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Neurological Diseases and Stem Cell Transplantation-Review Paper

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Abstract: Stem cells and their potentials for therapy are major areas of research. The literature on the subject is expanding at a very rapid pace and the great prospectives offered by these remarkable cells are continuously being unravelled. The use of stem cells vis-à-vis neurological diseases is of particular interest since many such diseases have a poor prognosis and generally decrease considerably the quality of life. Treatment of neurological diseases is complicated by the lack of regeneration of the nervous system, the neuroanatomical complexity, the presence of the blood-brain barrier and the possible spread of excitotoxicity. Hence, more effective ways how to intervene on these pathological processes are required. This review highlights, the fundamental aspects of stem cell biology, with a special reference to neural stem cells and their niches. Dysmyelinating diseases and invasive brain tumours are specifically considered since, they are presumed candidates for successful clinical application of stem cell therapy.

Key words: Stem cell biology, neural stem cells, neurological diseases, dysmyelinating diseases, genetic leukodystrophies, lysosomal storage diseases, invasive brain tumours, stem cell therapy

INTRODUCTION

In 1998, pluripotent stem cells were isolated for the 1st time from early human embryos and grown in culture (Thompson *et al*., 1998; Shamblott *et al*., 1998). The importance of the long-term proliferation of the Embryonic Stem Cells (ESCs) and Embryonic Germ Cells (EGCs) was immediately recognized because it offered the opportunity to study the genetic and molecular basis of proliferation with applications in the understanding of embryological development, drug development (assessment of teratogenicity and toxicity) and to yield sufficient quantities of cells for potential transplantation purposes.

BASIC PROPERTIES OF STEM CELL BIOLOGY

The 2 basic properties that characterize stem cells are the long-term self-renewal and multipotency i.e., the ability to differentiate into many specialized cell types (NIH Report, 2001a).

Self-renewal of stem cells involves mitotic division while maintaining pluripotency (Niwa, 2004). Self-renewal is postulated to be indefinite, but it is defined, in practice, as a proliferation ability, without oncogenic transformation, well beyond that of somatic cells. The extent of self-renewal depends on the type of stem cell and the culture conditions. There are 2 modes of self-renewal: symmetrical and asymmetrical. For instance, the ESC divides symmetrically to give 2 identical stem cells, while the Adult Stem Cell (ASC) divides asymmetrically to yield a stem cell and a progenitor cell.

Pluripotency implies the ability to differentiate into ectodermal, mesodermal and endodermal derivatives; this accounts to >200 cell types. Pluripotency can be demonstrated *in vitro* by the formation of embryoid bodies, which are masses of different types of progenitor cells from the 3 germ layers. An *in vivo* test for pluripotency is teratoma formation upon transplantation in immunodeficient mice. hESCs are the only stem cells able to form teratomas. This property implies that ESCs cannot be used directly for potential therapeutic applications.

Stem cells remain uncommitted until appropriate inducer substances cause induction and cell determination i.e., the commitment to differentiate. Once determined, stem cells give rise to progenitor/precursor cells. These are partially specialized cells, which divide asymmetrically to generate progenitor and more specialized cells. Neuroblasts are Neural Progenitor Cells (NPCs).

The proteome of stem cells is characterized by components of signalling cascades, cell cycle regulators, telomerase, chromatin remodelling enzymes, a specialized post-translatonal regulatory machinery (Vasa type RNA helicases) and stress-resistance proteins (Johnson and Andrews, 2004).

Oct-4 is a key transcription factor, which maintains stem cells in a proliferative, non-differentiated state

