Sources of Neural Stem Cells for Clinical Application

Cell replacement therapy could aid in alleviating symptoms or even reversing progression of neurodegenerative diseases. Over the last decade, convincing evidence has emerged of the capability of various stem cell populations to induce regeneration in animal models of Parkinson's disease (PD), Huntington's disease (HD) and Alzheimer's disease (AD) along with multiple sclerosis and cerebral ischemia [Gogel et al., 2011]. Neural stem cells (NSCs) hold tremendous potential for neurodegenerative diseases. This approach is not limited to the use of NSCs for transplantation, but includes the stimulation of endogenous stem cells and the multiple bioactive molecules that they express during reciprocal interactions with the diseased central and peripheral nerve systems. Several critical problems for NSC clinical application remain to be resolved: (i) the source of NSCs should be personalized; (ii) the source should be personalized for each patient; and (iii) the safety and efficacy of NSCs need to be ensured.
(ii) the isolation methods and protocols of human NSCs should be standardized; (iii) the clinical efficacy of NSC transplants must be evaluated in more adequate animal models and (iv) the mechanism of intrinsic brain repair needs to be better characterized. In addition, the ideal imaging technique for tracking NSCs would be safe and yield high temporal and spatial resolution, good sensitivity and specificity.

The ideal sources of NSCs for clinical application should better be personalized, tolerant to immune rejection, and resistant to tumorigenesis, easy to obtain and to amplify, effective in cellular or molecular replacement. The potential cell sources for central nervous system transplantation include fetal or adult NSCs [Tamaki et al., 2002; Uchida et al., 2000], embryonic stem cells (ESCs) and mesenchymal stem cells (MSCs). NSCs can be expanded over a long period of time and as they are already neuralized (for example, committed to a central nervous system cell fate) there is no need for recapitulating early developmental signals that lead to neuroectodermal commitment. However, the transplantation of fetal NSCs into the adult brain is accompanied by numerous ethical, scientific and legislative hurdles [Mathews et al., 2008]. In addition, the prolonged culturing of NSCs leads to an ever increasing glial differentiation pattern at the expense of neuronal differentiation, which significantly reduces the therapeutic potential of fetal NSCs [Anderson et al., 2007]. Human ESCs can be manipulated to generate defined neuronal and glial lineages, thereby offering a major opportunity to study neurodevelopment and model neurological disease in vitro, as well as potentially having direct therapeutic applications in the field of regenerative neurology. However, certain challenges remain to be resolved before the promise of ESCs for neurological diseases can be fully realized, including the need to optimize survival, fate and function of neural derivatives upon both neural conversion and long-term differentiation in vitro and in vivo. Current protocols for the derivation of NSCs from ESCs require defined conditions; however, these conditions lead to significant cell death, in which the production of reactive oxygen species has a central role. Thus, neutralization protocols often contain antioxidants (which may increase the propensity to accumulate genetic mutations), involve co-culture with stromal feeder layers or use B27 and/or N2 supplements. It is becoming increasingly clear that the more traditional stem cell systems using oxygen levels approximating room air (20%) are far from optimal, particularly with regard to neural specification and differentiation. Thus, recent protocol has been designed to generate NSCs and their regionally specified derivatives from ESCs using a physiological oxygen level of 3% (normoxia) [Stacpoole et al., 2011].

Flow cytometry and fluorescence-activated cell sorting (FACS) have been used successfully to resolve the complexity of lineage progress in the hematopoietic and other systems. Recently, FACS has been applied extensively in NSC biology, such as isolation of different precursor and progenitor populations from the central nervous system and peripheral nervous system. NSCs that had been isolated from brain tissues by cell-surface markers, such as CD133, or GFP expression driven by NSC-specific promoters. These promoters include Sox1, Sox2, Nestin and FGF11 [Hsu et al., 2009b; Lee et al., 2009]. NSCs thus isolated were cultured in the presence of growth factors and examined to determine whether they could expand to form neurospheres. The capacity to form neurospheres was defined as self-renewal. The potential for neural differentiation of these isolated cells upon withdrawal of growth factors or administration of inducing factors was used to determine multipotency.

