Human Neural Stem Cells for Brain Repair

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Cell replacement therapy and gene transfer to the diseased or injured brain have provided the basis for the development of potentially powerful new therapeutic strategies for a broad spectrum of human neurological diseases including Parkinson disease, Huntington disease, amyotrophic lateral sclerosis (ALS), Alzheimer disease, multiple sclerosis (MS), stroke, spinal cord injury and brain cancer. In recent years, neurons and glial cells have successfully been generated from neural stem cells, and extensive efforts by investigators to develop neural stem cell-based transplantation therapies have been carried out. We review here notable experimental and pre-clinical studies we have previously conducted involving human neural stem cell-based cell- and gene-therapies for Parkinson disease, Huntington disease, ALS, stroke and brain cancer.

Keywords: Stem cell, Human neural stem cell, Cell therapy, Gene transfer, Transplantation, Neurodegenerative diseases, Parkinson disease, Huntington disease, Amyotrophic lateral sclerosis, Stroke, Brain cancer

Introduction

Stem cells are the cells that have the ability to renew themselves continuously and possess pluripotent ability to differentiate into many cell types. Previously two types of mammalian pluripotent stem cells, embryonic stem cells (ESCs) and embryonic germ cells (EGCs), have been identified and these stem cells give rise to various organs and tissues (1, 2). Recently there has been an exciting development in generation of a new class of pluripotent stem cells, induced pluripotent cells (iPS cells), from adult somatic cells such as skin fibroblasts by introduction of embryogenesis-related genes (3-5). In addition to ESCs and iPS cells, tissue specific stem cells such as hematopoietic stem cells, bone marrow mesenchymal stem cells, adipose tissue-derived stem cells, amniotic fluid stem cells and neural stem cells (NSCs) could be isolated from various tissues. Existence of multipotent NSCs has been known in developing or adult rodent or human brain with properties of indefinite growth and multipotent potential to differentiate into three major cell types of CNS, neurons, astrocytes and oligodendrocytes (6, 7).

Recently continuously dividing immortalized cell lines of NSC have been generated by introduction of oncogenes, and these cells have emerged as highly effective source for cell- and gene-therapy in animal models of neurological disorders. We have previously generated immortalized cell lines of human NSC by infecting fetal human brain cells grown in primary culture with a retroviral vector carrying v-myc oncogene and selecting continuously dividing NSC clones. Both in vivo and in vitro these cells were able to differentiate into neurons and glial cells and populate the developing or degenerating CNS (6, 7). Cell replacement and gene transfer to the diseased or injured brain using NSC have provided the basis for the development of potentially powerful new therapeutic strategies for a broad spectrum of human neurological diseases including Parkinson disease (PD), Huntington disease (HD), Alzheimer disease (AD), amyotrophic lateral sclerosis (ALS),...
multiple sclerosis (MS), stroke, spinal cord injury (SCI) and brain cancer. In this review, I will focuses on the utility of stable immortal human NSC lines developed in my University of British Columbia (UBC) laboratory as substrates for structural and functional repair of the diseased or injured brain.

**Human neural stem cells**

Recently in our UBC laboratory, stable immortalized cell lines of human NSC have been generated by introduction of myc oncogene. These immortalized NSC lines have advantageous characteristics for basic studies on neural development and cell replacement or gene therapy studies (6, 7): (i) NSC cell line can be expanded to large numbers in culture in short time (24~48-h doubling time), (ii) NSC cells are homogeneous since they were generated from a single clone, and (iii) stable expression of therapeutic genes can be achieved readily. Immortalized NSCs have emerged as a highly effective source for genetic manipulation and gene transfer into the CNS ex vivo. Immortalized NSCs were genetically manipulated in vitro, survive, integrate into host tissues, and differentiate into both neurons and glial cells after transplantation into the intact or damaged brain (6-9).