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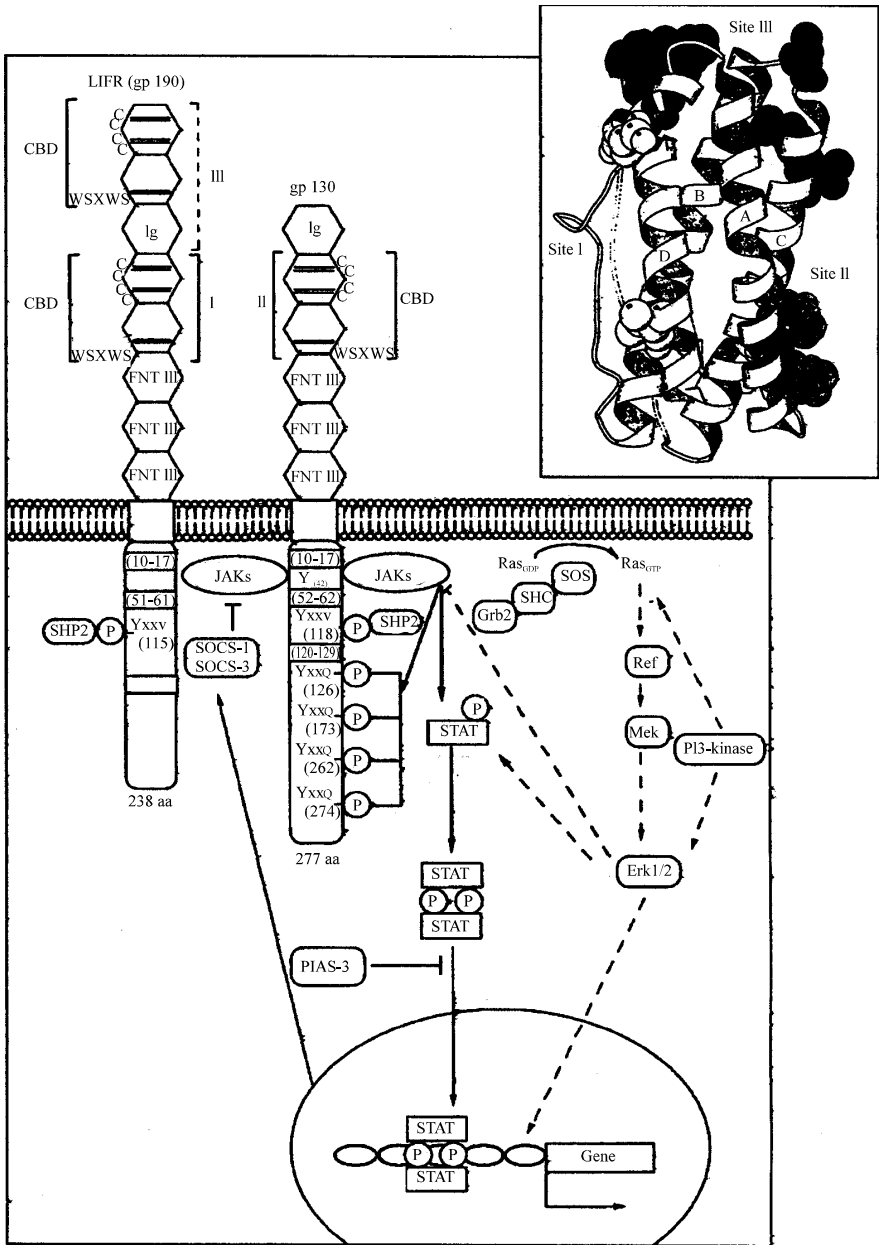

Fig. 1: LIF and the associated Jak/Stat mechanism. A: The 3D structure of murine LIF. It is a long-chain 4 "-helix bundle cytokine. LIF possesses 3 binding sites. Site I binds to the membrane-proximal Cytokine-Binding Domain (CBD) of LIFR, site II binds to the CBD of gp130 and site III also binds to the LIFR. B: The LIFR-gp130 complex and its molecular signalling pathways. Heterodimerization of the LIFR and gp130 upon LIF binding activates Jak activity, leading to phosphorylation of gp130 and the LIFR. Phosphorylated tyrosine residues on LIFR and gp130 provide docking sites stat proteins, which are subsequently phosphorylated. The pattern of Jak/Stat protein activation by LIF is cell type-specific (Auernhammer and Melmed, 2000)

(Pesce and Schöler, 2001). The levels of Oct-4 within a given cell seem to govern the commitment along a given fate; at baseline levels it induces self-renewal, at decreased levels it induces differentiation along the trophoectoderm lineage and at increased levels it induces differentiation along extra-embryonic endoderm and mesoderm lineages. An important co-determinant of pluripotency is the transcription factor Nanog

(Mitsui *et al*., 2003). Nanog is found in all pluripotent stem cells and endodermal commitment results in Nanog down-regulation.

Renewal is promoted by extrinsic factors which, via transcription factors such as Oct-4 and Nanog, modulate gene expression (Cavaleri and Schöler, 2004). Leukaemia Inhibitory Factor (LIF) in the only extracellular signal known to promote self-renewal (Fig. 1). However, it is not unique in this action. A medium conditioned by parietal endodermal cells can replace LIF. The exact signal responsible is not identified and was named ESC renewal factor (ESCF). Neither LIF, nor ESCF, is sufficient by itself in maintaining self-renewal. SCF, feeder cells, or high-density culture conditions (i.e., cell-cell interactions) are also required.

ESCs possess particular mechanisms of cell cycle control, different from those of somatic cells (Savatier and Malashicheva, 2004). Genes controlling G1 to S phase transition in response to mitogenic signals are likely to underlie proliferation properties of ESCs (Fig. 2). G1 phase in ESCs is very short (approximately 1.5 h). Hypophosphorylated retinoblastoma protein (pRb) is virtually undetectable in G1 phase. Therefore, in ESCs, pRB is likely to be rephosphorylated immediately after mitosis and does not seem to regulate ESC cycle. Evidence for this statement comes from the fact that ESCs are refractory to p16 (a CDK inhibitor) and inactivation of all three members of the pRb gene family does not compromise ESC proliferation. Phosphatidylinositol-3 kinase (PI3K) signalling contributes to ESC cycle regulation (Fig. 3). In fact, PI3K inhibition results in an

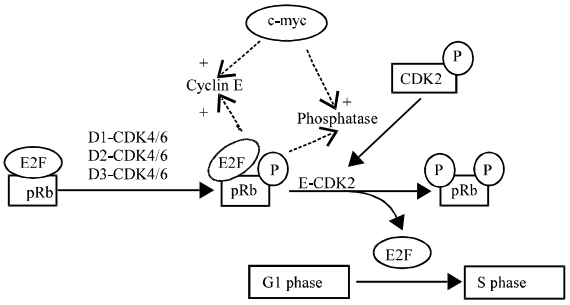

Fig. 2: Principal components of the G1 to S phase checkpoint. Multiple CDKs are involved in the initiation of DNA synthesis via the phosphorylation of pRb. D-type cyclins (D1-D3) and CDK4 and CDK6 (CDK4/6) form during the preceding G2 phase and their levels fall during S phase. When pRb is phosphorylated it releases E2F, a transcription factor which activates genes for enzymes involved in DNA synthesis. Partially phosphorylated pRb, in conjunction with c-myc, upregulates its own phosphorylation (Savatier and Malashicheva, 2004)

