

# Adult Stem Cell Plasticity Defined

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**Objectives** After completing this article, readers should be able to:

1. Define stem cell characteristics and the three separate classes of stem cells.
2. Define stem cell plasticity.
3. Describe four possible mechanisms of stem cell plasticity.
4. Describe clinical situations that may benefit from stem cell therapy.
5. Delineate areas of research that are needed to make feasible the use of adult stem cells for clinical therapy

## Introduction

Investigators have postulated since the turn of the century that some cells in the body appear to participate in the formation of new cells to replace injured, aged, or infected cells in tissues and organs. The concept that certain specialized cells within an organ (stem cells) could give rise to mature functioning cells in that same organ has been demonstrated most clearly in the hematopoietic system. Evidence for stem cell function in the hematopoietic system has come from controlled laboratory experiments and as a consequence of human exposure to massive irradiation during armed conflict.

World War II was brought to an end dramatically in August 1945 with the dropping of atomic bombs on the cities of Hiroshima and Nagasaki, Japan. In the wake of the devastation and human suffering of these events, several hundred thousand Japanese civilians and soldiers were exposed to lethal and sublethal doses of irradiation. Examination of the affected people included, but was not limited to, the ablative effects of irradiation on the body's hematopoietic system. Observation and treatment of these patients led to several scientific reports confirming the postulated existence of stem cells that are responsible for the continuous production of all circulating blood cells.

Subsequent studies performed on laboratory rodents detailed the detrimental effects of irradiation on the hematopoietic system as well as the ability of bone marrow transplantation to correct such anomalies. Hematopoietic repopulation was established by transplanting clonogenic marrow cells, which could be tracked to the spleen of the irradiated recipient following infusion. Thus, a single stem cell (clone) from the bone marrow could give rise to thousands of mature blood cells inside the splenic microenvironment within 8 days following transplantation. Over the past 30 years, additional animal and human studies have demonstrated the feasibility of successful transplantation of marrow stem cells into recipients, with reconstitution of the host blood cells with donor type cells and amelioration of congenital or acquired blood disorders.

Today we recognize that numerous adult stem cells operate in various capacities in postnatal humans, regulating

## Abbreviations

<b>BM:</b>	bone marrow
<b>ES:</b>	embryonic stem cell
<b>FAH:</b>	fumarylacetoacetate hydrolase
<b>FACS:</b>	fluorescence-activated cell sorting
<b>GFP:</b>	green fluorescence protein
<b>HSC:</b>	hematopoietic stem cell
<b>LacZ:</b>	beta-galactosidase
<b>Lin:</b>	mature blood cell lineage markers
<b>MAPC:</b>	multipotent adult progenitor cell
<b>MSC:</b>	mesenchymal stem cell
<b>NSC:</b>	neural stem cell
<b>NTBC:</b>	2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione
<b>SP:</b>	side population

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everything from blood cell production and the replenishment of the skin and gut epithelium to wound repair and regeneration of surgically removed liver tissue. Adult stem cells often are referred to as somatic or postnatal stem cells, and these terms can be used interchangeably. Some of the adult stem cells that have been identified include hematopoietic (HSC), retinal, skin, intestinal, liver (oval cells), neuronal (NSC), muscle (satellite cells), kidney, and mesenchymal (MSC). This article defines basic concepts of adult stem cell function and describes recent evidence for the developmental plasticity of adult stem cells and their use as potential therapies for tissue engineering and repair.

### Definition of Adult Stem Cells

Stem cells generally fall into one of three categories: totipotent, pluripotent, and multipotent. Totipotent stem cells are defined by their ability to differentiate into every cell type in a living being (including placenta, germ cells, and yolk sac), and because of this, only the fertilized ovum and the first few cleavage stage divisions of the zygote comprise this category. Pluripotent stem cells are capable of differentiation into multiple tissues of different embryonic origin (ie, endoderm-, ectoderm-, or mesoderm-derived tissues) and include such cells as the epiblast cells of the blastocyst or embryonic stem (ES) cells. Multipotent stem cells are described as stem cells that are capable of differentiation into specialized cells of a particular organ or system. Nearly all of the adult stem cell populations noted previously have been believed traditionally to represent multipotent stem cells. Some adult stem cell populations, such as muscle satellite cells, appear more restricted and give rise to a single type of differentiated cell. These adult stem cells reside in skeletal muscle and can be called on to proliferate and differentiate into multinucleated skeletal muscle cells following muscle injury.