**Introduction of Neurodegeneration**

The prevalence of neurodegenerative diseases is increasing rapidly. Neurodegenerative diseases such as AD, HD and PD trigger neuronal cell death through endogenous suicide pathways. Although progressive neuronal loss is a hallmark of neurodegenerative disorders, some neurological impairment may reflect dysfunction rather than loss of neurons. Abnormal protein assemblies seem to trigger vicious cycles of aberrant neuronal activity and compensatory alterations in neurotransmitter receptors and related signaling pathways that lead to synaptic deficits, disintegration of neural networks, and, ultimately, failure of neurological functions.

Recently, the U.S. President Obama administration plans to spend an additional $156 million over the next two years to help find an effective treatment for AD that affects more than five million Americans. The spending increase is intended to help in achieving a U.S. target set last month to find a way to treat or prevent AD by 2025. Current drugs help manage symptoms but so far no therapy can stop the progression of AD, which can start with vague memory loss and confusion before progressing to complete disability and death. Experts predict that without an effective treatment, the number of Americans with AD will double by 2050 and related healthcare costs could soar to more than $1 trillion a year.

Neurodegenerative diseases can disrupt molecular pathways, synapses, neuronal subpopulations and local circuits in specific brain regions, as well as higher-order neural networks. Abnormal network activities may result in a vicious cycle, further impairing the integrity and functions of neurons and synapses, for example, through aberrant excitation or inhibition. Neurodegenerative disorders such as AD and amyotrophic lateral sclerosis (ALS) are associated with microvascular dysfunction and/or degeneration in the brain, neurovascular disintegration, defective blood-brain barrier function and/or vascular factors. Microvascular deficits diminish cerebral blood flow and, consequently, the brain’s supply of...
oxygen, energy substrates and nutrients. Moreover, such deficits impair the clearance of neurotoxic molecules, non-neuronal cells and neurons. Recent evidence suggests that vascular dysfunction leads to neuronal dysfunction and neurodegeneration, and that it might contribute to the development of proteinaceous brain and cerebrovascular ‘storage’ disorders. Such disorders include cerebral β-amyloidosis and cerebral amyloid angiopathy, which are caused by accumulation of the peptide β-amyloid in the brain and the vessel wall, respectively, and are features of AD [Zlokovic, 2011].

PD is a degenerative disorder of the central nervous system. It was first described in 1817 by James Parkinson. Researchers believe that at least 500,000 people in the United States currently have PD, although some estimates are even higher. Society pays an enormous price for PD. The total cost to the nation is estimated to exceed $6 billion annually. The risk of PD increases with age, so analysts expect the financial and public health impact of this disease to increase as the population gets older. PD is characterized by an extensive loss of dopaminergic neurons in the substantia nigra, pars compacta, and their terminals in the striatum [Kish et al., 1988]. Although the etiology of idiopathic PD is not known, several predisposing factors for the dopaminergic depletion associated with the disease have been suggested, including programmed cell death, viral infection, and environmental toxins. As an effective treatment for PD, patients have been given L-dihydroxyphenylalanine (L-DOPA), a precursor of dopamine, but long-term administration of L-DOPA consequently produces grave side effects.

