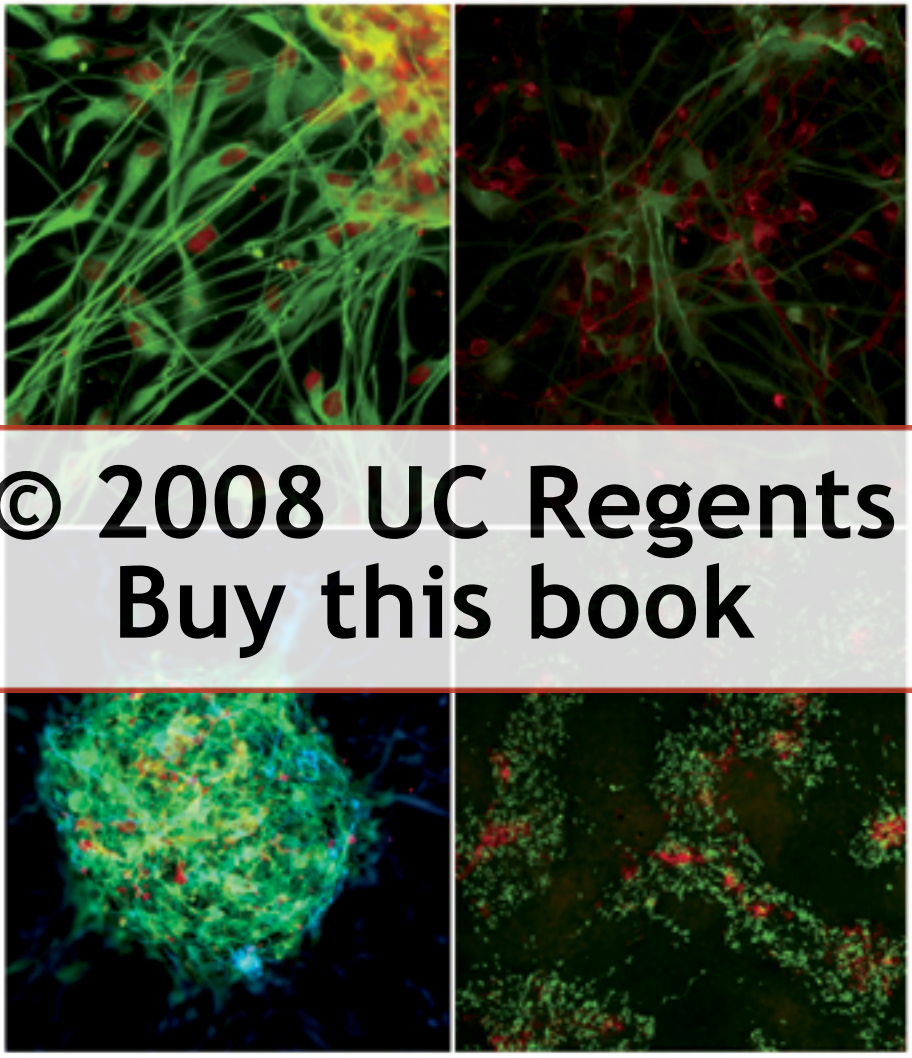
FUNDAMENTALS OF THE STEM CELL DEBATE



THE SCIENTIFIC, RELIGIOUS, ETHICAL & POLITICAL ISSUES

EDITED BY KRISTEN RENWICK MONROE, RONALD B. MILLER & JEROME S. TOBIS

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Contents

CHAPTER 1

Stem Cells

Peter J. Bryant and Philip H. Schwartz

WHAT ARE STEM CELLS?

Stem cells are undifferentiated cells found in the embryos and the later life stages of animals, including humans. They are recognized by their dualistic nature: they either can expand their numbers (self-renew) while remaining undifferentiated or can differentiate and contribute to the development or repair of tissues of the body. Some authors have added other criteria to the definition, including the ability to produce cells differentiating in different ways (multipotency); the ability of a single cell to proliferate into a population of similar cells (clone-forming ability); and the ability to keep dividing indefinitely (unlimited proliferative capacity)—the latter property distinguishing them from most other noncancerous cell types, which can undergo only a limited number of divisions. In most examples of stem cells only some of these properties have been demonstrated, and the term *stem cell* has been used fairly loosely. However, stem cells of many types are now being intensively studied by genetic and molecular methods, and biologists are developing more rigorous and convenient methods to identify them. They are recognized by their expression of certain genes, their production of characteristic proteins and antigens, and their responsiveness to certain growth factors.

