Prospects for Stem Cell-Based Therapy

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Resident pools of somatic stem cells in many organs are responsible for tissue maintenance and repair. The goal of regenerative medicine is to exploit these cells either by transplanting them from an exogenous source or by activating endogenous stem cells pharmacologically. For diseases caused by mutations in a single gene, the therapeutic goal is tissue replacement using stem cells engineered to correct the genetic defect. However, a number of technical hurdles must be overcome before therapies based on pluripotent human stem cells can enter the clinic.

Our aging human population is increasingly burdened by degenerative diseases that are largely treated by surgery and drugs designed to mitigate symptoms. Given their role in maintaining and replenishing tissues, stem cells represent a potential means of restoring tissue function and thereby treating the root cause of degenerative disease. Gene therapy for genetic diseases might also prove feasible when coupled to ex vivo repair of the patient's stem cells prior to autologous transplantation. The pharmaceutical industry is adept at medicinal chemistry and the manufacture of protein therapeutics with the same degree of precision as the manufacture of small molecules. However, industry has yet to master the production of pharmaceutical grade cells, and a business model whereby cells can be manufactured as products that can be purchased "off-the-shelf" has proven elusive (see Analysis by A. Parson in this issue of *Cell*). This raises central questions for the future of cell-based therapies, especially those based on stem cells. Must cell therapies always be customized for specific patients and thus remain a labor-intensive, individualized form of medical treatment? Or can cells be produced in bulk from master stocks of cells and be delivered directly from cell banks to patients in response to a physician's prescription? What are the prospects for delivering drugs that augment endogenous stem cell pools? The answers to these questions will dictate the manner in which stem cells impact regenerative medicine.

Stem Cells as Therapy

Stem cells can be used to restore tissue function either as integrated participants in the target tissue or as vehicles that deliver complex signals to a target tissue without actually integrating into the tissue itself. Transplantation of hematopoietic stem cells (HSCs) is the gold standard for restoring tissue function by engraftment of the stem cells directly into target tissue. In contrast, mesenchymal stem/progenitor cells (MSCs) appear to work by the second mechanism to improve damaged heart tissue.

MSCs offer a strategy for using stem cells as a platform to deliver drug-like effects as they do not appear to be incorporated into tissues in settings such as ischemic cardiac injury. This lack of incorporation and the complexity of measuring improved cardiac tissue performance have engendered controversy about their use, yet the number of studies that indicate at least a short-term beneficial effect is hard to dismiss. MSCs appear to provide some paracrine trophic effect that may in part be mediated by modulators of Wnt signaling such as *secreted frizzled related protein-2* (Mirotsou et al., 2007). Studies of other tissue injury models such as graft-versus-host disease or bleomycin-induced mouse lung injury also note amelioration of damage with infusions of MSCs, which they propose is due to anti-inflammatory effects (in the latter case through the release of *IL-1 receptor antagonist*; Ortiz et al., 2007). Although the immunologic or tissue trophic effects of MSCs are being elucidated, their real therapeutic potential remains unclear.

Driving Traffic in the Right Direction

If somatic stem cells are to serve as factor delivery vehicles or be incorporated into tissue, we need to know how they traffic to their targeted sites. All somatic stem cells migrate during development. Notably and of great therapeutic consequence, HSCs continue to migrate in the mature mammal and therefore hematologists/oncologists can collect HSCs for transplantation by pheresis. However, large numbers of HSCs must still be collected because we do not know the most efficient way to deliver them to their sites of action. There is ample evidence that molecules such as α4- and $\alpha 6\beta 1$-integrins, PSGL-1, and E- and L-selectin ligands participate in the interaction of HSCs with endothelium (see Review by D.J. Laird et al. in this issue). Also, the CXC chemokine, stromal derived factor-1 (SDF-1) or CXCL12, has been implicated in transmigration of HSCs to the bone marrow during development. However, SDF-1 and its receptor CXCR4 are not essential for bone marrow engraftment,

suggesting that there is much to learn about the localization and engraftment of HSCs even though they are the best understood and clinically applied of all stem cell types.

