

Adult Stem Cells

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Within just a few years, the possibility that the human body contains cells that can repair and regenerate damaged and diseased tissue has gone from an unlikely proposition to a virtual certainty. Adult stem cells have been isolated from numerous adult tissues, umbilical cord, and other non-embryonic sources, and have demonstrated a surprising ability for transformation into other tissue and cell types and for repair of damaged tissues. This paper will examine the published literature regarding the identity of adult stem cells and possible mechanisms for their observed differentiation into tissue types other than their tissue of origin. Reported data from both human and animal studies will be presented on the various tissue sources of adult stem cells and the differentiation and repair abilities for each source, especially with regards to current and potential therapeutic treatments.

Adult stem cells have received intense scrutiny over the past few years due to surprising discoveries regarding heretofore unknown abilities to form multiple cell and tissue types, as well as the discovery of such cells in an increasing number of tissues. The term "adult stem cell" is somewhat of a misnomer, because the cells are present even in infants and similar cells exist in umbilical cord and placenta. More accurate terms have been proposed, such as tissue stem cells, somatic stem cells, or post-natal stem cells. However, because of common usage this review will continue to use the term adult stem cell.

This paper will review the literature related to adult stem cells, including current and potential clinical applications (with apologies to the many who are not cited, due to the exponential increase in papers regarding adult stem cells and the limitations of this review.) The focus will be on human adult stem cells, but will also include results from animal studies which bear on the potential of adult stem cells to be used therapeutically for patients.

This paper will not attempt to review the literature related to hematopoietic stem cells, *i.e.*, the bone marrow stem cell that is the immediate precursor for blood cells, and the formation of typical blood cells. Nor will this paper review the substantial literature regarding clinical use of bone marrow or bone marrow stem cell transplants for hematopoietic conditions such as various cancers and anemias, nor the striking clinical results seen for conditions such as scleromyxedema, multiple sclerosis, systemic lupus, arthritis, Crohn's disease, etc.¹. In these instances, the stem cells are used primarily to replace the hematopoietic system of the patient, after ablation of the patient's own bone marrow hematopoietic system. Finally, multipotent adult progenitor cells (MAPC's), a bone marrow stem cell that has shown significant abilities at proliferation in culture and differentiation into other body tissues,² have been reviewed by Dr. Catherine Verfaillie in a separate paper for the President's Council on Bioethics, and the reader is directed to that review for more information.

Key questions regarding adult stem cells are: (1) their identity, (2) their tissue source of origin, (3) their ability to form other cell or tissue types, and (4) the mechanisms behind such changes in differentiation and effects on tissues and organs. Historically only a few stem cells were recognized in humans, such as the hematopoietic stem cell which produces all of the blood cell types, the gastrointestinal stem cell associated with regeneration of the gastrointestinal lining, the stem cell responsible for the epidermal

layer of skin, and germ cell precursors (in the adult human, the spermatogonial stem cell.) These stem cells were considered to have very limited repertoires, related to replenishment of cells within their tissue of origin. These limitations were considered to be a normal part of the developmental paradigm in which cells become more and more restricted in their lineage capabilities, leading to defined and specific differentiated cells in body tissues. Thus, discovery of stem cells in other tissues, or with the ability to cross typical lineage boundaries, is both exciting and confusing because such evidence challenges the canonical developmental paradigm.

STEM CELL MARKERS

Identification of cells typically relies on use of cell surface markers—cellular differentiation (CD) antigens—that denote the expression of particular proteins associated with genomic activity related to a particular differentiation state of the cell. Identification also has relied on morphological and molecular indications of function, such as expression of specific enzymes. Since stem cells by definition have not yet taken on a specific differentiated function, their identification has relied primarily on use of cell surface markers, and only secondarily on production of differentiated products in various tissues. One stated goal has been to isolate a single putative adult stem cell, characterized fully by specific markers and molecular characteristics, and then to follow the differentiation of this single cell (and/or its progeny) to show that it indeed has multipotent or pluripotent capabilities (clonogenic ability). For bone marrow stem cells, selection of putative adult stem cells has usually excluded typical markers for hematopoietic lineages (lin^-), CD45, CD38, with inclusion or exclusion of the hematopoietic marker CD34 and inclusion of the marker c-kit (CD117). Other proposed markers for adult stem cells are AC133-2 (CD133), which is found on many stem cell populations,³ and C1qR_p, the receptor for complement molecule C1q,⁴ found on a subset of CD34^{+/-} human stem cells from bone marrow and umbilical cord blood. When transplanted into immunodeficient mice, C1qR_p-positive human stem cells formed not only hematopoietic cells but also human hepatocytes. Other methods of isolation and identification include the ability of putative stem cells to exclude fluorescent dyes (rhodamine 123, Hoechst 33342), allowing isolation by fluorescence-activated cell sorter (FACS) of a “side population” of cells within a tissue that have stem cell characteristics. Expression of the *Bcrp1* gene (ABCG2 gene in humans) is apparently responsible for this dye exclusion, and could provide a common molecular expression marker for stem cells.⁵ A study of expressed genes from a single cell-derived colony of human mesenchymal stem cells identified transcripts from numerous cell lineages,⁶ and a similar attempt at profiling the gene expression of human neural stem cell in culture with leukemia inhibitory factor (LIF) has been done,⁷ perhaps providing an expressed molecular milieu which could identify candidate stem cells. Attempts to determine the complete molecular signature of gene expression common to human and mouse stem cells have shown over 200 common genes between hematopoietic and neural stem cells, with some considerable overlap with mouse embryonic stem cells as well.⁸ The function of many of these genes is as yet unknown, but may provide distinctive markers for identification of adult stem cells in different tissues.