All stem cells must display one important characteristic to be defined as a stem cell. Stem cells must undergo multiple and sequential self-renewing cell divisions. A self-renewing division means that following mitosis, each daughter cell retains all of the potential of the parental cell. These types of proliferative outcomes also are referred to as symmetric divisions. Symmetric stem cell divisions lead to an overall increase in the number of stem cells. In an asymmetric division, one daughter cell retains the parental potentiality while the other daughter cell becomes committed to a differentiation program, relinquishing some of the potentiality of the parental cell. Thus, asymmetric divisions maintain the stem cell population. In situations where both daughter cells differen-

tiate (another form of symmetric division), stem cell activity is lost, thereby exhausting the stem cell pool of an organ. At present, little is known of the molecular determinants that regulate stem cell self-renewal, but this property clearly distinguishes stem cells from other cells.

### Stem Cell Plasticity

In the past 5 years, a number of startling discoveries into the nature of adult stem cell differentiation potential have been reported. The dogma that adult somatic stem cells are restricted to differentiation into specialized cells that comprise the organ or tissue in which the stem cells reside is in question. Many studies investigating the phenomenon of stem cell plasticity have focused on testing adult bone marrow (BM) cell function. Several of these studies have reported that transplanted BM or BM HSC differentiate into neural tissue, liver tissue, skeletal muscle, and myocardium. BM cells also have been reported to differentiate into alveolar epithelial cells of the lung. Alternatively, a number of studies have noted evidence of several different stem cell types differentiating into the hematopoietic lineage. However, most studies of plasticity have met with criticism in the scientific community, in part due to lack of independent confirmation of the reported observations, the apparently low frequency with which various stem cell populations participate in plasticity events, and lack of evidence of the clonal origin of plasticity events (demonstration of the ability of an individual cell to differentiate into more than one tissue type). Critics also cite the lack of demonstrable mechanisms leading to stem cell plasticity as reason to be cautious about attributing new biologic functions to adult stem cells. Indeed, more evidence is needed to support most of the claims of stem cell plasticity. However, despite the current criticism, a growing body of evidence supports a broader differentiation potential of adult stem cells than previously recognized.

### Stem Cell Plasticity Mechanisms

If, in fact, the claims of adult stem cell plasticity are supported by reproducible evidence, it behooves us to contemplate possible mechanisms for pluripotent cell differentiation. As depicted in the Figure, at least four distinct mechanisms may be invoked to explain the observations of stem cell plasticity reported to date. The first explanation is straightforward: Multiple tissue-specific stem cells exist in different organs such that the harvesting of cells from one organ could yield two or more stem cell populations. Unless stem cell assays are performed using single cells (clonal analysis), this mechanism might confuse attempts to distinguish the cellular

origins of any differentiating cell following a tissue transplant.

A second mechanism to explain stem cell plasticity is that direct cell fusion of a less differentiated cell (stem cell) with a more differentiated cell results in a single tetraploid cell. This chimeric cell could express a variety of characteristics of either cell type. This mechanism was exposed in a series of in vitro experiments conducted to explain the findings that coculture of HSC or NSC with ES cells appears to lead to “transdifferentiated” HSC or NSC. To date, few reports of stem cell plasticity have excluded this possible mechanism to explain their experimental results. However, cell fusion has not been observed in any of the thousands of patients who have undergone bone marrow transplantation.

Another mechanistic possibility leading to apparent stem cell plasticity is cellular transdifferentiation or “dedifferentiation.” According to this theory, a stem cell directly morphs into a cell of another tissue type (transdifferentiation) or arrives at the same final mature cell identity by initially dedifferentiating into an intermediate precursor cell. It long has been known that the limbs of amphibians such as *Urodeles* undergo dedifferentiation and redifferentiation upon amputation. No evidence of such mammalian stem cell behavior has been reported yet.

Finally, true pluripotent stem cells may exist in the body, perhaps in multiple tissues. If so, such cells may display the same phenotypic and genetic signature and may be accessible for isolation. This potential mechanism to explain the reports of stem cell plasticity is the most appealing but difficult to defend with current concepts of cell commitment and specification during embryogenesis.

## Stem Cell Plasticity Criteria

Adult stem cell plasticity must be defined strictly. Some general principles include demonstrable evidence that different cell lineages can be derived from a single, identifiable, transplantable stem cell and that engraftment in a recipient is robust and persistent in the presence (and absence) of tissue damage. Furthermore, the transplanted stem cells must contribute to the physiologic function of the tissue or organ in which the cells have engrafted. The following sections review some of the current evidence for stem cell plasticity applying these principles.

