Versatile stem cells, young and old. A review

Sandro Eridani

Institute of Advanced Biomedical Technology, ITBA, the National Research Council, Milano, Italy
(E-mail: eridani@itba.mi.cnr.it; Fax: **39-02-26422770)

Received 6 June 2000; accepted 18 January 2001

Key words: cell culture, cell differentiation, cell transplantation, stem cells, transdifferentiation

Abstract

Both embryonic and somatic stem cells have been studied in recent years with particular regard to their differentiation potential. *In vitro* studies allow a considerable amplification of such cells in culture as well as the induction of commitment in different directions under proper stimulating factors. Moreover, a surprising versatility has been discovered, which makes possible a 'reprogramming' of stem cells into a lineage pathway which may be completely different from the expected direction: for instance, a production of brain cells from blood progenitors has been obtained. It is thus possible to envisage methods of producing in culture sufficient amounts of stem cells, committed to a certain pathway, which can be transplanted *in vivo* to replace damaged tissues and organs.

Introduction

Stem cells, by definition, must be capable of pluripotential differentiation as well as capable of self-renewal. Following early differentiation, the cells derived from such progenitors were, until recently, believed to progress irretrievably along a predetermined pathway, losing therefore any connection with other cell types, which would follow a parallel course along their own pathway of differentiation. Furthermore it was believed that stem cells, in each domain, would be able to differentiate only into that particular domain. Such concepts have been largely disproved by a succession of experimental data, which showed the occurrence of so-called 'trans-differentiation', namely the transition from one to another lineage, apparently not related.

We shall now attempt to review this topic, with special attention to certain types of stem cells, which have shown a great potential of evolution as well as transdifferentiation, both *in vitro* and *in vivo*. The evaluation of such amazing potential acquires a special relevance, as the possibility is emerging to use such progenitors for replacement of damaged cells and tissues in the human body.

Embryonic cells

A preliminary distinction should be made between germ cells and true stem cells: it is indeed accepted that the most primitive stem cells are the embryonic cells (ES cells), namely those derived from the blastocyst inner cell mass (Thompson et al., 1998). Such germ cells can be maintained in culture for an indefinite period of time if an appropriate cytokine, leukemia inhibitory factor (LIF) is present in the culture medium: in this condition they express the intrinsic transcription factor OCT 4 and may remain in an undifferentiated pluripotent state (Nichols et al., 1998; Niwa et al., 1998). If LIF is removed from such cultures, ES cells can be modulated to produce a variety of cell lines, from blood islands to neurons and epithelia (Bradley, 1990). They may therefore satisfy basic stem cell conditions, namely the capacity to divide symmetrically to expand their number and divide asymmetrically in order to self-renew and give rise to a differentiated progeny (Lajtha, 1979).

As for *in vivo* criteria for inclusion among true stem cells, there is no evidence that blastocyst cells self-renew *in vivo* and obviously they are not working over the entire lifespan of the organism, as stem cells should do: they cannot therefore be considered as

true stem cells, while among so called germ line stem cells (oocytes and sperm-producing cells) only male spermatogonial cells are present in mammals over the lifetime; oocytes are produced in a finite number shortly after birth and cannot therefore be classified as stem cells. It is therefore reasonable to apply the concept of germ line stem cells *in vivo* to cells capable of self-renewal for the entire lifespan of the organism, as well as of producing more differentiated cells (Van der Kooy and Weiss, 2000).

Somatic stem cells

Somatic stem cells seem to appear relatively late during development: haemopoietic stem cells (HSC) are first found in the yolk sac, then in the paraortic region, later in foetal liver, finally in spleen and bone marrow (B.M.) Among functional properties, repopulation of lethally irradiated animals is crucial: such repopulating stem cells (RHSC) tend to increase through successive sites (yolk sac, liver, B.M.) and there is evidence that fully functional RHSC are not the first ones to appear, but late-comers: for instance the order of appearance in mammalian embryo is as follows: first, one finds differentiated cells and their progenitors, while fully potent repopulating RHSC are found only later, a true reversal of normal 'adult' hierarchy (Dzierzak et al., 1998). It is thus possible that repopulating cells can originate from early differentiated precursors.

Adult stem cells are often situated in 'niches', where they are available to start a differentiation pathway; the environment of such niches seems able to exert a critical influence on the biochemical and developmental potential of such cells. Such influence becomes evident, for instance, when adult haemopoietic stem cells (HSC) from mouse bone marrow are injected into the inner mass of the mouse blastocyst: the haemopoietic cells developed from such chimera express foetal haemoglobin (HbF) instead of the adult haemoglobin (HbA) as expected from adult erythroid precursors (Geiger, 1998).