However, dependence on particular markers for prospective identification and isolation of adult stem cells seems unreliable. In particular, the use of specific hematopoietic markers such as the presence or absence of CD34, has yielded mixed results in terms of the identification of putative stem cells. There is evidence that the expression of CD34 and CD133 can actually change over time, and its expression may be part of a cycling phenomenon among human hematopoietic and mesenchymal stem cells in the bone marrow and peripheral blood, and perhaps in other tissues,⁹ *i.e.*, an isolated CD34⁺ cell

may become CD34⁻, and then reacquire CD34 expression. Likewise, a systematic analysis of the cell surface markers and differentiation potential of supposedly distinct isolated populations of human bone marrow stem cells revealed no differences in practice between the cell populations.¹⁰ Moreover, an analysis of genetic and ultrastructural characteristics of human mesenchymal stem cells undergoing differentiation and dedifferentiation has revealed reversibility in the characteristics studied.¹¹

Thus, any attempt to isolate a single type of adult stem cell for study may not actually capture the intended cell, or may, by using a particular set of isolation or growth conditions, alter its gene expression. This idea has been elaborated by Thiese and Krause,¹² who note that this “uncertainty principle” means any attempt to isolate and characterize a cell necessarily alters its environment, and thereby potentially its gene expression, identity, and potential ability to differentiate along various lineages. Likewise, the stochastic nature of cell differentiation in such dynamic and interacting systems means that attempts to delineate differentiation pathways must include descriptions of each parameter associated with the conditions used, and still may lead only to a probabilistic outcome for differentiation of a stem cell into a particular tissue. Blau *et al.*¹³ have raised the question of whether there may be a “universal” adult stem cell, residing in multiple tissues and activated dependent on cellular signals, *e.g.*, tissue injury. When recruited to a tissue, the stem cell would take its cues from the local tissue milieu in which it finds itself (including the soluble growth factors, extracellular matrix, and cell-cell contacts.) Examples of such environmental influences on fate choice have been noted previously.¹⁴ Thus, it may not be surprising to see examples of cells isolated using the same marker set showing disparate differentiative potentials,^{15, 16, 17, 18} based on the context of the isolation or experimental conditions, or to see cells with different marker sets showing similar differentiation. In the final analysis, description of a “stem cell”, its actual tissue of origin, and even its differentiation ability, may be a moving target describable only within the context of the particular experimental paradigm used, and may require asking the correct questions in context of the cell’s identity and abilities not clonally but rather within a population of cells, and within a certain environment.^{12, 19}

Given the uncertainties involved in isolating and identifying particular adult stem cells, Moore and Quesenberry²⁰ suggest that we consider an adult stem cell’s functional ability to be, at a minimum, taking on the morphology and cell markers of a differentiated tissue, supplemented by any further functional activity and interaction within a tissue. Certainly a physiological response by improvement of function in a damaged organ system is an indication of a functional response.^{19, 20} As will be discussed later, the function and therapeutic benefit may not necessarily require direct differentiation and integration of an adult stem cell into a desired tissue, but could be accomplished by stimulation of endogenous cells within the tissue.

DIFFERENTIATION MECHANISMS

Several possible mechanisms have been proposed for differentiation of adult stem cells into other tissues. One mechanism that has received attention lately is the possibility of cell fusion, whereby the stem cell fuses with a tissue cell and takes on that tissue’s characteristics. *In vitro* experiments using fusion of somatic cells with embryonic stem cells and embryonic germ cells²¹ have demonstrated that the cell hybrid can take on characteristics of the more primitively developed cell. However, given that such characteristics of spontaneous cell fusion hybrids *in vitro* have been known for quite some time,²² and that a cell fusion hybrid does not explain *in vitro* differentiation of adult stem cells unexposed to tissues, the experiments could not verify this as a possible mechanism for adult stem cell differentiation. More recently, *in vivo* experiments have

shown that for liver,²³ formation of a cell fusion hybrid is a viable explanation for some of the differentiation as well as repair of liver damage seen in these experiments. In an *in vitro* experiment where human mesenchymal stem cells were co-cultured with heat-shocked small airway epithelial cells, a mixed answer was obtained—some of the stem cells differentiated directly into epithelial cells, while others formed cell fusion hybrids to repair the damage.²⁴ The ability to form cell hybrids in some tissues may be a useful mechanism for repair of certain types of tissue damage or for delivery of therapeutic genes to a tissue.²⁵ The reprogramming of cellular gene expression via hybrids is not unlike a novel method reported recently for transdifferentiation of somatic cells. In this method, fibroblasts were soaked in the cytoplasm and nucleoplasm of a lysed, differentiated T lymphocyte cell, taking up factors from the exposed “soup” of the cellular contents of the differentiated cell, and began expressing functional characteristics of a T cell.²⁶

In contrast to the results discussed above, other experiments have shown no evidence that cell fusion plays a role in differentiation of adult stem cells into other tissue types. For example, using human subjects it was shown that human bone marrow cells differentiated into buccal epithelial cells *in vivo* without cell fusion,²⁷ and human cord blood stem cells formed hepatocytes in mouse liver without evidence of cell fusion.²⁸ In these cases it appears that the adult stem cells underwent changes in gene expression and directly differentiated into the host tissue cell type, integrating into the tissue. It is likely that the mechanism of adult stem cell differentiation may vary depending on the target tissue, or possibly on the state of the adult stem cell used, especially given that normal functioning liver typically shows cell fusion hybrids, with cell fusion functioning as a mechanism for most of the differentiation and repair in tissues such as liver, and direct differentiation (transdifferentiation) into other cell types functioning in other tissues. Much remains to be determined regarding the mechanisms associated with adult stem cell differentiation.

Keeping in mind the uncertainties noted above for identification of a particular adult stem cell and its initial tissue of origin, the majority of this review will focus on some of the evidence for adult stem cell differentiation into other tissues. The cells will be categorized based on general tissue of isolation, with the primary emphasis on human adult stem cells, supplemented with information from animal studies.

