Mesenchymal stem cells as a therapeutic approach to glomerular diseases: benefits and risks

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Most studies using adult stem cells (ASCs) and progenitor cells as potential therapeutics for kidney disorders have been conducted in models of acute kidney injury, where the damage mainly affects the tubulointerstitium. The results are promising, whereas the underlying mechanisms are still being discussed controversially. Glomerular diseases have not received as much attention. Likely reasons include the often insidious onset, rendering the choice of optimal treatment timing difficult, and the fact that chronic diseases may require long-term therapy. In this mini review, we summarize current strategies in adult stem cell-based therapies for glomerular diseases. In addition, we focus on possible side effects of stem cell administration that have been reported recently, that is, profibrotic actions and maldifferentiation of mesenchymal stem cells.

DIFFERENT APPROACHES TO STEM CELL-BASED RENAL THERAPIES

Several investigators have described distinct types of apparently endogenous, resident renal stem/progenitor cells. Others have investigated strategies to mobilize exogenous adult stem cells (ASCs) and enhance their engraftment in renal disease. Another approach is to isolate ASCs from extrarenal tissues, expand them in vitro, and inject them into the recipient, an approach that will be further elucidated in this review. Finally, given recent evidence that beneficial effects of ASCs are mostly paracrine, some investigators have tested whether the administration of a cell-free ‘cocktail’ of factors secreted by ASCs, that is, cell culture supernatants, might be equally effective as whole ASCs. One of the mechanisms herein has been suggested to be secreted microparticles enriched in pre-microRNAs, facilitating miRNA-mediated intercellular communication.

BONE MARROW IS A RESERVOIR OF REGENERATIVE CELLS FOR RENAL REPAIR

Bone marrow-derived (stem) cells contribute to cell turnover and repair in various tissues, including the kidneys. For example, differentiation of mouse and rat bone marrow cells into glomerular cell phenotypes was described in normal and diseased glomeruli. Cell culture experiments confirmed the ability of bone marrow cells to convert into mesangial-like cells on administration of platelet-derived growth factor-BB in the presence of type IV collagen. Two recent studies in chronic renal failure obtained promising results in ameliorating glomerulosclerosis and proteinuria by administering lineage-negative bone marrow cells 15 days after 5/6 nephrectomy or dedifferentiated fat cells with characteristics similar to mesenchymal stem cells (MSCs) in tenascin-C knockout mice with habu snake venom-induced nephritis.

Of the three major marrow-derived lineages, MSCs hold special promise for renal repair because nephrons are largely of mesenchymal origin. In a model of cisplatin-induced acute kidney injury (AKI), MSCs were more efficacious than hematopoietic stem cells in repairing damage. In athymic nude mice with glomerular injury, injected human MSCs localized to glomeruli and differentiated into mesangial-like cells. One recent publication reports on the protective effects of MSCs on coculture with adriamycin-treated...
podocytes in vitro (reduction of apoptosis), but injection of MSCs in vivo did not show regenerative effects in the adriamycin model of nephropathy.40

CHARACTERIZATION OF MSCS AND THEIR MAIN PROPERTIES
MSCs, or ‘multipotent mesenchymal stromal cell(s),’41 have to fulfill consensus criteria regarding their phenotype and biological behavior.42 Although undisputable criteria defining MSCs are still not available,43,44 the emerging data render them strong candidates for human therapies. Potentially more specific markers for human MSCs have been described.45–47

MSCs can be cultured without significant ethical concerns from adult bone marrow aspirates, adipose tissue, or umbilical cord blood,48 and can be expanded under inexpensive conditions in vitro. Their phenotypic stability is superior to that of embryonic stem cells, although cytogenetic aberrations in mouse MSCs after several passages in vitro and sarcoma formation of transduced MSCs in recipient mice have been observed.49 Others have found human MSCs to be very resistant against cytogenetic aberrations, probably because of their increased senescence and thus reduced proliferation rate under culture conditions.50

Another appealing aspect is that syngeneic MSCs can be obtained from a patient before a calculated medical risk, that is, major surgery, and later be re-administered in the case of organ failure (that is, AKI). In experimental AKI, MSC injection shortly after disease induction ameliorated the course of the disease.21,25,26,38,51–55 Even allogeneic transplantation of MSCs seems feasible given their tolerance-inducing effects and their ability to escape T-cell recognition (reviewed in McTaggart and Atkinson56). Several preclinical and clinical studies using MSCs are currently ongoing (reviewed in Giordano et al.57).

