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Neural Stem Cells—Trends and Advances

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ABSTRACT

For many years, accepted dogma held that brain is a static organ with no possibility of regeneration of cells in injured or diseased human brain. However, recent preclinical reports have shown regenerative potential of neural stem cells using various injury models. This has resulted in renewed hope for those suffering from spinal cord injury and neural damage. As the potential of stem cell therapy gained impact, these claims, in particular, led to widespread enthusiasm that acute and chronic injury of the nervous system would soon be a problem of the past. The devastation caused by injury or diseases of the brain and spinal cord led to wide premature acceptance that "neural stem cells (NSCs)" derived from embryonic, fetal or adult sources would soon be effective in reversing neural and spinal trauma. However, neural therapy with stem cells has not been realized to its fullest extent. Although, discrete population of regenerative stem cells seems to be present in specific areas of human brain, the function of these cells is unclear. However, similar cells in animals seem to play important role in postnatal growth as well as recovery of neural tissue from injury, anoxia, or disease. J. Cell. Biochem. 114: 764–772, 2013.

KEY WORDS: NEURAL STEM CELLS; MESENCHYMAL STEM CELLS; NEUROGENESIS; REGENERATION; BIOTHERAPEUTICS

The signals generated and transmitted in response to stimulus are necessary for communication between complex networks of neurons, which once disrupted, cannot be restored. In vitro, differentiation of neuronal–like cells from putative stem cells often is verified by morphology, wherein the cultured cells emit projections that resemble those of neurons derived from animals. However, it is now clear that many antigens considered specific for neural tissue could be expressed by many other cells, especially endothelial cells and monocytes [Vescovi et al., 1993]. Furthermore, nestin, the neuroepithelial stem cells marker seems to be expressed in most of the mitotically active cells. Suggestions regarding the existence of dividing cells in the postnatal central nervous system (CNS) were raised in 1901 by Hamilton. Ramon and Cajal [1913] suggested that neurons are generated exclusively during prenatal phase of development. Kaplan and Hinds [1977], and Kaplan and Bell [1984] proved that new born neurons in hippocampus survived for long periods of time, appeared to receive synaptic inputs and also extend projections to their target area. While, Reynolds and Weiss [1992] in 1992 isolated adult NSCs from adult CNS of rodents, Kukekov et al. [1999] isolated NSCs from human embryo. Belluzzi et al. [2003] showed that the new born neurons in adult mammalian CNS are indeed functional and synaptically integrated.

In 1983, Nottebohm et al. demonstrated the genesis of neurons in the telencephalon of adult male songbirds. In addition, it was found that acquisition of new neurons is hormonally controlled and therefore seasonally regulated, corresponding to the mating season of singing songbirds [Nottebohm, 1981]. The events may thus be independent but stimulated by common factors that arise during seasonal alterations of the environment.

Studies by the group of Stevens and Gage in 2002 indicated that adult NSCs indeed form functional neurons and do not simply express protein markers specific to differentiation. By recording electrical signals of the cultured cells, they showed that these fluorescently labeled precursors formed dendrites and synapses in the rat brain, challenging the dogma that neurons are not replaced in brain [Song et al., 2002]. The adult brain maintains discrete parts of neurogenesis, new neurons migrate from these parts and become integrated into the functional circuitry of the brain. These multipotent stem cells are present in various regions of the brain including the cortex [Marmur et al., 1998], the subventricular zone (SVZ) [Levison and Goldman, 1997] and the ventricular zone [Cai et al., 2002]. NSCs produce neuroblasts that migrate from the SVZ along a separate pathway, the rostral migratory stream; the mature neurons involved in the sense of smell are formed in to the olfactory bulb [Lennington et al., 2003].

Conflicts of interest: None.

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NEUROGENESIS AND TRANSDIFFERENTIATION IN BRAIN

Positional effects of NSCs can be appreciated by the observation that SVZ neuronal precursors have a bias to form olfactory bulb derivatives [Gritti et al., 2002] spinal cord neuron restricted precursors give rise to cholinergic neurons [Kalyani et al., 1998] and hippocampal neuronal progenitor cells develop CA1 and CA3 neurons [Regnell et al., 2012]. It is important to understand the timing of developmental restrictions that occur while identifying the optimal source of stem cells for transplantation. Such phenotypic restrictions occur early in development and cells can be isolated based on characteristics unique to that cell population which may vary according to applications for different neurological disorders.

