

Stem Cells: Their Definition, Classification and Sources

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Introduction

Stem cell biology has attracted tremendous interest recently. It is hoped that it will play a major role in the treatment of a number of incurable diseases via transplantation therapy. Several varieties of stem cells have been isolated and identified *in vivo* and *in vitro*. Very broadly they comprise of two major classes: embryonic/fetal stem cells and adult stem cells. Some scientists wish to pursue research on embryonic/fetal stem cells because of their versatility and pluripotentiality, while others prefer to pursue research on adult stem cells because of the controversial ethical sensitivities behind embryonic/fetal stem cells. However, both embryonic/fetal and adult stem cells are equally important and research on both types must be enthusiastically pursued since the final objective is the application of this technology for the treatment of a variety of diseases that plague mankind. It is very possible that the findings from one stem cell type may complement that of the other.

The word “stem cell” has also been loosely used by some scientists without the demonstration of stem cell markers or confirmation of stemness via transcriptome profiling. It is their ability to self-renew and differentiate that certain cells are termed stem cells both *in vivo* and *in vitro*. It is very crucial that the correct definition and proof of stemness through proper and accepted characterization tests be addressed before a particular cell type is classified as a stem cell. Stem cell therapy has already reached the bedside in some hospitals through the transplantation of donor bone marrow stem cells into the circulatory system of leukemic patients and the transfer of

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umbilical cord stem cells into the circulatory system of leukemic children or their siblings produced from the same mother who had previously stored her umbilical cord cells. However, the more challenging and impactful use of stem cells would come from the directed differentiation or transdifferentiation of stem cells into other cell types and tissues to help cure a plethora of incurable diseases. It would be tremendously useful if embryonic, fetal, adult or umbilical cord stem cells could be coaxed to produce islets cells for the treatment of diabetes or neurons for neurodegenerative diseases, cardiomyocytes for heart disease, and so on. This chapter attempts to define, classify and describe the sources of the various types of stem cells that have been isolated to date.

Definition of Stem Cells

Stem cells are unspecialized cells in the human body that are capable of becoming specialized cells, each with new specialized cell functions. The best example of a stem cell is the bone marrow stem cell that is unspecialized and able to specialize into blood cells, such as white blood cells and red blood cells, and these new cell types have special functions, such as being able to produce antibodies, act as scavengers to combat infection and transport gases. Thus one cell type stems from the other and hence the term “stem cell.” Basically, a stem cell remains uncommitted until it receives a signal to develop into a specialized cell. Stem cells have the remarkable properties of developing into a variety of cell types in the human body. They serve as a repair system by being able to divide without limit to replenish other cells. When a stem cell divides, each new cell has the potential to either remain as a stem cell or become another cell type with new special functions, such as blood cells, brain cells, etc.

Most tissue repair events in mammals are dedifferentiation independent events brought about by the activation of pre-existing stem cells or progenitor cells. By definition, a progenitor cell lies in between a stem cell and a terminally differentiated cell. However, some vertebrates such as salamanders regenerate lost body parts through the dedifferentiation of specialized cells into precursor cells. These dedifferentiated cells then proliferate and later form new specialized cells of the regenerated organ. In fact, some invertebrates such as the Planarian flatworm and the hydra regenerate tissues very quickly and with precision.^{1,2} The word “stem” actually originated from old botanical monographs from the same terminology as the stems of plants, where stem cells were demonstrated in the apical root and shoot

meristems that were responsible for the regenerative competence of plants. Hence also the use of the word “stem” in “meristem.”³ Today, stem cells have been isolated from preimplantation embryos, fetuses, adults and the umbilical cord and under certain conditions, these undifferentiated stem cells can be pluripotent (ability to give rise to cells from all three germ layers, viz. ectoderm, mesoderm and endoderm) or multipotent (ability to give rise to a limited number of other specialized cell types).