In the best-analyzed examples of stem cells in experimental organisms, self-renewal is accomplished through conventional symmetric cell division (figure 1), whereas differentiation is controlled through a specialized

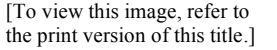

Figure 1. The fundamental characteristics of stem cells: *(A),* Symmetric cell division leads to self-renewal of stem cells; *(B),* Asymmetric cell division leads to replacement of the stem cell and production of a sister cell, exemplified here by a neural precursor, which may differentiate immediately or after one or a few divisions. Specifically expressed and localized stem cell determinants dictate the fate of the daughter cells.

mechanism called asymmetric cell division (ACD; figure 1). ACD results in the budding of a (usually) smaller cell from the larger stem cell (Potten 1997). Through this division the stem cell renews itself and can undergo more such divisions, while the other cell either begins to differentiate or undergoes a small number of additional divisions before the resulting cells differentiate.

When a cell begins the process of ACD, one set of specialized proteins accumulates on one side of the cell and another set accumulates on the other (figure 2). These proteins (and some messenger RNAs) are then included either in the stem cell or in the differentiating cell. Furthermore, experimental studies show that these localized molecules actually control whether the cell receiving them remains a stem cell or begins differentiating. The molecules are therefore called ACD determinants. Most of them have been identified through genetic studies of ACD during the development of the nervous system in the fruit fly *Drosophila.* In the absence of any one of the ACD determinants the asymmetry of division is disrupted, and this leads to abnormal cell proliferation and/or abnormal cell fates. Some of the ACD determinants control the localization of

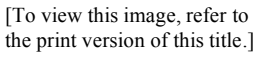

Figure 2. Fluorescently labeled ACD determinants during division of a neural stem cell in a fly embryo, showing the opposite localizations for ACD determinants in the stem cell and differentiating cell. The Miranda protein, stained red, marks the basal complex that determines the differentiating neural precursor and also includes Staufen, Prospero, Prospero mRNA, Numb, and Pon. The Pins protein, stained green, identifies the apical complex that determines the neural stem cell and also includes Atypical PKC, Gαi, Bazooka, and Insc. Image from Chris Doe, University of Oregon.

others, and the molecular interactions between them are under active study (Matsuzaki 2000).

Most of the proteins implicated in ACD in *Drosophila* have remarkably close mammalian and human counterparts (homologs), but there is only fragmentary evidence regarding the possible roles of these homologs in the control and division of mammalian stem cells. Much of the information comes from work on the formation of the nervous system in the mammalian embryo, where ACD has been demonstrated in the mouse (Shen et al. 2002) and ferret (Chenn and McConnell 1995). Preliminary studies have suggested that ACD during mammalian development is controlled by the homologs of some of the ACD determinants identified in *Drosophila,* including those named Numb, Numblike, Notch1 (Fang and Xu 2001; Justice and Jan 2002; Zhong et al. 1997; Zhong et al. 1996), and LGN (homolog of *Drosophila* Pins; Fuja et al. 2004; Mochizuki et al. 1996). In one of the most definitive studies, stem cells

were isolated from the living embryonic mouse brain and cultured through a division cycle, and the resulting cell pairs were stained using antibodies against the Numb protein (Shen et al. 2002). The protein often accumulated in one of the two daughter cells, and this accumulation was correlated with the subsequent fates of the daughter cells. The Notch signaling pathway, identified genetically in *Drosophila,* also seems to be involved in ACD of satellite cells during mammalian muscle development (Conboy and Rando 2002).

The fate of stem cells as well as the way they divide appears to be a function of their microenvironment, which in many cases is provided by a specialized structure known as the stem cell niche. At least in the hematopoietic (blood cell–forming) system, the niche develops independently and the stem cells migrate to and colonize the niche (Schofield 1983). It has been suggested that the niche controls the phenotype of the stem cell, including whether it undergoes self-renewal or ACD. Evidence suggesting the existence of stem cell niches has also been obtained for the epidermis, intestinal epithelium, nervous system, and gonads (Fuchs, Tumbar, and Guasch 2004), as well as in developing muscles (Venters and Ordahl 2005). Furthermore, some of the soluble growth factors mediating interaction between niche and stem cells have been identified (Hauwel, Furon, and Gasque 2005).

