Neural stem cell research: A revolution in the making

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Existence of stem cells capable of differentiating in all types of haemopoietic cells, red blood cells, white blood cells, platelets has been known for the last one decade. These have been isolated from bone marrow cultured and made to differentiate into specific cell types. It is, however, only in the last couple of years that totipotent cells isolated from human embryo at the blastocyst stage, have been shown to retain the potentials to differentiate into any type of adult cells including neuronal series. More or less simultaneously it was demonstrated that contrary to the prevailing belief, neurogenesis continues throughout life even in humans, at least in certain regions of the brain. Not surprisingly, this has lead to active research in the field with the hope of exploiting this knowledge for replacement of lost or degenerating neurons. This review is an attempt to summarize the current knowledge and future areas of research.

TWO independent publications[1,2] in November 1998 heralded the isolation of the human embryonic stem cells (ESCs) using two different approaches. These cells can not only differentiate into all types of tissue, but can, under carefully controlled conditions, be maintained continuously as undifferentiated cells in culture[3]. A year later Floyd Bloom[4] hailed it as the breakthrough of the year arguing that 'without question, the potential of embryonic stem cells again fulfills our definition of a breakthrough as a rare discovery that profoundly

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changes the practice or interpretation of science or its implications for society'. In contrast to the ESCs which are totipotent, there are pleuripotent stem cells in adult organs, which can divide repeatedly to replenish a tissue as in skin, intestinal epithelium or may remain quiescent as in mammalian brain[5]. In November 1998, Fred Gage[6] from California and Peter Eriksson *et al.*[7] from Göteborg published a remarkable observation that the mature human brain does spawn neurons routinely in at least one site – the hippocampus, an area important to memory and learning[8]. These neurons no doubt, would have differentiated from stem cells. Gage[9] reiterated that neural stem cells (NSCs) exist not only in the developing mammalian nervous system, but also in the adult nervous system of all mammalian organisms, including humans. He pointed out that the term 'neural stem cell' is used loosely to describe cells that (i) can generate neural tissue or are derived from the nervous system, (ii) have the capacity for self-renewal, and (iii) can give rise to cells other than themselves through asymmetric division. It is interesting to note that Kirschenbaum *et al.*[10] had already reported *in vitro* neuronal production and differentiation by precursor cells derived from adult human forebrain. It was soon realized that neuronal stem cell lines represent a homogenous source of cells for genetic, developmental, gene transfer studies as also for their clinical use for cell replacement therapy[11–16]. It may be mentioned that a type of human stem cell found in the bone marrow, which gives rise to the full range of cells in blood was already known since Irving Weissman of Stanford University discovered it in 1991 (ref. 17). Already in April 1997, McKay[18] from the Laboratory of Molecular Biology, National Institute of Neurological Disorders and Stroke, Bethesda had identified multipotent cells in the vertebrate central nervous system (CNS) and their ability to transform *in vitro* into the three major cell types (neurons, astrocytes, oligodendrocytes) of the adult brain under the influence of various growth factors. This paper was later reproduced in the section on The Best of Science: Neuroscience. It is not surprising that the ensuing two years have witnessed a flurry of scientific activity in this field (see Figure 1).

Definitions

Thompson *et al.*[1] whose paper aroused so much excitement stated that the essential characteristics of primate ESCs should include (i) derivation from the pre-implantation or per-implantation embryo, (ii) prolonged undifferentiated proliferation, and (iii) stable developmental potential to form derivatives of all three embryonic germ layers even after prolonged culture. It may be mentioned that they derived their pleuripotent cell lines from the totipotent cells from the inner lining of the