Classification and Sources of Stem Cells

Stem cells can be classified into four broad types based on their origin, viz. stem cells from embryos; stem cells from the fetus; stem cells from the umbilical cord; and stem cells from the adult. Each of these can be grouped into subtypes (Fig. 1). Some believe that adult and fetal stem cells evolved from embryonic stem cells and the few stem cells observed in adult organs are the remnants of original embryonic stem cells that gave up in the race to differentiate into developing organs or remained in cell niches in the organs which are called upon for repair during tissue injury.⁴

Embryonic stem cells

In mammals, the fertilized oocyte, zygote, 2-cell, 4-cell, 8-cell and morula resulting from cleavage of the early embryo are examples of totipotent cells (ability to form a complete organism).⁵ Proof that these are indeed totipotent cells comes from the fact that identical twins can be generated from splitting of the early embryo *in vitro* by micromanipulation in domestic animals. However, strictly speaking, the fertilized oocyte and blastomeres cannot be termed “stem cells” because the making of more of them is limited during early cleavage division. They, thus, cannot self-renew even though they have the potential to form a complete organism.

The inner cell mass (ICM) of the 5- to 6-day old human blastocyst is the source of pluripotent embryonic stem cells (hESCs). During embryonic development, the ICM develops into two distinct cell layers, the epiblast and hypoblast. The hypoblast forms the yolk sac which later becomes redundant in the human, and the epiblast differentiates into the three primordial germ layers (ectoderm, mesoderm and endoderm). Embryonic endoderm cells are rather restricted in their developmental pathways. A small population of multipotent cells, called the definitive endoderm, gives rise to all of the endoderm derived organs in the adult. The definitive endoderm is

