

Current Science of Regenerative Medicine with Stem Cells

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ABSTRACT

Regenerative medicine with stem cells holds great hope for the treatment of degenerative disease. The medical potential of embryonic stem cells remains relatively untapped at this point, and significant scientific hurdles remain to be overcome before these cells might be considered safe and effective for uses in patients. Meanwhile, adult stem cells have begun to show significant capabilities of their own in repair of damaged tissues, in both animal models and early patient trials.

Key Words: regenerative medicine, stem cells, stem cell transplant

Regenerative medicine holds great hope for millions of patients with degenerative diseases and injuries. Repair of damaged organs and tissues using stem cells could potentially address the needs of these patients, encompassing most of the top 15 leading causes of death in the United States. However, the emotional appeal of stem cells and the political debate in which the science is embroiled have clouded much of the actual results in this area. It is imperative that a complete review of the scientific results and potential promises be a part of any fully informed debate.

A stem cell has two chief characteristics: (1) it continues to proliferate so that a pool of cells is always available and (2) it responds to appropriate signals by differentiating into one or more specialized cell types (Figure 1A). Numerous sources of human stem cells exist, including those from early (5–7 day postconception) embryos, fetal tissues, umbilical cord blood and matrix, placental tissues, and most or all body tissues; postnatal sources are often grouped together under the term “adult stem cells” (Figure 1B). The “plasticity” of a stem cell, that is, its ability to form differentiated cell types, ranges from unipotent (able to form only one differentiated type), to multipotent (able to form multiple cell types), to pluripotent (able to form most or all tissues of the adult body), to totipotent (able to

form all postnatal and extraembryonic tissues, potentially able to regenerate a complete new embryo).

EMBRYONIC STEM CELLS

Mouse embryonic stem (ES) cells were first grown in culture in 1981,^{1,2} but human ES cells were not successfully cultured until 1998.³ Isolation of ES cells requires the disaggregation of the early embryo—hence the ethical debate regarding these cells. At about the same time, another team successfully cultured stem cells, termed embryonic germ cells, with similar properties from fetal primordial germ cells.⁴ ES cells are considered the archetypal pluripotent stem cell; they proliferate extensively in culture and, based on their normal function during development or results from reinsertion into another embryo, have the potential to form any tissue. Although this potential is attractive for treatment of degenerative disease, the results to this point have been modest, and there are still many scientific hurdles to overcome before ES cells might be used clinically, including generation of functional differentiated cells, tumor formation, and immune rejection.⁵ The best examples of potential success to date are in animal models of spinal cord injury and Parkinson’s disease. Keirstead and colleagues showed some success at ameliorating acute (although not chronic) spinal cord injury in rats, including improvement in locomotor activity,⁶ and

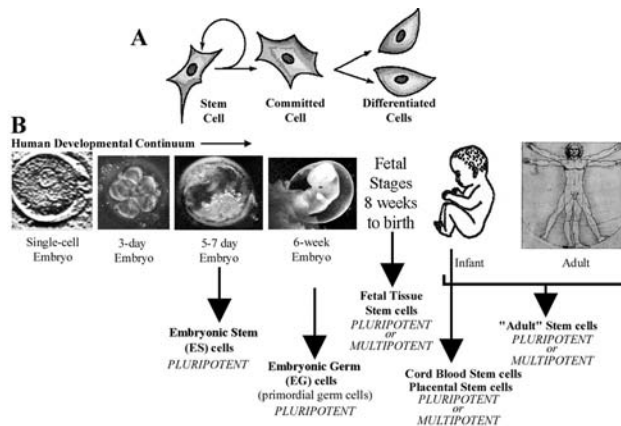


FIGURE 1 Characteristics and sources of stem cells. A), Stem cells maintain proliferation (circular arrow) and respond to differentiation signals (arrow to right). B), Sources include embryos, primordial germ cells, differentiated fetal tissue, and “adult” stem cells, including umbilical cord matrix and blood, placenta, and postnatal body tissues.