Older studies on MSCs focussed mainly on their ability to adopt non-mesenchymal phenotypes, that is, neural precursors and cardiomyocytes. However, methods used to verify transdifferentiation of MSCs (and ASCs in general) into other phenotypes in vivo are technically problematic and prone to misinterpretation.6,25,58–60 In addition, naturally occurring, but rare, fusion events of ASCs with injured kidney cells have been observed.61 More recent studies suggest that MSCs exert most of their effects through paracrine mechanisms.21,62–64

MSC INJECTION ENHANCES GLOMERULAR HEALING IN A MODEL OF ACUTE GLOMERULONEPHRITIS
Rat MSCs injected directly into a renal artery can accelerate recovery from mesangialytic damage and prevent transient AKI in a rat model of mesangiproliferative glomerulonephritis.63 In contrast, MSC injection into a tail vein was ineffective, which may be due to MSCs losing their homing capacity after in vitro culture.65 In inbred Lewis rats, anti-Thy1.1 nephritis follows an aggravated course with transient AKI. Again, AKI and mesangiolysis were ameliorated by syngeneic MSCs. MSCs largely failed to differentiate into endothelial, mesangial, or monocyte/macrophage-like cells. Rather, we found that MSCs secreted high amounts of vascular endothelial growth factor and transforming growth factor-β1, suggesting that MSCs likely exerted beneficial effects in glomeruli by paracrine effects.

TRANSPANTATION OF FULL BONE MARROW OR MSCS CAN AMELIORATE A MODEL OF CHRONIC GLOMERULONEPHRITIS
Anti-Thy 1.1 nephritis induced in uninephrectomized rats results in progressive renal failure.66 In this model, bone marrow contributes mainly to glomerular endothelial cell regeneration in the weeks following disease induction.67 In addition, infused bone marrow prevented death of the nephritic animals.68 Using the same progressive anti-Thy 1.1 nephritis, we investigated the long-term effects of early administration of syngeneic MSCs into the renal artery in chronic renal failure.69 Again, MSCs ameliorated early AKI and reduced glomerular adhesions. After 50 days, proteinuria had progressed in controls, but stayed low in MSC-treated animals. Renal function on day 60 in the MSC group was better than that in medium controls, more glomeruli had recovered from the initial injury and tubulointerstitial fibrosis was reduced.

MALDIFFERENTIATION OF MSCs DURING LONG-TERM FOLLOW-UP OF CHRONIC GLOMERULONEPHRITIS
In the above study,69 at day 60, about 20% of the glomeruli of MSC rats contained adipocytes apparently derived from the injected MSCs using various lines of evidence.69 Both adipocytes and their pronounced surrounding fibrosis severely distorted the normal glomerular morphology (Figure 1).

Despite the maldifferentiation of glomerular MSCs into adipocytes and the fibrotic response surrounding them, renal function was better preserved than in controls. This is likely the consequence of two counteracting effects of MSC treatment; that is, improved early preservation of glomeruli during mesangiolysis on one hand versus maldifferentiation and fibrosis on the other hand. However, the morphological aspect of glomeruli containing adipocytes strongly suggests that these glomeruli should exhibit a marked functional impairment and ultimately develop global glomerulosclerosis.

Other unwanted stem cell-associated phenomena include findings in murine lungs, where injected MSCs were trapped, and, similar to our findings in glomeruli, formed ‘cysts’ with adjacent collagen deposition, resulting in severe lung damage.70 Mouse MSC transplantation into infarcted hearts resulted in extensive bone formation in the myocardium.71 Earlier, less well-documented reports of such unwanted phenomena include bone marrow transplantation, leading to the formation of new bone in ‘inappropriate’ locations.72

At present, our novel observation of ‘orthodox MSC differentiation’ in an ‘unorthodox location’ raises considerable concerns about the safety of MSC-based cell therapies. Resolving these concerns will require extensive tests to evaluate how to prevent such unwanted differentiation.
survival of Alport mice, which has sparked a debate on the role of stem cells in the above studies. Interestingly, even mouse, only the normal allogeneic bone marrow with normal collagen production, or from another Alport with allogeneic unfractionated bone marrow from LacZ-mice ('Alport mice') were lethally irradiated and then transplanted with unfractionated wild-type blood into non-irradiated Alport mice.74,75 It was recruited from parietal cells.

So far, no unwanted differentiation of MSCs has been observed in animals with AKI at 3 months after systemic MSC injection.54 However, in that study, the MSCs did not localize to the kidney, but rather migrated to the bone marrow.