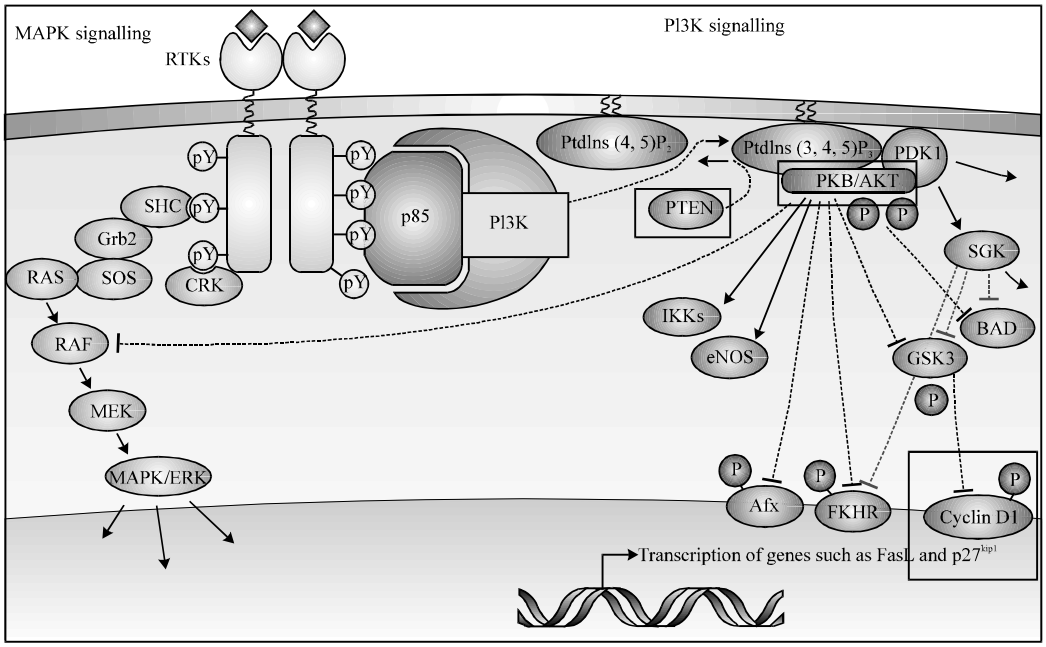

Fig. 3: The PI3K signal transduction pathway. The actions of PKB, activated via PI3K, include the decrease in the rate of degradation of cyclin D1 and the increase in the rate of degradation of p27, which inhibits entry into S phase. Therefore, the PI3K pathway promotes the G1 to S phase transition (Scheid and Woodgett, 2001)

increase in ESCs in G1 phase and phosphatase and tensin homolog (PTEN) tumour suppressor gene deficiency causes an increase in G1 transit (PTEN inhibits PI3K signalling). ESC PI3K activity may not depend on outside stimulation. It may be activated by E-ras; ESC growth is impaired by E-ras deficiency.

ESCs also show p53 dependent apoptosis in case of irreparable DNA damage. This prevents tumorigenesis and maintains clonality (Sabapathy *et al*., 1997).

TYPES OF STEM CELLS

Stem cells can be conveniently classified in accordance to their origin (NIH Report, 2001a). ESCs are derived from the inner cell mass of a 4-5 day blastocyst. hESCs are maintained in media with 20% foetal calf serum or serum replacement media supplemented with FGF2. They also require the presence of feeder cells or conditioned medium from feeder cells (Carpenter and Bhatia, 2004). Normal karyotype is maintained over a long period, but aneuploidy is observed at significant levels in hESC cultures (Roster *et al*., 2004). Markers used to characterize hESCs include alkaline phosphatase-related antigens, stage-specific embryonic antigens 3 and 4 (SSEA-3 and SSEA-4) and tumour rejection antigens 1-60 and 1-81 (Tra-1-60 and Tra-1-81; these are keratin sulphate-related antigens).

EGCs are derived from culturing of Primordial Germ Cells (PGCs) from the gonadal ridge of a 5-10 week foetus (McLaren, 2004). PGCs are not stem cells because, as they divide, they move along the germ cell lineage. Chromosomally stable EGCs are obtained from PGCs in culturing medium containing SCF, LIF and FGF2. The EGCs obtained are imprint-free, as opposed to the PGCs.

SSEA-1 positivity is generally used to differentiate hEGCs from hESCs. SSEAs are glycolipids of the globoside type i.e., a ceramide molecule linked to 2 or more sugar residues. It has been postulated that this difference may account for the different growth characteristics. Suggested biological roles of these glycolipids include regulation of cell growth, recognition and differentiation during development (Fenderson *et al*., 1990). Table 1 summarizes the fundamental distinguishing features between ESCs and EGCs.