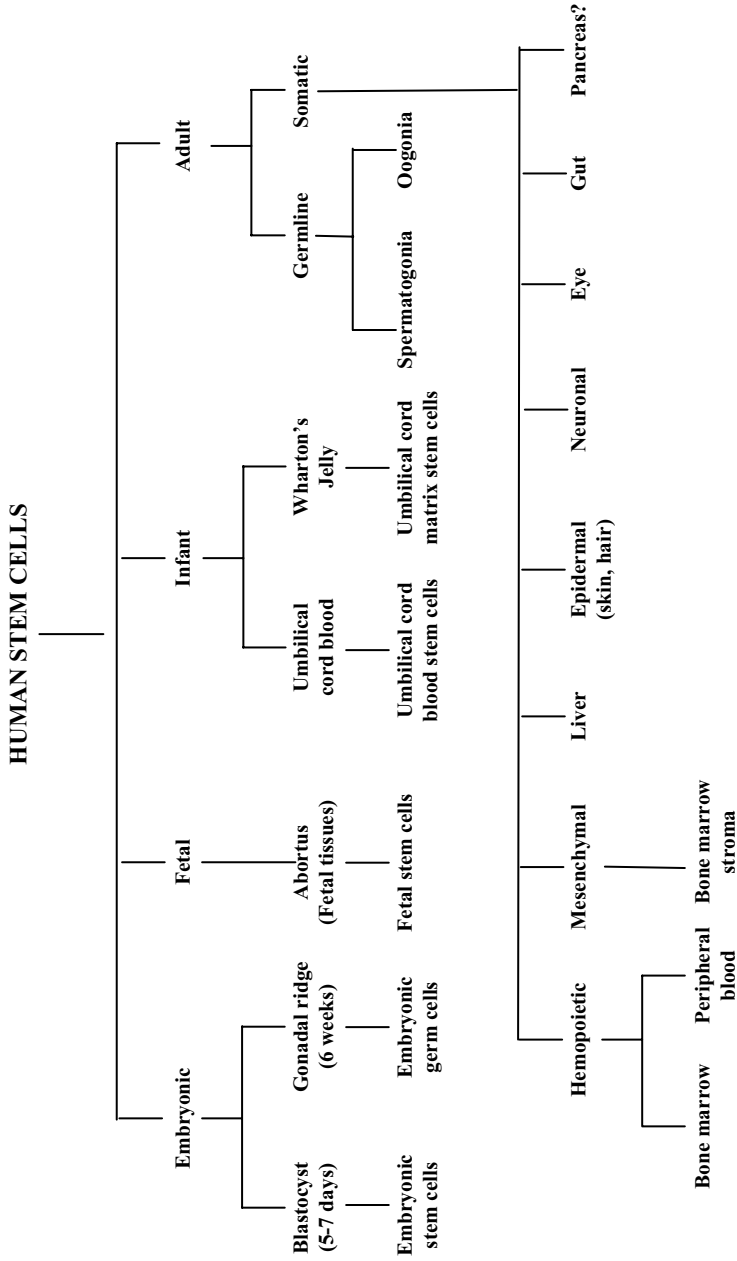


Figure 1. Classification of human stem cells.

separated from the pluripotent ICM during gastrulation immediately after implantation. The definitive endoderm comprises an epithelial sheet of approximately 600 cells that cover the ventral surface of the embryo. This sheet later forms the fore and hind gut. The fore gut later forms the lung, liver, stomach and pancreas, while its more posterior aspects gives rise to the intestines (mid-gut) and cloaca. The hind gut gives rise to the rectum and large intestine.⁶ Knowing what drives these developmental pathways is crucial to understanding the factors and events that lead to differentiation of embryonic stem cells to desirable tissues such as the pancreas. Pluripotent embryonic stem cells can give rise to many cell types *in vitro*, including cells specific to endodermal tissues. Advances in the understanding as to how ES cells differentiate should provide answers for re-programming of stem cells from adult tissues.

Embryonic germ cells

Primordial germ cells or diploid germ cell precursors transiently exist in the embryo before they closely associate with somatic cells of the gonads and then become committed as germ cells. Human embryonic germ cells (hEGCs) which are also stem cells, originate from the primordial germ cells of the gonadal ridge of 5- to 9-week old fetuses. hEGCs have been successfully isolated and characterized.⁷ These stem cells are pluripotent and are able to produce cells of all three germ layers.

Fetal stem cells

Fetal stem cells are primitive cell types found in the organs of fetuses. Neural crest stem cells, fetal hematopoietic stem cells and pancreatic islet progenitors have been isolated in abortuses.⁸ Fetal neural stem cells found in the fetal brain were shown to differentiate into both neurons and glial cells.^{9,10} Fetal blood, placenta and umbilical cord are rich sources of fetal hematopoietic stem cells.

Umbilical cord stem cells

Umbilical cord blood contains circulating stem cells and the cellular contents of umbilical cord blood appear to be quite distinct from those of bone marrow and adult peripheral blood.¹¹ The characteristics of hematopoietic stem cells in umbilical cord blood have recently been clarified. The frequency of umbilical cord blood hematopoietic stem cells equals or exceeds

that of bone marrow and they are known to produce large colonies *in vitro*, have different growth factor requirements, have long telomeres and can be expanded in long term culture. Cord blood shows decreased graft versus host reaction compared with bone marrow, possibly due to high interleukin-10 levels produced by the cells and/or decreased expression of the beta-2-microglobulin. Cord blood stem cells have been shown to be multipotent by being able to differentiate into neurons and liver cells.¹¹

While most of the attention has been on cord blood stem cells and more specifically their storage for later use, there have also been reports that matrix cells from the umbilical cord contain potentially useful stem cells.¹² This matrix termed Wharton's jelly has been a source for isolation of mesenchymal stem cells. These cells express typical stem cell markers, such as c-kit and high telomerase activity; have been propagated for long population doubling times; and can be induced to differentiate *in vitro* into neurons.

Adult stem cells

Hematopoietic stem cells (bone marrow and peripheral blood)

Bone marrow possesses stem cells that are hematopoietic and mesenchymal in origin. Hematopoiesis is the production and maintenance of blood stem cells and their proliferation and differentiation into the cells of peripheral blood. The hematopoietic stem cell is derived early in embryogenesis from mesoderm and becomes deposited in very specific hematopoietic sites within the embryo.¹³ These sites include the bone marrow, liver, and yolk sac. Hematopoietic stem cells can be purified using monoclonal antibodies, and recently, common lymphoid progenitor and myeloid-erythroid progenitor cells have been isolated and characterized.¹³ Bone marrow stem cells may be more plastic and versatile than expected because they are multipotent and can be differentiated into many cell types both *in vitro* and *in vivo*.