EMBRYONIC STEM CELLS (ESCS)

In the mammalian embryo, following fertilization of the egg by a sperm, several cell divisions take place without any growth in total volume (figure 3), so the cells (now called blastomeres) get progressively smaller. They also rearrange to form a hollow sphere of cells (blastocyst) surrounding a fluid-filled cavity called the blastocoel. The cells of the blastocyst then segregate into an outer layer, called the trophectoderm, and an inner cell mass (ICM). The cells of the trophectoderm (trophoblasts) become the fetal contribution to the placenta, while the ICM contains the embryonic stem cells (ESCs) that give rise to the tissues of the fetus (figure 4).

Isolation

Human ESCs (hESCs) are usually obtained from the ICM of embryos produced by in vitro fertilization (IVF). In this procedure, eggs are harvested

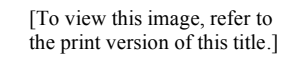

Figure 3. Early development of the human embryo. Embryonic stem cells are derived from the inner cell mass of the blastocyst. See text for explanation.

from a woman after she has been treated with follicular hormones to stimulate the ovaries. The eggs are fertilized either by combining them with sperm in a dish or by mechanically injecting the sperm into the egg (intracytoplasmic sperm injection). The latter technique has the advantage that every egg gets fertilized and that only one sperm enters each egg. The fertilized eggs are then incubated to allow them to develop into blastocysts. Then the trophectoderm is removed and the ICM is plated on to a "feeder layer" of mouse or human embryonic fibroblasts (Thomson et al. 1998), which is essential for the survival of the ICM (Cowan et al. 2004). The ICM then flattens into a compact colony of ESCs. ESC colonies are then

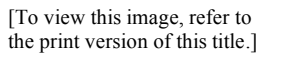

Figure 4. Products of the different cell types of the early blastocyst. The cells of the trophoblast give rise to the fetal component of the placenta, while the inner cell mass, the embryoblast, gives rise to every cell type and organ system of the body.

mechanically dissociated and replated several times to give rise to stable cell lines.

Properties

Under certain conditions hESCs can divide indefinitely while undifferentiated, but under other conditions they can differentiate into virtually any cell type in the body (Amit et al. 2000; Bodnar et al. 2004; Cowan et al. 2004; Odorico, Kaufman, and Thomson 2001; Thomson et al. 1998). When undifferentiated hESCs are transplanted into an animal, they often form a type of tumor called a teratoma (Altaba, Sanchez, and Dahmane 2002), which is unusual in that it contains cells representing all three germ layers (Trounson 2004). Indeed, the ability of hESCs to form a teratoma after injection is the accepted criterion for identifying hESCs as such.

When cultured in the laboratory, hESCs grow as compact colonies and usually require the presence of "feeder cells" for their survival (figure 5). The feeder cells are typically mouse fibroblasts that have been treated with mitotic inhibitors to prevent their proliferation. But to make hESCs safe for use in human cell therapy, methods are being developed in which the human cells have no contact with animal cells. Human feeder cells can be effective (Amit et al. 2000). Another possibility is to first condition the culture medium by incubating it with feeder cells, then

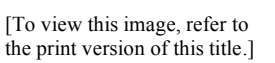

Figure 5. Human embryonic stem cells in culture. Phase-contrast photomicrographs taken at *(A)* 40x, *(B)* 100x, and *(C)* 400x magnification. Human ESCs appear as colonies of cells (white arrows) that are so tightly packed that individual cells are very difficult to discern, even at high magnification. The colonies are grown in the presence of a feeder layer of cells, in this case mouse embryonic fibroblasts (black arrowheads). Even when hESCs are grown under conditions that do not favor differentiation, they spontaneously differentiate and are then seen as groups of less tightly packed cells emanating from the sides of the colonies (white arrowheads).

remove the feeder cells and use the conditioned medium, presumably containing appropriate growth factors, for culturing the stem cells (Carpenter et al. 2004; Rosler et al. 2004; Xu et al. 2001).

Human ESCs have specific requirements for nutrients, including "serum replacement medium." Serum is a necessary component for survival and/or differentiation of many cell types, but it invariably induces differentiation of hESCs, so it cannot be used to promote their survival and/or proliferation. This problem has been overcome by the use of serum replacement medium, which has many of the supportive properties of serum but lacks the tendency to cause differentiation. Another feature of hESCs is their inability to divide and/or survive in low-density culture. When they are dissociated into a single cell suspension, these cells have a very low survival rate. Colonies are therefore usually mechanically dissected into smaller colonies, rather than dissociated into single cells, for propagation.