BONE MARROW STEM CELLS

Bone marrow contains at least two, and likely more,^{2,29} discernable stem cell populations. Besides the hematopoietic stem cell which produces blood cell progeny, a cell type termed mesenchymal or stromal also exists in marrow. This cell provides support for hematopoietic and other cells within the marrow, and has also been a focus for possible tissue repair.³⁰ Isolation is typically based on some cell surface markers, but also primarily on the ability of these cells to form adherent cell layers in culture. Human mesenchymal stem cells have been shown to differentiate *in vitro* into various cell lineages including neuronal cells,^{31,32} as well as cartilage, bone, and fat lineages.³³ *In vivo*, human adult mesenchymal stem cells transferred *in utero* into fetal sheep can integrate into multiple tissues, persisting for over a year. The cells differentiated into cardiac and skeletal muscle, bone marrow stromal cells, fat cells, thymic epithelial cells, and cartilage cells. Analysis of a highly purified preparation of human mesenchymal stem cells³⁴ indicated that they could proliferate extensively in culture, constitutively expressing the telomerase enzyme, and even after extensive culture retained the ability to differentiate *in vitro* into bone, fat, and cartilage cells. Isolated colonies of the cells formed bone when injected into immunodeficient mice. Expanding on their previous *in*

in vitro work with rat and human mesenchymal/stromal stem cells, Woodbury *et al.*³⁵ performed molecular analyses of rat stromal stem cells and found that the cells express genes associated with all three primary germ layers—mesodermal, ectodermal, and endodermal—as well as a gene associated for germinal cells. The gene expression pattern was also seen in a clonal population of cells, indicating that it was not due to an initial mixed population of cells, but was the typical gene expression pattern of the stromal cells. The results suggested that the stromal stem cells were already multidifferentiated and that switching to a neuronal differentiation pattern involved quantitative regulation of existing gene expression patterns. Koc *et al.*³⁶ have used infusion of allogeneic donor mesenchymal stem cells in an attempt to correct some of the skeletal and neurological defects associated with Hurler syndrome (mucopolysaccharidosis type-IH) and metachromatic leukodystrophy (MLD). A total of 11 patients received donor mesenchymal stem cells, expanded from bone marrow aspirate. Four patients showed significant improvements in nerve conduction velocities, and all patients showed maintenance or slight improvement in bone mineral density.

Bone marrow-derived cells in general have shown ability to form many tissues in the body. For example, bone marrow-derived stem cells *in vivo* appear able to form neuronal tissues,^{18,37} and a single adult bone marrow stem cell can contribute to tissues as diverse as marrow, liver, skin, and digestive tract.¹⁶ One group has now developed a method for large-scale generation of neuronal precursors from whole adult rat bone marrow.³⁸ In this procedure, treatment of unfractionated bone marrow in culture with epidermal growth factor and basic fibroblast growth factor gave rise to neurospheres with cells expressing neuronal markers.

In vivo studies using fluorescence and genetic tracking of adult stem cells in animals, and tracking of the Y chromosome in humans, has shown that bone marrow stem cells can contribute to numerous adult tissues. Follow-up of patients receiving adult bone marrow stem cell transplants has allowed tracking of adult stem cells within humans, primarily by identification of Y chromosome-bearing cells in female patients who had received bone marrow stem cells from male donors. Biopsy or postmortem samples show that some of the transplanted bone marrow stem cells could form liver, skin, and digestive tract cells,³⁹ as well as participate in the generation of new neurons within the human brain.⁴⁰ Bone marrow stem cells have also been shown to contribute to Purkinje cells in the brains of adult mice⁴¹ and humans⁴². Generation of this particular type of neural cell is significant in that new Purkinje cells do not normally appear to be generated after birth.

Regeneration or replacement of dead or damaged cells is the primary goal of regenerative medicine and one of the prime motivations for study of stem cells. It is thus of significant interest that bone marrow stem cells have shown the ability to produce therapeutic benefit in animal models of stroke. In mice, fluorescence-tracked bone marrow derived stem cells expressed neuronal antigens and also incorporated as endothelial cells, possibly producing therapeutic benefit by allowing increased blood flow to damaged areas of the brain.⁴³

In rats, intravenous (IV) administration of rat⁴⁴ or human⁴⁵ bone marrow stromal cells resulted in significant behavioral recovery after stroke. Interestingly, only a small percentage of the stromal stem cells appeared to incorporate into the damaged brain as neuronal cells (1-5% in the case of the human marrow stromal cells), but the levels of neurotrophin growth factors within the brains increased and were possibly the signal for repair of damaged brain tissue, perhaps by stimulation of endogenous neuronal precursors. It is also of interest that the marrow stromal cells were injected IV and not intracerebrally, indicating that the stem cells somehow “homed” to the site of tissue

damage. Most studies showing adult stem cell differentiation into other tissues show an increased incorporation of cells, or even an absolute requirement for differentiation, relying on tissue damage to initiate the differentiation. This may indicate that without a "need" for replacement and repair, there is little or no activation of adult stem cells. The recruitment and homing of adult stem cells to damaged tissues are fascinating but relatively unexplained phenomena. One report⁴⁶ indicates that recruitment of quiescent stem cells from bone marrow to the circulation requires release of soluble c-kit ligand (stem cell factor), but the range of factors necessary for recruitment and homing to organs other than bone marrow is unknown at this time and warrants increased investigation.

Bone marrow stem cells have also shown the ability to participate in repair of damaged retinal tissues. When bone marrow stem cells were injected into the eyes of mice, they associated with retinal astrocytes and extensively incorporated into the vascular (blood vessel) network of the eye.⁴⁷ The cells could also rescue and maintain normal vasculature in the eyes of mice with a degenerative vascular disease. In another animal study, bone marrow derived stem cells were observed to integrate into injured retina and differentiated into retinal neuronal cells.⁴⁸ Stromal stem cells have also shown capability in mice to repair spinal cord which was demyelinated.⁴⁹ One of the problems related to spinal cord injury is loss of the protective myelin sheath from spinal cord after injury. A mixed bone marrow stem cell fraction was injected into the area of damage in the spinal cord, and remyelination of the area was seen. In another mouse study, marrow stromal cells injected into injured spinal cord formed guiding strands within the cord;⁵⁰ interestingly, the effect was more pronounced when the stromal cells were injected 1 week after injury rather than immediately after injury.