**Introduction of Peripheral Nerve Injuries**

The incidence of peripheral nerve injury (PNI) in developed countries is estimated between 13 and 23 per 100,000 persons per year. Injuries to peripheral nerves result in partial or total loss of motor, sensory and autonomic functions in the involved segments of the body. Reinnervation of denervated targets can be achieved by regeneration of injured axons or by collateral branching of undamaged axons in the vicinity. Nevertheless, these mechanisms do not provide for satisfactory functional recovery, especially after severe injuries. Peripheral nerve problems are common and encompass a large spectrum of traumatic injuries, diseases, tumors and iatrogenic lesions. The incidence of traumatic injuries is estimated as >500,000 new patients annually in the world. PNI results in loss of neural control in denervated segments of the body, and severe disabilities for the patients. Nerve regeneration usually does not allow for adequate target reinnervation and functional restitution. Neuronal response and axonal regeneration imply a complex interaction of cell types and changes in the expression of many molecules. Many experimental models have been used to gain knowledge on nerve regeneration and to develop strategies to promote recovery.

The failure of axons to regenerate following PNI results from decreased intrinsic properties of the neurons [Kadoya et al., 2009], the absence of neurotrophic factors or the presence of inhibitory factors in the environment. After PNI, axons and myelin sheaths distal to the lesion are degraded. The degenerative products are eliminated by the cooperative action of denervated Schwann cells and infiltrating macrophages. Wallerian degeneration serves to create a microenvironment favoring axonal regrowth. Schwann cells within the endoneurial tubes of the distal nerve dedifferentiate towards a non-myelinating proliferative phenotype that over-express growth factors, cell adhesion molecules and extracellular matrices. The axotomized neurons shift from a ‘transmitter’ state to a ‘regenerative’ state, so their axons generate growth cones that progress from the proximal stump into the distal nerve. Axonal regeneration requires an adequate substrate of trophic factors, provided by reactive Schwann cells, macrophages and the extracellular matrix within the degenerated nerve. The regenerative process, however, does not usually reconstitute a normal nerve structure neither allows for normal distal reconnection after severe lesions. Neuronal response and axonal regeneration require a complex interaction of several cell types and changes in the expression of many molecules with variable spatial and temporal patterns. Therefore, a wide variety of methods are used in experimental studies, depending on the specific goals of each study [Navarro, 2009].

**Current Therapeutic Applications of NSCs in Neurodegeneration and Peripheral Nerve Injuries**

It is widely anticipated that transplantation of stem/progenitor cells will provide effective therapies for many neurological diseases and injuries [Trounson et al., 2011]. Numerous encouraging animal studies have shown that stem or progenitor cell treatments can rescue some degree of neurological function after injury. Current clinical trials of human NSCs and ESCs are shown in Table 1. We further summarize the preclinical studies evaluating NSC therapy for peripheral nerve repair in Table 2. Only few studies have demonstrated direct evidence of cell replacement in injury or disease models that clearly explains the benefits observed after cell therapy. Many positive outcomes after cell therapy appear to be attributed to rescue of pre-existing tissue rather than repair or cell replacement per se. The paracrine action of growth factors, cytokines, and hormones that are secreted or released by transplanted cells has been shown to provide most of the benefits after stem/progenitor cell administration. This can be seen as a problem, since for many years we missed paracrine activity as a principal mechanism in cell therapy and the paracrine mechanism may be complicated. Alternatively, the situation can be viewed as an exciting opportunity to the new cell replacement therapy. Notably, a research group in Taipei Veteran General Hospital developed a novel spinal cord injury repair strategy. Using peripheral nerve grafts and FGF1 improves hindlimb locomotor function in spinal cord-transected rats [Cheng et al., 1996; Lee et al., 2002; Tsai et al., 2008]. Repaired spinal cords induce the expression of the M2 macrophage marker arginase I six
to 14 days after repair and recruited large numbers of M2 macrophages to the graft area 10 days after repair [Kuo et al., 2007]. They further demonstrate that FGF1 induces IL-4 expression and that nerve grafts induce NGF and BDNF expression in transected spinal cords. A full repair strategy utilizes the beneficial effects of both FGF1 and nerve grafts simultaneously [Kuo et al., 2011].