Human ESCs in culture have a specific morphology, and they express characteristic surface antigens and nuclear transcription factors. The surface antigens include the stage-specific embryonic antigen SSEA-4 and the teratocarcinoma recognition antigens TRA-1–60 and TRA-1–81 (Carpenter et al. 2004). The transcription factors include the POU (pit-oct-unc)-domain transcription factor Octamer-4 (Oct-4), associated with the expression of particular elements of the embryonic genome (Thomson et al. 1998).

Differentiation

When undifferentiated hESC colonies are detached from the feeder layer and transferred into serum-containing medium, they form multicellular aggregates called embryoid bodies (EBs, figure 6), which can contain cell types representing all three germ layers of the body—endoderm, mesoderm, and ectoderm (figure 4). Many EBs tend to show cell types of only one or two germ layers, but in an unpredictable manner. Thus, with appropriate subculture conditions and physical removal of colonies showing specific morphologies, behaviors, or proteins, it is possible to establish cultures that are enriched for particular cell types or mixtures of cell types (figure 6; Carpenter et al. 2004). However, this cell behavior is unpredictable and the sorting is not completely effective. Many labs have therefore been trying to develop protocols for directly controlling the differentiation of hESCs.

Exogenous differentiating factors have been useful in favoring differentiation into specific derivatives: retinoic acid and nerve growth factor for neuronal differentiation (Schuldiner et al. 2001); basic fibroblast growth factor and platelet-derived growth factor for glial precursors (Brustle et al. 1999); 5-aza-2'-deoxycytidine for cardiomyocytes (Xu et al. 2002); bone

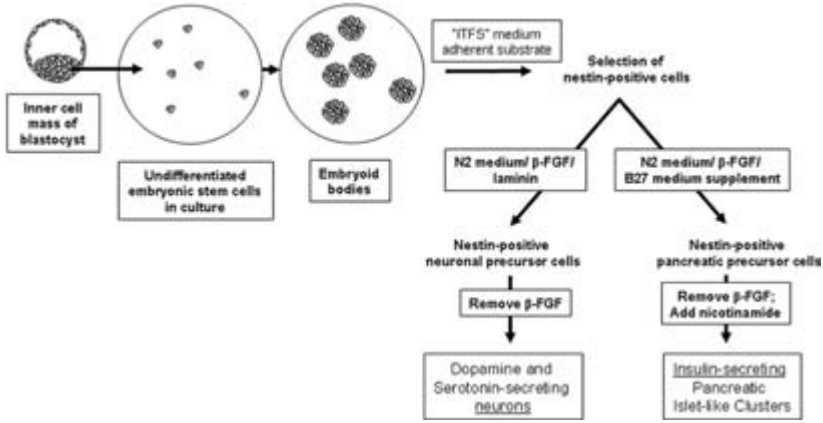

Figure 6. Harvesting and in vitro culture of embryonic stem cells for therapeutic use. Colonies of hESCs may be first differentiated into embryoid bodies, then encouraged to differentiate with specific media, selected according to the expression of specific proteins, behavior, or morphology, and then cultured using specific protocols to give rise to selected populations useful for a particular therapeutic application (Carpenter et al. 2004; He et al. 2003; Nistor et al. 2005; Perrier et al. 2004).

morphogenetic protein-4 and transforming growth factor-beta for trophoblast cells (Carpenter, Rosler, and Rao 2003); sodium butyrate for hepatocytes (Rambhatla et al. 2003); and various cytokines for hematopoietic cells (Zhan et al. 2004). Differentiation into particular tissue types can also be elicited by overexpressing genes encoding transcription factors that function in cell commitment during normal development: MyoD1 for skeletal muscle (Dekel et al. 1992) and Nurr1 for dopamine neurons (Kim et al. 2002). However, these methods still usually give only enrichment rather than total induction, so additional sorting is often necessary. This has been done on the basis of lineage-specific gene expression: PS-NCAM and A2B5 as cell-surface markers for neural precursors (Carpenter et al. 2001), or hygromycin resistance driven by a myosin heavy chain promoter for cardiomyocytes (Klug et al. 1996) (figure 6).