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DOI = 10.2310/6650.2005.05043

Nistor and colleagues showed remyelinating activity of human ES cells in a rat model.⁷ In animal models of Parkinson's disease, ES cells have been successfully transplanted and achieved dopamine secretion, alleviating some of the behavioral symptoms in monkeys⁸ and rats,⁹ although in the latter example, the ES cells stopped growth after 12 weeks. However, some experiments, although showing partial behavioral improvement, have also shown tumorigenesis of the injected ES cells.^{10,11} Tumor formation continues to be a problem for the potential clinical use of ES cells; the uncontrolled growth of native or even ES-derived progenitor cells is one factor that has so far precluded their use in humans.^{12,13} A few animal studies also show some ability of ES cells for cardiac repair,^{14,15} although in vitro studies have indicated potential problems with arrhythmia induced by ES-derived cardiac cells.¹⁶ Whereas some early work suggested possible use of ES cells for generation of insulin-secreting cells and diabetes treatment,^{17,18} more recent studies indicate that the previously observed insulin secretion was an artifact of insulin imbibed from the culture medium^{19,20} and that insulin-expressing cells derived from ES cells were not true beta cells, although they were still tumorigenic.¹² Thus far, it has been difficult to obtain a pure culture of ES-derived functional differentiated cells and to get physiologic integration into damaged tissues.

Another hurdle yet to be overcome in potential therapeutic use of ES cells is immune rejection. Animal studies have usually relied on immunosuppression or injection into immunoprivileged sites, such as the brain, and it is likely that such protocols would need to be followed for any human trials. Several possibilities have been proposed by Odorico and colleagues for overcoming potential rejection of ES cells, including genetic engineering of major histocompatibility complex (MHC) genes, induced hematopoietic chimerism, establishing "banks" of ES cell lines to match potential recipients, and somatic cell nuclear transfer (SCNT; so-called "therapeutic cloning").²¹ Zwaka and Thomson demonstrated that it is possible to do homologous recombination in human ES cells, similar to that routinely done in mouse ES cells, opening the possibility of engineering ES cells to match the MHC antigens of different patients.²² Transplant of ES-derived hematopoietic cells, producing an immune system chimerism, could potentially overcome immune rejection; the concept has already been demonstrated using adult stem cell bone marrow transplants followed by solid organ transplant.²³ Banks of human ES cells to match any patient might also be possible, although it is uncertain just how many ES cell lines would be required, with estimates ranging from 250 to 10,000 potential lines needed.

Therapeutic cloning has been hailed as a potential panacea for overcoming immune rejection. Theoretically, by creating an embryonic clone of the patient, from which matching ES cells could be harvested, patient-specific cell lines could be generated that would not be rejected. South

Korean researchers recently claimed creation of scores of cloned human embryos from patients and production of 11 ES cell lines.²⁴ These claims have now been proven fraudulent and the published paper withdrawn. It is still uncertain whether the cells would actually be accepted by the patient's immune system, and prominent ES cell researchers have questioned the efficiency of using therapeutic cloning for clinical use.^{25,26} In a previous experiment in mice, the cells from cloned embryos were rejected by the genetically matched host.^{27,28} Reports of successful matching of cells derived by SCNT cloning are so far dubious; the best results to date in animal studies actually come from gestating cloned animals to the fetal stage and then harvesting tissue stem cells.²⁹⁻³¹

ADULT STEM CELLS

Traditional dogma maintains that there are few adult (tissue or postnatal) stem cells present in the body and that they are difficult to isolate and grow in culture and extremely limited in their capacity to generate new cell types, being limited to forming more cells from their tissue of origin. However, an explosion in publications in the last few years is overturning this dogma and showing a remarkable flexibility for these cells.³² In a 2001 publication, evidence was presented that a single adult bone marrow stem cell could contribute not only to marrow and blood but also to formation of liver, lung, digestive tract, skin, heart, and muscle.³³ Several examples now exist of some adult stem cells with pluripotent flexibility, including cells from bone marrow,³⁴⁻³⁶ peripheral blood,³⁷ the inner ear,³⁸ umbilical cord blood,^{39,40} nasal mucosa,⁴¹ amniotic fluid,⁴² and the placental amniotic membrane.⁴³ Many of these published studies also document that these particular pluripotent adult stem cells can multiply in culture for extensive periods of time while still retaining their ability to differentiate and providing sufficient numbers of cells for clinical treatments.