Because bone marrow stem cells are of mesodermal lineage, it is not surprising that they show capabilities at forming other tissues of mesodermal origin. Human marrow stromal cells, which have been shown to form cartilage cells, have been used in an *in vitro* system to define many of the molecular events associated with formation of cartilage tissue.⁵¹ Bone marrow derived stem cells have also been shown capable of regenerating damaged muscle tissue.⁵² In an elegant study following genetically marked bone marrow stem cells in mice, LaBarge and Blau were able to document multiple steps in the progression of the stem cells to form muscle fibers and repair muscle damage.⁵³ The ability of human bone marrow derived stem cells to form muscle cells and persist in the muscle was recently documented. In this case, a patient had received a bone marrow transplant at age 1, and developed Duchenne muscular dystrophy at age 12. Biopsies at age 14 showed donor nuclei integrated within 0.5-0.9% of the muscle fibers of the patient, indicating the ability of donated marrow cells to persist in tissue over long periods of time.⁵⁴

Bone marrow stem cells have also shown capability at forming kidney cells. Studies following genetically marked bone marrow stem cells in rats⁵⁵ and mice⁵⁶ showed that the stem cells could form mesangial cells to repopulate the glomerulus of the kidney. In the mouse study, formation of cell fusion products was ruled out as a mechanism for differentiation of the bone marrow stem cells. Other animal studies have shown contribution of bone marrow stem cells to repair of damaged renal tubules in the kidney.^{57,58} taken together, animal studies indicate that bone marrow stem cells can participate in restoring damaged kidney tissue.⁵⁹

Liver was one of the earliest tissues recognized as showing potential contribution to differentiated cells by bone marrow stem cells. Bone marrow stem cells have been induced to form hepatocytes in culture⁶⁰ and liver-specific gene expression has been

induced *in vitro* in human bone marrow stem cells.⁶¹ *In vivo*, bone marrow stem cells were able to incorporate into liver as hepatocytes and rescue mice from a liver enzyme deficiency, restoring normal liver function.⁶² Bone marrow stem cells also repopulated liver after irradiation of mice to destroy their bone marrow.⁶³ Examination of livers of female patients who had received male bone marrow transplants, and male patients who had received female liver transplants, showed that similar repopulation of liver from bone marrow stem cells could take place in humans.⁶⁴ Examination of the kinetics of liver repopulation by bone marrow stem cells in a mouse model indicated that the replacement was slow, with only small numbers of cells replaced by the bone marrow stem cells.⁶⁵ As noted previously, two recent studies have found that replenishment of liver by bone marrow stem cells occurs primarily via cell fusion hybrid formation, even in repair of liver damage.²³ A side-population of stem cells has been identified in mouse liver, similar to that seen in bone marrow. This hepatic side-population, which contributes to liver regeneration, can be replenished by side-population bone marrow stem cells.⁶⁶

Pancreas and liver arise from adjacent endoderm during embryological development, and show relatedness in some gene expression and interconversion in some instances. Bone marrow derived cells have shown the ability to form pancreatic cells in animal studies. Mouse bone marrow stem cells containing a genetic fluorescent marker that is only expressed if insulin is expressed were transplanted into irradiated female mice.⁶⁷ Within 6 weeks of transplant, fluorescent donor cells were observed in pancreatic islets; donor cells identified in bone marrow and peripheral blood did not show fluorescence. *In vitro*, the bone marrow derived cells showed glucose-dependent insulin secretion as well. Bone marrow derived stem cells have also demonstrated the ability to induce regeneration of damaged pancreas in the mouse.⁶⁸ Mice with experimentally induced hyperglycemia from pancreatic damage were treated with bone marrow derived stem cells expressing the c-kit marker. Interestingly, only a low percentage of donor cells were identified as integrating into the regenerating pancreas, with most of the regeneration due to induced proliferation and differentiation of endogenous pancreatic cell precursors, suggesting that the bone marrow stem cells provided growth signals for the tissue regeneration.

Heart, as a mesodermally-derived organ, is a likely candidate for regeneration with bone marrow derived stem cells. Numerous references now document the ability of these adult stem cells to contribute to regeneration of cardiac tissue and improve performance of damaged hearts. In animal studies, for example, rat⁶⁹, mouse^{70.71.72} and human^{73.74} stem cells have been identified as integrating into cardiac tissue, forming cardiomyocytes and/or cardiac blood vessels, regenerating infarcted heart tissue, and improving cardiac function. In mice, bone marrow derived stem cells injected into old animals seems capable of restoring cardiac function,¹ apparently through increased activity for cardiac blood vessel formation. One fascinating study using xenogeneic (cross-species) transplants suggests that stromal cells may show immune tolerance by the host.⁷⁶ Mouse marrow stromal cells were transplanted into fully immunocompetent rats, and contributed formation of cardiomyocytes and cardiac vessels. Even after 13 weeks, the mouse cells were not rejected by the rat hosts. Evidence has accumulated from postmortem studies that bone marrow stem cells can contribute to cardiomyocytes after damage to the human heart as well.^{77.78}

The evidence has led numerous groups to use bone marrow derived stem cells in treatment of patients with damaged cardiac tissue.^{79.80.81.82} Results from these clinical trials indicate that bone marrow derived stem cells, including cells from the patients themselves, can regenerate damaged cardiac tissue and improve cardiac performance in humans. In terms of restoring angiogenesis and improving blood circulation, results in

patients are not limited to the heart. Tateishi-Yuyama *et al.*⁸³ have shown that bone marrow derived stem cells from the patients themselves can improve blood circulation in gangrenous limbs, in many cases obviating the need for amputation.

Bone marrow derived adult stem cells have also been found to contribute to various other adult tissues. Animal studies indicate evidence that bone marrow stem cells can contribute as progenitors of lung epithelial tissue⁸⁴, and mesenchymal stem cells can home to damaged lung tissue, engraft, and take on an epithelial morphology, participating in repair and reduction of inflammation.⁸⁵ Bone marrow derived stem cells also have been shown to contribute to regeneration of gastrointestinal epithelia in human patients.⁸⁶ A recent study in mice has indicated that bone marrow stem cells can also participate in cutaneous healing, contributing to repair of skin after wounding.⁸⁷

PERIPHERAL BLOOD STEM CELLS

There is abundant evidence that bone marrow stem cells can leave the marrow and enter the circulation, and specific mobilization of bone marrow stem cells is used to harvest stem cells more easily for various bone marrow stem cell treatments.⁸⁸ Therefore, it is not surprising that adult stem cells have been isolated from peripheral blood. Mobilized stem cells in peripheral blood have been administered intravenously in a rat model of stroke, ameliorating some of the behavioral deficits associated with the damaged neural tissue⁸⁹, leading to a proposal that stem cell mobilization in patients might be used as a treatment for stroke in humans.⁹⁰ Mobilized stem cells have also been used in cardiac regeneration in mice⁷². Two recent studies have found that human peripheral blood stem cells exhibiting pluripotent properties can be isolated from unmobilized human blood. One study showed that the isolated cells were adherent, similar to marrow mesenchymal cells, and could be induced to differentiate into cells from all three primary germ layers, including macrophages, T lymphocytes, epithelial cells, neuronal cells, and liver cells.⁹¹ The other study showed induction of the peripheral blood stem cells could produce hematopoietic, neuronal, or cardiac cells in culture.⁹² In the latter study, undifferentiated stem cells were negative for both major histocompatibility antigens (MHC) I and II, expressed high levels of the Oct-4 gene (usually associated with pluripotent capacity in other stem cells), and could form embryoid body structures in culture.