The human FGF1 gene was first cloned by us [Wang et al., 1989]. FGF1 is expressed in ventral cochlear neurons, olfactory bulbs and hippocampal neurons but not in glial cells [Alam et al., 1996]. The brain-specific FGF-1B promoter is active only in the brain [Chiu et al., 2001; Chiu et al., 2000]. Interestingly, it has been shown that FGF-1B mRNA is upregulated for the maintenance of NSCs in hippocampus dentate gyrus in response to activity-induced neurogenesis [Ma et al., 2009]. Furthermore, F1B-GFP-selected NSPCs from mouse brains were able to repair the damaged sciatic nerve of paraplegic rats [Hsu et al., 2009a; Lin et al., 2008].

Personalized Regenerative Medicine: Patient-specific Neural Stem Cells and Functional Neurons

The promising cell sources for personalized regenerative medicine is from patients' skin fibroblasts through transcription factors-mediated reprogramming, such as induced pluripotent stem (iPS) cells [Takahashi et al., 2007]. Development of iPS cells from patients with amyotrophic lateral sclerosis further attests the possibility of future cell-based therapy [Dimos et al., 2008]. However, recent studies using iPS cells have shown sizeable genetic and epigenetic abnormalities in iPS cells [Pera, 2011]. Recently, Vierbuchen et al. [Vierbuchen et al., 2010] found that a combination of just three factors (Ascl1, Brn2 and either Myt1l or Zic1) was sufficient to convert fibroblasts into neurons (iN). These iN cells expressed a variety of neuronal markers and were capable of firing action potentials. Furthermore, when cultured with mouse neural cells, the iN cells received both excitatory and inhibitory synaptic connections from the mouse neurons, and were able to form functional synapses with each other. Ascl1 alone was able to produce cells with immature neuronal features, but co-infection with Brn2 and Zic1 was required to produce cells with more mature neuronal features. In the past six months, six different laboratories independently demonstrated that adult human fibroblasts from a skin biopsy could be efficiently converted into functional neurons [Yang et al., 2011]. Although more accessible cell types such as peripheral blood could turn into iPS cells [Yamanaka, 2010], whether blood could be induced to neurons is yet unknown. In addition, transplantation

<table>
<thead>
<tr>
<th>COMPANY/ORGANIZATION</th>
<th>CELL PRODUCT</th>
<th>DISEASE</th>
<th>CLINICAL TRIAL STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>StemCells Inc., CA</td>
<td>HuCNS-SC® (fetal derived human NSCs)</td>
<td>Spinal cord injury</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>NeuroGeneration, CA</td>
<td>Autologous NSC-derived Neurons</td>
<td>Advanced PD</td>
<td>Phase I – completed</td>
</tr>
<tr>
<td>Neuralstem Inc., MD</td>
<td>Fetal derived spinal cord SCs</td>
<td>ALS</td>
<td>Phase II – clinical hold</td>
</tr>
<tr>
<td>ReNeuron, UK</td>
<td>ReN001 Immortalized huNSCs</td>
<td>Stroke</td>
<td>Phase I</td>
</tr>
<tr>
<td>City of Hope, CA</td>
<td>HB1.F3.CD Immortalized hu NSCs</td>
<td>Glioma</td>
<td>Phase I</td>
</tr>
<tr>
<td>Geron Inc.</td>
<td>Human ESC-derived oligodendrocyte progenitor cells (GRNOPC1)</td>
<td>Complete subacute thoracic spinal cord injuries. T3 to T10 segments between seven and 14 days after injury</td>
<td>Phase I</td>
</tr>
<tr>
<td>Advanced Cell Technologies</td>
<td>Retinal Pigment Epithelium derived from human ESCs.</td>
<td>Stargardt's Macular Dystrophy (juvenile macular degeneration)</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>California Stem Cell</td>
<td>Human motor neuron progenitor cells derived from human ESCs</td>
<td>Spinal muscular atrophy Type 1</td>
<td>Phase I: Currently on hold 2011</td>
</tr>
</tbody>
</table>