Stem cells also occur in adult tissues and these are collectively termed ASCs (Prentice, 2004). ASCs have limited renewal and differentiation potentials. Note that ASCs occur even in neonates, umbilical cord and placenta; thus, terms such as tissue/somatic/post-natal stem cells would be more accurate expressions to refer to such cells. Broadly speaking, ASCs yield cells of the tissue in which they reside. ASCs have been identified in the CNS, bone marrow, peripheral and umbilical cord blood, umbilical cord mesenchyme (Wharton's jelly), blood vessels, skin and gastrointestinal epithelia, liver, pancreas, heart, skeletal muscle, tendons, cartilage, synovial membrane, adipose tissue, corneal limbus, retina, testes, mammary glands, salivary glands, dental pulp, thymus and teratocarcinomas (NIH Report, 2001b). ASCs are quantitatively few and difficult to identify, isolate and purify. CD133 is generally used as a surface marker of these cells. Flow cytometry can be employed to isolate and identify ASCs, exploiting the ability of these cells to exclude fluorescent dyes via membrane-associated ABC transporters membrane-associated ABC transporters (Bunting, 2002).

Table 1: Differences between human embryonic stem cells and human embryonic germ cells (NIH Report, 2001a)

Feature	hESCs	hEGCs
SSEA-1	-	+
SSEA-3	+	+
SSEA-4	+	+
TRA-1-60	+	+
TRA-1-81	+	+
Alkaline phosphatase	+	+
4-Oct	+	Unknown
Telomerase activity	+	Unknown
Feeder cell dependence	+	+
Factors aiding renewal	FGF2	LIF, FGF2, forskolin
Growth characteristics *in vitro*	Flat loose aggregates; can form Ebs	Rounded multi-layered clumps; can form Ebs
Population doublings obtained ion cuture	300-450	40-80
Teratoma formation *in vitro*	+	-
Chimera formation	+	-

ASCs exhibit plasticity, they appear to have the ability of generating specialized cells of tissues other than their tissue of origin. This is termed transdifferentiation. Hence, the lineage restrictions of ASCs are not permanent. For example, haematopoietic stem cells have been shown to migrate into the brain and develop into neuron-like cells, oligodendrocytes and astrocytes (Mezey *et al*., 2000). Neural Stem Cells (NSCs) also show plasticity, they have been found to produce cells of the haematopoietic lineage after *in vivo* transplantation (Bjornson *et al*., 1999). There might also be a 'universal' ASC across tissues, which differentiates along tissue-specific lineages depending on the environmental cues within the niche in which, it is found (Blau *et al*., 2001).

While, ESCs exhibit clonality (i.e., they are genetically identical), ASCs from one particular tissue may not be clonally-derived (NIH Report, 2001b). This means that within a particular tissue, instead of having one stem cell type, which generates the multiple constituent cells, there may be multiple stem cell types to generate each of the different cell types. This means that ASC plasticity may actually be an artifact of poor purification methods.

NEURAL STEM CELLS

Neurogenesis in the adult brain has been shown to be persistent and functionally relevant (Taupin and Gage, 2002). For example, the Subventricular Zone (SVZ) has been shown to generate neuroblasts that undergo a 3-8 mm migration within the rostral migratory stream to the olfactory bulb (Luskin, 1993). Although, most precursors die during the migration or within the olfactory bulb, many survive, differentiate and functionally integrate as granule cells or periglomerular interneurons.

In the mammalian foetal brain, NSCs are concentrated in the olfactory bulb, ependyma of lateral ventricles, SVZ, hippocampus, cerebral cortex, cerebellum and spinal cord (NIH Report, 2001b). In the human adult, stem cells persist in the SVZ, subgranular zone and hilus of the dentate gyrus of the hippocampus and subcortical white matter (Fig. 4).

The SVZ of the lateral ventricles is the largest stem cell niche in the adult brain. The stem cell role is ascribed to the astrocytes just beneath the inner glial limiting membrane (Quiñones-Hinojosa *et al*., 2007). Such astrocytes acting as NSCs lack other vital astrocytic functions, such as the ability to regulate the extracellular concentration of neurotransmitters. Ultrastructural analysis has been used to classify the cellular constituents of the SVZ (Doetsch *et al*., 1997). Type B cells are the NSCs in the form of GFAP+ slowly dividing astrocytes; culture of these cells yields colonies with bipolar and multipolar neurons. The NSCs yield type C cells, which are an amplifying intermediate between NSCs and the type A cells, the neuroblasts, which are the most abundant, highly proliferative, migratory cells.

Hippocampal NSCs give rise to neuroblasts, which migrate into the granule cell layer of the dentate gyrus where they differentiate into granule cells and send axons to the CA3 region (Fig. 5). Unlike those in the SVZ, hippocampal stem cells are not associated with ependyma and they are not merely a source of new cells. The hippocampal astrocytes functioning as NSCs also have other astrocytic roles and may serve as a means of communication between the mature and germinal zones via processes, which penetrate the granule layer intercalating among mature granule cells. Hence, the rate of neurogenesis may be modulated as required. The neurons thus, generated are functional and may be involved for learning and memory (Praag *et al*., 2002). Stress, opiate abuse, seizures and other factors can affect the rate of neurogenesis in the dentate gyrus.