Primary cultures of fetal human telencephalon cells (at 15 weeks gestation) were infected with a retroviral vector carrying v-myc oncogene, and continuously dividing NSC clones were selected. HB1.F3 (F3), one of the newly generated human NSCs, is a clonally isolated, multipotential human NSC line, with the ability to self-renew and differentiate into cells of neuronal and glial lineages in vitro (7, 10-12). The cloned F3 cells are tripolar or multipolar in morphology with 8 μm in size. Cytogenetic analysis of F3 human NSCs showed normal karyotype of human cells with a 46, XX without any chromosomal abnormality. RT-PCR study indicates that F3 human NSCs grown in serum containing medium (10% fatal bovine serum) express transcript for nestin, cell type-specific markers for NSCs and neural progenitor cells, and transcripts for NF-L, NF-M and NF-H, cell type-specific markers for neurons, transcript for GFAP, structural protein and a cell type-specific marker for astrocytes, and transcript for MBP, structural protein and a specific cell type specific marker for oligodendrocytes. These results indicate that F3 cells grown in serum containing medium undergo asymmetrical division by which one daughter cell remains as NSCs and continues cell division while another one undergoes terminal differentiation into neurons or glial cells. Gene expression of neurotrophic factors in F3 NSCs as studied by RT-PCR indicates that the F3 NSCs express NGF, BDNF, NT-3, GDNF, CNTF, HGF, IGF-1, bFGF and VEGF. We also determined secretion of selected neurotrophic factors, NGF and BDNF, in F3 NSCs by ELISA quantification and the results indicate that the F3 NSCs constitutively secrete NGF and BDNF as high as 100 pg/10⁶ cells/day and 300 pg/10⁶ cells/day, respectively. An electrophysiological study has also demonstrated that F3 NSCs generate inward currents of voltage-activated sodium channels, which indicates that the neuronally differentiated F3 cells have electrophysiological characteristics of mature neurons (10).

Immunohistochemical determination of cell type specific markers for CNS cell types was performed using antibodies specific for neurons, astrocytes and oligodendrocytes. When F3 NSCs were grown in serum containing medium, there were more than 50% of total cells expressing neurofilament proteins (NF-L). In addition to neurons, 2~5% of F3 cells expressed GFAP, a cell type-specific marker for astrocytes, while much smaller number of galactocerebroside-positive cells, a surface antigen specific for oligodendrocytes, was found (<1%). These results indicate that F3 human NSCs are multipotent and capable of differentiation into neurons, astrocytes and oligodendrocytes under stable culture conditions. After brain transplantation, F3 human NSCs provide clinical improvement in the animal models of neurological disorders including neurodegenerative diseases, stroke, brain cancer (see next sections), epilepsy (13) and lysosomal storage disease MPS VII (14).

**Neurodegenerative diseases**

Cell replacement and gene transfer to the diseased or injured brain using NSCs have provided the basis for the development of potentially powerful new therapeutic strategies for a broad spectrum of human neurodegenerative diseases including Parkinson disease (PD), Huntington disease (HD), Alzheimer disease (AD), amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS).

Parkinson's disease (PD) is characterized by an extensive loss of dopamine neurons (DA) in the substantia nigra pars compacta and their terminals in the striatum (15, 16). Since late 1950s PD patients have been given L-dihydroxyphenyl alanine (L-DOPA), a precursor of dopamine, as an effective treatment for PD, but long-term administration of L-DOPA consequently produces grave side effects (17, 18). Since late 1980s, transplantation of human fetal ventral mesencephalic tissues (6~9 weeks gestation) into the patients' brain striatum has been adopted as a
successful therapy for PD patients (19-21). However, this fetal human tissue transplantation has grave problems associated with ethical, religious and logistical questions of acquiring fetal tissues. In addition, recent reports have indicated that the survival of transplanted fetal mesencephalic cells in the patients' brain was very low and it was difficult to obtain enough fetal tissues needed for transplantation (22). To circumvent these difficulties, utilization of neurons with DA phenotype generated from ESCs, MSCs or NSCs could serve as a practical and effective alternative for the fetal brain tissues for transplantation.

Recently we have transplanted immortalized human NSC cell line HBI.F3 (F3) in striatum of PD model rats, and induced functional improvement in rat model of PD following transplantation into the striatum. Intrastriatal transplantation of F3 human NSCs immediately after 6-OHDA lesion in rats reduced parkinsonian motor symptoms and preserved TH nigral neurons and striatal fibers. At 1 month after transplantation, some grafted F3 cells expressed neuronal and synaptic markers, accompanied by enhanced neurogenesis in SVZ adjacent to the transplant site. Neuroprotective and anti-apoptotic effects of F3 human NSCs induced by secretion of neurotrophic factors (stem cell factor and BDNF) were demonstrated in vivo and in vitro models of PD (23). Previous studies have used ex vivo gene transfer approach to generate dopamine cells by transferring tyrosine hydroxylase (TH) gene, a rate-limiting step enzyme in catecholamine biosynthesis process, into fibroblasts and then implant these cells into the brain of PD animal models (24, 25). However, fibroblasts expressing TH gene produced low level dopamine since these cells did not carry tetrahydrobiopterin (BH4), a co-presence TH gene produced low level dopamine since the pathogenesis of HD remain largely unknown and thus hamper effective therapeutic interventions. Transplantation of fetal human brain tissue has reported improvements in motor and cognition performance in HD patients following fetal cell transplantation (31). An ideal source of cell transplantation in HD is human NSCs which could participate in normal CNS development and differentiate into regionally-appropriate cell types in response to environmental factors. Rodents and primates with lesions of the striatum induced by excitotoxins kainic acid (KA), or quinolinic acid (QA) have been used to simulate HD in animals and to test efficacy of experimental therapeutics and neural transplantation (32).