Numerous studies have been performed on adult tissue to understand how the microenvironment of the 'niches' exerts such influence on stem cell commitment. Skin stem cells have thus been accurately characterized as far as their content in adhesion molecules (cadherins, integrins and so on) is concerned: it appears that such cells have a reduced content of intercellular junction proteins, allowing their rapid

mobilization and exit from the niche (Moles and Watt, 1997).

Adhesion proteins seem therefore to have an important effect not only on cell interconnection, but also on the activation of signals, which are essential to promote lineage differentiation (Nusse, 1999). Among the factors which are believed to be relevant in this process are certain secretion products, like the TGF-beta and Wnts families of transcription factors, highly conserved between species and between self-renewing tissues.

Neural stem cells

As for the CNS, evidence for the presence of adult stem cells dates back to the 60'ties, when postnatal neurogenesis was found in rat and guinea pig hippocampus (Altman and Das, 1965). Further research indicated other regions like the forebrain ventricular wall, harbouring neural stem cells. More precisely, in the subventricular zone, facing the lumen, a layer of proliferating sub-ependymal cells may be a candidate either for a stem cell compartment or for a stream of progenitor cells in transit (Doetsch et al., 1997): the subependyma actually persists during the whole adult life as a population of undifferentiated heterogeneous cells, some of which have the capacity of self-renewal and, to some extent, to differentiate *in vitro* into neurons and glia (Lois and Alvarez Buylla, 1993); it seems moreover that depletion of such proliferating cells is followed by a complete repopulation of the subependyma by a second population of relatively quiescent stem cells present *in situ* (Moorshead et al., 1994). It is therefore possible that such cells may serve as an endogenous source for new neurons and glia in the adult mammalian forebrain.

The philosophical implications of the persistence of stem cells in the adult brain have been the subject of recent discussions and it is suggested that a slow, but continuous replacement of neural stem cells is a distinct possibility, so far overlooked: young sphere-forming cells (SFC), a population of neural precursors, may be produced at different stages of embryo development and may persist, as a 'leftover supply' in the brain and other tissues, remaining there until the necessity arises to proliferate and differentiate (Sheffler et al., 1999). Little is known about the proliferative potential of neurons in adult life: it is however of high interest the suggestion that restriction of neuronal growth and its subsequent arrest may be related

to up-regulation of Notch gene activity, while inhibition of Notch signaling seems to be associated with neurite extension (Sestan et al., 1999). It is thus possible that changes in such activity may explain the loss or the acquisition of neuronal capacity to grow and differentiate.

Neural stem cells can also be immortalized in culture and, when exposed to proper stimulating factors, can give rise *in vitro* both to neurons and glial cells (Gritti et al., 1996). It thus becomes apparent that adult CNS stem cells can be preserved in culture as a reservoir for producing differentiated cells of the same lineage (as in Figures 1 and 2), which can be used to replenish depleted tissues (Vescovi et al., 1999). As for the requirement to obtain *in vitro* a stable human neural stem cell line, recent studies have shown that *v-myc*, a fusion protein derived from the avian retroviral genome, seems to be a very effective factor. As expected, the presence of mitogens like FGF2 and EGF in the medium is also necessary for immortalization and in these conditions a monoclonal human stem cell line has been obtained, capable of producing all differentiated elements of CNS (Villa et al., 2000). It is also worth noting that treatment for Parkinson's disease includes foetal cells which have undergone an initial neural differentiation. Such treatment however requires such an excessive amount of foetal tissue that it is impracticable on a large scale, and attention is therefore presently focused on stem cells in culture for this purpose as well (Barinaga, 2000).

Haemopoietic stem cells

Many classes of haemopoietic stem cells have been identified, each capable of giving rise to different sets of blood cells: among the earliest to be proposed were the so-called CFU-S (colony-forming-units-spleen), capable to induce in lethally irradiated mice a complete repopulation of the haemopoietic system. An analogous class of cells in human beings, including a subset of long-term self-renewing cells (LT-HSC), have been shown able to reconstitute haemopoiesis in patients who have received lethal doses of radiation or chemotherapy as a treatment for blood neoplasias and other tumors as well (Keating et al., 1984; Osawa et al., 1996).