Localization is even murkier with other stem cell types such as MSCs. Reportedly, MSCs have been isolated from the circulation and, along with other tissue stem cells, are believed to migrate to sites of injury (Palumbo et al., 2007; Wu et al., 2003). MSC migration appears to depend on molecules involved in immune cell localization such as the CC chemokine MCP-3 (Schenk et al., 2007) and β1 integrins (Ip et al., 2007). Whether SDF-1 or CXCR4 participate in localizing MSCs is controversial. A deeper investigation into homing has led to a new strategy for applying MSCs therapeutically. Ex vivo modification of the glycans on CD44, which is expressed by MSCs, modulates their interaction with receptors known to be expressed by the bone marrow microvasculature, dramatically increasing the efficiency of MSC delivery to bone (Sackstein et al., 2008). Alternative strategies that target the site of desired localization are also being tested in the hopes of enriching the bed into which stem cells are recruited. Protease-resistant chemokines tethered to self-assembling peptides injected into a desired site of recruitment in the damaged heart led to improvements not only in cell localization but also functional outcomes (Segers et al., 2007).

Stem Cells and Tissue Engineering

HSCs face the lowest hurdles for stem cell delivery because the liquid nature of hematopoiesis permits ready engagement of the bone marrow. Clearly, there are very different requirements for the replacement of cardiac or neural tissue. Replaying morphogenic programs to drive complex tissue relationships is a daunting prospect, casting doubt on these efforts. Recent evidence, however, suggests that recapitulating tissue structure may be possible. For example, vascular cells derived from human embryonic stem cells (ESCs) can self-assemble into blood-carrying conduits in vivo and spontaneously enact anastamoses with the host vasculature (Levenberg et al., 2002). Thus, there may be some degree of intrinsic morphogenic capability that will assist efforts to use cell replacement therapy or to create tissue constructs. This capability may be assisted by engineered scaffolds. For example, cardiomyocytes assemble into functional units that coordinate synchronous impulse propagation on biocompatible thin films in vitro and can be shaped into 3D structures (Feinberg et al., 2007). Also, matrices can be generated with graded concentrations of signaling and tethering molecules that enable heterologous cells to assemble in an organized manner. Combining cell-intrinsic morphogenic properties and engineered scaffolds to assist them offers the potential for more complex tissue reconstruction in the future. However, we need to better understand organ morphogenesis to fully exploit this possibility. The interface of developmental and stem cell biology with tissue engineering ultimately promises to transform regenerative medicine.

In Situ Stem Cells as Targets for Therapy

Cell transplantation is but one of several ways in which somatic stem cells may contribute clinically. With the burgeoning awareness of immature cell populations in many tissues that participate in maintenance and, under particular conditions, also repair damaged tissue, activating endogenous stem cells in situ has broad applications. The key is to identify which signals guide the behavior of these cells and to determine whether those signals can be pharmacologically modulated to induce a more vigorous reparative response. This requires a deeper understanding of the stem cell microenvironment or niche (see Review by S.J. Morrison and A.C. Spradling in this issue). Defining the components of that niche and how the niche translates tissue state into stem cell behavior will provide a rational basis for developing drugs to target endogenous stem cells.

One such example is the recognition that osteoblasts—a type of mesenchymal cell in the bone marrow niche—regulate the number of HSCs. However, the molecules involved, the requirement for physical interaction, and whether all or just a subset of osteoblasts participate remain to be determined (Calvi et al., 2003; Kiel et al., 2005; Zhang et al., 2003). But what has been shown is that activation of the parathyroid receptor on the osteoblast can change HSC behavior resulting in increased stem cell numbers, enhanced tolerance to cytotoxic injury, and improved engraftment efficiency in animal models (Adams et al., 2007). These studies have now entered the clinical arena to determine whether parathyroid hormone can improve the outcome of patients receiving umbilical cord blood transplants.

Direct pharmacologic targeting of stem cells in vivo has some experimental support. For example, prostaglandin E2 was shown to increase zebrafish and mouse HSCs and improve the outcome of mouse bone marrow transplantation (North et al., 2007). Also, proteasome inhibitors were shown to prompt increased MSC production of osteoblasts, in part by upregulating the transcription factor Runx-2 (Mukherjee et al., 2008). This strategy has been applied to a mouse model of osteoporosis to improve bone density.

The potential for modifying stem cells in situ is best illustrated by a bold set of experiments pairing animals of different ages by surgical connection of their circulatory systems. This parabiosis model was used to test whether material in the circulation might alter the aged phenotype (Conboy et al., 2005). Comingling the circulatory systems of an aged mouse and a young mouse reverted impaired muscle stem cell activity in the older animal to a younger phenotype. The improved phenotype was observed in molecular terms (increased Notch signaling) and in repair of muscle injury. Wnt regulating factors in serum have been implicated in the improved phenotype as increased Wnt signaling in aged animals favors a fibrotic rather than a myogenic response (Brack et al., 2007). These studies have clear implications for drug-based enhancement of stem cell function and tissue repair.