Thus, there is a significant amount of NSCs in the adult brain, especially when considering the size of the human lateral ventricles. However, in neurological diseases, spontaneous regeneration of structure and function are generally ineffective. Nonetheless,

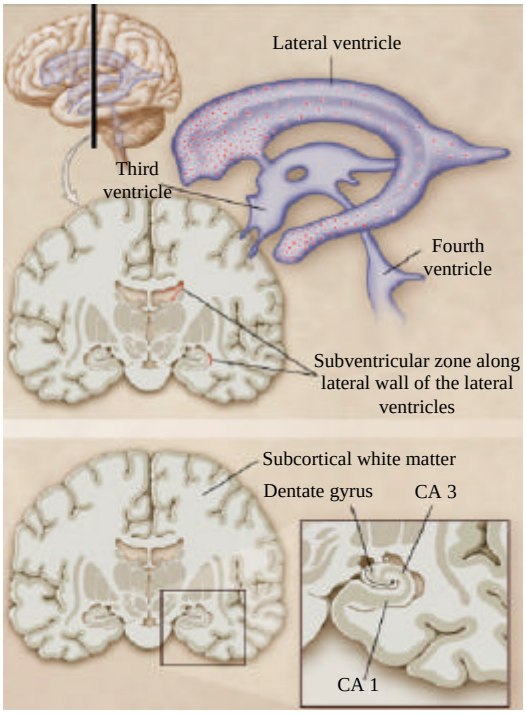

Fig. 4: Neural stem cell niches in the adult brain (Sanai *et al*., 2005)

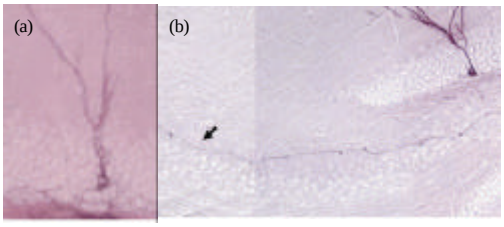

Fig. 5: Demonstration of neurogenesis in the hippocampal subgranular zone. A: Immature granule neurons start appearing in the granular layer 8 days after labelling subgranular zone astrocytes. B: After 30 days, labelled granule neurons in the dentate gyrus show the characteristic dendritic arborisations in the molecular layer and an axon (arrow) that projects to the CA3 region (Seri *et al*., 2001)

NSCs can be a source of progenitor cells for future neuroregenerative therapy. NSCs may also be a potential source of brain tumours (Sanai *et al*., 2005).

THERAPEUTIC POTENTIALS

Cell replacement therapy may take the form of an *in vitro* expansion and transplantation or the induction of

migration of endogenous NSCs towards areas of damage or cell loss. NSCs and neuroblasts may show low immunogenicity, thus being immunoprivileged on transplantation, allowing the possibility for using NSCs allografts to treat degenerative brain conditions (Hori *et al*., 2003).

However, given the complexity of the cytoarchitecture of the CNS, one must assess the real potentials and feasibility of stem cell therapy for neurological disorders (Schwartz, 2007). It is important not to have a simplistic idea that these cells might offer a fast and easy treatment. Rather than replacing lost neurons in order to re-establish a particular function, stem cell therapy can become more clinically relevant if it is aimed to prevent or minimise a particular disease process.

Various encouraging but inconsistent results have been obtained for Amyotrophic Lateral Sclerosis (ALS), Parkinson's Disease (PD) and stroke, for instance (Kerr *et al*., 2003; Akerud *et al*., 2002; Lévesque and Neuman, 2002).

Kerr *et al*. (2003) have directed the differentiation of hESCs and hEGCs to neuron-like cells by exposing the stem cells to specific growth factors (e.g., retinoic acid, EGF, BMP4, FGF2) to induce differentiation along the ectodermal lineage. The neuron like cells were identified and injected into the central canal of the spinal cord of a murine model of ALS. A significant functional recovery was observed.

NSCs have been employed in models of PD and have shown a capability to rescue and prevent further degeneration of dopaminergic neurons (Akerud *et al*., 2002). It seems that the transplanted NSCs themselves do not give rise significantly to dopaminergic neurons but, via the secretion of neuroprotective and growth factors, stimulate the endogenous NSCs. On this principle, GDNF was directly infused into the putamen of 5 PD patients in a phase I trial (Gill *et al*., 2003). After 1 year a 61% increase in the Barthel's Index of activities of daily living (BAI) score was noted. At 18 months PET showed a 28% increase in putamen dopamine storage. Autologous NSC transplantation in a PD patient gave a reduction of 80% in symptoms after 1 year (Lévesque and Neuman, 2002). This latter approach eliminates immune reactions at the site of implantation; thus, it improves the likelihood of survival of the surgically implanted cells and one can do without immunosuppressants or steroids. It also minimizes risks of transmission of infectious disease.

Animal studies have revealed that adult NSCs can participate in repair of damage after stroke, either via endogenous NPCs or transplanted NSCs (Arvidsson *et al*., 2002; Riess *et al*., 2002). In both cases, significant neuronal differentiation was observed and postulated to offer promises for future therapy.