NEURONAL STEM CELLS

One extremely interesting finding of the past few years has been the discovery of neuronal stem cells, indicating that cell replenishment was possible within the brain (something previously considered impossible.) Neuronal stem cells have been isolated from various regions of the brain including the more-accessible olfactory bulb⁹³ as well as the spinal cord⁹⁴, and can even be recovered from cadavers soon after death.⁹⁵ Evidence now exists that neuronal stem cells can produce not only neuronal cells but also other tissues, including blood and muscle.^{96,97,98,99,100,101} Animal studies have shown that adult neural stem cells can participate in repair of damage after stroke, either via endogenous neuronal precursors¹⁰² or transplanted neural stem cells.^{103,87} Evidence indicates that endogenous neurons and astrocytes may also secrete growth factors to induce differentiation of endogenous precursors.¹⁰⁴ In addition, two studies now provide suggestive evidence that neural stem cells/neural progenitor cells may show low immunogenicity, being immunoprivileged on transplant,¹⁰⁵ and raising the possibility for use of donor neural stem cells to treat degenerative brain conditions.

Pluchino *et al.*¹⁰⁶ recently used adult neural stem cells to test potential treatment of multiple sclerosis lesions in the brain. Using a mouse model of chronic multiple

sclerosis—experimental immune encephalitis—they injected neural stem cells either intravenously or intracerebrally into affected mice. Donor cells entered damaged, demyelinated regions of the brain and differentiated into neuronal cells. Remyelination of brain lesions and recovery from functional impairment were seen in the mice. Neural stem cells have also been used to investigate potential treatments for Parkinson's disease. Using experimentally-lesioned animals as models for Parkinson's disease, human neural stem cells have been observed to integrate and survive for extended periods of time.¹⁰⁷ Dopaminergic cells (the cells degenerated in Parkinson's disease) can be induced in these systems,¹⁰⁸ and neural stem cells are capable of rescuing and preventing the degeneration of endogenous dopaminergic neurons,^{109,110} also producing improved behavioral performance in the animals. In these studies, the data suggest that the transplanted neural stem cells did not participate to a large extent in direct formation of dopaminergic neurons, but rather secreted neuroprotective factors and growth factors that stimulated the endogenous neural cells. In this respect, infusion of transforming growth factor into the brains of Parkinson's mice induced proliferation and differentiation of endogenous neuronal precursors in mouse brain.¹¹¹ Following this potential for stimulation of endogenous neuronal cells, Gill *et al.* recently reported on a Phase I trial in which glial derived neurotrophic factor (GDNF) was infused into the brains of five Parkinson's patients.¹¹² After one year there was a 61% increase in the activities of daily living score, and an increase in dopamine storage observed in the brain. In a tantalizing clinical application with direct injection of neural stem cells, a Parkinson's patient was implanted with his own neural stem cells, resulting in an 80% reduction in symptoms at one year after treatment.¹¹³ Further clinical trials are underway.

The olfactory ensheathing glial (OEG) cell from olfactory bulb has been used extensively in studies regarding spinal cord injury and axon regrowth. Human OEG cells can be expanded in number in culture and induced to produce all three main neural cell types.⁹³ Transplant of the cells into animal models of spinal cord injury has shown that the cells can effect remyelination of demyelinated spinal cord axons,¹¹⁴ and provide functional recovery in paraplegic rats,¹¹⁵ including in transected spinal cords.¹¹⁶ Another study has found that infusion of growth factors such as GDNF can stimulate functional regeneration of sensory axons in adult rat spinal cord.¹¹⁷ Interestingly, one group has made use of the similarities between enteric glial cells and OEG cells, and shown that transplanted enteric glial cells can also promote regeneration of axons in the spinal cord of adult rats.¹¹⁸ Clinical trials are underway to test the abilities of OEG cells in spinal cord injury patients. Finally, a significant impediment to recovery from spinal cord injury is the formation of a glial/astrocyte scar at the site of injury, which can prevent growth of axons no matter what the source of the cells. Menet *et al.* have shown, using a mutant mouse model, that much of the scar can be prevented by inhibition of glial fibrillary acidic protein and vimentin.¹¹⁹ In mutant mice that lacked these genes, there was increased sprouting of axons and functional recovery after spinal cord injury. Thus, endogenous neural cell growth and reconnection might suffice for repair of damage if inhibitory mechanisms can be removed from neural systems.

hNT CELLS

Embryonal carcinoma (EC) cells can be derived from teratocarcinomas of adult patients, and show multipotent differentiation abilities in culture. From one such isolation, a "tamed" (non-tumorigenic) line of cells with neuronal generating capacity has been developed, termed hNT (NT-2) cells. Because of their capacity to generate neuronal cells, these cells have been studied for possible application in regeneration of neuronal tissues. The hNT neurons show the ability to generate dopaminergic neurons,¹²⁰ and have shown some benefit of transplantation in animal models of amyotrophic lateral sclerosis (ALS,

Lou Gehrig's disease).¹²¹ Early clinical trials using hNT neurons transplanted into stroke patients have shown initial positive results.¹²²

MUSCLE STEM CELLS

Muscle contains satellite cells that normally participate in replacement of myoblasts and myofibers. There are also indications that muscle additionally may harbor other stem cells, either as hematopoietic migrants from bone marrow and peripheral blood, or as intrinsic stem cells of muscle tissue. Muscle appears to contain a side population of stem cells, as seen in bone marrow and liver, with the ability to regenerate muscle tissue.¹²³ Muscle derived stem cells have been clonally isolated and used to enhance muscle and bone regeneration in animals.¹²⁴ An isolated population of muscle-derived stem cells has also been shown to participate in muscle regeneration in a mouse model of muscular dystrophy.¹²⁵ Stimulation of muscle regeneration from muscle-derived stem cells, as observed in other tissues, is greatly increased after injury of the tissue.^{126,127}