In some transplant experiments, it has been shown that CNS stem cells will not populate the peripheral nervous system (PNS) and vice versa [Moreno and Fraser, 2002]. NSCs are part of CNS that have the capacity to self-renew and give rise to astrocytes, neurons, and oligodendrocytes. Oligodendrocyte precursor cells and Schwann cells of the CNS have the capability to remyelinate the injured CNS axon. In contrast, remyelination of injured axon in multiple sclerosis (MS) is limited. In addition to this, boundary cap cell which is the type of PNS stem cell population can differentiate into CNS as well as PNS lineage [Reynolds and Weiss, 1992; Fawcett and Asher, 1999; Zujovic et al., 2011]. Neural crest stem cells (NCSCs) are likely regionally specified and the differentiated progeny of NCSCs and olfactory stem cells appears to be capable of enhancing CNS repair and regeneration [Bunge, 2002]. Indeed dramatic results have been reported with OEC (olfactory ensheathing glia) transplants in spinal cord injury models and it is likely that these cells can be harvested from the adult neuroepithelium and amplified in vitro. Undifferentiated embryonic stem (ES) cells may have limited use for therapy due to their propensity to form teratomas while the ES cells derived differentiating neural progenitors and also the matured ones can be valuable for therapy provided they are properly depleted of undifferentiated cells. Depending on the role of transplanted cells, one would choose a cell type based on their properties and ease of availability and isolation. While attempting to replace neurons, as in the case of ALS, one would choose a neuronal precursor, a multipotent stem cell, or a glial cell. Using the human ESC-based ALS model, the Eggan's group has revealed that inhibition of signaling through the classic prostaglandin D2 receptor suppresses the toxic effect of SOD1 glia on motor neurons, hence providing a target for developing new methods of cure for ALS [Giorgio et al., 2008].

It is generally believed that the adult bone marrow cells can bring about the required changes in the tissue adjacent to the site of implantation repopulating the cell lineages during lifetime. A plethora of recent results from many groups suggests that adult stem cells may have a broader differentiation capacity than expected and that their fate may not be as tissue specific as once thought. It has been shown that adult NSCs can differentiate into a broad range of cells of different sources when introduced into myogenic cells and blastocyst. Moreover, skeletal muscle, brain and hepatic cells can give rise to bone marrow stem cells, whereas blood cells can generate from muscle precursors [Vescovi et al., 2002]. However, many argue that original source of tissue used in these studies was contaminated by blood and the hematopoietic stem cells in that blood gave rise to the blood cells formed. There are some reports which indicate that the structures do not develop from a single primordial stem cell but arise from the coordinated and dynamic interactions of many stem cells in what amounts to a ''stem system,'' similar to primitive buds that give rise to limbs amputated from primitive animals. Others believe that in response to distal injury, cells of the sub lamina undergo reverse differentiation and then differentiate to form viable tissues. Lately, induced pluripotent stem cells (iPSCs), are a type of artificially derived pluripotent stem cell from a non-pluripotent cell (from adult somatic cell), by providing the inducing medium to ''forced'' expression of specific genes, have been using for the same.

