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STEM CELLS IN NEUROLOGY

T. R Raju, Mansoor Ali Khan, Phalguni Anand Alladi
Dept of Neurophysiology, NIMHANS,
Bangalore - 29 India.

INTRODUCTION: Stem cells are operationally defined as undifferentiated cellular entities that can give rise to plethora of cellular phenotypes found in the body, originally defined in haematological system and now demonstrated to be present at multiple sites including brain and share the properties of self-renewal and multipotentiality *(Gage et al, 1995)*. 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 *(Pincus et al, 1998)*.

Sources of stem cells:

Stems cells are of four major types, each type offering advantages and disadvantages.

1. *Embryonic stem (ES) cells*:
   These cells are derived from human embryos that are a few days old at the blastocyst stage (between 50-150 cells) and have the potential to become almost any type of cell. The issues concerning to process of Embryonic Stem Cell (ESC) creation are ethically contentious as the embryo is destroyed. In order to overcome this serious ethical issue, researchers are exploring alternative techniques of obtaining embryonic stem cells by extraction using a single-cell biopsy method, which does not interfere with embryo's developmental potential and does not involve cloning or destruction of the embryo *(Klimanskaya et al., 2006)*.
2. *Foetal stem cells:*
   Derived from aborted human fetuses, they are multipotent-have the potential to become many of the cell types. There are serious ethical issues to be considered and there is a limited availability of foetuses.
3. *Cord blood and placental stem cells:*
   These are derived from umbilical cord blood and placentas. They are already in use for a variety of therapies, to treat Gunther's disease, Hunter syndrome, Hurler syndrome, acute lymphocytic leukemia and other blood and immune disorders occurring mostly in children. They are easily extractable from the source but studies have shown that they can only form a limited number of cell types and are available in low concentrations.
4. *Adult stem cells:*
   Found in all humans as resident population in specific brain regions. Many of the therapies involving transplantation strategies are already underway in animals and to an extent in humans. The concern is of its access, extraction and making them

differentiate to different phenotypes, and the current research demonstrates only limited number of cell types can be derived from this stem cell population.

Few of the neurological disorders where stem cells can potentially find significant application are listed in table 1.

Table 1: Applications of stem cells in certain neurological diseases

Disease	Principle function required of stem cells
Parkinson's disease	Nigrostriatal dopamine neurons
Huntington's disease	GABAergic striatal projection neurons
Alzheimer's (and other dementias)	Diffuse neuronal replacement, including basal forebrain cholinergic
Multiple system atrophy (MSA)	Nigrostriatal and striatal output neurones
Hippocampal damage	Hippocampal neurones especially those of CA1
Focal ischaemic damage	Broad phenotypes required; dependent on site
Traumatic brain injury	Broad phenotypes required; dependent on site
Spinal injury	Projection neurones (glutamate); remyelination
Amyotrophic-lateral sclerosis	Replacement of alpha motorneurons
Multiple sclerosis and other demyelinating conditions	Remyelination through oligodendrocytes

(Barker, 2003)

The reasons for stem cells receiving such attention for the treatment of neurological disorders relates to their:

(a) Capacity to proliferate in culture with the prospect to obtain plethora of cells from a limited source;
(b) Potential of harvesting them from the patients themselves;
(c) Ability to migrate and disseminate following implantation within the adult central nervous system (CNS);
(d) Possible tropism for areas of pathology;
(e) Ease of manipulation using viral and non-viral gene transfer methods;
(f) Ability to better integrate into normal brain cytoarchitecture with the potential for physiologically regulated release of substances (*Barker et al, 2003*).

It has long been thought that functional regeneration of the injured CNS is impossible, as Santiago Ramon y Cajal described in the early 20th century, ''once the development was ended, the fonts of growth and regeneration . . . dried up irrevocably''. This contention has been refuted since the first landmark report by Candadian scientists Ernest A. McCulloch and James E. Till in 1960s. Several other studies have demonstrated the suitability of stem cells in therapeutics.

Embryonic Stem Cells derived from the inner cell mass of the embryonic blastula and are pluripotent with great proliferative potential, although tagged with the possible risk of teratomas. Human embryonic stem cells have now been isolated and grown in

culture with enrichment for neuronal lineages, possible through exposure to a combination of growth factors and mitogens *(Kim et al, 2002)*.