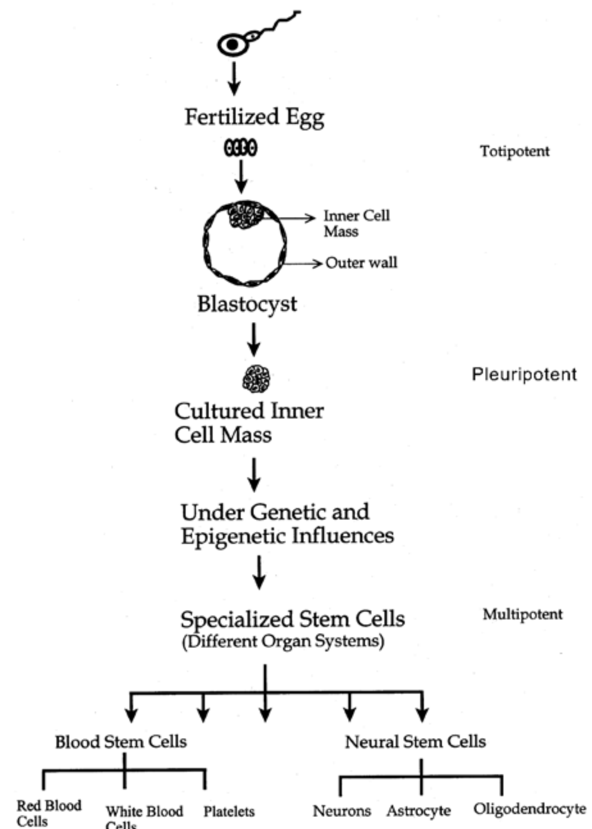

Figure 1. Developmental stages of stem cells.

Box 1. Definitions

Stem cells: Cells which are able to reproduce themselves throughout the life span of the animal and are able to give rise to differentiated cells. They have the ability to divide for indefinite periods in culture and give rise to specialized cells.

Embryonal stem cells: Cells derived from embryo – pro or post implantation prior to their differentiation into specific cell types.

Totipotent cells: Cells which have the potential to differentiate into derivatives of all three embryonic germ layers, i.e. ectoderm, mesoderm and endoderm. In addition they can also specialize into extraembroynic membrane and tissues.

Pleuripotent cells: Cells which can give rise to different types of cells representing derivatives of two different germ layers, e.g. skin (ectoderm) and muscle (mesoderm).

Neural stem cells: Cells which can generate neural tissue either any one or both neuron and glia (astrocytes, oligodendrocytes). The term is also used for stem cells, derived from the embryonic or adult nervous system which normally differentiate into nervous tissue. These cells remain undifferentiated for long periods of time while retaining potential to differentiate into nervous tissue.

Progenitor cells: Cells with a more restricted potential than a stem cell, and generally destined to give rise to a specific cell type.

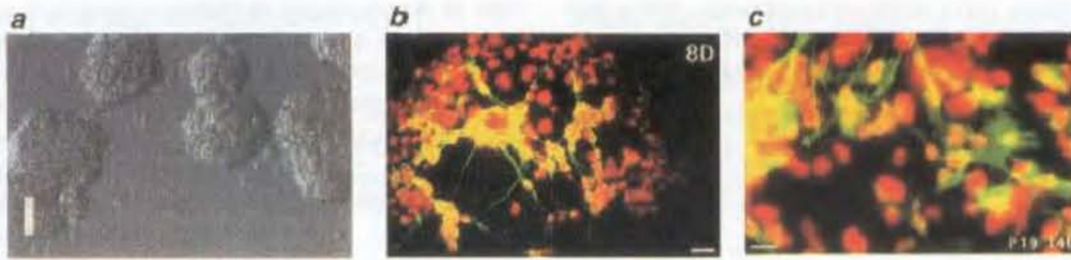

Figure 2. P19 embryonal carcinoma cells can give rise to neurons and glia in culture. P19 EC cells were aggregated in culture in the presence of retionoic acid for four days (panel ***a***, bar = 150 μm). Cells were dissociated and plated. They were fixed four days later and stained with neuron-specific tubulin (green) and propidium idodie (red). Neuronal cell bodies in ganglion-like structures (yellow) from which long neurites extend (green) can be seen (panel ***b***, bar = 40 μm). Cells were fixed ten days after plating and stained with glial specific GFAP (green) and propidium iodide (red). Differentiaiton of cells into glia can be seen (panel ***c***, bar = 20 μm). (By courtesy of S. Mani.)