Several groups (Brustle et al. 1999; Reubinoff et al. 2001; Tabar et al. 2005; Wernig et al. 2004) have produced neuronal precursors from either mouse or human ESCs and tested them by injection into the developing brain of newborn mouse or embryonic rat. The transplanted cells were incorporated into the host brain, migrated along appropriate tracks, differentiated into neurons in a region-specific manner, and made synaptic contacts with host neurons. In some cases the transplanted cells also gave rise to glia and astrocytes. This procedure has been shown to promote recovery in animal models of Parkinson's disease and spinal cord injury (Shufaro and Reubinoff 2004).

NEURAL CREST STEM CELLS

A peculiar and heterogeneous population of migratory precursor cells, called neural crest cells, originates during fetal development from the neural folds at the dorsal side of the neural tube. These cells migrate through the embryo to differentiate into a bewildering collection of derivatives, including most of the neurons, Schwann cells, and glia of the peripheral nervous system; most primary sensory neurons; some endocrine cells in the adrenal and thyroid glands; smooth muscle associated with the heart and great vessels; pigment cells of the skin and internal organs; and bone, cartilage, and connective tissue of the face and neck (Le Douarin and Dupin 2003). The migrating cells include multipotential neural crest stem cells, but the population becomes progressively restricted, and terminal differentiation usually ensues soon after the cells reach their targets (Baroffio, Dupin, and Le Douarin 1991). However, some studies show that neural crest–derived stem cells can still be identified in adult organs, including the

central nervous system (Altman 1969; Doetsch et al. 1999; Eriksson et al. 1998; Gould et al. 1999; Johansson et al. 1999; Palmer, Takahashi, and Gage 1997; Reynolds, Tetzlaff, and Weiss 1992) and the hair follicle (Sieber-Blum et al. 2004). Some of the other reported examples of adult stem cells, described below, have not yet been adequately tested to see whether they might also have a neural crest origin. Neural crest–derived cells can be identified by the expression of the neural crest marker Sox-10 (Sieber-Blum et al. 2004).

ADULT STEM CELLS

Classical embryologists developed the concept that, as mammals developed, their cells became progressively more determined for a certain tissue fate and the tissues progressively lost the potential for repair or regeneration. However, recent work has shown that many mammalian tissues contain stem cells that can mobilize, proliferate, and differentiate in response to wounding or disease. These cells can be isolated and grown in culture, and during propagation they retain the ability to differentiate into one or a few tissue types appropriate to their original site. Their potential for self-renewal, their multipotentiality, and their lack of differentiation until they receive the appropriate environmental signals have led to their designation as adult stem cells, although they are sometimes designated more conservatively as progenitor cells. They are referred to as adult stem cells to distinguish them from embryonic stem cells, even if they are taken from fetal or neonatal sources.

Adult stem cells appear to be involved in the normal tissue renewal that occurs in many organ systems, including bone marrow, skin, gut lining, blood vessels, heart, kidney, endocrine glands, liver, pancreas, mammary gland, prostate, lung, retina, and parts of the nervous system (Sell 2003). Some of the stem cell populations also appear to be able to "transdifferentiate" into other tissue types depending on their location in the body. These findings, of course, raise tremendous possibilities for cell-based therapy of many disorders, especially those involving tissue losses.

Bone Marrow: Hematopoietic Stem Cells

Bone marrow contains some of the most complex, but nevertheless best-understood, stem cell populations in the body, including the cell populations responsible for maintaining blood cells, which constitute one of the most rapidly replaced tissues in the body. Most circulating blood

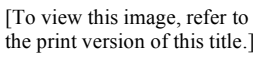

Figure 7. Bone marrow is the source of hematopoietic stem cells. This well-understood stem cell type gives rise to red blood cells and platelets as well as white blood cells (B cells and T cells) that function in the immune system.

cells cannot proliferate, so replacement of blood cells is dependent on the activity of precursors in the bone marrow (and elsewhere) called hematopoietic stem cells (Ponting, Zhao, and Anderson 2003). In a process called hematopoiesis, the stem cells give rise to several blood cell populations, including erythrocytes (red blood cells), leukocytes (white blood cells, including neutrophils, eosinophils, and basophils), monocytes, and platelets (figure 7). The bone marrow also produces all of the cells of the immune system, including B cells for the circulation and the lymph nodes and spleen; T cells for the thymus; and macrophages and dendritic cells.