Table 1. Current Clinical Trials of Human Neural Stem Cells and Embryonic Stem Cells
experiments will be necessary to see whether iN cells can integrate into the damaged tissues and ameliorate disease in animal models [Nicholas and Kriegstein, 2010]. A way of expanding cell numbers is also required. Thus, it is expected to know that reprogramming allows the conversion of mouse fibroblast to induced NSCs. It is also noted that FGFs have been shown to be essential in culturing ESCs and NSCs. FGF1 has been shown to regulate cell proliferation, cell division and neurogenesis. Cheng et al. [Cheng et al., 1996] and Lee et al. [Lee et al., 2002] showed that the combination of FGF1 treatment and peripheral nerve grafts could restore hind limb function in adult paraplegic rats. More recently, it was shown that similar treatments benefited the patients with common peroneal nerve lesions as well [Tsai et al., 2009]. Thus, in addition to the stem cell-based therapy, direct clinical application of FGF1 will likely benefit the patients. Given the significance of FGF1 in the treatment of spinal cord injury and PNI, future efforts generating FGF1-expressing NSCs or iN will have promising potential in regenerative medicine and in the treatments of central and peripheral nerve system diseases.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>INJURY TYPE/ANIMAL MODEL</th>
<th>CELLULAR TYPE/FACTOR</th>
<th>MAJOR FINDINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dezawa et al. (2001)</td>
<td>Sciatic nerve injury in rats (1.5 cm gap)</td>
<td>Bone marrow MSCs differentiated into Schwann-like cells suspended in Matrigel</td>
<td>Successful nerve regeneration and myelination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>injected into hollow fibers</td>
<td></td>
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<tr>
<td>McKenzie et al. (2006)</td>
<td>Sciatic nerve crush injury in myelin-deficient mice</td>
<td>Skin-derived precursors differentiated into Schwann cells</td>
<td>Remyelination and functional recovery</td>
</tr>
<tr>
<td>Marchesi et al. (2007)</td>
<td>Sciatic nerve injury in rats (1.6 cm gap)</td>
<td>Guides filled with skin-derived stem cells</td>
<td>Functional recovery and myelination</td>
</tr>
<tr>
<td>Lin et al., 2008, Hsu et al. (2009a)</td>
<td>Sciatic nerve injury in rats (1.0 cm gap)</td>
<td>Nerve conduit seeded with F1BGFP(+) mouse neural stem cells</td>
<td>Axon regeneration</td>
</tr>
<tr>
<td>Chen et al. (2010)</td>
<td>Acutely distracted sciatic nerves in rabbits</td>
<td>Neural stem cells</td>
<td>Functional recovery and nerve regeneration</td>
</tr>
<tr>
<td>Gu et al. (2010)</td>
<td>Sciatic nerve injury in rats (1.0 cm gap)</td>
<td>Rat fetal neural stem cells</td>
<td>Transplanted neural stem cells differentiated into neurons in peripheral nerves that synthesize and secreted synaptophysin</td>
</tr>
<tr>
<td>Cheng et al. (2010)</td>
<td>Sciatic nerve crush injury in rats</td>
<td>human amniotic fluid-derived mesenchymal stem cells and</td>
<td>Functional recovery and nerve regeneration</td>
</tr>
<tr>
<td>Di Summa et al. (2010)</td>
<td>Sciatic nerve injury in rats (1.0 cm gap)</td>
<td>Nerve fibrin conduits seeded with adipose derived stem cells</td>
<td>Enhanced peripheral nerve repair</td>
</tr>
<tr>
<td>Reid et al. (2011)</td>
<td>Sciatic nerve injury in rats (1.0 cm gap)</td>
<td>Adipose derived stem cells</td>
<td>Dorsal root ganglia protection from apoptosis</td>
</tr>
</tbody>
</table>

Table 2. Preclinical Studies Evaluating Stem Cell Therapy for Peripheral Nerve Repair

References


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