

Renal Therapy by Stem Cells: Outsource or In-House?

Dale R. Abrahamson and Brooke M. Steenhard

Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, Kansas

J Am Soc Nephrol 17: 12–14, 2006. doi: 10.1681/ASN.2005111189

Therapeutic stem cell research is among the most exciting topics in biomedicine today. Many compelling studies have shown that mouse and human embryonic stem (ES) cells in culture are multipotent and can differentiate into a wide variety of cell types, depending on culture conditions (1). Applications of ES cells *in vivo* for tissue repair have also shown much therapeutic promise, although many theoretical and practical problems need to be overcome before this technology is fully robust. Nevertheless, several experiments in animals have shown that ES cells have the capacity for organotypic cell differentiation (2–4). For example, reasoning that the developing kidney microenvironment might provide appropriate cues for entry of ES cells into a nephrogenic program, we microinjected mouse ES cells that were tagged with a lineage marker into embryonic day 12 to 13 metanephroi (5). After 3 to 5 d in organ culture, ES cell derivatives predominantly differentiated into lumenized tubules with apical junctional complexes and primary cilia and assembled a basement membrane around their basal aspects. Many of these structures also expressed proximal tubule markers and Na⁺/K⁺ ATPase, all of which are signatures of a highly differentiated epithelium. Conversely, we found only rare evidence for integration of injected ES cells into the glomerular epithelial tufts that form in organ culture (5).

Owing in part to various difficulties with the few human ES cell lines that are available to most researchers in the United States and the ongoing debate regarding generating new lines of human ES cells, considerable efforts have been devoted to exploring adult somatic stem cells as alternatives, especially those derived from bone marrow (BMSC). These cells can be harvested not only from marrow chambers but also from umbilical cord or peripheral blood and therefore are readily available. Of course, grafting of autologous BMSC is a well-established procedure for restoring hematopoietic tissues in patients who have undergone intensive radiation and chemotherapies.

Beyond marrow reconstitution, there have been numerous reports during the past several years for the experimental repopulation of injured tissues by BMSC in diverse organs (cardiac muscle cells [6], central nervous system astrocytes and microglia [7], and hepatocytes [8]), including kidney. Similar to

our findings with mouse ES cells, when human BMSC are injected into kidneys of intact mouse embryos during whole-embryo culture, BMSC differentiate into complex renal structures (9). Several studies showed recently that when BMSC are injected into mice that are undergoing experimental models of acute renal failure, stem cell derivatives are localized within the renal parenchyma, and, under certain conditions, they help to restore renal function (10–12). However, whether large numbers of extrinsic BMSC differentiate into functional renal cells *in vivo* was questioned recently. A careful study measured compartmentalization of systemically injected BMSC into mouse kidneys that were regenerating from postischemic injury (13). These experiments showed that BMSC derivatives accounted only for 11% of the proliferating epithelial cells of regenerating tubules, whereas 89% of reparative epithelial cells were derived from an intrinsic source. Moreover, >80% of the BMSC localized to the interstitium, where they appeared to be profibrotic and proinflammatory, actually worsening the recovery from acute renal failure (13).

Several obstacles confront potential therapies using extrinsic stem cells: Selection of appropriate cells, differentiation into harmful phenotypes, and delivery into discrete tissue compartments. Perhaps one reason that so many BMSC develop into interstitial cells rather than tubular epithelium in postischemic kidney is that this represents a default pathway adopted by the majority of these cells. Alternatively, the interstitium may represent a more permissive environment for BMSC survival/proliferation. One way to avert such difficulties may be to condition or otherwise manipulate stem cells before their delivery *in vivo*. In this issue of the *JASN*, canine BMSC were cultured on plastic, type I collagen matrices, or type IV collagen NC1 hexamers and compared with the behavior of an immortalized podocyte cell line (14). Like the cultured podocytes, BMSC expressed highest levels of $\alpha 1$, $\alpha 2$, and $\alpha 5$ chains of type IV collagen and redistributed CD2AP into punctuate patterns only when cells were cultured on NC1 hexamer substrates. The authors conclude that culturing BMSC on type IV collagen substrates, which resembles matrix ordinarily seen by podocytes *in vivo*, promotes their partial differentiation *in vitro* into a podocyte-like phenotype (14). Whether these cultured cells could ever become functional podocytes *in vivo* is unknown, but this *in vitro* conditioning strategy with stem cells clearly underscores the importance of cell–matrix interrelationships in achieving appropriate phenotypes.