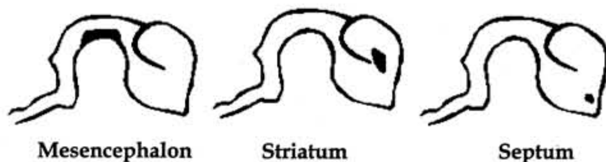

Figure 3. Examples of regions on the developing mammalian CNS from which stem cells can be isolated. Modified from ref. 85.

human embryos produced by *in vitro* fertilization cultured up to the blastocyst stage. These ESCs must be differentiated from terato carcinoma-derived pleuripotent embryonal carcinoma cells[19], as also from cells derived from the embryonic germ cells[20] and pleuripotential stem cells isolated from specific organs like bone marrow, liver and brain, which unlike ESCs are not totipotent, but produce a narrow range of cells (see Figure 2). Svendsen *et al.*[21] in a review claimed that recent evidence suggests that NSCs exist in both the developing and adult human CNS. These cells can be grown *in vitro* and remain undifferentiated for long periods of time, while retaining potential to differentiate into nervous tissue (Figure 3). In the adult human brain, both neuronal and oligodendroglial precursors have been identified and methods for their harvesting and enrichment have been established[11]. However, Murphy *et al.*[22], Bjornson *et al.*[23], Clarke *et al.*[24], Kooy and Weiss[5] have reported surprising plasticity of adult NSCs to differentiate into cells of other lineage like skin, hematopoietic tissue, etc. at least in rodents. Bjornson *et al.*[23] transplanted genetically-labelled NSCs into irradiated hosts. These were found to produce a variety of blood cell types, including myeloid and lymphoid cells as well as early hematopoietic cells. On the basis of their observation they concluded: 'Thus, neural stem cells appear to have a wider differentiation potential than previously thought'. Clarke *et al.*[24] demonstrated that NSCs from adult mouse brain have a very broad developmental capacity and may give rise to cells of all germ layers.

Various definitions seem to have been adopted for stem cells by different authors, but a consensus definition is likely to include at least two features: stem cells are able to reproduce themselves throughout the lifespan of the animal and they are able to give rise to differentiated cells[25]. Vescovi and Snyder[16] recognized that the debate is still open concerning the most appropriate definition of a stem cell and on how to identify, characterize and manipulate it, but expected these to manifest evidence of plasticity both *in vitro* and *in vivo*. McKay[18] indicated that to be considered a stem cell in the CNS, a cell must have the potential to differentiate into neurons, astrocytes and oligodendrocytes. The term 'progenitor' refers to a cell with a more restricted potential than a stem cell. 'Precursor' is a less stringent term that refers to any cell that is earlier in a developmental pathway than another (Box 1).

Kooy and Weiss[5] suggested that even as the identification of structural attributes of stem cells at the morphological or molecular levels become possible, the definition of stem cells must be on a functional basis. Functionally stem cells are the multipotential, self-renewing cells that sit at the top of the lineage hierarchy and proliferate to make differentiated cell types of a given tissue *in vivo*. The developmental potential of stem cells is generally restricted to the differentiated elements of the tissue in which they reside. However, there is evidence to suggest that certain cells may differentiate into quite different cell types. Unlike many other tissues, stem cells in mammalian brain are generally quiescent or at best have limited potentials for multiplication except at certain locations[26]. It may be mentioned that Gage[9] debated about the function of the stem cells in the adult nervous system and wondered, if these were vestiges of the evolution or possessed limited capacity for self-renewal. He pointed out that it was unknown whether or not stem cells from different re-

gions of the brain carry different constraints. NSCs that have the capacity to self-renew and differentiate into neurons and glia have been isolated from adult brain and cultured[27–30].

Neurogenesis in adults

Till very recently, scientists firmly believed that neurons in adult mammals are devoid of any capability to multiply once brain development had ended (Box 2). No doubt vigorous regeneration of damaged nervous tissue was well known in lower species like lizards.