Mesenchymal stem cells (bone marrow stroma)

Mesenchymal stem cells (MSCs) are found postnatally in the non-hematopoietic bone marrow stroma. Marrow stromal tissue is made up of a heterogenous population of cells, which include reticular cells, adipocytes, osteogenic cells, smooth muscle cells, endothelial cells and macrophages.¹⁴ In a steady state or in response to injury, turnover of stromal tissue and

repair occurs through the participation of a population of stem cells found in the stromal tissue.¹⁵ Apart from bone marrow stroma, MSCs can also be derived from periosteum, fat and skin. MSCs are multipotent cells that are capable of differentiating into cartilage, bone, muscle, tendon, ligament and fat.¹⁶ There is some recent evidence that there is a rare cell within MSC cultures that is pluripotent and can give rise not only to mesodermal but to endodermal tissues.¹⁷ The authors have called this a Multipotent Adult Progenitor Cell.

Gut stem cells

The gastrointestinal epithelial lining undergoes continuous and rapid renewal throughout life. Differentiation programs thus exist in specific regions of the tract. Epithelial renewal is sustained with populations of multipotent stem cells residing in distinct anatomic sites governed by niches.¹⁸ A major challenge is to identify these niches, the properties of these stem cells and the molecular mechanisms underlining their fate decisions in appropriate developmental pathways. These answers will provide clues as to why some patients infected with *Helicobacter pylori* are at risk in developing gastric adenocarcinoma. Many patients harbor *H. pylori* in their stomachs but only a percentage goes on to develop pathology.¹⁹

Epithelial cell renewal in the intestine is sustained by multipotent stem cells located in the crypts of Lieberhahn. In the small intestine, epithelial cells of enterocytic, goblet and enteroendocrine origin differentiate as they migrate from a crypt up an adjacent villus and leave the intestine once they reach the villus tip. In the colon, it is different. Epithelial cells migrate from the crypt to a flat surface cuff that surrounds its opening. The stem cell hierarchy in the gut and the fact that stem cells and their progeny are located in well defined anatomic units make the gut an ideal *in vivo* model for stem cell research.²⁰

Liver stem cells

Mammals are said to survive surgical removal of at least 75% of the liver by regeneration. The original tissue can be restored in 2–3 weeks. This is in contrast to most other organs such as the kidney or pancreas. Recent evidence strongly suggests that different cell types and mechanisms are responsible for organ reconstitution, depending on the type of liver injury. In the case of the liver, regeneration must be distinguished by transplantation (repopulation) with donor cells.²¹

Bone and cartilage stem cells

Mesenchymal Stem Cells in bone marrow can differentiate into bone and cartilage under appropriate conditions. However, if bone or cartilage is injured, are there stem cells inherent in bone or cartilage to participate in the repair process? Bone itself has been found to have both uncommitted stem cells as well as committed osteoprogenitor cells.^{22,23} In addition, when bone is fractured, there is exposed marrow and abundant bleeding with hematoma formation in the marrow space, which results in good repair potential. *In vivo*, articular cartilage has a very limited capacity for repair if injured. It is currently not clear whether there is a committed chondrocyte progenitor cell located within cartilage. In the presence of injury to cartilage, stem cells do participate in the repair process. The numbers, however, are small and the regulatory factors are limited.^{24,25} It is postulated that these cells may be derived from surrounding tissues such as muscle, bone or other non-cartilaginous tissues.²⁶

Epidermal stem cells (skin and hair)