Patient-Specific Stem Cell Therapies

Like organ transplants, stem cell transplants confront an immune barrier, which requires either that transplants be autologous (derived from "self-tissues") or that patients take immunosuppressive drugs if the transplants are allogeneic (that is, from unrelated donors). To provide wider access to cell therapies and to avoid the need for immune suppression, one of the

ambitions of regenerative medicine is to produce genetically equivalent (isogenic) cells. This can be achieved by producing pluripotent ESCs from adult somatic cells by somatic cell nuclear transfer (SCNT; in which an adult somatic cell nucleus is injected into an enucleated oocyte) or by direct reprogramming of the adult somatic cells back to a pluripotent state using a transcription factor cocktail.

Pluripotent Stem Cells by SCNT

Pluripotent ESCs created by SCNT (so-called ntESCs) can be used both to model diseases and as rejection-proof "autologous" tissues for cell replacement therapies. This theoretical approach has been reduced to practice in mouse models to treat genetic immunodeficiency with HSCs (Rideout et al., 2001) and to treat a Parkinson's-like syndrome with dopaminergic neurons (Barberi et al., 2003). Although cloned organisms resulting from SCNT have health defects due to faulty reprogramming, ntESCs seem to be equivalent to ESCs derived from naturally fertilized embryos (Brambrink et al., 2006). Apparently the process of establishing ESCs in culture entails a winnowing of incompletely reprogrammed clones and selection for those clones that have sustained effective reprogramming and pluripotency. Although ntESCs can be readily generated from the mouse, only recently have two ntESC lines been derived from rhesus macaques (Byrne et al., 2007). This report suggests that SCNT might indeed be feasible in humans, and as long as healthy oocytes are available for research, it is only a matter of time before cloned human blastocysts and human ntESC lines are generated by SCNT (French et al., 2008).

Pluripotent Stem Cells by Reprogramming

Given the hardships involved in donating oocytes, alternatives that avoid the need for oocytes altogether are ultimately preferable but depend on being able to recapitulate the reprogramming process in vitro. The groundbreaking work of Shinya Yamanaka has shown that retroviral transduction of only four genes encoding the transcription factors c-Myc, Klf4, Oct4, and Sox2 is sufficient to induce a state of pluripotency in adult murine and human fibroblasts (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). This work has been corroborated by others in the murine (Maherali et al., 2007; Wernig et al., 2007) and human (Park et al., 2008; Yu et al., 2007) systems. The four transcription factors are introduced by retroviral infection and appear to mediate the reprogramming process over several weeks in culture, resulting in induced pluripotent (iPS) cells that closely resemble ESCs (see Review by R. Jaenisch and R. Young in this issue). In all cases, the retroviruses are silenced, and the pluripotent state of the reprogrammed cells ultimately hinges upon the activity of endogenous genes. Unraveling the mechanisms of reprogramming should identify ways to make this process more efficient. However, there are challenges if iPS cells are to be used therapeutically because three of the reprogramming factors—Myc, Klf4, and Lin28 (or close relatives)—have been linked with oncogenesis, and although Myc is not essential for reprogramming, retroviral insertion alone can cause deleterious and cancer-causing mutations. Indeed, the reversion of mesenchymal cells (fibroblasts) to iPS colonies expressing cadherins mimics the mesenchymal-to-epithelial transition characteristic of malignant transformation in some tissues, suggesting that reprogramming and tumorigenesis may entail a similar dedifferentiation process. By reprogramming adult somatic cells from patients to iPS cells that can then be differentiated into a variety of tissues in vitro, the goal is to generate patient-specific tissues for regenerative medicine. An important next step will be to evaluate whether methods for transient expression of the three or four factor reprogramming cocktail might be sufficient to induce iPS cell formation, and whether small molecules can replace these transcription factors. It is appealing to imagine that therapeutic reprogramming strategies might ultimately be aimed at diverting one adult somatic cell type directly into another, without reverting all the way back to a pluripotent state.

Single-gene disorders may be the best targets for combined gene repair and cell replacement therapy using pluripotent stem cells. Such cells are immortal in culture, which facilitates precise gene repair and characterization of the cells for safety. A recent study demonstrates the feasibility of this approach (Hanna et al., 2007). These investigators reported amelioration of symptoms in a mouse model of sickle cell anemia after transplant of HSCs derived from iPS cells (prepared from skin cells of these mice) in which the genetic defect had been repaired. Although promising, both direct reprogramming and reprogramming by SCNT remain highly cumbersome, labor-intensive, and inefficient processes and present enormous practical barriers to their widespread use to treat disease.