It is worth noting that already in 1960s Altman and Das[31–33] demonstrated that rats make new brain cells throughout life. However, no one took them seriously, probably because these observations were against the prevailing dogma. Kaplan and Hinds[34] independently confirmed neurogenesis in the adult rat using electron microscopy. It was nearly 15 years later that Nottebohm[35] demonstrated that birds do continually make new brain cells which replace old dying cells in a programme of constant brain rejuvenation. During a meeting convened by him entitled 'Hope for a new neurology', Nottebohm expressed confidence that the adult human brain could also create neurons. Progressively evidence of neurogenesis in birds, rats and mice continued to accumulate[34,36–41]. It was reported that the generation of new neurons occurs in just two regions of the adult brain. The first is the subventricular zone (SVZ) in the wall of the lateral ventricle, where new interneurons were generated for the olfactory bulb. The second is the subgranular zone of the dentate gyrus. In these areas, there is seemingly a continuous turnover of interneurons and granule cells, implying that the newborn neurons replace dying cells[42]. The increased neurogenesis in the hippocampus has also been demonstrated in mice living in an enriched environment[43]. In addition, increased neurogenesis has been observed in the dentate subgranular zone following seizures and inadequate blood supply[42,44]. It may be mentioned that in addition to SVZ and hippocampus, stem cells have also been demonstrated in the septum, striatum and even the spinal cord of rodents. However, these do not appear to produce new neurons under normal condition[8,45].

It was in 1998 that Elizabeth Gould and colleagues for the first time established the addition of new neurons in hippocampus in higher mammals, i.e. adult macaques. And a year later they extended these observations and reported that new neurons were added to prefrontal, inferior temporal and posterior parietal neocortical association areas, but not to a primary sensory area (striate cortex). These new neurons appeared to originate in the SVZ and to migrate through the white matter to the neocortex where they differentiated into mature neurons, as established by demonstration of specific markers. They suggested that these new neurons, which are continually added in adulthood, might play a role in the functions of association neocortex like learning and memory, though direct evidence was not available and is still lacking[46,47].

Box 2. Neurogenesis

Generation of new neurons; till recently believed to occur only during embryonic development, now unequivocally demonstrated to occur throughout life even in humans.

As mentioned earlier, Eriksson *et al.*[7] demonstrated the same phenomenon in human hippocampus. Human hippocampal tissue obtained at autopsy from patients suffering from oro-pharyngeal cancer, who had earlier been given the thymidine analogue, bromodeoxyuridine (BrdU) to assess the proliferative activity, was submitted to immunofluorescent labelling for BrdU and for one of the neuronal markers, NeuN, Calbindin or neuron specific enolase (NSE). New neurons generated from dividing progenitor cells were seen in the dentate gyrus in all the specimens. The authors concluded that the human hippocampus retains the ability to generate neurons throughout life. They also studied the SVZ adjacent to the caudate nucleus. While they observed BrdU positive cells, but these did not co-express the cell-specific markers GFAP or NeuN. This suggested that the human SVZ contains progenitor cells that migrate from SVZ before they differentiate.

The exact phenotype of the most primitive cell in SVZ and subgranular layer of the dentate gyrus is not yet known with certainty, but there is evidence to indicate that these are really stem cells. Johansson *et al.*[45] pointed out that new neurons are continuously generated in specific regions of the adult mammalian brain. These neurons are derived from multipotent stem cells, the identity of which has been enigmatic. On the basis of immunohistological and cell sorting techniques using markers for nestin and Notch 1, they claimed that (in rats) ependymal cells are NSCs. However, a few months later Doetsch *et al.*[48] contradicted these observations and provided quite convincing evidence that the NSCs residing in the SVZ of the adult mammalian brain are really astrocytes, which continually generate new neurons destined for the olfactory bulb. A definitive identification will require phenotypic markers that discriminate between different cell types or different states of a common cell. Defining the factors that initiate the 'arousal' of the quiescent cells to multiply, migrate and acquire the ultimate fate of these cells remains the most active and exciting area of developmental biology[9]. This knowledge no doubt will be invaluable for future therapeutic applications.

Factors influencing multiplication and differentiation of stem cells

A variety of genetic, environmental and molecular factors influence neurogenesis, both in the developing embryo and adult brain[9,49]. This is not surprising when one realizes that the extraordinary diversity of adult vertebrate nervous system is generated from a single sheet of epithelial cells. Many of the molecules that have been found to be important in the developing brain persist in adult brain. McKay[18] demonstrated that multipotent cells could be isolated and cultured from foetal as well as adult vertebrate CNS. Some of the factors that control their differentiation into neurons and glia have been defined *in vitro*. However, it is obvious that we need to know more about the intrinsic controls that keep the stem cell quiescent or direct them along differential pathways[26]. As mentioned earlier, normally neurogenesis occurs only in limited areas of the adult mammalian brain – the SVZ, the dentate nucleus of the hippocampus and only at low levels in some regions of macaque cortex[47]. Magavi *et al.*[50] demonstrated that endogenous neural precursors can be induced *in situ* to differentiate into mature neurons, in regions of adult mammalian neocortex that do not normally undergo any neurogenesis. This differentiation occurred in a layer- and region-specific manner and the neurons formed appropriate cortico-thalamic connections.