The human skin comprises the outer epidermis and underlying dermis. Hair and sebaceous glands also make up the epidermis. The most important cell type in the epidermis is the keratinocyte which is an epithelial cell that divides and is housed in the basal layer of the epidermis. Once these cells leave the basal layer they undergo terminal differentiation resulting in a highly specialized cell called a squame which eventually forms either the hair shaft or the lipid-filled sebocyte that form an outer skin layer between the harsh environment and underlying living skin cells. The epidermis houses stem cells at the base of the hair follicle and their self-renewing properties allow for the re-growth of hair and skin cells that occurs continuously. New keratinocytes are produced continuously during adult life to replace the squames shed from the outer skin layers and the hairs that are lost. Stem cells differentiate into an intermediate cell called the “transient amplifying cell” which gives rise to the more differentiated cell types inclusive of the keratinocytes and sebocytes.²⁷

Neuronal stem cells

It has been suggested that a continuous neurogenic turnover occurs in some limited areas of the central nervous system (CNS). Two neurogenic regions

of the adult mammalian CNS are supposed to be involved in this process: the subventricular zone (SVZ) of the forebrain^{28–30} and the dental gyrus of the hippocampus^{31,32} which are considered reservoirs of new neural cells. Thus, neural stem cells (NSCs) are known to reside in these two areas and they consistently generate new neurons.^{33–35} *In vivo*, endogenous NSCs seem to be able to produce almost exclusively neurons, while a single NSC *in vitro* is competent to generate neurons, astrocytes and oligodendrocytes.³⁶ NSCs are multipotent progenitor cells that have self-renewal activity. Although it seems clear at present that the bona fide NSC is the subventricular zone B cells, the search for self-renewing, multipotent NSCs is in progress and conflicting information is available in the literature. There has been data to suggest that the SVZ NSC is an ependymal cell,³⁷ while others have demonstrated that the SVZ astrocyte is the NSC.³⁸ It was also demonstrated that ependymal cells were unipotent giving rise to only glial cells, whereas SVZ astrocytes were able to produce multipotent neurospheres that yielded both neurons and glia.³⁹ The final fate of the NSC is under tight environmental control and a stem cell niche has been postulated for the adult mammalian brain.

Pancreatic stem cells

There has been controversy as to whether the pancreas contains true stem cells. It was reported that the endocrine cells of the rat pancreatic islets of Langerhans, including insulin-producing beta-cells, turn over every 40–50 days by processes of apoptosis and the proliferation and differentiation of new islet cells (neogenesis) from progenitor epithelial cells located in the pancreatic ducts. The administration to rats of glucose or glucagon-like peptides resulted in the doubling of the islet cell mass, suggesting that islet progenitor cells may reside within the islet themselves.⁴⁰ The same authors showed that rat and human pancreatic islets contained an unrecognized population of cells that expressed the neural stem cell-specific marker nestin. These nestin-positive cells were distinct from ductal epithelium. These nestin positive cells, after isolation, had an unusually extended proliferative capacity *in vitro*, could be cloned repeatedly and appeared to be multipotential. They were able to differentiate *in vitro* into cells that expressed liver and exocrine pancreas markers. The authors proposed that these nestin-positive islet derived progenitor cells were a distinct population of cells that resided within the pancreatic islets and participated in neogenesis of islet endocrine cells.⁴⁰

More recently, however, in an effort to pin down the source of new b cells, Dor *et al.*⁴¹ designed transgenic mice in which insulin-producing cells were prompted to produce HPAP that is detected by blue staining. When the mice were 6–8 weeks old, the HPAP gene was turned on. Once the HPAP gene was turned on, b cells were expected to pass on the gene to daughter cells. If the new b cells came from stem cells, then they should not be labeled by the stain. After 12 months, the percentage of blue cells was higher than that in 6-week-old mice, suggesting that the b cells replicate themselves and that the pancreas is unlikely to harbor stem cells that produce large numbers of new b cells. Later, Seaberg *et al.*⁴² exposed pancreatic cells to culture media that encourage growth of neural stem cells. One out of every 5000 cells quickly multiplied into groups of cells. The authors suggested that this grouping was characteristic of stem cells. Additionally, the authors demonstrated the formation of a variety of cell types from these cell groups when the culture medium was changed to encourage the cell groups to differentiate. The cell milieu comprised neurons and pancreatic cells inclusive of b cells based on gene profiling. The b cells secreted insulin and when sugars were added to the culture medium, the b cells put out more than twice as much of insulin. The unequivocal demonstration of the existence of stem cells in the pancreas was, however, not proven.