An interesting use of muscle-derived stem cells has been the regeneration and strengthening of bladder in a rat model of incontinence.¹²⁸ Because of the similar nature of muscle cells between skeletal muscle and heart muscle, muscle-derived stem cells have also been proposed for use in repairing cardiac damage,¹²⁹ with evidence that mechanical beating is necessary for full differentiation of skeletal muscle stem cells into cardiomyocytes.¹³⁰ At least one group has used skeletal muscle cells for clinical application to repair cardiac damage in a patient, with positive results.¹³¹

LIVER STEM CELLS

As noted before, there are similarities between liver and pancreas which could facilitate interconversion of cells between the two tissues. This concept has been demonstrated using genetic engineering to add a pancreatic development gene to liver cells, converting liver to pancreas.¹³² Rat liver stem cells have been converted *in vitro* into insulin-secreting pancreatic cells.¹³³ When transplanted into immunodeficient mice which are a model for diabetes, the converted liver stem cells were able to reverse hyperglycemia in the mice. One other interesting observation regarding liver stem cells has been the possible formation of myocytes in the heart by liver stem cells. A clonal cell line derived from adult male rat liver and genetically tagged was injected into female rats, and marked, Y-chromosome bearing myocytes were identified in the host hearts after six weeks.¹³⁴

PANCREATIC STEM CELLS

Interconversion between pancreas and liver has also been demonstrated starting with pancreatic stem cells, in which mouse pancreatic cells repopulated the liver and corrected metabolic liver disease.¹³⁵ For pancreas, however, the possibility of solutions to the scourge of diabetes has been a driving force in efforts to define a stem cell that could regulate insulin in a normative, glucose-dependent fashion. The success of the Edmonton protocol,¹³⁶ where cadaveric pancreatic islets are transplanted into patients, has provided a glimmer of hope, but more readily-available sources of insulin-secreting cells are needed. Fortunately, there seems to be no shortage of potential candidates that can form insulin-secreting cells. The pancreas itself appears to contain stem/progenitor cells that can regenerate islets *in vitro* and *in vivo*. Studies indicate that these pancreatic stem cells can functionally reverse insulin-dependent diabetes in mice.¹³⁷ Similar pancreatic stem cells have been isolated from humans and shown to form insulin-secreting cells *in vitro*,¹³⁸ the hormone glucagon-like peptide-1 appears to be an important inducing factor

of pancreatic stem cell differentiation. Interestingly, the same hormone could induce mouse intestinal epithelial cells to convert into insulin-producing cells *in vitro*, and the cells could reverse insulin-dependent diabetes when implanted into diabetic mice.¹³⁹ Besides pancreatic and intestinal stem cells, other adult stem cell types showing the ability to secrete insulin and regenerate damaged pancreas include bone marrow^{57,58} and liver.¹³³ Genetic engineering of rat liver cells to contain the pancreatic gene PDX-1 has also been used to generate insulin-secreting cells *in vitro*; the cells could also restore normal blood glucose levels when injected into mice with experimentally-induced diabetes.¹⁴⁰

CORNEAL LIMBAL STEM CELLS

Corneal limbal stem cells have become commonly used for replacement of corneas, especially in cases where cadaveric donor corneas are insufficient. Limbal cells can be maintained and cell number expanded in culture,¹⁴¹ grown on amniotic membranes to form new corneas, and transplanted to patients with good success.¹⁴² A recent report indicates that human corneal stem cells can also display properties of functional neuronal cells in culture.¹⁴³ Another report found that limbal epithelial cells or retinal cells transplanted into retina of rats could incorporate and integrate into damaged retina, but did not incorporate into normal retina.¹⁴⁴

MAMMARY STEM CELLS

Reports have indicated that mammary stem cells also exist. Isolated cells from mouse could be propagated *in vitro* and differentiated into all three mammary epithelial lineages.¹⁴⁵ Clonally-propagated cells were induced in culture to generate complex three-dimensional structures similar to that seen *in vivo*. Transcriptional profiling indicated that the mammary stem cells showed similar gene expression profiles to those of bone marrow stem cells. In that respect, there is a report that human and mouse mammary stem cells exist as a side population, as seen for bone marrow, liver, and muscle stem cells.¹⁴⁶ When propagated in culture, the isolated mammary side population stem cells could form epithelial ductal structures.

SALIVARY GLAND

A recent report indicates that stem cells can be isolated by limiting dilution from regenerating rat salivary gland and propagated *in vitro*.¹⁴⁷ Under differing culture conditions, the cells express genes typical of liver or pancreas, and when injected into rats can integrate into liver tissue.

SKIN

Multipotent adult stem cells have been isolated from the dermis and hair follicle of rodents.¹⁴⁸ The cells play a role in maintenance of epidermal and hair follicle structures, can be propagated *in vitro*, and clonally isolated stem cells can be induced to form neurons, glia, smooth muscle, and adipocytes in culture.

Dermal hair follicle stem cells have also shown the ability to reform the hematopoietic system of myeloablated mice.¹⁴⁹

TENDON

A recent report notes the isolation of established stem cell-like lines from mouse tendon. The cells exhibited a mesenchymal morphology, and expressed genes related to osteogenic, chondrogenic, and adipogenic potential, similar to that seen in bone marrow mesenchymal stem cells.¹⁵⁰

SYNOVIAL MEMBRANE

Stem cells from human synovial membrane (knee joint) have been isolated which show multipotent abilities for differentiation, including evidence of myogenic potential.¹⁵¹ These stem cells were used in a mouse model of Duchenne muscular dystrophy to test their ability to repair damaged muscle. Stem cells injected into the bloodstream could engraft and incorporate into muscle, taking on a muscle phenotype, and with evidence of muscle repair.¹⁵²