However, the most likely disorders to give successful results are genetic leukodystrophies, Lysosomal Stroage Diseases (LSDs) and invasive brain tumours (Schwartz, 2007). In such disorders, the restoration of neuronal connections is not required and this makes it more likely that a positive therapeutic outcome is obtainable.

DYSMYELINATING DISEASES

Myelination is essential for normal nervous functions. Myelin insulates the axolemma and increases the nerve conduction velocity by as much as 5-50 fold by increasing the length constant and giving rise to saltatory conduction (Guyton and Hall, 2006). In addition, this saltatory conduction involves less ionic disturbances when compared to continuous conduction since action potentials occur only at the nodes of Ranvier; hence, less metabolic energy is necessary for maintaining ionic concentration differences. Myelination also allows repolarisation to be achieved with small ionic transfers since the myelin decreases membrane capacitance by around 50 fold.

Dysmyelinating diseases include genetic leukodystrophies and LSDs. These represent primary and secondary defects in myelination, respectively. Common neurological diseases, such as cerebral palsy, may also by attributable to a perinatal loss of oligodendrocytes and their precursors, which are particularly vulnerable to insult (Kaye, 2001).

Oligodendrocyte Progenitor Cells (OPCs) can remyelinate axons in dysmyelination (Windrem *et al*., 2004). The adult forebrain white matter can be a source of OPCs, which are identified as A2B5+, PSA-NCAM cells. Windrem *et al*. (2004), transplanted OPCs to newborn shiverer mice (Fig. 6). Shiverer mice are a model of demyelination and show a generalised tremor on locomotion (Chernoff, 1981). The outcome of OPC transplantation differed between foetal and adult OPCs (Table 2). Within white matter OPCs developed mostly as oligodendrocytes, while in grey matter astrocytes were predominantly obtained. The resultant myelination suggested normal structure and function. For instance, the paranodal region of the nodes of Ranvier exhibited Caspr, a member of the neurexin superfamily of transmembrane proteins which mediate junction formation between the axon and the paranodal loops of the myelinating glia (Einheber *et al*., 1997). These junctional complexes serve as attachment sites between the myelin sheath and the axon, electrically insulating the periaxonal space under the myelin from the ionic fluxes at the nodes and preventing the lateral diffusion of membrane proteins confining them to their appropriate membrane domain.

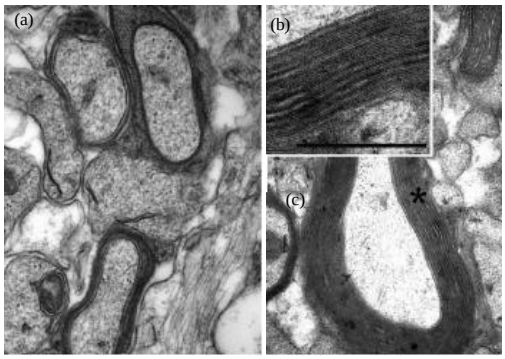

Fig. 6: Electromicrographs of saggital sections through the corpus callosum of adult shiverer mice. A: Axons of shiverer mice typically have a single loose wrapping of uncompacted myelin. B and C: Sections of 16 week old shiverer mice implanted with human OPCs shortly after birth. Dense compact myelin sheaths with major dense lines are now apparent (Windrem *et al*., 2004)

Table 2: Comparison between foetal and adult OPC properties upon transplantation (Winderm *et al*., 2004)

Feature	Foetal OPCs	Adult OPCs
Rate of myelination	Slow	Fast
Time required for dense myelination	3 months	1 month
Oligodendrocytes generated/OPC	Less	More
Extent of migration	Widespread, penetrating grey matter to yield astrocytes	More restricted
Ideal applications	Treatment of demyelination due to enxzymatic deficiency due to widespread dispersal and grey matter invasion	Replacing oligoden dro-cytes after acute loss as in sub-cortical infarts and post-inflammatory demyelinated lesions

Hence, OPCs offer an important treatment possibility especially for genetic leukodystrophies such as Pelizaeus-Merzbacher disease.

LSDs result in death of oligodendrocytes due to the toxic metabolites emanating from enzyme-deficient cells (Windrem *et al*., 2004). Transplanted OPCs might be able to infiltrate grey matter differentiating into astrocytes and conveying the deficient enzyme via mannose-6-phosphate receptor-mediated endocytosis. Even small increases in lysosomal enzyme activity thus obtained can be enough to prevent or slow down the disease process (Jeyakumar *et al*., 2005). Hence, LSDs promise to be ideal candidates of such gene therapy. Success of these treatments postulates an early diagnosis and intervention before substantial neurodegeneration occurs. Various experiments on animal models of LSDs are documented in the literature.

Niemann-pick disease results in lipid accumulation in the brain, bone marrow, lungs, liver and spleen (National Institute of Neurological Disorders and Stroke, 2007a). Types A and B of the disease are due to sphingomyelinase deficiency. Type A is the commonest and occurs in infants. Neurological signs may include incoordination and learning difficulties. Management and prognosis are very poor; type A disease has no effective treatment and is invariably fatal. In a murine model of the disease, mesenchymal stem cells engineered to over-express acid sphingomyelinase have been employed successfully to delay neurological abnormalities and extend lifespan (Jin *et al*., 2002).