Adult stem cells are a limited population of undifferentiated cells that reside in a differentiated tissue. Studies suggest that at least some adult stem cells are multipotent. Adult stem cells are rare, difficult to identify or purify and when grown in culture are difficult to maintain in the undifferentiated state. Embryonic stem cell research is still in the basic research phase as these were first isolated in 1998 (at least for humans), whereas adult stem cells have been studied since the 1960s. New neurons are derived in adulthood from a population of adult neural precursor cells (NPCs), which are primarily found in the subependymal layer of the ventricular zone and the dentate gyrus of the hippocampus, although they are also found in other sites. However, the behavior of the NPCs found in all these sites is different, and may relate as much to the environment in which they find themselves as to their intrinsic properties. For example, nigral NPCs appear to only differentiate into astrocytes in situ or when grafted to the adult nigra, but when they are cultured in vitro or transplanted into the hippocampus they can form neurons *(Lie et al, 2002)*. The function of these newborn neurons in the adult CNS is not known but they do have the characteristics of mature neurons with appropriate neurophysiological properties and evidence of integration into neuronal networks with functional synaptic transmission and behavioural effects *(Song et al, 2002)*.

Two of the potential methods that could probably result in effective application of stem cell technology include:

1. Transplantation of stem cells
2. Mobilization of resident endogenous stem cells

Literature abounds with transplantation studies with various modalities including stem cells, cell lines and differentiated cells (both quasi-differentiated and fully differentiated). The behaviour of embryonic neural stem cells (NSCs) following transplantation varies depending on the source of cell and animal model. In the case of human Expanded Neuronal Phenotypes (ENPs) and the intact adult brain, it has been shown that they are able to generate neurons in vivo in regions of active neurogenesis such as the SVZ and hippocampus *(Barker et al, 2003)*, but not when implanted/transplanted in areas such as the striatum. The situation may be different in the diseased or damaged CNS. Several studies have attempted use of stem cells in certain neurodegenerative diseases.

Parkinson's disease:

The pathological hallmark of Parkinson's disease (PD) is a gradual loss of nigrostriatal dopamine-containing neurons, but degeneration also occurs in systems of non-dopaminergic neurons. Clinical trials of the transplantation of human fetal dopaminergic neurons have shown that cell replacement can produce major long lasting improvements in some patients (*Lindvall et al., 2004)*. Early transplant studies using human ENPs showed some survival and dopaminergic differentiation, but the numbers were low. This may relate to the fact that in vitro, NPCs derived from the developing

ventral mesencephalon lose the ability to spontaneously differentiate into dopaminergic cells after only a few divisions. Thus, "pre-differentiation" of the ENPs prior to implantation would seem logical and this approach has been adopted with some success by Studer and colleagues *(Studer et al., 1998)*. An alternative approach has been to employ *ex vivo* genetic techniques to modify cells prior to implantation to express tyrosine hydroxylase, which again has met with some success.

Huntington's disease:

Huntington's disease (HD) is a fatal, intractable disorder that is characterized by chorea (excessive spontaneous movements) and progressive dementia. It is caused by the death of projection neurons in the striatum. To date, studies using NSCs in this disorder are limited but there is some evidence of appropriate neuronal differentiation with human NSCs - a strategy that might be insufficient because patients also suffer progressive neocortical degeneration. Human NS cells implanted into the brains of rats were recently found to reduce motor impairments in experimental HD through trophic mechanism *(McBride et al., 2004)*.

Cerebral ischemia:

Stroke is caused by blockage of a cerebral artery, leading to focal ischemia, loss of neurons and glial cells, and motor, sensory or cognitive impairments. Cell replacement therapy for ischemic injury has experimentally shown some promise. Most of the grafts used in stroke patients were to provide trophic support to enhance cell survival and function. One study showed the migration of transplanted human fetal stem cells in the brains of stroke-damaged rats towards the ischemic region *(Kelly et al., 2004).*To improve the survival strategies and other functions therapeutic strategies have been tried like over-expression of anti-apoptotic gene Bcl-2 *(Wei et al, 2005)*.

Amyotrophic Lateral Sclerosis:

In amyotrophic lateral sclerosis (ALS), dysfunction and degeneration of motor neurons occur not only in the spinal cord (lower motor neurons) but also in the cerebral cortex and brainstem (upper motor neurons). Muscle weakness progresses rapidly and death occurs within a few years. There is no effective treatment. Recent reports have shown that it is possible to generate lower motor neurons in vitro from stem cells of various sources, including ES cells and those from the fetal CNS *(Wichterl et al., 2002)*. Mouse ES-cell-derived motor neurons establish functional synapses with muscle fibres in vitro *(Harper et al., 2004)* and extend axons to ventral roots after transplantation into adult rats. But whether these neurons can integrate into existing neural circuitries and restore motor function has not been established.