Eye stem cells

Stem cells have been identified in the adult mouse eye.⁴³ Single pigmented ciliary margin cells were shown to clonally proliferate *in vitro* to form sphere colonies of cells that can differentiate into retinal-specific cell types, including rod photoreceptors, bipolar neurons and Muller glia. The adult retinal stem cells were localized to the pigmentary ciliary margin and not to the central and peripheral retinal pigmented epithelium.

REFERENCES

1. Wolpert L, Hicklin J, Hornbruch A. (1971) Positional information and pattern regulation in regeneration of hydra. *Symp Soc Exp Biol* **25**: 391–415.
2. Brockes JP. (1997) Amphibian limb regeneration: Rebuilding a complex structure. *Science* **276**: 81–87.
3. Kiessling AA, Anderson SC. (2003) Human embryonic stem cells. Boston: Jones and Bartlett.
4. Anderson DJ, Gage FH, Weissman IL. (2001) Can stem cells cross lineage boundaries? *Nature* **7**: 393–395.

5. Bongso A, Richards M. (2004) History and perspective of stem cell research. In *Best Practice & Research Clinical Obstetrics & Gynaecology*, eds. N. Fisk & J. Itskovitz, London: Elsevier Ltd.
6. Stem Cells and the Future of Regenerative Medicine. (2001) *Comm Biol Biomed Appl Stem Cell Res*. Board of Life Sciences, NRC. Washington DC: National Academy Press.
7. Shambloott MJ, Axelman J, Wang S, *et al.* (1998) Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci USA* **95**: 13726–13731.
8. Beattie GM, Otonkoski T, Lopez AD, *et al.* (1997) Functional beta-cell mass after transplantation of human fetal pancreatic cells: Differentiation or proliferation? *Diabetes* **46**: 244–248.
9. Brustle O, Choudary K, Karram K, *et al.* (1998) Chimeric brains generated by intraventricular transplantation of human brain cells into embryonic rats. *Nat Biotech* **16**: 1040–1044.
10. Villa A, Snyder EY, Vescovi A, *et al.* (2000) Establishment and properties of a growth factor dependent perpetual neural stem cell line from the human CNS. *Exp Neurol* **161**: 67–84.
11. Rogers I, Casper RF. (2004) Umbilical cord blood stem cells. In *Best Practice & Research Clinical Obstetrics & Gynaecology*, eds: N. Fisk & J. Itskovitz, London: Elsevier Ltd.
12. Mitchell KE, Weiss ML, Mitchell BM, *et al.* (2003) Matrix cells from Wharton's jelly form neurons and glia. *Stem Cells* **21**: 50–60.
13. Stem Cell and Developmental Biology Writing Group's Report. (2004) *Natl Inst Diabetes & Digestive & Kidney Dses, NIH*. 1–27.
14. Bianco P, Riminucci M. (1998) The bone marrow stroma *in vivo*: Ontogeny, structure, cellular composition and changes in disease. In *Marrow Stromal Cell Culture. Handbooks in Practical Animal Cell Biology*, ed. J.N. Beresford and M.E. Owen, p. 1025. Cambridge, UK: Cambridge University Press.
15. Owen ME. (1988) Marrow stromal stem cells. *J Cell Sci Suppl* **10**: 63–76.
16. Caplan AI. (1994) The mesengenic process. *Clin Plast Surg* **21**: 429–435.
17. Jiang Y, Jahagirdar BN, Reinhardt RL, *et al.* (2002) Pluripotency of mesenchymal stem cells derived from adult bone marrow. *Nature* **418**: 41–49.
18. Wright NA. (2000) Epithelial stem cell repertoire in the gut: Clues to the origin of cell lineages, proliferative units and cancer. *Int J Exp Pathol* **81**: 117–143.
19. Burdick JS, Chung E, Tanner G, *et al.* (2000) Treatment of Menetrier's disease with a monoclonal antibody against the epidermal growth factor receptor. *New Eng J Med* **343**: 1697–1701.
20. Alison MR, Poulosom R, Forbes S, *et al.* (2002) An introduction to stem cells. *J Path* **197**: 419–423.
21. Alison MR, Vig P, Russo F, *et al.* (2004) Hepatic stem cells: From inside and outside the liver? *Cell Prolif* **37**: 1–21.