As mentioned earlier, the ESCs may be totipotent, pleuripotent or specific to the organ from which these are derived, i.e. blood, brain, liver, etc. What determines this potential is not well understood. It is believed that as these cells divide, sub-divide and migrate their potential for differentiation becomes more and more restricted. However, some of the cells derived from a specific tissue have been shown to retain pleuripotent potential. Thus bone marrow-derived stem cells have been grown in culture to develop neurons and vice versa[23]. It is not necessary to summarize all the intrinsic and extrinsic factors that have been identified to be responsible for *in vivo* and *in vitro* multiplication, differentiation, axon generation, migration and integration; only some examples are mentioned. It is, however, important to realize that the necessary requirements though similar during foetal development and adult life are not identical. There is obviously a chain of events requiring involvement of different factors at different stages. Similarly factors required *in vivo* may not be the same for *in vitro* manipulation.

Tissue-specific stem cells themselves are a result of differentiation of the ESCs. Tuszynski and Gage[51] claimed that multiple genetic and epigenetic events determine neuronal phenotype during nervous system development. After the mature mammalian neuronal phenotype has been determined it is usually static for the remainder of life, unless an injury or degenerative event occurs. Every lineage is controlled by unique combinations of such factors, each of which may be expressed individually in several lineages[26]. There is abundant evidence that transcription factors control stem cell fate. A large number of evolutionarily conserved transcription factors have already been implicated. These in turn are dependent on a hierarchy of genes. There are still conflicting reports whether neuronal precursor cells are irreversibly committed to distinct regional fate. Observations on developing brain, e.g. in chick indicate that single factors like FG F8 are sufficient to bias the differentiation cascade and establish major regional features of the CNS. On the other hand, postnatal transplantation studies using genetically-labelled mouse telencephalic neural cells, which were simply deposited in the ventricles demonstrated large numbers of grafted cells incorporated into many sites in the host brain. They migrated in accordance with known pathways and incorporated into telencephalic, diencephalic and mesencephalic regions[18]. Wichterle *et al.*[52] observed that neural precursors from embryonic medial ganglionic eminence but not from lateral ganglionic eminence or neocortex, dispersed and differentiated into neurons in multiple adult brain regions.

Besides intrinsic controls there are a number of factors available or secreted in the microenvironment that serve as external controls. For example, at least two members of the TGFβ family of signalling proteins, have been found to regulate differentiation of neural crest cells. Epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) have been used as mitogens to expand CNS stem cells *in vitro*[53]. Ciliary neurotrophic factor (CNTF) directs the multipotent stem cell to adopt an astrocytic lineage, while thyroid hormone (T3) causes the stem cells to become lineage-restricted progenitors for oligodendrocytes[18]. Retinoic acid – which helps drive nervous-system development in the embryo, when added to the undifferentiated stem cell *in vitro* culture converts 90% of the cells to adopt the neural lineage pathway[13,27,30,48]. Svendsen *et al.*[54] isolated precursor cells from the developing human cortex and cultured these in presence of EGF and FGF-2. They achieved a 1.5 million-fold increase in precursor cell number over a period of less than 200 days. Similarly, Carpenter *et al.*[55] described *in vitro* propagation of a continuously dividing multipotent population of progenitor cells in the human embryonic forebrain. They used a serum-free medium containing bFGF, leukaemia inhibitory factor (LIF) and EGF. The cells remained multipotent for at least 1 year *in vitro*, during which period they increased in number to 10^7. Upon differentiation they formed neurons, astrocytes and oligodendrocytes. This opens up immense potential for therapeutic applications[49,56].

Not only could one multiply and induce differentiation of stem cells into neurons *in vitro*, these could be

experimentally induced to produce neuritis so as to become integrated with the host nervous system. Shetty and Turner[57] demonstrated that application of brain-derived neurotrophic factor (BDNF) promoted neurite outgrowth.