Multiple Sclerosis:

Multiple sclerosis (MS) is caused by the inflammation-induced destruction of the myelin sheath that surrounds axons, leading to conduction deficits and a variety of neurological symptoms. Axonal loss as a consequence of acute inflammation or chronic demyelination is an important cause of functional deterioration. Immunomodulatory and immunosuppressive treatments are only partially effective. An important area of research is that focused on finding ways to enhance remyelination from the available oligodendrite

progenitor cells (OPCs) that are abundant in adult human brain, and identifying the factors that lead to a failure of cells to produce myelin in the first place. Human adult *(Windrem et al., 2004)* and ES-cell-derived *(Nistor et al., 2005)* OPCs have been shown to myelinate dysmyelinated mouse brain and spinal cord after transplantation.

Spinal cord lesions:

Spinal cord injuries interrupt ascending and descending axonal pathways, and cause a loss of neurons and glia, inflammation and demyelination. The lesions lead to a loss of movement, sensation and autonomic control below the site of injury. There is no cure, and the most common treatment of high-dose methylprednisolone is of questionable value. Various transplantation strategies have been attempted with functional benefits *(Ogawa, Y. et al, 2002)* primarily through trophic factor secretion or remyelination of rescued axons.

Mobilization of endogenous stem cells:

The microenvironment influences on stem cell generation, maintenance, and various aspects of integration with host tissue are well recognized *(Scadden, 2006).* Understanding and simulating the microenvironment is an alternative mode of approach for interfering with the pathological area in brain would be to invoke the resident stem cells in the affected site or if available in the vicinity. The migratory abilities of endogenous and exogenous neural stem cells are well known, and the properties can be employed to deliver new cells to replace the degenerating, apoptosing cells. The gradients of factors such as vascular endothelial growth factor (VEGF) or stromal cell-derived factor 1 (SDF1), which emanate from distant brain lesions, may act as attractants for stem cells. Several reports published suggest neural stem cells engineered to have chemotherapeutic qualities might be used to track down and destroy malignant cells *(Aboody et al, 2000).* This opens up a possible new realm for stem cell therapy. While attempting to predict the behaviour of stem cells in the brain, it is important to consider both endogenous stem cells and those that are exogenously transplanted into the brain. Endogenous and transplanted neural stem cells are often found to respond similarly to pathology, but there are some important differences to bear in mind. The cultured neural stem cells used for transplantation are expanded in culture well beyond their expected proliferative capacity in vivo, since the culture conditions have a strong influence on the phenotype of cells, it could markedly alter the cells response to their environment when reintroduced in vivo.

Table 2: Disease models with neural stem cell tropism [*Müller F-J et al. (2006)*]

ANIMAL MODEL	HUMAN DISORDER
Chronic / degenerative lesion	
Intrastriatal 6-OHDA lesion model	Parkinson's disease
MPTP-lesioned aged mice	Parkinson's disease
Mutant superoxide dismutase 1 transgenic mouse	Amyotrophic Lateral Sclerosis
Acute/traumatic lesion	
Middle cerebral artery occlusion	Stroke
Rice-Vanucci model	Neonatal stroke
Traumatic brain injury	Traumatic brain injury
Inflammatory lesion	
MHV-MS	Multiple Sclerosis
Experimental allergic encephalomyelitis	Multiple Sclerosis

Major advantages of utilizing endogenous pool of stem cells include no use of fetal tissue and absence of immune rejection. Disadvantages constitute a major part indicating a gap in our understanding of the areas which are neurogenic per se like sub-ventricular zone, hippocampus and olfactory bulb. These involve not only genesis of the neurons but also migration and establishment of the functional circuitry. Thus the microevnvironment components (includes cytokines, neurotrophic factors and the extracellular matrix molecules), guidance cues (both attractants and repellants) need to be understood in greater detail in the neurogenic areas and the non-neurogenic so as to simulate the niche facilitating the phenomena of neurogenesis and in turn neuronal replacement.