At a less primitive level there are classes of oligopotent stem cells, which can produce either a common lymphoid progenitor or a common myeloid precursor, which in turn may give rise to an erythroid or mega-

karyocytic or a granulo-monocytic precursor. Furthermore a number of so-called colony-forming cells are demonstrable, which can produce colonies of a restricted type, like BFU-Es and CFU-Es, producing differentiated cells of the erythroid lineage, CFU-GMs for granulocytes and monocytes, and CFU-MKs for megakaryocytes (Eridani and Morali, 1993).

Finally, another restricted-type stem cell acting as a lymphoid progenitor, has been recently demonstrated (Kondo et al., 1997). The potential of such haemopoietic stem cells has already been exploited for many years in a variety of clinical applications, not only for the correction of blood neoplastic disorders, but in a number of genetic diseases of the immune system as well (Thomas, 1991).

Transdifferentiation

Recent studies have shown how versatile adult stem cells can be. There is ample evidence for a surprising variety of modulation from one cell type to another: neural stem cells, for instance, have been able to transform under selective stimulation into different types of blood cells (Bjornson et al., 1999). On the other hand HSC's, injected into irradiated hosts, have produced a number of differentiated cell types, ranging from glial cells (Eglitis and Mezey, 1997) to hepatic oval cells (Petersen et al., 1999). Moreover human mesenchymal stem cells have differentiated in culture into adipocytes, chondrocytes and osteocytes (Pittenger et al., 1999).

Such wide spectrum of possible differentiation illustrates the impressive potential of early stem cells, which can be modulated not only by the surrounding cells and tissues, but may exceed the influence of the micro-environment.

It is therefore clear that adult stem cells can be manipulated to differentiate towards a direction different from the expected lineage. The next question is: how crucial is the establishment of such cells in culture and how long this permanence should be in order to allow a process of dedifferentiation and subsequent shift of lineage. A rather long passage in culture seems indispensable in some experiments with neural stem cells, sometimes up to 2 years (Suhonen et al., 1996), while from other studies it does not appear to be so: for instance marrow stromal cells, grown for 5 days only and injected into the forebrain of neonatal mice, were able to differentiate into mature astrocytes and, possibly, into neurons as well (Kopen et al., 1999).

Figure 1. Neural cell precursors (microspheres) obtained in culture from adult mouse stem cells of brain subventricular region.

It also appears that the differentiation potential of stem cells in the haemopoietic system is not confined to blood cell types, but, as we have already described, may involve completely different cell lineages.

How frequent are such versatile stem cells in the bone marrow? They have been reckoned to be rare and that may explain the necessity to have a considerable *in vitro* expansion in order to produce them in sufficient amounts. According to Reyes and Verfaillie (1999) there is a mesodermal progenitor cell (MPC) in adult B.M. capable of proliferating for 7 months *in vitro*, which can give rise to osteoblasts, chondroplasts, adipocytes, skeletal and cardiac myocytes; such precursors are more frequent in children than in adults, although they are found in 40–50-year-old people as well.

Stem cell transplantation

Already in use

Transplantation of human haemopoietic stem cells, obtained from different sources, has already be-

come an established practice for certain therapeutic strategies (Emerson, 1996). In order to obtain a sufficient amount of haemopoietic cells for transplantation, expansion of these cells *in vitro* is normally accomplished through the addition of a cocktail of cytokines (Piacibello et al., 1997).

How frequent are such progenitors in blood or blood-forming cells? The exact number is still uncertain, but a comparison between the frequency of early clonogenic cells from different sources (B.M., peripheral blood and cord blood) has shown a significantly higher amount of such precursors in cord blood samples, while the peripheral blood gives the smallest yield (Eridani et al., 1998). It is also well known that cord blood clonogenic cells have a fast *in vitro* expansion and have been used already to treat patients with haemopoietic ailments, including genetic disorders and leukemia (Gluckman et al., 1997; Long et al., 1999).

It appears however of special relevance to avoid early depletion of the stem cell pool in culture and attention has been recently focused on the action of negative regulators of haemopoiesis, like macrophage inflammatory protein 1-alpha (MIP-1-alpha), tumor

Figure 2. Fully differentiated neurons developed in culture from precursors, as in Figure 1.

necrosis factor – alpha, and others, which may allow stem cell protection from cytotoxic agents (Parker and Pragnell, 1995). MIP-1-alpha and LIF have actually been shown to protect the repopulating ability of purified haemopoietic stem cells, and preliminary clinical trials to test the tolerance to the former compound are in progress (Marshall et al., 1998; Tanosaki et al., 1999). It is also worthwhile to mention a report on a cyclin-dependent kinase inhibitor, p21, which seems to protect the stem cell pool from premature exhaustion (Cheng et al., 2000).