HEART

Beltrami *et al.* analyzed the hearts of post-mortem patients who succumbed 4-12 days after heart attack, and found evidence of dividing myocytes in the human heart. While it is unclear from the study whether the cells were originally cardiomyocytes or were other stem cells which had homed to damaged heart tissue, such as bone marrow stem cells, the evidence indicated dividing cells within the heart.¹⁵³

CARTILAGE

Human cartilage biopsies placed into culture show apparent dedifferentiation into primitive chondrocytes with mesenchymal stem cell appearance.¹⁵⁴ These chondrocytes have been used for transplants to repair articular cartilage damage, and in treatment of children with osteogenesis imperfecta.^{155,156,157}

THYMIC PROGENITORS

Bennett *et al.* have reported the isolation of thymic epithelial progenitor cells.¹⁵⁸ Ectopic grafting (under the kidney capsule) of the cells into mice allowed production of all thymic epithelial cell types, as well as attraction of homing T lymphocytes. In separate experiments, Gill *et al.* also isolated a putative thymic progenitor cell from mice and were able to use these cells to reform miniature thymuses when the cells were transplanted under mouse kidney capsule.¹⁵⁹

DENTAL PULP STEM CELLS

Stem cells have been isolated from human adult dental pulp that could be clonally propagated and proliferated rapidly.¹⁶⁰ Though there were some similarities with bone marrow mesenchymal stem cells, when injected into immunodeficient mice the adult dental pulp stem cells formed primarily dentin-like structures surrounded by pulpy interstitial tissue. Human baby teeth have also been identified as a source of stem cells, designated SHED cells (Stem cells from Human Exfoliated Deciduous teeth).¹⁶¹ *In vitro*, SHED cells could generate neuronal cells, adipocytes, and odontoblasts, and after injection into immunodeficient mice, the cells were indicated in formation of bone, dentin, and neural cells.

ADIPOSE (FAT) DERIVED STEM CELLS

One of the more interesting sources identified for human stem cells has been adipose (fat) tissue, in particular liposuctioned fat. While there is some debate as to whether the cells originate in the fat tissue or are perhaps mesenchymal or peripheral blood stem cells passing through the fat tissue, they represent a readily-available source for isolation of potentially useful stem cells. The cells can be maintained for extended periods of time in culture, have a mesenchymal-like morphology, and can be induced *in vitro* to form adipose, cartilage, muscle, and bone tissue.¹⁶²

The cells have also shown the capability of differentiation into neuronal cells.¹⁶³

UMBILICAL CORD BLOOD

Use of umbilical cord stem cells has seen increasing interest, as the cells have been recognized as a useful source for hematopoietic transplants similar to bone marrow stem cell transplants, including for treatment of sickle cell anemia.¹⁶⁴ Cord blood shows decreased graft-versus-host reaction compared to bone marrow,¹⁶⁵ perhaps due to high interleukin-10 levels produced by the cells.¹⁶⁶ Another possibility for the decreased rejection seen with cord blood stem cell transplants is decreased expression of the beta-2-microglobulin on human cord blood stem cells.¹⁶⁷ Cord blood can be cryopreserved for over 15 years and retain significant functional potency.¹⁶⁸ Cord blood stem cells also show similarities with bone marrow stem cells in terms of their potential to differentiate into other tissue types. Human cord blood stem cells have shown expression of neural markers *in vitro*,¹⁶⁹ and intravenous administration of cord blood to animal models of stroke has produced functional recovery in the animals.^{89,170} Infusion of human cord blood stem cells has also produced therapeutic benefit in rats with spinal cord injury,¹⁷¹ and in a mouse model of ALS.¹⁷² A recent report noted establishment of a neural stem/progenitor cell line derived from human cord blood that has been maintained in culture over two years without loss of differentiation ability.¹⁷³ Several reports also note the production of functional liver cells from human cord blood stem cells.¹⁷⁴ Additional differentiative properties of human umbilical cord blood stem cells are likely to be discovered as more investigation proceeds on this source of stem cells.

UMBILICAL CORD MESENCHYME (WHARTON'S JELLY)

While most of the focus regarding umbilical cord stem cells has focused on the cord blood, there are also reports that the matrix cells from umbilical cord contain potentially useful stem cells. Using pigs, this matrix from umbilical cord, termed Wharton's jelly, has been a source for isolation of mesenchymal stem cells. The cells express typical stem cell markers such as c-kit and high telomerase activity, have been propagated in culture for over 80 population doublings, and can be induced to form neurons *in vitro*.¹⁷⁵ When transplanted into rats, the cells expressed neuronal markers and integrated into the rat brain, additionally without any evidence of rejection.¹⁷⁶

AMNIOTIC STEM CELLS

Amniotic fluid has also been found to contain stem cells that can take on neuronal properties when injected into brain.¹⁷⁷ These stem cells were recently isolated from human amniotic fluid,¹⁷⁸ and were found to express Oct-4, a gene typically associated with expression in pluripotent stem cells.

MESANGIOBLASTS

Mesangioblasts are a multipotent stem cell that has been isolated from large blood vessels such as dorsal aorta.¹⁷⁹ The cells show long term proliferative capacity in culture as well as the capability of differentiation into most mesodermally derived types of tissue. In a recent report, the cells were injected into the bloodstream of mice that are a model for muscular dystrophy,¹⁸⁰ and participated in repair of the muscle tissue.

Adult stem cells in other tissues very likely exist, but this survey of many of the known adult stem cells and their capacities for differentiation and tissue repair can serve as a beginning point for discussion regarding the progress as well as potential of adult stem cells. Some final thoughts on current and potential utilization of adult stem cells follow.

