronic kidney injury in humans.

Despite this potential, numerous challenges still remain. The survival of the delivered cells is limited, and the mechanisms and signaling pathways involved in MSC-mediated renal repair...
are poorly understood. Moreover, the risks associated with cell therapy cannot be ignored. In a rat model of glomerulonephritis, intrarenal injection of MSCs resulted in healthier kidneys and reduced proteinuria after 50 days of treatment. However, at day 60, MSCs appeared to have developed adipogenic maldifferentiation, causing glomerular sclerosis (66). Thus, the use of undifferentiated cells might not be suitable if they open up the chances for cancer. An additional report (28) has suggested that the injection of MSCs is associated with high risks in clinical settings. The functional incompetence of MSCs derived from patients with CKD appears to be another barrier for autologous cell therapy (86). Finally, despite strong preclinical data, most of the studies rely on rodent MSCs or rodent disease models. To explore the translation potential of MSC-based renal therapy, further research for human MSCs, as well as for finding strategies to avoid the above-mentioned risks, is greatly needed.

HSCs. Despite extensive preclinical studies on the therapeutic potential of BMSCs and MSCs, the role of HSCs in renal repair and regeneration has been less investigated. HSCs are capable of self-renewal and can give rise to all hematopoietic lineages. In the adult, they reside mainly in the bone marrow (0.01% of total nucleated cells in the marrow) (79), but they can also be found in cord blood and in the circulation, especially after stimulation with proper cytokines. As in the case of MSCs, HSCs are characterized by the expression of certain markers, such as c-Kit, Sca1, CD150, and CD45 (79).

Early HSC mobilization experiments using granulocyte colony-stimulating factor or macrophage colony-stimulating factor have yielded conflicting results showing either beneficial or detrimental effects on kidney repair, depending on the injury model used (38, 64, 97, 124). In one of the first studies, Prodomidi et al. (97) used the Alport syndrome genetic mouse model described above to compare the effects of whole BMSCs versus bone marrow-derived MSC transplantation. These investigators found that MSC-treated animals did not show signs of improvement compared with BMSCs and suggested that HSCs are the effective component of the bone marrow involved in glomeruli regeneration and the improvement of renal function in this model. Similarly, Fang et al. (38) used a mouse model of HgCl2-induced tubular injury to test whether bone marrow-derived HSCs or MSCs were important for kidney regeneration. After transplantation, both types of stem cells stably engrafted in the bone marrow and spleen, but only the HSCs were engrafted in renal tubules. Importantly, these cells were able to undergo DNA synthesis after acute renal injury, suggesting not only engraftment but also the capacity to get activated. In a study by Li et al. (70), human peripheral HSCs were isolated from healthy donors and injected in mice 1 or 2 days after ischemia-reperfusion injury. Labeled HSCs were found engrafted in the bone marrow after transplantation. These cells were also found in the injured kidney but not in other organs or kidneys from sham-operated animals, suggesting injury-induced HSC mobilization and migration into the kidney. The recruitment of HSCs to the kidney correlated with decrease mortality, improvement of kidney function, vascular and tubular regeneration, and long-term fibrosis. Interestingly, HSCs were found around the renal vasculature but not in the glomerular capillaries; yet improved vascular and tubular regeneration was observed, suggesting that the renal repair was mediated by a paracrine mechanism rather than direct regeneration of the vasculature. This hypothesis is further supported by the finding that recruited HSCs expressed markers similar to circulating EPCs and also high levels of proangiogenic cytokines, which promote vascular and tubular regeneration (97). More recently, Burst et al. (13) injected Lin−CD90+ HSCs in a mouse model of ischemic mouse injury and reported that the cells showed integration within the kidney but did not seem to contribute repair and regeneration. These discrepancies between studies could be possible be explained by differences in the injury model, the source of HSCs, and/or the methods used to identify each cell lineage.

EPCs. EPCs are circulating progenitor cells that can differentiate into endothelial cells and contribute to the formation of new vascular tissue. A variety of markers have been used to define those cells, including expression of CD34, CD133, and VEGF receptor-2 (95).