Experimental

As for other tissues and organs, experimental studies are available concerning the CNS: a widely used model in experimental animals is the lesioned striatum, in which cells at different stages of maturation have been inserted (Sabata et al., 1995). The availability of practically immortal human nervous stem cell lines is actually the basis for attempting experimental transplants into areas of the brain. For instance, long-term cultured human CNS stem cells were implanted into the striatum of immuno-deficient mice

and were detected within the host parenchyma up to 1 year after grafting (Vescovi et al., 1999). Presently, implantation of dopamine-producing cells has been successfully tried not only in mice, but also in patients with Parkinson's disease (Olanow et al., 1997; Gage, 2000); transplants of ES-derived, as well as adult neural stem cells have also been successfully performed in laboratory models of demyelinating diseases, like multiple sclerosis (Brustle et al., 1999; Zhang et al., 1999). Such procedures, however, have to be evaluated against the possibility to reactivate quiescent cells *in situ*.

Speculative

Attempts at stem cell transplantation and modulation can be considered in a variety of other clinical conditions. The turning point has recently been the recognition and identification of stem cells in tissues which so far had not been accessible for such purpose, for instance muscle stem cells, hepatic precursors and even pancreatic islet progenitors (Kritzik et al., 1999). Moreover, a surprising plasticity has been found, which allows transdifferentiation processes:

B.M. cells for instance, may be capable of producing epidermal cells, skeletal muscle cells and even hepatic oval cells, precursors of mature liver cells (Petersen et al., 1999).

Such a process is presently the subject of intensive studies: it is now believed that, when stem cells are introduced in a new 'niche', they can undergo a process of reprogramming, which is promoted, for instance, by the influence of neighbouring cells, differentiating at the same time, as well as by *ex novo* activation of surface receptors for a range of signals, present and active in the new micro-environment (Watt and Hogan, 2000). It is of interest that stem cells with similar properties can be isolated with the same procedure from different tissues like bone marrow and muscle (Gussoni et al., 1999), suggesting a critical role of the environment for their ultimate differentiation and fate.

It has already been shown, indeed, that in areas of muscle regeneration there is normally an influx of progenitor cells from other sources and that even marrow-derived cells, transplanted into immunodeficient mice can migrate into such areas, undergo myogenic differentiation and originate fully differentiated muscle fibers (Ferrari et al., 1998). This may suggest a tentative strategy, utilizing genetically modified myogenic precursors, to treat degenerative disorders like muscular dystrophies.

New evidence has also been obtained very recently about the surprising potential of adult neural stem cells: such cells from mouse brain have been shown indeed endowed with a 'repertoire' of differentiation very close to that of embryonic cells (Clarke et al., 2000), providing a further stimulus to investigations in this area (Snyder and Vescovi, 2000).

Another type of adult stem cells, which are easily obtainable and show a high proliferation potential are those from the skin. They can actually be propagated and amplified in culture, and their biochemical features have already been discussed before. It is possible to envisage that such cells may not only be used to obtain epidermal sheets to be used to repair skin lesions, but that they can be 'reprogrammed' to other somatic stem cell types, which may be transplanted into different tissues or organs (Fuchs and Segre, 2000).

Such speculation opens the way to many experimental attempts of cell transplants for a variety of clinical conditions, ranging from muscular dystrophy to liver failure, diabetes and so on.

Numerous biotechnology companies have already started ambitious programmes, which are designed to use both embryonic and adult stem cells, manipulated

in order to restore damaged tissues in a variety of clinical conditions. It has been stressed, anyway, that adult stem cells seem to attract far more investments: intensive research is indeed currently in progress (Marshall, 2000). Mesenchymal cells seem to attract considerable attention, so that systems for isolation and production of such cells have already been patented. Preliminary clinical trials are also contemplated, mainly aiming to replace damaged cartilages or repair injured tendons; other attempts are being made to grow neural stem cells, to be transplanted into the brain or the spinal cord of patients with severe CNS damage (Vescovi et al., 1999; Eridani, 1999). Altogether this field has become very exciting and new interesting developments can be expected.