Therapeutic potentials of NSCs

As already mentioned, NSCs in the adult mammalian (including human) brain remain dormant, except in the SVZ and hippocampus. What triggers these to regeneration is obviously an important issue, with implications for therapy. It has been proposed that when regeneration is required there must be local chemical signals released in the tissue. However, little is known about them at present[9,25]. Enrichment of the environment[8,41,43,58], apoptosis[50,59], seizures[44] and reduction in blood supply[42] have been shown to trigger the dormant cells. The extent of this regeneration is obviously limited, otherwise one would observe much greater recovery of function following an insult. In contrast to these positive influences, Gould *et al.*[46] demonstrated that single exposure to stressful experience resulted in a significant reduction in the number of proliferating cells in the dentate gyrus of adult monkeys. The possibility of utilizing the inherent potentials of NSCs either by manipulating the local microenvironment using growth factors, transplantation of genetically modified cells secreting these factors or use of *in vitro* multiplied NSCs for therapy appears to be real in view of the findings in experimental animals[42].

Based on rapidly accumulating evidence from diverse sources it is being proposed that multipotent human NSCs, successfully isolated and conditionally perpetuated, maintained in culture by genetic and epigenetic means, may serve as a cellular vehicle for molecular therapies as well as for cell replacement in the human CNS[6,16,60–62]. Snyder and Macklis[63] had commented on a possible tropism of transplanted NSCs for neurodegenerative environment. Gray *et al.*[64] demonstrated that a clonal line of conditionally immortalized NSCs, MHP36, derived from mouse embryo (E14) hippocampal analage implanted above the damaged CA1 region of adult rats, migrated to the damaged area, and reconstituted the gross morphology of pyramidal layer, manifested both neuronal and glial phenotypes and gave rise to cognitive recovery. This found confirmation in the work of Auerbach *et al.*[65] who demonstrated for the first time that *in vitro* expanded CNS precursors, upon transplantation into the brain of rats, formed electrically active and functionally connected neurons. These neurons exhibited spontaneous and evoked postsynaptic events and responses to focal glutamate application. They had observed the same in marmoset monkeys also[14].

A rat model of Huntington's disease was utilized by Armstrong *et al.*[66] to transplant epigenetically propagated human neural precursor cells. The cells survived transplantation and large numbers differentiated to express neuronal antigens, including some that expressed DARPP-32, indicating transformation to a mature striatal phenotype. Similarly Rosario *et al.*[67] demonstrated that approximately 75% of progenitors transplanted into the anterior cerebellar lobe of newborn *mea*-mutants (meander tail mice known to be deficient in granule cells) differentiated into granule cells. Clonal, multipotent neural precursor cells transplanted into regions of adult mouse neocortex undergoing selective neuronal degeneration of layer II/III pyramidal neurons, integrated into the regions of selective neuronal death, extended axons and dendrites and established different synapse contacts[59]. Wagner *et al.*[68] were able to manipulate an immortalized multipotent NSC line to a ventral mesencephalic dopaminergic phenotype. In contrast to this highly type-specific differentiation, Yandava *et al.*[15] used a model of global dysfunctional disorder-shiverer mouse to evaluate the possible beneficial effect of NSCs. They transplanted clonal NSCs into the ventricle, which resulted in widespread engraftment with MBP-expressing oligodendrocytes myelinating up to 52% of host neuronal processes. Attempts have also been made to induce myelination in the demyelinating lesions in the spinal cord[69–71].

While existence of NSCs in rodents and lower mammals was known and their isolation, culture and transplantation had been tried in early 1990s, it is only more recently that similar efforts have been directed towards human neuronal stem cells. In the present state of knowledge no attempt has, however, been made to transplant these in human, but only in rodents[6,20,72]. Rubio *et al.*[73] transplanted cells from a multipotent cell line of human NSCs (HNSC100) into the striatum and substantia nigra of the adult intact rat brain. One week after transplantation the cells had already integrated in a nondisruptive manner into the surrounding tissue and migrated to different distances depending upon the graft location. The engrafted cells completely down regulated the stem cell marker nestin and differentiated and expressed mature neural markers.