Overcoming the Immune Response

We assume that tissue histocompatability is a prerequisite for cell replacement therapy, but there is a remarkable vacuum of knowledge about the immune responses directed against most classes of stem cells. Some data suggest that human ESCs may be less susceptible to immune attack either because of low-level expression of class I HLA molecules or active suppression of the immune response through uncertain mechanisms (Drukker et al., 2006; Li et al., 2004). Study of the host immune response to transplanted stem cells needs to be given more prominence in the stem cell field.

Because of the cost and inconvenience of customized patient-specific therapies, an appealing strategy is to engineer a pluripotent cell line that is invisible to the immune system and can thereby serve as a universal donor. Such a cell might have to be rendered deficient in class I and II HLA genes and ligands that activate cytotoxicity receptors on natural killer (NK) cells, to express class I mimics such as HLA-G (Mandelboim et al., 1997), and to engage inhibitory NK cell receptors. Generating such a cell is daunting given that it might entail genetic manipulation of scores of loci. An alternative strategy might be to express proteins in transplanted cells that would actively antagonize invading immune cells. Expression of indoleamine dioxygenase, an enzyme that degrades tryptophan, an essential amino acid necessary for T cell function, is believed to be an important feature of the immune privilege of the invading trophoblast tis-

sue of the developing embryo (Munn et al., 1998; see Essay by J. Rossant in this issue). Indeed, a better understanding of the immune mechanisms operating at the maternal-fetal interface may help to define ways to achieve universal donor capability from a single or very limited number of human pluripotent cell lines.

Prospects for Stem Cell Banking

International registries of bone marrow donors and public cord blood banks enable the transplantation of allogeneic HSCs to treat a variety of blood cancers and genetic diseases, but will such registries and banks be required for the widespread application of stem cell transplants? Interestingly, computer simulations that match individuals in transplant donor registries with potential recipients have shown that a large but feasible number of carefully selected cell lines could be banked and could provide a productive tissue match for large segments of the population (if we accept the need for immunosuppressive medication). In a study to identify histocompatibility between 10,000 potential donors and some 6500 renal allograft recipients, only 150 donors were needed to identify a perfect 6/6 antigen match for nearly 20% of the recipients; when the criteria were loosened to allow a single antigen match only, the same number of donors matched nearly 85% of recipients (Taylor et al., 2005). Importantly, donors with homozygosity, which present only 3 rather than 6 distinct histocompatiblity antigens, were far more likely to yield productive matches with recipients. Indeed, as few as 10 donors produced perfect matches for nearly 38% of recipients and productive single antigen matches for 67% of recipients. A simulation performed within a Japanese cohort concluded that 100 homozygous donors could match 80% of the recipients (Nakajima et al., 2007). Both of these studies highlight the feasibility of creating banks of stem cell lines for tissue transplantation.

Humans with homozygous HLA haplotypes are rare in the population but might be identified from transplant registries and approached to donate tissue for generating pluripotent stem cells. Alternatively, pluripotent stem cells with homozygous HLA might be generated from parthenogenetic embryos (pESCs; Kim et al., 2007a; Revazova et al., 2007). Primate pESCs have been derived from cynomolgous monkeys (Cibelli et al., 2002), and the first human ESC line purportedly made by SCNT has actually proven to be the first human pESC line (Kim et al., 2007b). Recently, an independent group has confirmed the isolation of 10 human pESCs at robust efficiency, including 4 that show HLA homozygosity (Revazova et al., 2007). Generating pESCs from even modest numbers of human oocytes appears practical and could be the basis for building banks of stem cells that could provide a source of tissues for transplantation. Because of aberrant imprinting, tissues from pESCs might not grow or function properly, but stable and functional hematopoietic engraftment has been reported from parthenogenetic cells in mice (Eckardt et al., 2007) and in a rare human parthenogenetic chimera (Strain et al., 1995). If pESC-derived tissues prove safe and effective after careful functional analyses, then pESCs might represent a favorable resource for stem cell banking and "off-the-shelf" tissue replacement therapies.

Unanticipated challenges in safety or efficacy might render stem cells or their progeny less than ideal for cell replacement therapies. Nevertheless, insights gleaned from stem cell biology may facilitate classical drug development and will no doubt accelerate progress in affiliated fields like tissue engineering, physiology, systems biology, and developmental biology. Even in the unlikely case that stem cells fail to realize their promise for tissue replacement therapy, their value for in vitro discovery will forever remain unchallenged.

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