Acknowledgement

The author thanks Dr Angela Gritti, of the Stem Cell Research Institute at DIBIT, S. Raffaele Hospital, in Milan, for kindly providing the pictures included.

References

- Altman J and Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rat. *J Comp Neurol* 124: 319–335.
- Barinaga M (2000) Fetal neuron grafts pave the way for stem cell therapies. *Science* 287: 11–12.
- Bjornson CRR, Rietze RL, Reynolds BA, et al. (1999) Turning brain into blood: A haemopoietic fate adopted by adult neural stem cells *in vivo*. *Science* 283: 534–537.
- Bradley A (1990) Embryonic stem cells: Proliferation and differentiation. *Curr Opin Cell Biol* 2: 1013–1017.
- Brustle O, Jones KN, Learish RD, et al. (1999) Embryonic stem cell-derived glial precursors: A source of myelinating transplants. *Science* 285: 754–756.
- Cheng T, Rodrigues N, Shen H, et al. (2000) Haematopoietic stem cell quiescence maintained by p21-cip1/waf1. *Science* 287: 1804–1808.
- Clarke DL, Johanson CB, Wilbertz J, et al. (2000) Generalized potential of adult neural stem cells. *Science* 288: 1660–1663.
- Doetsch F, Garcia-Verdugo M and Alvarez-Buylla A (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci* 17: 5046–5061.
- Dzierzak E, Medvinsky A and De Bruijn M (1998) Qualitative and quantitative aspects of haematopoietic cell development in the mammalian embryo. *Immunol Today* 19: 228–235.
- Eglitis MA and Mezey E (1997) Haematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci USA* 94: 4080–4085.
- Emerson SG (1996) *Ex vivo* expansion of haematopoietic precursors, progenitors and stem cells: The next generation of cellular therapeutics. *Blood* 87: 3082–3088.