22. Gronthos S, Zannettino AC, Graves SE, *et al.* (1999) Differential cell surface expression of STRO-1 and alkaline phosphatase antigens on discrete developmental stages in primary cultures of human bone cells. *J Bone Miner Res* **14**: 47–56.
23. Nuttall ME, Patton AJ, Olivera DL, *et al.* (1998) Human trabecular bone cells are able to express both osteoblastic and adipocytic phenotype: Implications for osteopenic disorders. *J Bone Miner Res* **13**: 371–382.
24. Metsaranta M, Kujala UM, Pelliniemi L, *et al.* (1996) Evidence for insufficient chondrocytic differentiation during repair of full thickness defects of cartilage. *Matrix Biol* **15**: 39–47.
25. Nakajima H, Goto T, Horikawa O, *et al.* (1998) Characterization of cells in the repair tissue of full thickness articular cartilage defects. *Histochem Cell Biol* **109**: 331–338.
26. Shapiro F, Koide S, Glimcher MJ. (1993) Cell origin and differentiation in the repair to full thickness defects of articular cartilage. *J Bone Joint Surg Am* **75**: 532–553.
27. Blanpain C, Lowry WE, Geohagan A, *et al.* (2004) Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* **118**: 530–532.
28. Reynolds BA, Weiss S. (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255**: 1707–1710.
29. Luskin MB. (1993) Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* **11**: 173–189.
30. Lois C, Alvarez-Buylla A. (1993) Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc Natl Acad Sci USA* **90**: 2074–2077.
31. Seaberg RM, Van der Kooy D. (2002) Adult rodent neurogenic regions: The ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors. *J Neurosci* **22**: 1784–1793.
32. Palmer TD, Ray J, Gage FH. (1995) FGF-2 responsive neuronal progenitors reside in proliferative and quiescent regions of the adult rodent brain. *Mol Cell Neurosci* **6**: 474–486.
33. Mckay R. (1997) Stem cells in the central nervous system. *Science* **276**: 66–71.
34. Gage FH. (2000) Mammalian neural stem cells. *Science* **287**: 1433–1438.
35. Temple S. (2001) The development of neural stem cells. *Nature* **414**: 112–117.
36. Bottai D, Fiocco R, Gelain F, *et al.* (2003) Neural stem cells in the adult nervous system. *J Hematother Stem Cell Res* **12**: 655–670.
37. Johansson CB, Momma DL, Clarke DL, *et al.* (1999) Identification of a neural stem cells in the adult mammalian central nervous system. *Cell* **96**: 25–34.
38. Doetsch F, Caille DA, Lim JM, *et al.* (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**: 703–716.

39. Laywell ED, Rakic P, Kukekov VG, *et al.* (2000) Identification of a multipotency astrocytic stem cell in the immature and adult mouse brain. *Proc Natl Acad Sci USA* **97**: 13883–13888.
40. Zulewski H, Abraham EJ, Gerlach MJ, *et al.* (2001) Multipotential nestin positive stem cells isolated from adult pancreatic islets differentiate *ex vivo* into pancreatic endocrine, exocrine and hepatic phenotypes. *Diabetes* **50**: 521–533.
41. Dor Y, Brown J, Martinez OI, *et al.* (2004) Adult pancreatic b cells are formed by self-duplication rather than stem cell differentiation. *Nature* **429**: 41–46.
42. Seaberg RM, Smukler S, Kieffer TJ, *et al.* (2004) Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. *Nature Biotech* **22**: 1115–1124.
43. Tropepe V, Coles BLK, Chiasson BJ, *et al.* (2000) Retinal stem cells in the adult mammalian eye. *Science* **287**: 2032–2036.