SOURCES OF NSCS

Large-scale resources of NSCs are crucial for both basic research and for the development of novel approaches for treating neurological disorders. NSCs primarily arise from embryonic ectoderm that forms neuroepithelial cells. The neuroepithelial cells generate radial glia that produces fetal and adult NSCs within CNS [Weiner, 2008]. The alternative sources of neural stem cells are shown in Table I [Nakatsu et al., 2005; Ryan et al., 2007; Robertson et al., 2008; Amit et al., 2010; Polo et al., 2010; Julius et al., 2011; Uri and Benvenisty, 2011]. Cells including multiple subtypes of CNS and PNS neurons, as well as oligodendrocytes, Schwann cells, and astrocytes, are modeled in these large-scale sources. Although most cell lines were initially from rodents, their human counterparts are being characterized and discovered. The prominent regions in the mammalian brain that have a reservoir of stem cells include the ependymal lining, SVZ and the olfactory bulb [Gritti et al., 2002]. The ciliary margin and the limbal regions of the retina have also been shown to be rich in NSCs which have been expanded in culture and shown to grow into neurospheres, many of which have been implanted in animal models to differentiate into retinal neurons. Until recently, it was widely believed that the marrow did not contain any non-hematopoitic cells. Recently, these cells have been recognized as the colony forming fibroblasts and mesenchymal cells. The latter type of cells have also been shown to be expressed in Umbilical Cord, cord blood as well as other tissues and have been found to be useful in neural regeneration [Song and Ramos, 2008]. Mesenchymal stem cells (MSCs) are multipotent stem cells possessing the intrinsic ability to differentiate into different types of cells that include osteocytes, adipocytes, and chondrocytes. MSCs have been postulated to generate cells of the mesoderm, endoderm and ectoderm, including neurons in culture depending of the inducing agents used. Interestingly, progeny of human MSCs infused after ectodermal differentiation has been identified in brains of mice and other animals. Identification of these cells as human is evident by a marker, only the infused cells possess due to their tagging with fluorescent reporter genes such as green fluorescent protein. Furthermore, the validation of these human cells as neural cells relies on immune localization of specific antigens such as nestin, S-100β, Sox2, Map2, GFAP, etc. In vitro MSCs have been

TABLE I. Comparison Between Different Types of NSCs Cells

Types of stem cells	Sources	Advantages	Disadvantages	Refs.
Embryonic NSCs	Embryonic CNS	Non-tumorigenic, committed neural lineage, and regionally specific	Difficulty in long-term preservation and ethical considerations	Julius et al. [2011]
NSCs derived from ES cells	Blastocyst inner cell mass	These cells have the pluripotent, unlimited differentiation, and stable karyotype	Tumorigenicity, ethical considerations and need to commit as neural specification	Amit et al. [2010], Uri and Benvenisty [2011]
Non-NSCs	Umbilical cord, blood, skin, bone marrow, etc.	No ethical consideration, abundant available supply and generate autologous cells	Require neural specification and restricted potential to differentiate in different cells	Stewart and Przyborski [2002]
Adult NSCs	Subventricular zone of Hippocampus	Committed neural lineage	Restricted potential to differentiate, limited availability and difficulty in long-term preservation	Robertson et al. [2008]
Induced pluripotent stem cells (iPSCs)	Adult somatic cells	Genetically matched cell lines, No ethical consideration, easier to create	Patient specific cell lines	Polo et al. [2010]
Mesenchymal stem cells (MSCs)	Wharton's jelly, bone marrow, periosteum, trabecular bone, adipose tissue, synovium, skeletal muscle and deciduous teeth and umbilical cord	No ethical issues, Immuno-privileged and therefore most beneficial for allogenic transplantation, reduced risks of rejection and complications of transplantation, isolated and easily expandable	Require neural specification, and highly heterogeneous nature isolates	Ryan et al. [2007]
Oncogene immortalized cell lines	PC-3, HeLa and Jurkat cell lines, cancer patients	Organ-specific genes which work as the sensitive or resistant factors	Contain numerous genetic mutations and exhibit an unstable genotype	Nakatsu et al. [2005]

shown to express properties of neuroectodermal cells by researchers and in vivo after transplantation into spinal cord and the brain [Mazzini et al., 2010]. NSC transplantation may be hampered by the limited number of donors available and by the toxicity of immunosuppressive regimens that may be needed after allogeneic transplantation. These limitations may be avoided if NSCs can be generated from clinically accessible sources, such as bone marrow (BM) and peripheral blood samples that are suitable for autologous transplantation. Fu et al. [2008] have reported that NSCs can be generated from human BM-derived mesenchymal stem cells (MSCs). When cultured in NSC culture conditions, 8% of MSCs were able to generate neurospheres. These MSC-derived neurospheres expressed characteristic NSC antigens, such as nestin and musashi-1, and were capable of self-renewal and multilineage differentiation into neurons, astrocytes, and oligodendrocytes. More recently, dental pulp was shown to possess a pool of stem cells that were expanded in culture which upon differentiation acquired a neural fate [Huang et al., 2008]. These had earlier been used for regeneration of dental and craniofacial cells. Spinal cord-derived NSCs have also been isolated and characterized and search for new sources continue to add to an increasing knowledge base in this area.