Experiments along these lines for the treatment of Krabbe's disease have also been performed (Pellegatta *et al*., 2006; Escolar *et al*., 2005). The disease involves a galactocerebrosidase deficiency resulting in various neurological symptoms, generally of infantile onset (National Institute of Neurological Disorders and Stroke, 2007b). There is no cure and the infantile disease is generally fatal before the 2nd year of life. Neural progenitor cells overexpressing galactocerebrosidase were transplanted into twitcher mice (a murine model of Krabbe's disease). The neuroblasts showed positive tropisms to sites of active demyelination. Unfortunately, the cells were unable to survive at such foci due to the inflammation. Conversely, Escolar *et al*. (2005) obtained very positive results with allogenic umbilical cord blood stem cell transplantation to infants with Krabbe's disease. Donor cell engraftment and survival were both 100% in asymptomatic patients who thus, exhibited a slower disease progression, with most of them showing normal cognition and language skills. Similar treatment of symptomatic patients resulted in 43% donor cell survival with minimal neurologic improvement. This confirms that such treatments should be initiated as early as possible in order to achieve clinical benefits.

Similarly, a presyptomatic murine model of Metachromatic Leukodystrophy (MLD) responded positively to OPC engraftment (Givogri *et al*., 2006). MLD is caused by a deficiency of arylsulfatase A (National Institute of Neurological Disorders and Stroke, 2007c). The late infantile form of MLD is the most common and symptoms include muscle wasting, weakness and rigidity. This form of MLD is fatal by around the fifth year of life. In the experiment by Givogri *et al*. (2006), the OPCs incorporated within the white matter as myelinating oligodendrocytes. The arylsulfatase A levels were thus enhanced with a complete prevention of the characteristic motor symptoms.

Much work is being focused on Multiple Sclerosis (MS) due to its high incidence and prevalence (Riuzi and

Agius, 2004). In Northern Europe, North America and Australasia, about one of every 1000 citizens suffers from MS; its typical juvenile-onset and prolonged course result in such a high frequency of the disease. Current treatment of MS involves management with six possible approved pharmacological interventions, which do not reverse damage already done by the disease process but slow down relapses (FDA, 2005). The multifocal nature of the disease makes it a difficult target for cell therapy. However, NSCs were successfully integrated in an animal model of MS reducing astrogliosis, causing axonal remyelination and slowing of further axonal loss, hence, leading to a functional recovery (Pluchino *et al*., 2004). It seems that the NSCs give rise to OPCs and also remain undifferentiated to act as local immune suppressants by inducing apoptosis of T_H cells, which enter the CNS (Pluchino *et al*., 2004). This effect decreases disease progression by preventing the triggering cause of the demyelination and axonal loss. Thus, MS offers therapeutic potential but its intermittent nature, variable anatomical disease localisation and accompanying axonal damage are major hurdles to overcome. The capability of stem cells to home to areas of injury is an important asset in this regards.

In order to bring stem cell therapy into standard practice in the treatment of dysmyelination, one has to establish how much cells need to be used, where they are to be delivered and the time frames for treatment (Windrem *et al*., 2004).

INVASIVE BRAIN TUMOURS

Brain tumours account for 85-90% of all primary CNS tumours (National Cancer Institute, 2008). According to the, Central Brain Tumour Registry of the United States, the incidence rate of malignant brain tumours is 7.4/100,000 person years (Schiff and O'Neill, 2005). The ratio of brain metastases to primary neoplasms is around 10-1; 20-40% of cancer patients develop brain metastases and brain metastases are multiple in more than 70% of cases. From the epidemiology and end results registry 1973-2002 data, the 5 year relative survival rates following diagnosis of malignant CNS tumour are 28.1% for males and 30.5% for females.

Tumours of the nervous system cannot be classified according to the TNM system, since the tumour size is relatively irrelevant, lymphatic spread cannot occur and death generally ensues before metastatization can occur (National Cancer Institute, 2008). The WHO classification is employed whereby the tumours are classified primarily by their cellular origin and graded according to the level of malignancy from grade I to IV, grade IV being anaplastic and highly infiltrative, invading deep into the parenchyma. The most common type of brain tumour is the glioblastoma multiforme. It is an astrocytic grade IV tumour and accounts for around 15% of brain tumours. Its peak incidence age range is 45-70 and survival with this disease is less than a year.

Hence, brain neoplasia generally offers a bleak prognosis in spite of radical resection techniques with possible adjuvant radio-and chemotherapy. In addition, the latter therapies are generally non-specific and cause significant toxicity and potential cognitive impairment, especially with supratentorial irradiation (Vigliani *et al*., 1999).

Endogenous neural progenitors may be involved in the defence against neoplasia. It has been demonstrated that these cells migrate towards, surround and infiltrate an experimentally induced primary tumour mass and tumour satellites, decreasing the tumour mass and enhancing survival (Glass *et al*., 2005). A decrease in this tropism, as occurs with advancing age, was correlated with a decreased survival.