Recently we have injected F3 human NSCs intravenously to counteract neural degeneration in QA-HD model and demonstrated functional recovery in grafted animals (33, 34). The systemic transplantation of NSCs via intravascular route is probably the least invasive method of cell administration (34). Neural transplantation into striatum requires an invasive surgical technique using a stereotaxic frame. Non-invasive transplantation via intravenous routes, if it may be effective in human, is much more attractive.

Systemic administration of 3-nitropropionic acid (3-NP), a mitochondrial toxin, in rodents leads to metabolic impairment and gradual neurodegeneration of the basal ganglia with behavioral deficits similar to those associated with HD (35). We have investigated the effectiveness of transplantation of human NSCs in adult rat striatum prior to striatal damage induced by the mitochondrial toxin 3-NP (36). Animals receiving intrastriatal implantation of human NSCs one week prior to 3-NP treatments exhibited significantly improved motor performance and increased resistance to striatal neuron damage compared with control sham injections. In contrast, transplantation of human NSCs at 12 hr following 3-NP administration did not show any protective effects against 3-NP-induced behavioral impairment and striatal neuronal damage (36). The neuroprotection shown by the proactive transplantation of human NSCs in the rat HD model is contributed by transplanted human NSCs' secretion of brain-derived neurotrophic factor (BDNF). Active production of BDNF by human NSCs in vivo and in vitro was firmly established by studies using RT-PCR, immunocytochemistry, dot-blot, and ELISA. Since genetic screening of HD gene and neuroimaging could determine the diagnosis in "predisposed" HD patients, the results of our study suggest that early intervention using brain transplantation with human
NSCs expressing neurotrophic factors should provide an effective stem cell-based therapy for "predisposed" HD patients. We have recently written an extensive review that focuses on the stem cell-based therapy for HD and investigators who wish to learn more about the subject are referred to the review article (37).

Amyotrophic lateral sclerosis (ALS), known as Lou Gehric disease, is a relentlessly progressive, adult onset neurodegenerative disorder characterized by degeneration and loss of motor neurons in the cerebral cortex, brain stem and spinal cord, leading to muscle wasting and weakness, and eventually to death within five years after the onset of its clinical symptoms (38, 39). To date there is no effective treatment for patients suffering from ALS.

Several recent studies have demonstrated that delivery of vascular endothelial cell growth factor (VEGF) significantly delayed disease onset and prolonged the survival of ALS animal models (40, 41). VEGF is one of growth factors that can be used in combination with transplanted stem cells to improve therapeutic efficiency of cellular transplantation in ALS animals. VEGF is an angiogenetic growth factor acting as a potent mitogen and survival factor of endothelial cells, and also known for neurotrophic and neuroprotective effects against brain injury. Recently we have demonstrated that spinal intrathecal transplantation of human NSCs over-expressing VEGF (F3.VEGF) in transgenic SOD1/G93A mouse model of ALS (42) significantly delayed disease onset for 7 days and prolonged the survival of animals for 15 days (43). Our results suggest that this treatment modality using human NSCs might be of value in the treatment of ALS patients without significant adverse effects.

**Stroke**

Stroke represents the second highest among the causes of death in Asia including Korea, China and Japan. There are two major types of stroke and they are ischemia and intracerebral hemorrhage (ICH). Ischemic stroke caused by abrupt and near-total interruption of cerebral blood flow, and ICH by breakdown of intracerebral blood vessels, produces ischemic changes in the striatum and cortex, leading to a long-term sensorimotor deficit (44). Since current medical therapy against stroke shows only limited effectiveness, an alternative approach is required, such as stem cell-based cell therapy (45).