DETECTION AND MANIPULATION OF NSCS

Mice and humans studies have exposed a important developmental occurrence of aneuploid NSCs, whereas other chromosomal defects, such as inter-chromosomal translocations and partial chromosomal deletions/insertions, are extremely rare.

"Cre-Lox" systems in mice combined with other genetic markers empower researchers to track differentiation markers that are expressed in the growing brain. Neurosphere formation, which is characterized by aggregates of similar looking cells in culture, is another established system of screening the NSCs by virtue of their immunoreactivty with markers specific to NSCs although absence of differentiation markers doesn't necessarily indicate absence of differentiation or vice versa [Yang et al., 2005]. However, because this assay may choose and enlarge a heterogeneous stem/progenitor cell population, rigorous clonal, and serial subcloning analyses are required to detect and document stem cell activity and to unequivocally identify bona fide stem cells (see Fig. 1). Oncogene immortalized cell lines are the beginner's tool to dissect the proliferation and differentiation cues in culture. In fact, recent development of a magnetic bead based assay (Milteny Biotech, Inc.) allows sorting of putative NSCs using a cocktail of antibodies specific for markers expected to be selectively expressed by these cells. Similarly, the study of migration of these cells requires accurate mapping of the traffic of NSCs in the mammalian brain. The migration of stem cells to an injured or infarcted region of the brain requires mobilization, and it is imperative to mark these donor cells in order to differentiate them from resident stem cells. Various methods of labeling exist that include the use of genetically tagged NSCs or Y chromosome labeling that employs donor NSCs from male cells transplanted into female recipients. Lately, several dyes are being used for such types of labeling. Prominent among these is the CFDA-SE label. CFDA is an ester which diffuses into the cell where it