Neural progenitors for delivering therapeutic gene products

In addition to their use for replacement of lost or degenerating nervous tissue, NSCs may be useful in delivering therapeutic gene products directly into the brain. Neural progenitors are considered ideal for genetic manipulation and may be engineered to express exogenous genes for neurotransmitters, neurotrophic factors and metabolic enzymes[11,12,72]. Snyder *et al.*[74] reported trans-

Box 3. Potential uses of stem cells

- For replacement of lost or degenerating nervous tissue.
- Genetically engineered to express exogenous genes for neurotransmitters, neurotrophic factors and metabolic enzymes.
- For *ex vivo* gene therapy, e.g. engineered to transfer genes to suppress tumour growth.
- For purposes of exploring the normal process of neuronal development.

plantation of beta-glucuronidase-expressing neural progenitors in the cerebral ventricles in a mouse model of Sly disease. Donor-derived cells were found throughout the neuraxis expressing the appropriate enzyme, resulting in correction of lysosomal storage in neurons and glia in affected mice. Similar results were obtained by Sabate *et al.*[75] using *in vitro* genetically modified human neural progenitor cells transplanted to rat brain. Borlongan *et al.*[19] and Andsberg *et al.*[76] demonstrated the neuroprotective action of genetically modified NGF-secreting, immortalized NSCs transplanted into a rat model of cerebral ischaemia. Raymon *et al.*[77] utilized CNS-derived neural progenitors for *ex vivo* gene therapy in an animal model of Parkinson's disease. Whittemore[78] found CNS-derived, neuronal precursor cell line RN33B to be a useful source for cell replacement following spinal injury. The transplant-derived cells showed remarkable plasticity to respond to local micro-environmental cues. They differentiated to give rise to cells morphologically indistinguishable from the endogenous neurons at the site of transplantation. Neural stem/progenitor cells engineered by retrovirus-mediated transfer of the gene for interleukin-4 have been shown to suppress tumour growth and prolong survival in a mouse model of glioblastoma[79]. No doubt there are a number of problems which need to be resolved before it could be introduced for clinical trials[80] (Box 3).

Concluding remarks

The remarkable progress that has been made in the field of NSC research, including isolation and *in vitro* cultivation of human NSCs, has no doubt generated high expectations for its clinical application for treatment of a host of currently incurable degenerative and metabolic disorders of the CNS. Similarly, demonstration of neurogenesis, at least in a few restricted areas of the brain, prompts us to find ways to induce these existing stem cells to produce useful numbers of functional nerve cells following pathological insults – trauma, stroke, degeneration, etc. However, it is obvious that in the present stage of knowledge we still have to go a long way before the current laboratory research can reach the clinics.

First of all the question of the source of the stem cells itself poses practical and ethical problems. The two sources utilized by Thomson and colleagues and Snyder and colleagues were the blastocysts from unused embryos developed for purposes of *in vitro* fertilization and aborted foetuses. Both these sources raised ethical issues, so much so that the US congress enacted a ban on embryo research in 1996 and renewed it every year[81]. A notification to exempt stem cell research, under careful ethical supervision by the Department of Health and Human Services in February 1999, has prompted seventy-seven anti-abortion members of the congress criticizing this decision. This has initiated a national public debate[82]. Harold Shapiro (National Bioethics Advisory Commission, USA) in a report delivered to President Clinton on 13 September 1999, recommended that research in which cadaveric foetal tissue is used or research using or deriving ESCs remaining from *in vitro* fertilization, should under appropriate conditions be permitted. In that case the rules that should govern the donation of unneeded and unimplanted embryos from *in vitro* fertilization would have to be formulated taking into consideration public opinion and religious sentiments in a given society[83]. It was hoped that lines of embryonic germ (EG) cells taken from aborted foetal tissue could be used instead. DFG, the main research funding agency in Germany, where production of human ESCs is banned, advised its researchers to use EG cells for their research. However, strong doubts have already been cast on the assumption that EG cells can simply be substituted for ESCs[84].

Even if one was permitted to use such cells for clinical purposes, the questions of safety and possibility of immune-rejection would have to be resolved first.