The complex cell production machinery in the bone marrow involves several stem cell populations with intermediate levels of multipotency, and many of the lineage relationships between these different levels of progenitors have been worked out, but some remain hypothetical (Sell 2003). At an early point in the pathway, progenitor cells have been shown to undergo ACD in which one of the two daughters retains stem-cell properties and the other shows restriction to a smaller range of differentiation potential (Takano et al. 2004).

Bone Marrow: Mesenchymal Stem Cells

In addition to the hematopoietic system, bone marrow contains a supporting tissue called stroma. This was originally thought to simply provide a structural framework for the hematopoietic system, but it has now been found to contain several cell types with other functions and potentials. Most importantly, it contains a population of mesenchymal stem cells (MSCs; Dennis and Caplan 2003), which are strongly adherent and can therefore be isolated by culturing marrow on an appropriate substrate and washing other cells off. MSCs can give rise to many kinds of connective tissue cells, including those responsible for remodeling of cartilage, bone, fat, and vascular tissue (Pittenger et al. 1999). They also produce the essential microenvironment necessary to support the hematopoietic stem cells in the bone marrow (Dennis and Caplan 2003).

The results of bone marrow transplantation studies have led to the conclusion that this remarkable tissue can also produce cells that can circulate to various other sites in the body and contribute to even more tissues, including endothelium, muscle, liver, pancreatic islets, heart, brain, lung, kidney, and retina (Huttmann, Li, and Duhrsen 2003; Sell 2003). Some of this evidence comes from postmortem studies on women who had received bone marrow transplants from male donors. The presence of a Y chromosome provided a reliable marker for cells from the donor, even when the cells were present only in very small numbers. These studies showed evidence for bone marrow cells producing neurons in the brain (Mezey et al. 2003), as well as cells in the liver and buccal epithelium (Theise et al. 2000). Similar studies, using markers recognizing either the X or the Y chromosome, showed that bone marrow could contribute to muscle cells in the heart (Thiele et al. 2002). However, whether these cells functioned appropriately for the new site could not be determined from these studies.

Experimental studies on mice have also suggested that cells from transplanted bone marrow can contribute to other tissues, including the epithelia of the gastrointestinal and respiratory systems (Krause and Gehring 1989), skeletal muscle (Gussoni et al. 1999), heart muscle, endothelium and smooth muscle (Orlic et al. 2001), and liver (Lagasse et al. 2000; Petersen et al. 1999; Wang et al. 2003). In the studies on contribution of transplanted bone marrow to infarcted heart muscle, it has been shown by several laboratories that the damaged tissue is repaired and that heart function is improved (Mathur and Martin 2004). In most

of the other cases, as with the human studies, it is not clear whether the transformed bone marrow cells improve the function of the organ in which they reside.

Some of the results in mice may reflect the directed change in the differentiation program of the bone marrow–derived cells by the tissue microenvironment. However, at least in the case of liver and muscle, some of the differentiated products from bone marrow cell transplantation may be derived by fusion of the transplanted cells with differentiated tissue cells of the host, rather than by directed differentiation of the transplanted cells. It is also possible in some cases that the transplanted bone marrow may not have been a pure cell population but may have included some stem cells of different potential. For example, it may have included multipotential MSCs or some tissue-specific stem cells that had circulated from mature organs into the bone marrow. Finally, in the studies showing improved heart function following bone marrow transplantation, much of the improvement may have been due to stimulation of the formation of new blood vessels rather than the direct contribution of the transplanted cells to muscle regeneration (Mathur and Martin 2004).

In the transplantation studies it is usually difficult to identify the factors controlling the differentiation of the transplanted cells. However, it has recently been shown that appropriate combinations of growth factors can cause the efficient conversion of stromal cells from human adult bone marrow into a population closely resembling neural stem cells (Hermann et al. 2004), which are described below. The transformed cells grow as balls called neurospheres, express neural-specific genes at high levels, and differentiate into the three main derivatives of neural stem cells: neurons (nerve cells), astrocytes (star-shaped cells with a variety of functions), and oligodendrocytes (which are responsible for generating the myelin sheath that surrounds the axons of neurons). The discovery of this expanded potential of bone marrow cells could open up many important new avenues for stem cell therapy, using a patient's own bone marrow as a convenient source of genetically compatible cells.