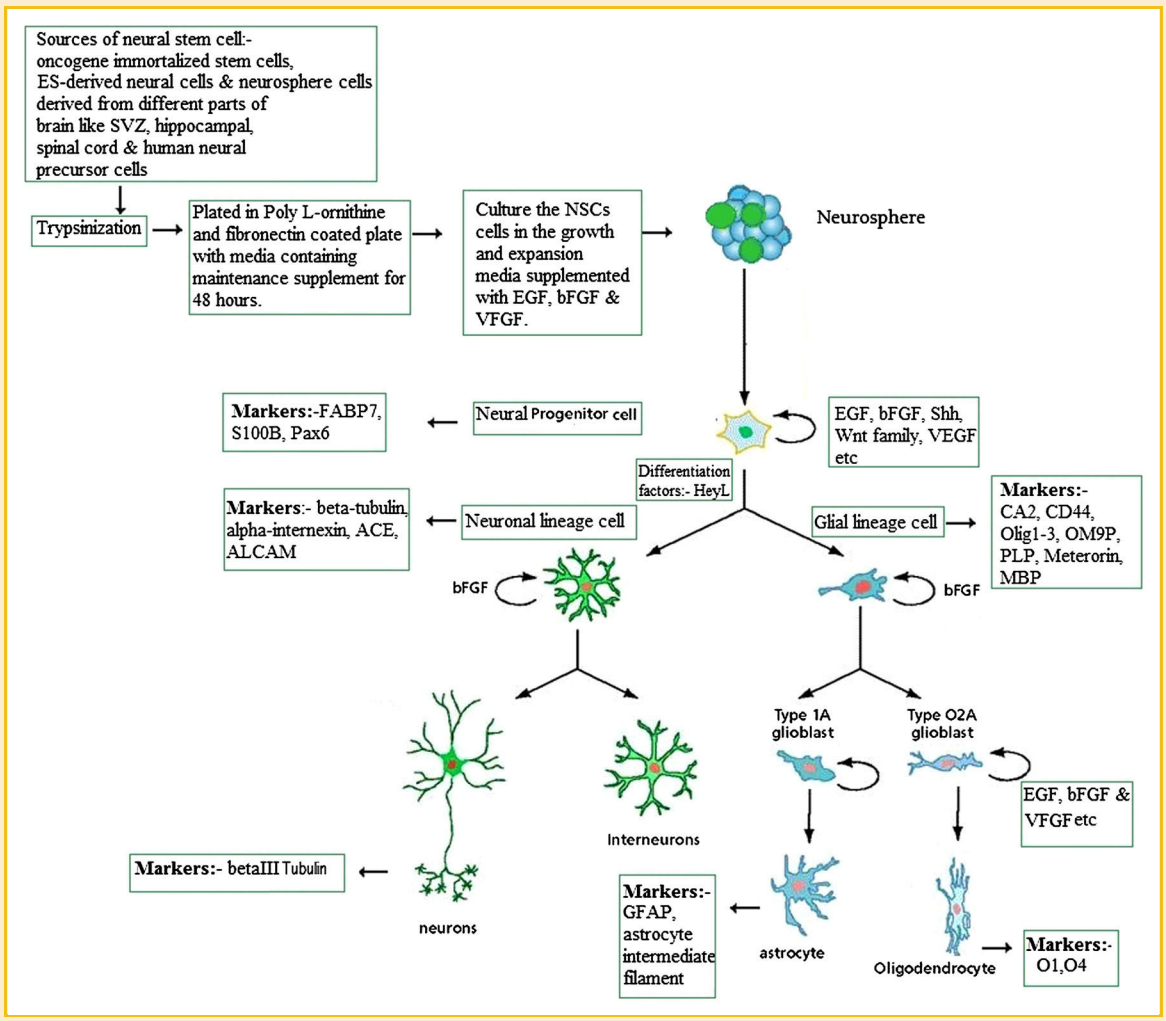

Fig. 1. Schematic representation of manipulation of neural stem cells. SVZ, subventricular zone; NSCs, neural stem cells; EGF, endothelial growth factor; bFGF, basic fibroblast growth factor; VFGF, viral fibroblast growth factor; Shh, sonic hedgehog; VEGF, vascular endothelial growth factor; FABP, fatty acid binding protein 7; S100B, S100 calcium binding protein B; PAX6, paired box protein Pax-6; ACE, angiotensin I-converting enzyme; ALCAM, activated leukocyte cell adhesion molecule; A2B5, type 2 astrocyte precursor marker; Olig1, oligoden drocyte transcription factor-1; Olig2, oligoden drocyte transcription factor-2; Olig3, oligoden drocyte transcription factor-3; PLP, proteolipid protein; MBP, myelin basic protein; O2A, oligodendrocyte-type-2 astrocyte; GFAP, glial fibrillary acidic protein; O1, oligodendrocyte marker O1; O4, oligodendrocyte marker O4; CA2, carbonic anhydrase 2.

is acted upon by endogenous esterases which liberate secondary products that interact with amines and fluoresce at the similar wavelength as GFP and imparts green fluorescence to the transplanted cell. There has been an equally rapid development in the field of cell marker analysis which characterize particular stem cells and enables sorting of these cells from various sources, based on the expression of neural antigens such as nestin and musashi. Two photon microscopy also has been used and represents a powerful tool that has greatly empowered analysis by allowing in vivo imaging of cells [Wang et al., 2006]. Many argue that the neuronal regeneration has no meaning unless there is functional revival of the affected region of brain. Functional MRI and electrophysiological measurements are powerful tools which allow localization of functional neurons in vivo and in vitro, respectively. Investigators have shown that human NSCs express both outward and inward K(+) currents with no evidence of Na(+) currents [Cho et al., 2002] and are useful in evaluating the potential for clinical translation. Similarly, electroretinograms enable assessment of regenerative capacity of stem cells in the eye and are very effective in strengthening the detection of regenerating neurons induced pharmacologically.

REGULATION OF NEURAL STEM CELLS: INTRINSIC CUES VERSUS GROWTH FACTORS

The reductionist view is the heart of breakthroughs in stem cell biology as it allows the clear definition of stem cell characteristics using genetic and molecular tools. The NSCs acquire a neural fate in response to environmental signals, leading to migration or differentiation into defined phenotypes. Apart from genetic signals that shape the pluripotency of these cells, cytokines and growth

factors, particularly bFGF, EGF, and VEGF play prominent role in proliferation and differentiation of the neural proginator cells. Lately, several researchers have provided credible evidence that angiogenesis and neurogenesis engage in a cross talk [Carmeliet and Tessier-Lavigne, 2005; Sharma et al., 2009]. Nakatomi et al. [2002] have shown that the infusion of EGF and FGF-2 into the lateral ventricle of the rat model of ischemia, in which CA1 neurons are selectively lost, leads to recovery of memory and learning functions with concomitant regeneration of pyramidal neurons due to neurogenesis. This has facilitated the discovery of factors that would enable desirable neurogenesis (or angiogenesis). For example, Gage's group showed that enriched environment and exercise improves neurogenesis and learning lending credence to the hope that environment factors can greatly influence the rate of neurogenesis [Van Praag et al., 2005].