### Clonal Demonstration of Plasticity

Because a wide variety of cell types can be harvested from any particular organ at any one time, it becomes necessary to sort through these populations prospectively to identify a stem cell. Only by observing single cells under-

going pluripotent differentiation can one be sure that no contaminating cells are altering the observation of such events. It is well known that BM contains both HSC and MSC, and muscle contains both satellite cells and HSC. Implied in the identification of a single stem cell capable of undergoing pluripotent differentiation is a means to characterize that cell and uniquely identify it.

Adult HSC have been characterized highly and represent the paradigm for isolation of adult stem cells from any tissue or organ. One method for purifying HSC and other adult stem cells is based on the ability of the cells to exclude a DNA dye (Hoechst 33342) that can be reversed by the multidrug resistance protein inhibitor verapamil. These quiescent cells exist in a “side population” (SP) that is revealed on fluorescence-activated cell sorting (FACS) and, therefore, can be purified. Although this technique has been successful in isolating noncycling stem cells, it has not been successful in excluding the simultaneous presence of more than one noncycling stem cell population. Success in enriching for stem cells also has been reported by using culture methods to derive pure populations of stem cells that involve either a preplating technique designed to separate adherent from nonadherent cells over a period of several days or a lengthy culture of cells to purify senescence-resistant stem cells capable of long-term expansion.

Other methods include the use of monoclonal antibodies and FACS to identify hematopoietic-specific cell surface markers to attempt to purify populations of both BM- and nonBM-derived stem cells with properties of plasticity. One particularly interesting study used FACS to isolate a single “homed” HSC from a population of engrafted cells within a few days of transplantation. Murine BM cells initially were allocated into cells expressing mature lineage cell surface markers ( $\text{Lin}^+$ ) and lineage-depleted marrow cells ( $\text{Lin}^-$ ). After allowing transplanted  $\text{Lin}^-$  BM cells to home to the marrow of a lethally irradiated host mouse for 2 days, the marrow was recovered, and homed cells were apportioned into single-cell samples using limiting dilution. These single cells then were reinjected into secondary recipient mice, and multiorgan engraftment was analyzed. Single cells gave rise to progeny that restored the hematopoietic system in the secondary mice, but they also could be found at low frequency in epithelia of liver, gut, lung, and skin, where the cells acquired not only morphologic but phenotypic characteristics of epithelium. In contrast, evidence has been presented that single HSC do not differentiate into epithelium. Using an extensive set of hematopoietic-specific markers to isolate single transplantable cells by FACS, a separate study found that little

multitissue engraftment other than high hematopoietic reconstitution occurred when sorted marrow HSC were transplanted into lethally irradiated mice. These observations underscore the importance of conducting more extensive and reproducible experiments to prove that single, identifiable stem cells can undergo pluripotent differentiation on transplantation.

### Demonstration of Function

In addition to pluripotential differentiation of single stem cells and homing to multiple host organs in a transplant setting, the differentiated progeny of plastic adult stem cells must demonstrate the ability to participate in the normal functions of the organs in which they engraft. Most of the published reports of stem cell plasticity have demonstrated only the acquisition of tissue-specific phenotypic or morphologic characteristics by donor stem cells; they have not proven that the cells contribute to the viable function of recipient tissues or organs. However, in one exceptionally noted study, non-purified murine BM cells were transplanted into a mouse model of hereditary tyrosinemia caused by the deletion of the fumarylacetoacetate hydrolase (FAH) gene. Mice lacking expression of the FAH gene fail to survive into young adulthood due to loss of liver and kidney function after accumulation of metabolic toxins. Affected mice may be kept alive, however, by administration of the drug 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). The experimental design called for donor BM cells to be isolated from Rosa-26 transgenic mice expressing the bacterial beta-galactosidase gene (LacZ). Each of the donor BM cells expressing LacZ could be visualized *in vivo* by staining tissues and organs with the histochemical agent X-gal. The authors reported that the transplanted cells repopulated the host BM and formed LacZ<sup>+</sup> repopulating nodules of hepatocytes in the livers of the FAH-deficient hosts. In addition, many of the FAH-deficient mice could be weaned from NTBC following transplantation, thereby demonstrating functional differentiation of the donor BM cells into normal liver. Subsequent studies have confirmed that transplanted donor BM cells also repopulate the kidneys of FAH-deficient mice.