Another approach involves enrichment of certain stem cells from a larger, mixed pool. Considerable evidence has accumu-

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. Dale R. Abrahamson, Department of Anatomy and Cell Biology, University of Kansas Medical Center, 3901 Rainbow Boulevard, MS 3038, Kansas City, KS 66160. Phone: 913-588-7000; Fax: 913-588-2710; E-mail: dabrahamson@kumc.edu

lated showing that BMSC and peripheral blood contain a relatively small number of endothelial progenitor cells (EPC) that express a restricted set of surface markers (CD31, CD34, and vascular endothelial growth factor receptor 2, among others) (15). Under certain conditions, these EPC-enriched fractions from blood exhibit properties that are similar to those of embryonic angioblasts and contribute to the endothelium of growing vessels and those that are undergoing repair. But these findings, too, are controversial. For example, others have shown that conditioned medium from BMSC/EPC and not the cells themselves are important for certain vascular therapies (16). Indeed, in mice with hind limb ischemia and reconstituted green fluorescent protein (GFP)-positive bone marrow, GFP cells did not co-localize with collateralizing endothelium or smooth muscle but instead accumulated as monocytes/macrophages in vascular peripheries. Nevertheless, these experiments have imposed some new thinking on roles for BMSC in vessel repair. Rather than direct incorporation of BMSC into endothelium, paracrine signals that are transmitted from stem cell derivatives seem to promote vascular growth (16).

The accessibility and microanatomy of certain target organs may present significant barriers to extrinsic stem cell therapy. This may be particularly true for the nephron, which contains dozens of morphologically and functionally distinct epithelial cells, all separated from the vasculature by continuous sheets of basement membrane. For stem cells that circulate in the blood to reach nephron epithelia directly (including glomerular podocytes), this basement membrane barrier would have to be breached, which may lead to a number of poor outcomes. Retrograde, ureteral administration of stem cells might result in repopulation of collecting duct or distal tubular epithelial cells but would require a long, tortuous journey to the proximal and glomerular compartments.

Problems of extrinsic cell access might be overcome through the stimulation of intrinsic stem cell beds, which of course exist in bone marrow, as we have been discussing, but also in other organs, including skin and gastrointestinal tract. A recent study reported that the adult renal papilla harbors an apparently large population of intrinsic stem cells (17). These cells were identified after the administration of bromodeoxyuridine in mice and shown to re-enter the cell cycle after ischemic injury. Whether these papillary cells specifically are the primary or sole source for cell renewal after renal injury remains to be proved. Nevertheless, these intriguing results are also consistent with evidence discussed earlier arguing for a primarily intrinsic, not extrinsic, origin of cells in regenerating kidney (13). Because these papillary stem cells are located in both tubular epithelial and interstitial compartments, therapeutic strategies that selectively stimulate either population may make basement membrane transgression unnecessary. Indeed, for kidney and other organ systems, technologies that target intrinsic stem cells may be our best bet for developing realistic, organotypic stem cell therapies *in vivo*.

Although intrinsic stem cells may ultimately prove to be an optimal target for regenerative medicine, in many cases, these cells are very poorly understood currently. Whether every organ system contains such populations is not known, and how

to promote their differentiation selectively and otherwise manipulate them also are problematic. Therefore, much continued work on the biology of ES cells and BMSC are clearly needed to understand basic questions such as the composition and regulation of their respective niches and control of mobilization, proliferation, and differentiation of their derivatives. Among other approaches, continued experimentation with these cells through conditioning their microenvironment *in vitro*, such as the provision of various culture substrates as discussed by Perry *et al.* (14), should yield much valuable information on mechanisms that dictate stem cell fate. All of this information will be essential before the promises of stem cell research are fulfilled.