Nevertheless, a recent case report on a patient with severe lupus nephritis, who had received percutaneous intrarenal injections of autologous peripheral stem cell preparations in a private clinic, seems to confirm the possibility of stem cell maldifferentiation in humans: The cell injections apparently led to formation of solid renal (and extrarenal) masses showing angiomyeloproliferative and myeloproliferative components.76

MSCs in Genetic Renal Diseases: Experiences from Alport Mice

When mice deficient of the collagen α3(IV)-chain ('Alport mice') were lethally irradiated and then transplanted with allogeneic unfractionated bone marrow from LacZ-mice with normal collagen production, or from another Alport mouse,74,75 only the normal allogeneic bone marrow improved renal function and diminished fibrosis. LacZ-positive cells constituted about 10% of the glomerular cells and were found in podocyte and mesangial cell locations. In a third study,64 weekly injections of MSCs in the Alport mice prevented loss of peritubular capillaries and reduced interstitial fibrosis. However, irradiation alone also increases survival of Alport mice,76 which has sparked a debate on the role of stem cells in the above studies. Interestingly, even transfusion of unfractionated wild-type blood into non-irradiated Alport mice improved both renal phenotype and survival, as shown by LeBlu and Kalluri.77

In another study, human fetal MSCs were transplanted intrauterinally into mice deficient for collagen type I α2, a condition that induces abnormal progressive collagen deposition in glomeruli.78 Renal engraftment of fetal human MSCs was only about 1% of total kidney cells, but it reduced the abnormal collagen type I deposition in 4- to 12-week-old transgenic mice.78

MSCs Can Influence Fibrotic Processes

We provided first evidence for both a pro- and anti-fibrotic role of MSCs in renal disease.63,69 Others have described that bone marrow-derived mesangial cell progenitors from ROP Os/+ mice, a model of spontaneous glomerulosclerosis, can transmit glomerulosclerosis when transplanted into congenic +/+ mice.29 In another study, bone marrow-derived cells differentiated into renal tubulointerstitial myofibroblasts after ischemia/reperfusion injury.79 There are also a number of studies documenting the acquisition of a myofibroblast-like MSC phenotype in chronically injured livers,80 chronic heart allograft failure,81 and ovarian cancer.82

Other ASCs Within the Glomerulus

Recently, it was noted that human parietal epithelial cells express the stem cell markers CD24, CD133, CD106, and stem cell-specific transcription factors.7 On injection into severe combined immunodeficiency mice, these cells, termed 'adult parietal epithelial multipotent progenitors,' were capable of ameliorating AKI and differentiated into podocytes.83 Using a novel transgenic mouse model, parietal epithelial cells were genetically tagged. With this approach, we could provide the first definitive clues that in young developing mice, parietal cells migrate onto the capillary tuft and differentiate into podocytes (Figure 1). Studies using human adult parietal epithelial multipotent progenitors showed similar findings in adult mice.84 Understanding the regulation of this process offers exciting new aspects to approach progressive disease.
glomerular diseases. Nevertheless, in this context, it appeared as if progenitor cells once again showed unwanted effects: Smeets et al. 11 could demonstrate a contribution of glomerular progenitor cells to hyperplastic lesions in crescentic glomerulonephritis.

In addition to adult parietal epithelial multipotent progenitors, extravascular mesangial progenitor cells can invade the glomerulus after damage from the hilar pole juxtaglomerular region and contribute to mesangial restitution. 85 Whether these cells represent ASCs is currently unknown.

CONCLUSION

MSCs have now been firmly established as sources for protective factors mediating paracrine effects, 21,62–64 supporting the evolving concept of protection rather than differentiation. 18 Unwanted side effects include the potentially proangiogenic role of MSCs in tumor formation and adoption of unwanted phenotypes (‘maldifferentiation’), 69,71,73 which need to be investigated more systematically in the future. Alternatively, instead of administering cultured MSCs, enhanced recruitment of endogenous MSCs might help to avoid maldifferentiation. 71 Once MSC trafficking is more completely understood, other strategies aiming at increasing/inducing homing of endogenous MSCs to nephritic glomeruli should be assessed. 86 Another approach is to manipulate MSCs in vitro. For example, hypoxic preconditioning augments the angiogenic potential of cultured MSCs through increased vascular endothelial growth factor expression. 87

Despite the above concerns, the high potential of MSCs for solid organ and glomerular repair, in particular, cannot be denied.

DISCLOSURE

All the authors declared no competing interests.

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