Indian Scenario:

Stem cells research has been spawned by many premier research institutes in India, few of them were listed in the 'Map of World Stem Cell Labs' that includes NCBS, Bangalore, NCCS at Pune and CCMB at Hyderabad. A lot many labs have initiated work on various aspects and applications of stem cells in diverse tissues. Among the leading researchers working in various aspects of stem cell biology applications in neurology include:

- **Mitradas Panicker** (NCBS, Bangalore): Studying the role of the serotonin 5-HT2A receptor in embryonic stem cells *in vitro* neurogenesis.
- **Satish Totey** (Manipal Hospital, Bangalore) works with human embryonic stem (ES) cell establishment and differentiation, and their dual ability to proliferate indefinitely and differentiate into multiple tissue types so as to provide an unlimited supply of tissues for transplantation. Reliance Life Sciences, where he worked has produced dopamine-secreting cells in the lab.
- **Nibedita Lenka** (NCCS, Pune) uses murine ES cell system as a model to investigate the early neurogenic proceedings in vitro and its efficacy in generation of functional neuronal progenitors and dopaminergic neurons.

- **Bindu M Kutty** (NIMHANS, Bangalore) in collaboration with Mitradas Panicker, NCBS work with immortalized neural stem cell lines (hippocampal cell lines-H3, GFP H3 and P40H1) derived from discrete brain regions in neural replacement studies. The transplants have shown extensive migratory properties to the site of lesion which promises the potential use in the repair of CNS leading to functional recovery.
- **Prakash Babu** (UOH, Hyderabad) is working for stem cell therapy after stroke using stem cells from cord blood and bone marrow.
- **Shyamala Mani** (NBRC, Gurgaon) works on the regulation of neuronal differentiation into specific subtypes of neurons, sonic hedgehog (shh)-mediated neuronal differentiation and the upregulation of genes in response to shh.
- **TR Raju** (NIMHANS, Bangalore): He works with Olfactory bulb as a paradigm facilitating neurogenesis, migration and integration of the newly generated neurons from sub-ventricular zone (SVZ) via rostral migratory stream (RMS).

Challenges and Prospects:

Although much of the work has been done in rodents, there is now evidence of neurogenesis in the adult human hippocampus, with cells being grown from the adult human CNS *(Palmer et al, 2001)*. Therein the potential for autologous grafts, assuming that the NPCs are not themselves involved in the disease process has indeed already been attempted in one patient with Parkinson's disease. *(Levesque et al, 2002)*. The regulatory aspects of stem cell biology are least explored and that includes intrinsic controls that keep stem cells as stem cells or direct them along particular differentiation pathways. Such intrinsic regulators are, in turn, sensitive to the influences of the microenvironment, or niche, where stem cells normally reside also known as 'Garden of Eden' from where stem cell descendants are evicted to face differentiation and death *(Watt, 2000)*.

The excitement and the controversies accompanying stem cells research and applications have spawned many symposia and conferences. A workshop held on 'Stem Cells and the Future of Regenerative Medicine' in June 22, 2001 provided an independent report encompassing critical comments that will assist institutional standards of objectivity, evidence and responsiveness to the study charge. The following are the recommendations by Committee on the Biological and Biomedical Applications of Stem Cell Research:

1. Studies with human stem cells are essential to make progress in the development of treatments for human disease.
2. Studies of both embryonic and adult human stem cells will be required to most efficiently advance the scientific and therapeutic potential of regenerative medicine.
3. Genetic and biological properties of the stem cell lines available and used for stem cell trials / therapy necessitate continued monitoring and efforts towards development of new stem cell lines in the future should be enhanced.
4. Open scientific exchange, peer review, and public oversight offers the most efficient and responsible means to fulfill the promise of stem cells to meet the need for regenerative medical therapies.

5. A national advisory group composed of exceptional researchers, ethicists, and other stakeholders should be established to oversee research on human embryonic stem cells. The group should evaluate the technical merit of any proposed research on human embryonic stem cells including evaluation of potential risks to research subjects and ensuring compliance with all legal requirements and ethical standards.
6. Research on approaches that prevent immune rejection of stem cells and stem cell-derived tissues should be actively pursued. These scientific efforts include the use of a number of techniques to manipulate the genetic makeup of stem cells, including somatic cell nuclear transfer.

Central nervous system stem cells have become the subject of many laboratories efforts, presentations, and publications. Yet, in the stem cell world, CNS cells are viewed with skepticism. This is likely due to a dearth of biology *(in vivo* function) to accompany a flurry of phenomenological and restorative neurology studies.

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