Adipose tissue–derived mesenchymal stem cells: a fat chance of curing kidney disease?

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Abstract

Many kidney diseases are associated with inflammation and altered immune response. Mesenchymal stem cells (MSCs) are known for their anti-inflammatory properties and immune modulation. Demonstration that the phenotype and immunosuppressive ability of adipose tissue–derived MSCs are not affected by human kidney disease or uremic serum might have clinical significance if autologous adipose tissue–derived MSCs can be tested to prove their long-term safety and efficacy in treating kidney disease.

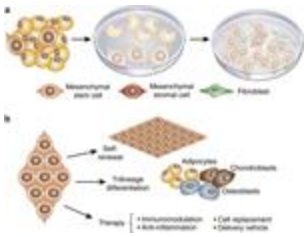
Mesenchymal stem cells (MSCs) are adult stem cells with the capacity of self-renewal and trilineage differentiation into adipocytes, chondroblasts, and osteoblasts. They were first isolated from the bone marrow of guinea pigs in 1970 by Friedenstein and colleagues on the basis of their properties of adherence to plastic and formation of fibroblast colonies.[1] MSCs have since been isolated from virtually every organ in mice, including fat, liver, spleen, pancreas, kidney, lung, muscle, and brain. In humans, MSCs have also been isolated from umbilical cord tissue and cord blood, placenta, and joints. However, there is no unique cell-surface marker distinguishing MSCs from other stem cells. The International Society for Cell Therapy has suggested the following minimal criteria to define human MSCs: 'First, MSC must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*.'[2] Although these criteria have been adopted to identify MSCs isolated from other animal species, murine MSCs are known to express a different set of markers. As cells are expanded in culture dishes, the surface molecules and their level of expression may change as well.

Bone marrow-derived MSCs (BM-MSCs) constitute the major source of MSCs and are the best studied. The widely used method for isolation of BM-MSCs involves density gradient centrifugation to obtain nucleated cells, separation of non-adherent hematopoietic cells from plastic-adherent MSCs, and expansion of MSCs in culture. A similar method is used to isolate and expand MSCs from other organs. The resulting cells are usually heterogeneous with variable self-renewal and differentiation capacities. Many of the cells represent mesenchymal stromal cells, thus raising the concern of 'stemness' or true stem-cell property in the mixed population. Although MSCs isolated from different sources share a considerable degree of overlap in their surface expression profile, they are known to have variations in the pattern and level of expression at different times in culture. For example, MSCs isolated from human adipose tissue are known to express CD34 initially, but CD34 expression is downregulated in culture. Presently, there are no uniform markers that identify MSCs isolated from all sources.

Roemeling-van Rhijn *et al.*[3] (this issue) isolated MSCs from the adipose tissue of humans with end-stage renal disease (MSCs-RD) (mean glomerular filtration rate of 10.3 ml/min/1.73 m^2) and control

subjects (mean glomerular filtration rate of 76.8 ml/min/1.73 m^2) using a culture method (Figure 1a). The immunophenotype, adipogenic and osteogenic differentiation, immunomodulation, and genetic stability of MSCs-RD were compared with those of control MSCs. The authors did not find any differences in the above characteristics. One interpretation of the results would be that the biological properties of adipose tissue–derived MSCs are not affected by renal failure. However, derivation of MSCs requires weeks of cultures in medium containing 15% fetal bovine serum, so it is also possible that cells from either source may have undergone adaptations in culture. To exclude this possibility, the authors performed flow cytometric analysis on freshly isolated $CD34^+$ and $CD73^+$ non-hematopoietic and non-endothelial cells from adipose tissue. No difference in the expression of CD90, CD105, and CD166 was detected between MSCs isolated from controls and patients with renal disease, providing some reassurance in surface molecular expression. It remains unknown whether freshly isolated MSCs from either renal patients or control subjects exhibit self-renewal and trilineage differentiation. The small number of cells before culture expansion limits further studies to answer this question.

Figure 1.



Isolation, characterization, and potential therapeutic use of mesenchymal stem cells. (**a**) Adipose tissue–derived stem cells can be isolated by culture expansion of plastic-adherent cells followed by cell characterization. Note that cells in culture are heterogeneous. They include mesenchymal stem cells, stromal cells, and fibroblasts. (**b**) Mesenchymal stem cells are characterized by their properties of self-renewal and differentiation into adipocytes, chondroblasts, and osteoblasts. The cells could potentially be used to treat diseases by providing immunomodulation, anti-inflammatory actions, and cell replacement as well as delivering therapeutic agents.