In another study, investigators employed FACS and monoclonal antibodies to isolate c-kit<sup>+</sup> BM cells, transduced the cells with the green fluorescent protein (GFP), and transplanted them into the heart muscles of mice recovering from an experimentally induced left ventricular infarction (ligation of the left descending coronary artery). Injected GFP<sup>+</sup> donor cells repopulated the injured myocardial and endocardial tissue and improved

left ventricular function, with higher measured ejection volumes compared with control animals. Although these results were not performed with single, injected GFP<sup>+</sup> donor cells, improvement in whole organ function was achieved using an injected population of BM cells.

One example of widespread donor cell engraftment in nearly all organs has been reported recently. Multipotent adult progenitor cells (MAPC) were isolated from murine BM, brain, and muscle by culturing these tissues in specific *in vitro* conditions. MAPC can achieve at least 75 population doublings, a remarkable number that exceeds Hayflick's limit. When single LacZ<sup>+</sup> MAPC were injected into murine blastocysts, embryos recovered from pregnant, implanted mice revealed up to 45% whole animal chimerism. Remarkably, based on visual examination of LacZ<sup>+</sup> donor cells in host tissue sections, every organ system and tissue type was represented by donor cell engraftment and differentiation. In essence, these cells, although adult in origin, behaved as though they were ES cells, known to possess the ability to outcompete some of the inner cell mass cells of the developing host blastocyst and contribute to nearly all tissues in developing embryos. Although the function of these cells was not analyzed, the embryos all appeared normal and in good health at the time of sacrifice. Some examples of tissue and organ function of transplanted donor cells have been reported, but no one study has yet been reported that incorporates a study design to test all of the strict principles of stem cell plasticity as defined previously.

### Adult Stem Cells as Therapy for Genetic and Degenerative Diseases

We currently stand at the gate of potentially groundbreaking stem cell therapies for genetic and degenerative diseases. With much attention being paid to the ethical challenges presented by ES cell isolation, additional focus has been directed toward understanding the nature of adult stem cell plasticity. Further studies are warranted to determine if any of the adult stem cell populations currently under investigation display clinically relevant therapeutic potential.

One essential requirement for the eventual therapeutic use of adult stem cells in a clinical setting is that the donor cells engraft and differentiate to such a robust extent that the cells assume the entire physiologic functions of that tissue or organ. At this point in time, most studies of adult stem cell plasticity have yielded less than 1% engraftment of donor cells into tissues other than their origin. The primary means to achieve higher levels of engraftment have required ablation of host tissue or organ function. For example, robust engraftment of the

liver in FAH-deficient mice by BM-derived cells was achieved only when NTCB was withheld. High-level BM engraftment in a standard BM transplant generally requires ablation of host marrow. Thus, much additional research is required to understand how to manipulate the proliferation and differentiation of transplanted adult stem cells following transplantation to achieve the desired cell mass and functional level.

Although the use of adult stem cells as a therapy for tissue regeneration or repair may be many years from realization, stem cells could be currently useful for drug discovery and drug toxicity screening in *ex vivo* systems. The field of pharmacogenomics has revolutionized our understanding of the vast array of genetic variations that result in the heterogeneous absorption, distribution, metabolism, response, and elimination of medications in humans. A number of these processes may be testable *in vitro* using tissue-specific cells derived from stem cells of patients of different genetic backgrounds and provide new insights for derivation of improved medications.

Finally, little direct comparison of adult stem cell and ES cell properties with respect to similarities and differences in basic cell biology has been performed. What are the molecular mechanisms regulating self-renewal divisions or commitment decisions leading to cell specification? Recent preliminary comparisons of the transcriptional activity of adult stem and ES cells suggest the possibility of a basic stem cell signature. Further studies designed to capitalize on advances in gene microarray and proteomic approaches to characterize the *in situ* state of limiting numbers of stem cells as they undergo proliferation, differentiation, and elimination (apoptosis) may permit a clearer understanding of how to manipulate stem cell behavior to develop large numbers of mature cells that could be implanted into humans to alleviate pain and suffering.

### Suggested Reading

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## NeoReviews Quiz

3. Stem cells fall into one of three categories: totipotent, pluripotent, and multipotent stem cells. Of the following, the formed tissue *most* likely to indicate a totipotent stem cell differentiation is the:
- A. Ectodermal tissue.
  - B. Endodermal tissue.
  - C. Mesodermal tissue.
  - D. Placental tissue.
  - E. Specialized organ-specific tissue.