Liver Hematopoietic Stem Cells

The liver is the major site of blood cell formation in the mammalian embryo. Stem cells isolated from this site proved to be capable of remarkable transdifferentiation into myocytes (muscle precursor cells) following transplantation into a mouse heart that had been subjected to a myocardial infarction (Lanza et al. 2004). In this experiment the stem

cells had been modified by nuclear transfer so that they were genetically identical to the host and were therefore not recognized as foreign by the host immune system. The transplanted cells contributed substantially to regeneration of the heart muscle, and the regenerated muscle replaced 38 percent of the scar after one month. Furthermore, in this report, unlike many of the reports with bone marrow transplantation, the transplanted cells appear to have clearly transformed into heart muscle, and this did not involve fusion with host muscle cells. The transplanted cells also contributed directly to the formation of new blood vessels, which connected to the host circulatory system and functioned normally. The beneficial effects on heart muscle regeneration obtained in this study were far superior to those obtained with bone marrow transplantation, suggesting that further studies on the properties of fetal liver stem cells would be very worthwhile.

Neural Stem Cells

Neural stem cells, defined by their clone-forming ability, self-renewal capability, and multipotency, were first isolated from embryonic and adult mice (Reynolds and Weiss 1996), and their origin during development (Temple 2001) and distribution in the adult (Garcia-Verdugo et al. 1998; Morshead et al. 1994) has since been analyzed in detail. Similar cells have been found in fetal, neonatal, and adult human brains (Palmer et al. 2001), where they are localized in the hippocampus and subventricular zone (SVZ) in stem cell niches (Doetsch 2003). Up to 100 million cells can easily be harvested from a single human neonatal brain (P. Schwartz et al. 2003), and these can easily be proliferated thirty-thousand-fold, yielding 3×10^{12} cells from a single brain. Single neural progenitor cells divide and, in the absence of a substrate, gradually grow into balls of 10,000 to 15,000 undifferentiated cells called neurospheres. Neural precursor cells migrate out from the neurospheres (figure 8) and can give rise to neurons, astrocytes, and oligodendrocytes (Brewer and Cotman 1989; Gage 1998; McKay 1997; Palmer et al. 2001; P. Schwartz et al. 2003; Uchida et al. 2000; Zhang et al. 2001).

The presence of neural stem cells in the adult brain accounts for the finding that neurons are generated constantly, even into adulthood, in many regions of the brain, including the SVZ of the anterior lateral ventricles and the dentate gyrus of the hippocampus (Chiasson et al. 1999; Clarke et al. 2000; Lu, Jan, and Jan 2000; Roy et al. 2000). Stem cells in the SVZ give rise to neuroblasts that migrate to the olfactory bulb and

Figure 8. Human neural stem cells in culture. Photomicrographs are taken through the fluorescence microscope with different colors representing different proteins that are expressed by the cells. Some cells express multiple proteins while others express fewer. *(A)* Cells streaming out from a neurosphere (clump of cells in upper right corner). The green staining is nestin, a filamentous protein present in the cytoplasm of neural stem cells, while the red staining is Sox2, a transcription factor present in both embryonic and neural stem cells. These protein markers are commonly co-expressed. *(B)* Neural cell adhesion molecule staining (NCAM, red) and glial fibrillary acidic protein staining (GFAP, green) predominate in different subpopulations of cells and demonstrate the heterogeneity of the cultures. *(C)* Doublecortin (DCX, red), vimentin (green), and nestin (blue) staining in a neurosphere demonstrate the intimate commingling of the cells in a sphere as well as expression of multiple markers both in the same cells and in different cells. *(D)* These cells, grown from the neural retina, show staining common to brain neural stem cells (DCX, red) and staining found only in neural stem cells derived from the retina (recoverin, green). This shows that neural stem cells harvested from different parts of the nervous system may have certain intrinsic differences.

differentiate there (Gage 2002; Lu, Jan, and Jan 2000; Piper et al. 2000). In the developing cerebral wall of embryonic rodents, the cells at the ventricular surface generate their progeny by ACD (Miyata et al. 2004).

Human neural stem cells have been recovered from brain tissue removed from patients undergoing lobectomy (Johansson et al. 1999) and from donated fetal tissue (Flax et al. 1998; Svendsen, Caldwell, and Ostenfeld 1999; Tamaki et al. 2002; Vescovi et al. 1999). They can also be recovered from cadavers even as late as twenty hours after death (Palmer et al. 2001; P. Schwartz et al. 2003). These cells can proliferate for long periods in culture and can be grown in adherent monolayers or as neurospheres, depending on the conditions. They express immature neurodevelopmental markers including nestin (Frederiksen and McKay 1988; Lendahl, Zimmerman, and McKay 1990), Sox2 (Cai et al. 2002; Han et al. 1993; Zappone et al. 2000), and nucleostemin (Tsai and McKay 2002).