Are we entering an era where cellular therapy may finally be able to reverse some of the disorders of brain. NSCs are becoming attractive tools for advancement of cellular therapy in neurodegenerative disorders. NSCs derived from fetuses have also been successfully used for symptomatic treatment after long-term follow-up in PD (Parkinson's disease) patients and functional improvement in patients who received fetal striatal grafts for treatment of Huntington's disease was remarkable [Claire et al., 2008]. PD is the neurodegenative disorder of CNS resulting from the death of substantia nigra (SN) which is located in midbrain. The consequence of SN cell death results in dopamine deficits with accompanying symptoms like movement rigidity, dementia, sleep disorders, and psychological problems, etc. So far, the most effective transplantation strategies concern the paracrine systems in which the affected cells exert modulatory actions on target circuits such as in the case of PD. More requirements have to be met in cases such as focal ischemia where it may be possible to rebuild the anatomical matrix. However, a long distance connection may be difficult to recreate. As a consequence, the behavioral benefits may be attributed to the tropic effects of transplants as the rewiring of the disrupted circuits may be necessary but not sufficient. Even the long-term follow-up of PD patients has shown that the limited functional recovery of such patients comes long after anatomical repair [Gogel et al., 2010]. Cell transplantation should be more accessible where degeneration affects a restricted area, such as in the case of PD, where transplants distributed across large areas require multiple transplantation approaches and have to rely on targeted migration of transplanted cells.

INJURY INDUCES STEM CELL RECRUITMENT

One important property that NSCs possess is migration. They were once thought to be more suited to deliver substances to specific sites in the brain than for regeneration. They appear to home to ischemic and neoplastic areas of brain and at least three physiological processes such as angiogenesis, reactive astrocytosis and inflammation invite their presence. Chemokines such as VEGFR1/R2,VEGF, Ccl2, and cKIT have been reported to be involved in NSC tropism [Chen et al., 2011] and studies have shown that VEGF mediated homing of cells may play a prominent role in the same [Chyi et al., 2010]. There is growing evidence to suggest that there is intimate relationship between CNS morphogenesis and endothelial cells; the basal lamina produced by endothelial cells contains many components that are supposed to be important for the maintenance of a neurogenic niche. Even SDF1 is expressed by both endothelial cells and astrocytes in stroke lesions and could be important for NSCs mobilization. Animal studies discussed below show how the lesion or damaged brain or retina mobilizes stem cells to damaged areas.