ADULT STEM CELL MOBILIZATION FOR TISSUE REPAIR

An important point to consider as we look ahead regarding utilization of adult stem cells for tissue repair is that it may be unnecessary first to isolate and culture stem cells before injecting them back into a patient to initiate tissue repair. Rather, it may be easier and preferable to mobilize endogenous stem cells for repair of damaged tissue. Initial results regarding this possibility have already been seen in some animal experiments, in which bone marrow and peripheral blood stem cells were mobilized with injections of growth factors and participated in repair of heart and stroke damage.^{72,89,90} The ability to mobilize endogenous stem cells, coupled with natural or perhaps induced targeted homing of the cells to damaged tissue, could greatly facilitate use of adult stem cells in simplified tissue regeneration schemes.¹⁸¹

GENE THERAPY APPLICATIONS WITH ADULT STEM CELLS

Adult stem cells can provide an efficient vehicle for gene therapy applications, and engineered adult stem cells may allow increased functionality, proliferative capacity, or stimulatory capability to these cells. The feasibility of genetically engineering adult stem cells has been shown, for example, in the use of bone marrow stem cells containing stably inserted genes. The engineered stem cells when injected into mice could still participate in formation and repair of differentiated tissue, such as in lung.¹⁸² As another example, engineered stem cells containing an autoantigen, to induce immune tolerance of T cells to insulin-secreting cells, were shown to prevent onset of diabetes in a mouse model of diabetes,¹⁸³ a strategy that may be useful for various human autoimmune diseases. Introduction of the PDX-1 gene into liver stem cells stimulated differentiation into insulin-producing cells which could normalize glucose levels when transplanted into mice with induced diabetes.¹⁴⁰ Simply engineering cells to increase their proliferative capacity can have a significant effect on their utility for tissue engineering and repair. For example, McKee *et al.*¹⁸⁴ engineered human smooth muscle cells by introducing human telomerase, which greatly increased their proliferative capacity beyond the normal lifespan of smooth muscle cells in culture, while allowing retention of their normal smooth muscle characteristics. These engineered smooth muscle cells were seeded onto biopolymer scaffolds and allowed to grow into smooth muscle layers, then seeded with human umbilical vein endothelial cells. The resulting engineered arterial vessels could be useful for transplants and bypass surgery. Similarly, human marrow stromal cells that were engineered with telomerase increased their proliferative capacity significantly, but also showed enhanced ability at stimulating bone formation in experimental animals.¹⁸⁵ Genetically-engineered human adult stem cells have already been used in successful treatment of patients with genetic disease. Bone marrow stem cells, from

infants with forms of severe combined immunodeficiency syndrome (SCID), were removed from the patients, a functional gene inserted, and the engineered cells reintroduced to the same patients. The stem cells homed to the bone marrow, engrafted, and corrected the defect.^{186,187,188}

Adult stem cells could also be used to deliver stimulatory or protective factors to tissues and endogenous stem cells. This would utilize the innate homing ability of adult stem cells, but would not necessarily rely on differentiation of the stem cells to participate in tissue replenishment. For example, Benedetti *et al.* utilized the homing capacity of neural stem cells in brain by engineering mouse neural stem cells with the gene for interleukin-4. Transfer into brain glioblastomas in mice led to the survival of most of the mice, and imaging analysis documented the progressive disappearance of large tumors.¹⁸⁹ Likewise, engineered mesenchymal stem cells were transplanted into the brains of mice that are a model of Niemann-Pick disease; the enzyme acid sphingomyelinase is lost in the disease, resulting in neurological damage and early death. The mesenchymal stem cells were engineered to overexpress the missing enzyme. When injected into brains of the mouse model, the mice showed a delay in onset of neurological abnormalities and an extension of lifespan, suggesting that the stem cells delivered and secreted the necessary enzyme to the brain tissue.¹⁹⁰ Muscle-derived stem cells that were engineered to express the growth factor bone morphogenetic protein-2 were used to stimulate bone healing in mice with skull bone defects. While the muscle-derived stem cells did show differentiation as bone cells, the results indicated that the critical factor was delivery of the secreted growth factor by the stem cells to the areas of bone damage, allowing much more rapid healing than in control animals.¹⁹¹ As noted previously, neural stem cells show an ability to rescue degenerating neurons, including the dopaminergic neurons whose loss is associated with Parkinson's disease. The delivery of neuroprotective substances is postulated as the most likely explanation for this phenomenon, rather than substantial differentiation by the injected neural stem cells.¹⁰⁹ In support of this hypothesis, when neural stem cells were specifically engineered to overexpress a neurotrophic factor similar to glial derived neurotrophic factor, degeneration of dopaminergic neurons was prevented.¹¹⁰

STIMULATING ENDOGENOUS CELLS

The indications from the previous examples suggest that direct stimulation of endogenous stem cells within a tissue may be the easiest, safest, and most efficient way to stimulate tissue regeneration. Such stimulation need not rely on any added stem cells. This approach would circumvent the need to isolate or grow stem cells in culture, or inject any stem cells into the body, whether the cells were derived from the patient or another source. Moreover, direct stimulation of endogenous tissue stem cells with specific growth factors might even preclude any need to mobilize stem cells to a site of tissue damage. A few experimental results suggest that this approach might be possible. One group has reported that use of glial derived neurotrophic factor and neurotrophin-3 can stimulate regeneration of sensory axons in adult rat spinal cord.¹¹⁷ Administration of transforming growth factor to the brains of Parkinson's mice stimulated proliferation and differentiation of endogenous neuronal stem cells and produced therapeutic results in the mice,¹¹¹ and infusion of glial derived neurotrophic factor into the brains of Parkinson's patients resulted in increased dopamine production within the brain and therapeutic benefit to the patients.¹¹² And, Zeisberg *et al.* have found that bone morphogenetic protein-7 (BMP-7) can counteract deleterious cell changes associated with tissue damage. In this latter study, a mouse model of chronic kidney damage was used. Damage to the

tissue causes a transition from epithelial to mesenchymal cell types in the kidney, leading to fibrosis. The transition appears to be initiated by the action of transforming growth factor beta-1 on the tissues, and BMP-7 was shown to counteract this signaling *in vitro*. Systemic administration of BMP-7 in the mouse model reversed the transition *in vivo* and led to repair of severely damaged renal tubule epithelial cells.¹⁹² These experiments indicate that direct stimulation of tissues by the correct growth factors could be sufficient to prevent or repair tissue damage. The key to such treatments would be identification of the correct stimuli specific to a tissue or cell type.

In summary, our current knowledge regarding adult stem cells has expanded greatly over what was known just a few short years ago. Results from both animal studies and early human clinical trials indicate that they have significant capabilities for growth, repair, and regeneration of damaged cells and tissues in the body, akin to a built-in repair kit or maintenance crew that only needs activation and stimulation to accomplish repair of damage. The potential of adult stem cells to impact medicine in this respect is enormous.

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