A healthy renal microvasculature is crucial for preventing the progression of renal damage after acute kidney injury as well as for regeneration (74). Since EPCs are already committed to the endothelial lineage, they represent a potentially more efficient approach to regenerate the microvasculature in renal diseases, especially those involving transplantation, renovascular disorders, and pathologies directly affecting microvascular integrity. In one of the first reports using EPCs for kidney repair (125), intrarenal delivery of EPCs in the rat model of anti-Thyl-induced nephritis 1 day after anti-Thyl treatment protected against endothelial cell loss and decreased macrophage infiltration. Furthermore, 16% of EPCs incorporated in the glomeruli and expressed the endothelial marker rat endothelial cell antigen-1, suggesting endothelial differentiation. Although the cells were shown to secrete VEGF, its role in the regeneration process was not investigated. Similar results were observed when EPCs were delivered at early stages of aminoglycoside-induced injury in the mouse (137). Importantly, the benefit of EPC treatment was confirmed when the cells were delivered at later stages of disease progression, supporting that EPCs promote regeneration regardless of any potential early ant apoptotic or anti-inflammatory effects. Indeed, in a swine model of atherosclerotic renal artery stenosis, autologous EPC injection directly into the renal artery 6 wk after the stenosis resulted in a significant restoration of the cortical microvasculature together with an improvement of renal blood flow and glomerular filtration rate (18). Four weeks after delivery, ~12% of the total number of injected EPCs appeared to localize into vascular and tubular structures. In addition, VEGF was significantly elevated, and oxidative stress was diminished. EPCs also reduced glomerulosclerosis, tubulointerstitial perivascular fibrosis, and renal expression of TNF-α. Similarly, Sangidorj et al. (108) detected transplanted EPCs in a 5/6 nephrectomy mouse model of chronic renal failure 8 wk postinjury. Labeled EPCs could be found in both the glomeruli and tubulointerstitial area. Again, deterioration of renal function was lower in EPC-treated animals along with decreased proteinuria, preservation of kidney structure, and renal transforming growth factor-β. Finally, VEGF expression in the kidney of recipient mice was also elevated compared with untreated mice (108).

Although none of the above studies followed up the animals for an assessment of survival, these data illustrate that delivery of a few EPCs can lead to significant changes within the local...
environment of the injured renal tissue to promote repair. EPCs can influence the renal environment by forming direct interactions and/or directly contribute to the generation of new vascular tissue (137). Alternatively, EPCs can contribute to renal repair by the release of paracrine molecules, especially angiogenic factors. Indeed, increased levels of angiogenic VEGF-A and endothelial nitric oxide synthase have been reported after EPC administration (17). Moreover, EPCs could modify the local levels of cytokines involved in inflammation or activate endogenous renal progenitor cells via paracrine mechanisms. Intriguingly, recent studies have provided evidence showing that EPCs promote kidney regeneration by different mechanisms than MSCs. In a report by Zhu et al. (143), the authors compared the efficacy of adipose tissue derived-MSCs versus cultured EPCs to regenerate kidney structure and function in the swine model of renal artery stenosis. The reduced glomerular filtration rate was significantly increased by intra-arterial cell therapy of either MSCs or EPCs. However, MSCs seemed to have a greater effect than EPCs. Interestingly, EPCs stimulated renal growth factor expression and decreased oxidative stress, whereas MSCs more significantly attenuated renal inflammation, endoplasmic reticulum stress, and apoptosis. These data suggest that, at least in this model of renal injury, EPCs and MSCs affect tissue repair by different mechanisms. Unfortunately, the effect of combination therapy was not investigated, as it would be of interest to know if delivery of both cell types has additive beneficial effects.

Embryonic stem cells, amniotic fluid stem cells, and induced pluripotent stem cells. As mentioned above, the ideal candidate for kidney stem cell therapy would be a multipotent progenitor with the capacity to regenerate all the different types of renal cells. Currently, such a cell type has not been identified in the adult. In contrast, embryonic stem cells (ESCs) are multipotent, and a recent report (72) has shown that they can be manipulated to differentiate toward renal lineages. Still, the use of ESCs for human therapy raises several safety and ethical concerns.

In that context, human amniotic fluid stem cells (AFSCs) can offer a potentially more attractive alternative as these cells represent a new pluripotent cell source that can obtained with amniocentesis during gestation (105). These cells can be stimulated to give rise to renal cells and participate in neofrogenesis in an ex vivo culture system (91). Recent studies have also suggested that their transplantation after injury can lead to tissue repair. Intravenous injection of human AFSCs into nonimmune-competent mice with glycerol-induced acute kidney injury resulted in cytoprotection, increased tubular cell proliferation, and normalization of renal function as measured by serum creatinine and blood urea nitrogen level (45). In another study (92), injection of CD117-positive human AFSCs into the kidneys of mice with rhabdomyolysis-related acute tubular necrosis induced by glycerol rescued them from cell damage. This cell treatment was able to mediate a protective effect, possibly through modulation of the inflammatory response (92). Using a mouse model of Alport syndrome, the same group has more recently shown that AFSC injection ameliorates the acute phase of acute tubular necrosis and delays the progression of renal fibrosis. While promising, further studies are required to fully explore the therapeutic potential of this stem cell population for renal disease as well as to compare them with other stem cell sources.