The next technical issue that needs still further research is to fully understand the mechanism involved in prompting pleuripotent stem cells in culture to differentiate into specific cell types. It is still not clear which is the appropriate cell to transplant, the NSC, the progenitor or the required neurons[62]. The factors that influence regulation of *in vivo* neurogenesis – both positive and negative – are still being investigated. Once identified, these at best could be utilized for locations where neurogenesis has been shown to occur in adult human beings.

In conclusion it could be safely stated that NSC research needs to be pursued with vigour for it to be of clinical use. While there is lot of hope, one should not be carried away by the hype and prematurely raise the expectations among those most in need of it.

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Low dose radiobiology: Mechanistic considerations

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The question of whether very small doses of ionizing radiation really exert stochastic effects (i.e. induce harmful genetic effects, including cancer in a probablistic manner) has not been unequivocally settled. The much relied upon linear, no-threshold (LNT) hypothesis does not have convincing experimental evidence. As there are practical difficulties in generating data on genetic effects at very low doses and low dose rates, the conceptual development of the LNT hypothesis has depended upon background extrapolations from observations at high doses to low dose-regions. The dose, dose rate as well as the quality of radiation exposures in the case of atomic-bomb survivors and their descendants are fraught with uncertainties. With data accumulating on radiation-induced gene expression, the basic concepts of how cell death is caused are undergoing significant changes. Many reported phenomena such as 'radiation hormesis' and 'radioadaptive response' could no more be outright rejected and these challenge the LNT hypothesis. The fact remains that the LNT is an over simplistic 'biophysical model' to explain radiation action on the DNA of living cells and organisms. It truly masks the whole lot of physical, physicochemical, biochemical and metabolic events involving not just the DNA but also the myriads of small and large molecules, which characterize the various organelles. Most fundamentally, the LNT ignores repair processes, immune reactions and the role of apoptosis. The purpose of this review is purely to address this issue from a scientific point of view and *not* to deal with implications for radiological protection standards.

THE twin discoveries of X-rays and radioactivity, made only a few months apart, a few years before the close of the nineteenth century and the observation of carcinogenicity as early as 1902, were the commencing events in the evolution of a radiation paradigm. It was in 1925 that the first protective limits were suggested for the safety of nuclear workers. For three decades these limits were based on the concept of a tolerance dose, which if not exceeded, would result in no demonstrable harm and implicitly assumed a threshold dose below which radiation effects would be absent. Based on 'deterministic effects' such as epilation, reddening of skin, cataract formation, etc. tissue tolerance dose limits were prescribed. Hence prior to 1950, it was accepted that a 'threshold' existed. It was after World War II that epidemiological studies on the occurrence of leukaemia in children exposed to atomic-bombs of Hiroshima and Nagasaki, led to a notion that for genetic effects there was probably no threshold and that there possibly exists a linearity between dose of exposure and cancer incidence. Radiation protection limits until then were expressed in terms of risk based on maximum permissible dose that clearly implied a threshold. The observations of H. J. Muller in 1927, that X-rays induced sex-linked recessive lethal mutations (now known as deletions) in fruit flies were viewed with serious concern. In general many studies then were believed to suggest that the induced mutation rate was independent of dose-rate, mutation was a single hit process with no threshold, and that the mutagenic effect of radiation was cumulative over a lifetime. An observation of perhaps even greater significance was the X-ray-induced somatic mutations, which offered a plausible explanation for the carcinogenicity of ionizing radiation and also was consistent with the long latency period associated with the production of cancer[1]. Hence the so-called 'stochastic' effects which were believed not to have any threshold dose constituted the backbone of the current radiation paradigm based on the linear, no threshold (LNT) model. Since the dose response curve could not be determined at low dose levels, a backward extrapolation from the high to low regions of the dose effect curve had been adopted, although without valid scientific experimental evidence.

LNT hypothesis, the current radiation paradigm

For at least four decades, the fundamental underpinning of the standards of radiation protection has been the LNT dose response model. The LNT model is based on three fundamental assumptions: (1) any radiation dose, no matter how small is harmful (2) the probability of health outcome is linearly related to the absorbed dose; if the absorbed dose is double, so is the risk, and (3) effects other than those observed at high doses will not occur at low doses[1]. Figure 1 shows the LNT model along with the threshold model, where deterministic effects such as epilation, reddening of the skin, cataract

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