MECHANISMS INVOLVED IN NSC BASED REGENERATION

Neural stem cells (NSCs) are heterogeneous population of cells which are mitotically active. These cells have self-renewing and multipotent capacity which shows the differential pattern of gene expression at different times at various damaged regions of Brain [Gage, 2000; Temple, 2001; Ivanova et al., 2002]. Understanding the mechanisms of NSCs are important because these may be critical for driving clinical applications. NSC based investigations for different CNS disorders, for example, PD [Tonnesen et al., 2011], Huntington's disease [Connor, 2011], MS [Carbajal et al., 2010], retinal ganglion cell degeneration [Bull and Marti, 2011] and spinal cord injury (SCI) [Abematsu et al., 2010] have been studied in various animal models. The molecular mechanism of NSCs during recovery of injury induced inflammation, like rolling, adhesion, and extravasations into damaged CNS regions are sequentially mediated by constitutive expression of cell adhesion molecules (e.g., CD44) [Pluchino et al., 2003; Haas et al., 2005; Wang et al., 2010], integrins (such as α4, FAK, β1) [Campos et al., 2004; Leone et al., 2005; Pluchino et al., 2005; Campo et al., 2006; Wang et al., 2010; Battiste et al., 2011], chemokines receptors (Such as CCR3, CCR1, CCR2, CCR4, and CCR5) [Imitola et al., 2004; Ji et al., 2004; Wang et al., 2010; Andres et al., 2011a; Choi and An, 2011] on the surface of NSCs. These factors work as chemoattractive gradient, which leads to specific homing of NSCs in inflammatory regions of the brain. The recruited NSCs could exert bimodal effect depending on the CNS resident cells (such as microglia and astrocytes) which are reactive to pathological insults. First, neuroprotective effect offered by NSCs is accompanied by increased expression of neurotrophins such as brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), nerve growth factors (NGF), and glial-derived neurotrophic factor (GDNF) which has been demonstrated in experimentally induced neurodegenerative CNS disorder in rodents [Teng et al., 2002; Lu et al., 2003; Chu et al., 2004; McBride et al., 2004; Ryu et al., 2004; Richardson et al., 2005; Lee et al., 2007; Tamaki et al., 2009; Jaderstad et al., 2010]. Secondly, the recruited NSCs might promote immunomodulation by releasing chemokines or cytokines [Pluchino et al., 2003, 2009] and express relevant receptors (such as chemokines receptors and cell adhesion molecules), which are able to change the inflammatory responses. NSCs mediated mechanism is accelerated by pro-inflammatory cytokines (such as INFγ, IL-1β, Thelper 1-like, and TNFα). These recruited NSCs can significantly and specifically reduce the effector functions of inflammatory T-cells as well as macrophages [Einstein et al., 2003].

NSC transplantation also promises new hope in stroke by enhancing the axonal transport and structural plasticity in cerebral ischaemia [Andres et al., 2011b] and infiltration of mononuclear cells has been found to be decreased at the lesion site of ischaemic areas in the CNS where recruited NSCs accumulate in stroke animal model [Park et al., 2002; Kelly et al., 2004].

NEURAL STEM CELLS AND ANIMAL MODELS

Retina is the extension of the central nervous system which provides a convenient tool to examine the complicated nervous system. It can be manipulated with relative ease, making it feasible to test the regenerative potential of different types of stem cells, pharmacological compounds, and neurotropic factors. Human embryonic stem cell-derived retinal pigment epithelium (RPE) has been reported to rescue the visual function in an animal model of retinal disease [Lund et al., 2006]. When fetal neurons were assessed, they appeared to survive transplantation surgery better than adult neurons [MacLaren and Taylor, 1997], highlighting the value of fetal derived NSCs. Improvement in visual performance was twice that of untreated controls (spatial acuity was approximately 70% that of normal non-dystrophic rats) without evidence of untoward pathology [Lund et al., 2006]. Therefore, stem cells applications in the eye have become a center of hope for therapeutic use in regeneration and repair of damaged retina and possibly other neural tissue. The search for additional foci of NSCs led to their localization at the junction of retina and ciliary bodies, which is the remnant of ciliary marginal zone (CMZ) [Mayer et al., 2003]. CMZ is proliferative region at the periphery of the retina where the retinal stem cells are located. Thereafter, it was shown that NSCs could be isolated from mouse, rat, rabbit and human pigmented ciliary epithelium [Tropepe et al., 2000; Tsonis and Rio-Tsonis, 2004]. Under in vitro conditions, these cells can differentiate into retinal neurons such as photoreceptors, bipolar cells and muller glial cells. It has been shown that extrinsic factors strongly influence the progeny of retinal cells [Ezzeddine et al., 1997].

Further experiments in this direction have tested the in vivo potential of the retinal stem cells and their progeny, human retinal sphere cells, in eyes of postnatal (day 1) NOD-SCID mice and in embryonic chicks [Brenda et al., 2004]. RSCs progeny were able to survive, integrate, migrate, and differentiate into the neural retina, especially photoreceptors. The integration and differentiation of stem cells derived from human ciliary epithelium suggests that these cells finally may be precious in treating human retinal diseases [Brenda et al., 2004]. Chacko et al. have also isolated stem cells/progenitors from peripheral nerve type 1 (PN1, which is expressed in high levels throughout the PNS) rat retina and adult ciliary and limbal epithelium and used them for transplantation experiments in 10-day-old rat eyes. These cells survived and differentiated into photoreceptor-like cells expressing opsin but did not integrate into the existing retina. Postnatal PN1 retinal progenitors when transplanted into host retinas where mechanical damage was induced proved that retinal damage was essential for retinal integration [Chacko et al., 2004].