Finally, recent advances in the field of cell transdifferentiation have opened new possibilities of using induced pluripotent stem cells (iPSCs) for kidney regeneration. These cells offer many advantages (47), as they can be generated from

![Fig. 1. Potential strategies of stem cell-based therapy for kidney repair. A: adult and embryonic stem/progenitor cells can be isolated, expanded, and/or differentiated in vitro for subsequent transplantation. B: delivery of transcription factors and/or microRNAs into the kidney could be used to induce cellular reprogramming from one mature cell type to another. C: administration of humoral factors (such as VEGF) can be used to stimulate kidney-resident stem activation and cell differentiation. In addition, such factors can be used to direct homing of circulating progenitors into the injured tissue (D).](http://ajprenal.physiology.org/2013/00238/0033_AJP_RenalPhysiol_F18 STEM CELLS FOR KIDNEY REPAIR)
somatic cells, thereby avoiding the ethical issues relevant to ESCs; 2) they originate from somatic cells that are later to be transplanted to the same individual, avoiding immune rejection-associated problems; 3) they have the potential to regenerate all cell types within the kidney; and 4) the careful selection of factors to induce pluripotency could potentially decrease the associated oncogenic risk of pluripotent cells.

Protocols for the generation of iPSCs have already been established from various types of adult mammalian cells, including human cells (136). The original protocol was based on the combined overexpression of four specific transcription factors [Oct3/4, sex-determining region Y-box (Sox)2, Myc, and Kruppel-like factor (Klf)4] (120) using viral delivery, although new approaches using nonviral methods and/or small molecules or microRNAs in place of transcription factors have been developed (40, 118).

Interestingly, a recent report by Zhou et al. (142) demonstrated that human iPSCs can be generated by reprogramming renal cells obtained from urine samples. For this, cultured renal cells were infected with retroviruses expressing Sox2, Klf4, Oct4, and c-Myc. Small colonies were found after 11 days after transfection, and most of them adopted human ESC morphology and the expression of stem cell markers. These derived iPSCs have the advantage of being induced from somatic cells collected noninvasively. Importantly, since the somatic cells used were originally committed to the kidney, they might have a higher kidney regenerative capacity, presumably due to the retention of epigenetic memory. Indeed, several studies have suggested that the source of origin of the somatic cell to be reprogrammed affects the behavior as well as the differentiation potential of the derived iPSCs (40).

Still, the delivery of undifferentiated iPSCs back into a patient poses the risk of teratoma formation, and, therefore, directed differentiation of the iPSCs before transplantation would be preferred. Since kidney regeneration requires the restoration of the many functionally distinct cell types of the nephron, differentiation to renal progenitor lineages would offer the advantage of transplanting cells with the capacity to regenerate more than one cell type of the adult kidney, including “de novo” nephrogenesis. Still, to our knowledge, the sets of genes or factors needed to force this phenotype have not yet been identified. Alternatively, direct in situ conversion (reprogramming) from one somatic cell type to another without involving a pluripotent intermediate state could be envisioned (107). This could also circumvent the need of cell delivery and directly deliver reprogramming factors (genes, molecules, or microRNAs) to generate reprogrammed renal or renal progenitor cells in the injured tissue. Although this approach is being investigated for neuronal (99, 122) and cardiac injury (12), its possibilities for the generation of patient-derived renal progenitors in vitro or directly in the injured kidney.

Similar challenges also remain for the use of nonrenal stem cells for kidney repair. First, whether autologous transplantation of isolated and expanded cells versus matching donor cell transplants are best in humans remains to be investigated. Second, the protocols for the ex vivo manipulation of those cells are not streamlined yet. Third, the cell type that would be more appropriate for each diseased condition remains to be determined. Finally, the homing, survival, and engraftment of the transplanted cells are still very low, limiting their effects to transient release of paracrine factors. Further studies as well as clinical trials documenting the feasibility of each therapeutic intervention and assessment of adverse events are expected to provide more insights in the potential use of these stem cells to treat kidney disease.

ACKNOWLEDGMENTS

The authors thank Dr. Jeff Schneckpeper and Alan Payne for a critical reading of the manuscript and proofreading, respectively.

GRANTS

M. M. Herrera is supported by National Heart, Lung, and Blood Institute Grant K99-HL-109167.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.H. and M.M. prepared figures; M.H. and M.M. edited and revised manuscript; M.H. and M.M. approved final version of manuscript.

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