Stem cell therapy might offer a targeted treatment alternative (Mapara *et al*., 2007). NSCs reach the neoplastic cells even when transplanted at a distant location from the tumour, such as in the contralateral lateral ventricle/hemisphere parenchyma or even intravascularly outside the CNS. This strong homing ability is preserved with engineered cells expressing a foreign gene of a potential therapeutic product such as cytosine deaminase (Fig. 7). It is suggested that the trophic signals are generated by the tumour itself since, it was observed that NSC placement within the primary tumour mass resulted in lack of NSC migration beyond the tumour borders. Soluble proteins, cell adhesion molecules and extracellular matrix components are probably involved in the migratory ability of the stem cells.

For example, the CXCR4 is expressed by the NSCs and SDF-1, a CXCR4 ligand, is produced by brain tumour cells in correlation to their invasiveness (Ehtesham *et al*., 2004). In fact, NSC migration is inhibited by neutralising SDF-1 and NSCs weakly immunopositive for CXCR4 are non-migratory. Interestingly, migrating NSCs are immature astrocytes, while NSCs, which remain within the primary tumour are more differentiated since they express a proteome typical of functional astrocytes, including EAAT1 and EAAT2.

The literature shows several instances whereby, the survival of mice with intracranial glioma was enhanced by transplantation of cytokine secreting NSCs. For example, Benedetti *et al*. (2000) employed IL-4 secreting NSCs, while IL-12 was used by Ehtesham *et al.* (2002) IL-12 caused more CD4+ and CD8+ T-cells to reach the dispersed tumour cells and was also shown to have a long-term anti-tumorigenic effect.

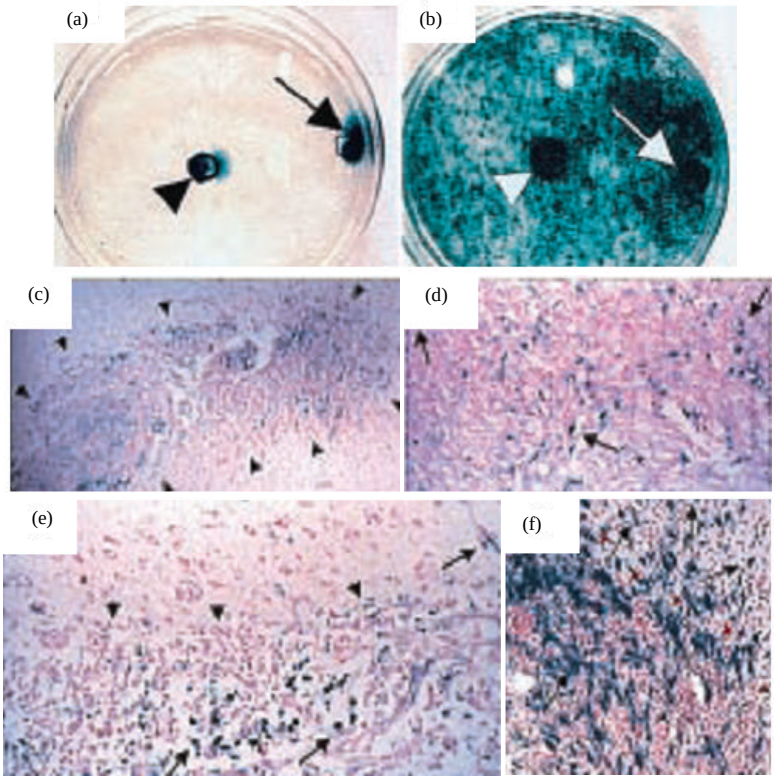

Fig. 7: The migratory capacity of NSCs *in vitro* and *in vivo.* A and B: Glioblastoma cells were plated at the periphery of 2 plates. Fibroblasts (A) and NSCs (B), engineered to express a green fluorescent protein, were placed at the centre (arrowheads). After an incubation of 5 days NSCs migrated widely, compared with fibroblasts (A), which remained localized to their area of initial seeding C and D. Sections of brain under low (C) and high (D) power from an adult rat killed 48 h after NSC injection into an established glioma; arrowheads demarcate approximate edges of the tumour. NSCs (arrows) can be seen extensively distributed throughout the mass, interspersed among the tumour cells. E: After 10 days, the NSCs (arrows) fail to penetrate beyong the tumour borderd (arrowheads). However, NSCs appear to follow the invading tumour cell into surrounding tissue (upper right arrow). F: NSCs migrate extensively following the invading glioblastoma cells

Another successful approach has employed cytosine deaminase expressing NSCs. Such cells would convert the systemically administered pyrimidine analogue 5-fluorocytosine to 5-fluorouracil in very close proximity to the tumour cells. A reduction of the tumour mass by 80% was achieved after 2 weeks of 5-fluorocytosine administration.

Mapara *et al*. (2007), also review other possible treatment possibilities, such as virus and pro-apoptotic protein delivery. Replication-conditional (i.e., able to replicate and hence kill, only actively dividing cells) HSV-1 and TNF-Related Apoptosis Inducing Ligand (TRAIL) are considered to be promising possible approaches.

CONCLUSION

Stem cell research is likely to translate into new therapeutic strategies. As regards neurological diseases, very promising results have already been obtained; many diseases, which at present have a poor prognosis and treatment might be satisfactorily managed with stem cell therapy. For this to become a reality, the properties of stem cells have to be unravelled to a greater depth, more clinical trials need to be initiated to test the potentiality of these novel approaches and ethical issues have to be circumvented.

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