- Eridani S and Morali F (1993) Identification of haemopoietic stem cells. *Cytotechnology* 11: 101–106.
- Eridani S, Mazza U, Massaro P, et al. (1998) Cytokine effect on *ex vivo* expansion of haemopoietic stem cells from different human sources. *Biotherapy* 11: 291–296.
- Eridani S (1999) Replacement of damaged neural cells: A mirage? *J R Soc Med* 92: 502–504.
- Ferrari G, Cusella Deangelis G, Coletta M, et al. (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 279: 1528–1530.
- Fuchs E and Segre JA (2000) Stem cells: A new lease of life. *Cell* 100: 143–155.
- Geiger H, Sick S, Bonifer C and Muller AM (1998) Globin gene expression is reprogrammed in chimeras generated by injecting adult hemopoietic stem cells in mouse blastocyst. *Cell* 93: 1055–1055.
- Gluckman E, Rocha V, Boyer A, et al. (1997) Outcome of cord blood transplantation from related and unrelated donors. *N Engl J Med* 337: 373–381.
- Gritti A, Parati FA, Cova L, et al. (1996) Multipotent stem-like cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. *J Neurosci* 16: 1091–1100.
- Gussoni E, Soneoka Y, Strickland CD, et al. (1999) Dystrophin expression in the *mdx* mouse restored by stem cell transplantation. *Nature* 401: 390–394.
- Keating A, Powell J, Takahashi M and Singer JW (1984) The generation of human long-term marrow cultures from marrow depleted of Ia positive cells. *Blood* 64: 1159–1162.
- Kondo M, Weissmann IL and Akashi K (1997) Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 91: 661–672.
- Kopen GC, Prockop DJ and Phinney DG (1999) Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brain. *Proc Natl Acad Sci USA* 96: 10711–10716.
- Kritzik MR, Jones E, Chen Z, Krakowski M, Krah T, Good A, Wright C, Fox H and Sarretnick N (1999) PDX 1 and Msx2 expression in the regenerating and developing pancreas. *J Endocrinol* 163: 523–530.
- Lajtha L (1979) Stem Cell Concepts. *Differentiation* 14: 23–34.
- Lois C and Alvarez-Buylla A (1993) Proliferating sub-ventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc Natl Acad Sci USA* 90: 20074–20077.
- Long G, Madan B, Kurtzberg J, et al. (1999) Unrelated umbilical cord blood transplants in patients with hematologic malignancies and genetic disorders. *Blood Nov Suppl Abstract* 2544.
- Marshall E (2000) The business of stem cells. *Science* 287: 9–11.
- Marshall E, Howell AH, et al. (1998) Clinical effect of human macrophage inflammatory protein 1-alpha administration in humans: A phase I study in cancer patients and normal healthy volunteers with the genetic variant BB 10010. *Eur J Cancer* 34: 1023–1029.
- Moles GP and Watt FM (1997) The epidermal stem cell compartment: Variation in expression levels of E. cadherin and catenins within the basal layer of human epidermis. *J Histochem Cytochem* 45: 867–864.
- Morshead CM, Reynolds BA, Craig CG, et al. (1994) Neural stem cells in the adult mammalian forebrain: A relatively quiescent subpopulation of subependymal cells. *Neuron* 13: 1071–1082.
- Nichols J, Zevnik B, Anastassiadis K, et al. (1998) Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor OCT 4. *Cell* 95: 379–391.
- Niwa H, Burdon T, Chambers I and Smith A (1998) Self-renewal of pluripotent embryonic stem cells is modulated via activation of STAT 3. *Genes Dev* 12: 2048–2060.
- Nusse R (1999) WNT targets. Repression and activation. *Trends Genet* 15: 1–3.
- Olanow CW, Freeman TB and Kordower CW (1997) Neural transplantation as a therapy for Parkinson's disease. *Adv Neurol* 74: 249–269.
- Osawa M, Hanada K, Hamada H and Nakauchi H (1996) Long term haemopoietic reconstitution by a single CD34-low negative haematopoietic stem cell. *Science* 273: 242–244.
- Parker AN and Pragnell IB (1995) Inhibitors of haemopoiesis and their potential clinical relevance. *Blood Rev* 9: 226–233.
- Petersen BE, Bowen WC, Patrene KD, et al. (1999) Bone marrow as a potential source of hepatic oval cells. *Science* 284: 1168–1174.
- Piacibello W, Sanavio F, Garetto L, et al. (1997) Extensive amplification and self-renewal of human haematopoietic stem cells from cord blood. *Blood* 89: 2644–2653.
- Pittenger MF, Mackay AM, Beck SC, et al. (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143–146.
- Reyes M and Verfaillie CM (1999) Characterization of multilineage mesodermal progenitor cells in adult bone marrow. *Blood Nov Suppl, Abstract* 124.
- Sabata O, Horellu P, Vigne E, et al. (1995) Transplantation to the rat brain of human neural progenitors that were genetically modified using adenovirus. *Nat Genet* 9: 256–260.
- Sestan N, Artavanis-Tsakonas S and Rakic P (1999) Contact-dependent inhibition of cortical neurite growth mediated by Notch signaling. *Science* 286: 741–746.
- Scheffler B, Horn M, Blumke I, et al. (1999) Marrow mindedness: A perspective on neurogenesis. *Trends Neurosci* 22: 348–357.
- Snyder EY and Vescovi A (2000) The possibilities/perplexities of stem cells. *Nat Biotechnol* 18: 827–828.
- Suhonen JO, Paterson DA, Ray J and Gage FH (1996) Differentiation of adult hippocampus-derived progenitors into olfactory neurons *in vivo*. *Nature* 383: 624–627.
- Tanosaki R, Ashihara E, et al. (1999) MIP-1-alpha and LIF protect the repopulating ability of purified murine haemopoietic stem cells in serum-deprived cultures stimulated with SCF and IL-3. *Ann Ist Superiore Sanita'* 35: 553–562.
- Thomas ED (1991) Frontiers in bone marrow transplantation. *Blood Cells* 17: 259–267.
- Thompson JA, Itskovitz-Eldor J, Shapiro SS, et al. (1998) Embryonic stem cells derived from human blastocysts. *Science* 282: 1142–1145.
- van der Kooy D and Weiss S (2000) Why stem cells? *Science* 287: 1439–1441.
- Vescovi AL, Parati EA, Gritti A, et al. (1999) Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. *Exp Neurol* 156: 71–83.
- Villa A, Snyder E, Vescovi A and Martinez-Serrano A (2000) Establishment and properties of a growth factor dependent, perpetual human stem cell line from the human CNS. *Exp Neurol* 161: 67–84.
- Watt FW and Hogan BLM (2000) Out of Eden: Stem cells and their niches. *Science* 287: 1427–1430.
- Zhang SC, Ge B and Duncan ID (1999) Adult brain retains the potential to generate oligodendroglial progenitors with extensive myelination capacity. *Proc Natl Acad Sci USA* 96: 4089–4084.