Neural stem cells in vitro show asymmetric localization of LGN (homolog of the *Drosophila* ACD determinant Pins; Fuja et al. 2004), but the consequences of this localization and the behavior of other ACD determinants have not been tested. These cells are generally considered to be derived from the SVZ, but in vivo the SVZ cells do not show any signs of ACD (Gleason et al. n.d.). However, we have recently shown that cells of the ependymal layer, which overlie the SVZ at the ventricular surface and are generally considered to be postmitotic in the adult, can be activated to proliferate by injury and that they show clear asymmetric localization of ACD markers. It therefore seems likely that the ependymal cells are true stem cells as defined by ACD and that they give rise to the SVZ cells, which proliferate further before they differentiate.

Other Mesenchymal and Tissue-Specific Stem Cells

In addition to bone marrow, other tissues contain stem cell populations that are capable of differentiating into mesenchymal derivatives and that are therefore called MSCs (Jiang et al. 2002; R. Schwartz et al. 2002). These cells have been found in periosteum, trabecular bone, adipose tissue, synovium, skeletal muscle, lung, and deciduous teeth, and most of them can differentiate into several tissue types.

Skin and Hair

Human skin consists of two distinct layers, each with different populations of stem cells. The lower 90 percent of skin, the dermis, provides

most of the structural support and contains fibrous components (collagen and elastin) as well as ground substance, blood vessels, and nerves. Most of the cells found in dermis are fibroblasts, but multipotent stem cells have been isolated from the dermis of mice (Toma et al. 2001), and clones derived from these cells were shown to differentiate in vitro into neurons, glia, smooth muscle cells, and adipocytes (fat cells). The fact that these cells can produce both neural and mesodermal derivatives led to the suggestion that they may provide an easily accessible source of stem cells for therapeutic purposes.

The outer layer of skin, the epidermis, is continuous with the epithelial sheath of the hair follicles, and stem cells capable of producing both epidermis and hair follicles are located in a niche, called the bulge, at the base of each follicle in the outer root sheath (Amoh et al. 2004). These cells are identified as stem cells because of their slow cycling (shown by long-term retention of labeled precursors in DNA) and the presence of stem cell markers, including nestin. There may also be some stem cells, possibly with more limited potential than the bulge cells, between follicles (Ma, Yang, and Lee 2004). Genetically marked individual cells taken from the follicle bulge in a normal mouse, mixed with dermal cells, and grafted onto an immune-deficient mouse were able to form epidermis, outer root sheath, inner root sheath, hair shaft, and sebaceous gland (Morris et al. 2004), showing that they can produce all the cell types of the epidermal layer. Recently the bulge has been shown to also contain a distinct population of stem cells derived from the neural crest (Sieber-Blum and Grim 2004), which retain the ability to differentiate into known neural crest derivatives, including neurons, Schwann cells, smooth muscle cells, melanocytes, and chondrocytes.

The identification of stem cells in both dermis and epidermis marks a major advance in the effort to produce complete artificial skin, which would find enormous applications in treatment of burn injuries. The ability of bulge cells to regenerate hair structures also suggests that this kind of research could lead to treatments for hair loss (DeNoon 2004).

Stem Cells from Other Tissues

In addition to the examples cited above, several other organ systems have been investigated as possible sources of stem cells. These include intestinal mucosa (Marshman, Booth, and Potten 2002; Potten et al. 2003), liver (Xiao et al. 2004), lung (Kotton, Summer, and Fine 2004), heart (Hughes

2002), and skeletal muscle (Chen and Goldhamer 2003; Morgan and Partridge 2003). In all of these cases some troubling questions have arisen with respect to the origin of the stem cells. It is often very difficult to determine whether the stem cells are authentic components of the organ system where they are found or cells that have migrated from another source such as the bone marrow. These questions are under active investigation in many laboratories.

CONCLUSION

The human body is turning out to have many more stem cell populations than previously recognized, and many of them seem to have more developmental potential than expected. It seems very likely that in the near future we will see the discovery of methods to control the proliferation and differentiation of many kinds of stem cells, and technologies are already being developed for replacing the nuclei of stem cells with those of prospective patients so that immune rejection can be avoided. The enormous opportunities and challenges in the development of stem cell therapy are the subject of the following chapter.

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