Though retinal stem cells exist in the mammalian eye throughout life, these cells proliferate embyonically and help to build the retina only in the initial phase, but in postnatal mammals they do not proliferate to regenerate the retina in response to injury [Ezzeddine et al., 1997]. However, van der kooy and coworkers [Brenda et al., 2006] reported that there was 3–8-fold increase in stem cell population in the region of ciliary margin in chx10 orj/orj and Mitf mi/mi mutant mice [Coles et al., 2006]. This indicates that loss of the neural retina or RPE progenitor populations results in increase in the resident stem cell population in pigmented ciliary epithelium. Such findings are important in the context of localizing stem cell progenitors.

To evaluate the morphological integration and host photoreceptor rescue as well as the impact on visual behavior, progenitor cells from neural retina of postnatal Day 1 EGFP transgenic mice were transplanted into the C57BL/6 rho $-/-$ mice at 4 weeks of age ($n = 12$) or C3H rd mice at 4 weeks of age [Klassen et al., 2004]. Brain- and retina-derived stem cells transplanted into adult retina have shown slight evidence of being able to differentiate into new photoreceptors and integrate into the outer nuclear layer. Sun et al. [2010] hypothesized that committed precursor or progenitor cells at later ontogenetic phases might have a greater probability of success upon transplantation. They showed that donor cells from the developing retina can integrate into the adult or degenerating retina at a time coincident with the peak of rod genesis. These transplanted cells differentiate, integrate into rod photoreceptors, form synaptic connections, and improve the visual function. Furthermore, they used genetically tagged post-mitotic rod precursors expressing the transcription factor Nrl (neural retina leucine zipper) to show that successfully integrated rod photoreceptors can not be derived from proliferating progenitor, these are derived from immature post-mitotic rod precursors. These results define the ontogenetic phase of donor cells for successful rod photoreceptor transplantation [MacLaren et al., 2006].

The works discussed here has demonstrated that progenitor transplantation can achieve limited photoreceptor replacement in the mammalian retina in rodents; however, replication of these findings on a clinically relevant scale requires large animal models. Large animal models like caprine model, horse, pig, etc. have been successfully used for such proginator transplantation experiments [Wanga et al., 2007; Revishchin et al., 2008]. In order to investigate this, some groups have propagated such cells from the brain, retina, and corneo-scleral limbus and have genetically modified human NSCs so that GDNF could be expressed upon transplantation into the spinal cord of SOD 1 mutant rat model. It was found that there was significant migration of cells into degenerate areas with remarkable early and end stages of the disease within chimeric regions. Combined with this study another group showed that neural stem cell fractions could bring benefits through neurogenesis and release of growth factors in a population double positive for Lewis X and the chemokine receptor CXCR4. The Seventy-day-old transgenic SOD1-G93A mice were transplanted with Le + CX + NSCs (20,000 cells) or only with vehicle (saline solution). There was generation of cholinergic motor neuron-like cells upon differentiation. The transplanted mice survived longer than controls at 23 days [Corti et al., 2007]. A cursory analysis of these reports reveals that fewer

studies have analyzed the dose response or comparative efficacy of these cells when implanted through different routes of administration. Therefore such approaches may aptly supplement the pace with which the field is growing.

CONCLUSIONS

There is plenty of data to suggest that much of what is considered as promise for regeneration may not be limited to neurons but also include myelin-forming cells; however, whether the intact nervous system can be successfully reconstituted remains hotly debated. It is difficult to reconcile the two disparate thoughts and only further advancement of our knowledge in clinical translation studies combined with use of primate models of research will uncover the promise held by demolishing the Cajal's myth that brain cells do not divide. With ever increasing funding in stem cell research and sudden increase in impact factor of stem cell journals, it is the time for disease model specialists to collaborate with those that specialize in in vitro manipulations so that side by side comparisons on potential and efficacy of variety of stem cells from different sources, stages of development, administration routes and doses between species can be appropriately evaluated. This will not only accelerate the pace of clinical translation and consequent stem cell entrepreneurship for societal benefit but also improve the unmet requirements of current healthcare delivery systems.

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