Using mixed lymphocyte cultures, the authors showed that MSCs-RD inhibited the proliferation of activated peripheral blood mononuclear cells. To address whether this immunosuppressive ability was affected by uremia, the authors tested the effects of MSCs-RD in the presence of uremic serum and discovered that cell proliferation was inhibited similarly in the presence of uremic serum or control serum. This result could have clinical significance if autologous MSCs are used to induce or modulate immunosuppression in renal failure patients who need kidney transplantation. Animal and human studies have shown that the number and function of endothelial progenitor cells are reduced in renal failure.[4] Although studies presented by Roemeling-van Rhijn *et al.*[3] did not examine whether uremic condition influenced the initial number of MSCs, the cells isolated from controls and patients with renal failure showed similar population-doubling time when both were maintained in culture conditions up to 70 days. Taking a step further, the authors tested the population-doubling time of MSCs-RD in cultures supplemented with 10% control human serum or sera obtained from predialysis and dialysis patients. No difference in the proliferative capacity was detected, further supporting the conclusion that uremia does not affect the growth of MSCs in culture. Although the cells could be maintained in culture for months, the studies presented by Roemeling-van Rhijn *et al.*[3] did not address whether they were true stem cells that could self-renew and proliferate from a single cell colony.

The physiological function of BM-MSCs is to provide extracellular matrix, cytokines, and growth factors that are needed for the normal development, maintenance, and differentiation of hematopoietic stem cells. MSCs are a rare population in the bone marrow, representing less than one in 30,000 nucleated cells. Under culture conditions, MSCs can be expanded within weeks to achieve the numbers needed for potential clinical application. In the past two decades, studies have explored the use of MSCs as immunomodulators, cell replacement agents, or delivery vehicles for therapeutic purposes (Figure 1b). There are currently 225 registered clinical trials (using MSCs to treat various conditions, including wound healing, bone defects, graft-vs.-host disease, inflammatory diseases, organ ischemic injury, and diabetes. To date, most trials are in the recruitment phase and have not had sufficient data to demonstrate sustained effects. Animal studies have shown that administration of MSCs improves renal structure and function after acute kidney injury. The mechanism of renal protection is largely due to paracrine effects that inhibit proinflammatory cytokines and stimulate anti-inflammatory cytokines.[5] Although differentiation of MSCs into tubular epithelial cells has been reported,[6] most studies indicate that intravenously injected MSCs show minimal homing to renal tubules and have limited survival in the kidney environment.[7] A possible approach to enhance the therapeutic value of MSCs would be to increase homing to the kidney, for example, by overexpressing CD44.[8] Another interesting area of research is the use of MSCs in conjunction with induction of immunosuppression in renal transplant recipients to modulate immunity. A pilot study of two patients who received intravenous injection of autologous bone marrow-derived mesenchymal stromal cells 7 days after living-related kidney transplantation showed increased engraftment of regulatory T cells in the peripheral blood and modulation of memory CD8 T-cell function. After one year of follow-up, both patients have stable renal function, and a protocol biopsy in one patient showed normal graft.[9] Long-term studies in larger populations are required to confirm safety and efficacy.

Safety is a key issue in developing MSC-based therapies. Intra-arterial injection of BM-MSCs in a rat model of anti-Thy1.1 mesangioproliferative glomerulonephritis results in the appearance of adipocytes in 20% of glomeruli. The affected glomeruli show increased matrix deposition and sclerosis.[10] The undesired adipocyte differentiation in the kidney offsets the initial beneficial effects in preserving glomerular structure and reducing proteinuria. Like many other reports, this animal study raises important safety concerns that must be addressed before MSCs are used in clinical trials. Furthermore, injected MSCs have been shown to lead to tumor formation in multiple organs, which is thought to be due to chromosomal abnormalities that arise during expansion in culture. Roemeling-van Rhijn *et al.*[3] examined three samples of culture-expanded MSCs-RD using single-nucleotide polymorphism-based whole-genome analysis and did not detect any significant changes. Fluorescence *in situ* hybridization of MSCs isolated from five controls and three renal failure patients indicated that more than 95% of the cells had a normal karyotype. However, a small number of tetraploid cells were detected after ten population doublings, an amount of expansion that is often required to obtain a sufficient number of cells for clinical applications.

The studies by Roemeling-van Rhijn *et al.*[3] demonstrated that the phenotype of adipose tissue–derived MSCs isolated from renal failure patients and control subjects is similar. While maintaining enthusiasm in using our own fat-tissue-derived MSCs for potential treatment of kidney disease, it is critical that we perform thorough testing in animal models before conducting human studies and continue long-term monitoring for safety and efficacy.

Disclosure

The author declared no competing interests.

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