References

1. Loebel DA, Watson CM, De Young RA, Tam PP: Lineage choice and differentiation in mouse embryos and embryonic stem cells. *Dev Biol* 264: 1–14, 2003
2. Bjorklund LM, Sanchez-Pernaute R, Chung S, Andersson T, Chen IY, McKnaught KS, Brownell AL, Jenkins BG, Wahlestedt C, Kim KS, Isacson O: Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc Natl Acad Sci U S A* 99: 2344–2349, 2002
3. Blyszczuk P, Czyz J, Kania G, Wagner M, Roll U, St-Onge L, Wobus AM: Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. *Proc Natl Acad Sci U S A* 100: 998–1003, 2003
4. Chinzei R, Tanaka Y, Shimizu-Saito K, Hara Y, Kakinuma S, Watanabe M, Teramoto K, Arii S, Takase K, Sato C, Terada N, Teraoka H: Embryoid-body cells derived from a mouse embryonic stem cell line show differentiation into functional hepatocytes. *Hepatology* 36: 22–29, 2002
5. Steenhard BM, Isom KS, Cazcarro P, Dunmore JH, Godwin AR, St John PL, Abrahamson DR: Integration of embryonic stem cells in metanephric kidney organ culture. *J Am Soc Nephrol* 16: 1623–1631, 2005
6. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD: Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 105: 93–98, 2002
7. Kopen GC, Prokop DJ, Phinney DG: Marrow stromal cells migrate through forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci U S A* 96: 10711–10716, 1999
8. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M: Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. *Nat Med* 6: 1229–1234, 2000
9. Yokoo T, Ohashi T, Shen JS, Sakurai K, Miyazaki Y, Utsumomiya Y, Takahashi M, Terada Y, Eto Y, Kawamura T, Osumi N, Hosoya T: Human mesenchymal stem cells in rodent whole-embryo culture are reprogrammed to contribute to kidney tissues. *Proc Natl Acad Sci U S A* 102: 3296–3300, 2005
10. Poulosom R, Forbes SJ, Hodivala-Dilke K, Ryan E, Wyles S, Navaratnarajah S, Jeffery R, Hunt T, Alison M, Cook T, Pusey C, Wright NA: Bone marrow contributes to renal

- parenchymal turnover and regeneration. *J Pathol* 195: 229–235, 2001
11. Lin F, Cordes K, Li L, Hood L, Couser WG, Shankland SJ, Igarashi P: Hematopoietic stem cells contribute to the regeneration of renal tubules after renal ischemia-reperfusion injury in mice. *J Am Soc Nephrol* 14: 1188–1199, 2003
 12. Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, Abbate M, Rottoli D, Angioletti S, Benigni A, Perico N, Alison M, Remuzzi G: Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. *J Am Soc Nephrol* 15: 1794–1804, 2004
 13. Lin F, Moran A, Igarashi P: Intrarenal cells, not bone marrow-derived cells, are the major source for regeneration in postischemic kidney. *J Clin Invest* 115: 1756–1764, 2005
 14. Perry J, Tam S, Zheng K, Sado Y, Dobson H, Jefferson B, Jacobs R, Thorner PS: Type IV collagen induces podocyte features in bone marrow stromal cells in vitro. *J Am Soc Nephrol* 17: 66–76, 2006
 15. Hristov M, Weber C: Endothelial progenitor cells: Characterization, pathophysiology, and possible clinical relevance. *J Cell Mol Med* 8: 498–508, 2004
 16. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE: Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circ Res* 94: 678–685, 2004
 17. Oliver JA, Maarouf O, Cheema FH, Martens TP, Al-Awqati Q: The renal papilla is a niche for adult kidney stem cells. *J Clin Invest* 114: 795–804, 2004

See related article, "Type IV Collagen Induces Podocyte Features in Bone Marrow Stromal Cells *In Vitro*," on pages 66–76.