Relevant to their potential use in clinical therapies, there have been numerous reports of the effectiveness of adult stem cells in treating animal models of disease. In stroke models, adult stem cells have provided therapeutic benefit.⁴⁴⁻⁴⁶ Interestingly, in some experiments, the cells showed a "homing" ability to the site of tissue damage. There is some evidence that c-kit ligand (stem cell factor) may be important for this homing behavior⁴⁶; although this phenomenon is still not completely understood, it provides an intriguing possibility for targeting of regenerative stem cells. For spinal cord injury, adult stem cells have promoted neuronal growth and therapeutic benefit in rodent models.⁴⁸⁻⁵⁰ A recent result that brings into focus some of the unexpected problems potentially faced with regenerative medicine was the discovery that, in successful transplants, the new nerve growth could result in increased pain; however, this could be managed by directed differentiation of the stem cells before trans-

plant.⁵¹ Initial clinical trials in Portugal are under way with approximately 36 patients.⁵² In animal models of Parkinson's disease, adult stem cells have shown effectiveness at stimulating dopamine secretion and decreasing behavioral symptoms.^{53,54} One patient received a transplant of his own neural stem cells, resulting in decreasing the symptoms of Parkinson's disease.⁵⁵ In a study designed not to transplant stem cells but rather to stimulate endogenous adult stem cells for repair, five patients were injected with glial cell-derived neurotrophic factor, resulting in an average 61% decrease in symptomatology.⁵⁶ Follow-up pathology with one patient showed that the growth factor stimulated sprouting of new neurons.⁵⁷

Adult stem cells have also been effective at ameliorating retinal degeneration in animal models,⁵⁸⁻⁶⁰ raising hopes for possible treatments for diabetic retinopathy and age-related macular degeneration. Regarding diabetes, several examples now exist showing generation of insulin-secreting cells from various adult stem cells, including the liver,⁶⁰ bone marrow,^{62,63} and pancreas.⁶⁴ In some experiments, it appears that it is not the adult stem cells that form new beta cells but rather that the injected cells stimulated endogenous precursors within the pancreas to accomplish regeneration.⁶⁵ Using spleen cells, one group was able to achieve permanent disease reversal and now has approval from the US Food and Drug Administration to begin human trials for juvenile diabetes.⁶⁶

Use of adult stem cells from bone marrow or mobilized into peripheral blood has become relatively common as an adjunct for cancer chemotherapy to replace the patient's hematopoietic system or for anemias. Similar techniques to replace the immune system are now being tested with some success in patients for various autoimmune conditions, such as scleromyxedema,⁶⁷ multiple sclerosis,⁶⁸ and Crohn's disease.⁷⁰ Such treatments have also shown promising results for metabolic disorders, such as Krabbe's disease.⁷¹ Adult stem cells have also been used in bone repair protocols.⁷¹ Repair of cardiac damage in patients has also moved to the clinical trials stage, with several reports of early success in repair of infarct damage.⁷²⁻⁷⁴

The mechanism for these regenerative results is still unclear. Adult stem cells in some cases appear to be capable of interconversion between different tissue types, known as transdifferentiation. In some tissues, adult stem cells appear to fuse with the host tissue and take on that tissue's characteristics, facilitating regeneration. In some studies, the adult stem cells do not directly contribute to the regenerating tissue but instead appear to stimulate the endogenous cells of the tissue to begin repair. Whatever the mechanism, adult stem cells are successful at regenerating damaged tissue.

In summary, a great deal of work remains to be done before widespread clinical application of stem cells for regenerative medicine. Given the scientific hurdles that yet remain to be overcome for ES cells, they may be less well suited for clinical applications than for basic scientific studies. Recent results from animal studies and early clin-

ical trials indicate that adult stem cells, in contrast to previous theories, have significant capacities for repair of damaged cells and tissues, somewhat like a native repair kit. The flexibility and potential of these adult stem cells to impact disease appear to be enormous.

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