We have previously demonstrated intravenously transplanted F3 human NSCs could selectively migrate into lesioned brain sites, differentiate into new neurons and glial cells, and improve the functional deficits in rat stroke models of focal ischemia (46, 47) and cerebral hemorrhage (48-50). NSCs can circumvent blood-brain barrier and migrate to the specific pathologic areas of brain with home-in property. We introduced F3 immortalized human NSCs intravenously via tail vein, F3 NSCs migrated into the adult rat brain with focal cerebral ischemia or with cerebral hemorrhage, and induced marked improvement in sensorymotor functions. Transplanted F3 human NSCs migrated to the lesion sites, differentiated into neurons and astrocytes, and a large number of the grafted NSCs survived in the lesion sites for up to 12 weeks. Three-12 weeks post-transplantation, functional improvement was observed in the transplanted animals on rotarod and limb placement tests (46-50).

Previous studies have reported that the combined administration of NSCs and VEGF resulted in improved structural and functional outcome from cerebral ischemia. In our study, F3 human NSCs transduced with a retroviral vector encoding human VEGF were transplanted into cerebral cortex overlying ICH lesion, and at 2–8 weeks post-transplantation, there were improved survival of grafted NSCs, increased angiogenesis and behavioral recovery in mouse ICH model. ICH was induced in adult mice by unilateral injection of bacterial collagenase into striatum. F3.VEGF human NSCs produced an amount of VEGF four times higher than parental F3 cells in vitro, and induced behavioral improvement and 2–3 fold increase in cell survival at 2 weeks and 8 weeks post-transplantation (50).

In a recent study, F3 human NSCs were treated with an iron chelator (deferoxamine, DFX) to stabilize Hypoxia-inducible factor-1 (HIF-1) in NSCs and proactively transplanted into the rat brain 7 days prior to MCA occlusion-induced ischemia (51). HIF-1 is known to inhibit cerebral ischemic lesion (52). In ischemia animals with DFX-treated NSCs, the infarct volume was reduced indicating that proactively implanted HIF-1 stabilized human NSCs provide neuroprotection against ischemic injury in animals (51).

Intravenous transplantation of F3 human NSCs during the hyperactive stage of ICH (at 2 hr after ICH) induces anti-inflammatory and anti-apoptotic properties, reduced brain edema and reduction in expression of inflammatory cytokines in the brain and spleen. It is interesting to note that the spleen participates in cerebral inflammation during the ICH bout as splenectomy reduced cerebral edema and inflammatory cell count. These results indicate that the early intravenous administration of F3 human NSCs provides anti-inflammatory functionality that promotes neuroprotection in ICH lesion, by blocking splenic in-
**Brain cancer**

Malignant brain cancer such as glioblastoma multiforme remains virtually untreatable and lethal (54). Similarly in childhood brain cancer, medulloblastoma is the most common and incurable (55). Multimodal treatment including radical surgical resection followed by radiation and chemotherapy, have substantially improved the survival rate in patients suffering from these brain cancers; however, it remains incurable in large proportion of patients (56). Therefore, there is substantial need for effective, low-toxicity therapies for patients with malignant brain cancers, and gene therapy approach targeting brain cancer should fulfill this requirement.

During the last 20 years gene therapy research has advanced greatly. According to a recent study, over 1340 gene therapy clinical trials have been completed, or are ongoing worldwide in 28 countries, and more than 70% of these trials are in cancer gene therapy (57). In the brain cancer gene therapy, one of recent approaches is to use neural stem cells (NSCs) as a reliable delivery vehicle to target therapeutic gene products to primary and secondary invasive glioma, medulloblastoma, melanoma brain metastases and neuroblastoma throughout the brain and extracerebral loci (58, 59).

Human NSCs possess an inherent tumor tropism that supports their use as a. In recent studies, we have utilized the F3 immortalized human NSC line that stably expresses therapeutic genes designed to treat animal models of brain cancers via suicide gene therapy and immunotherapy approaches (60-69).

We have demonstrated that the human glioma cell lines produce HGF and VEGF, which act as potent chemotactants for HB1.F3 human NSCs (60, 61, 66). These growth factors, HGF and VEGF, stimulate receptor tyrosine kinase signaling that leads to the activation of phosphoinositide 3-kinase (PI3K), which has been shown to be an important regulator of directed cell migration (61). Inhibition of the PI3K pathway significantly inhibited the chemotactic cell migration towards all growth factors tested (HGF, VEGF and EGF), suggesting that the growth factors produced by brain tumors converge on the PI3K signaling pathway.

Suicide gene therapy for brain cancer is based on the conversion of non-toxic prodrugs into active anticancer agents via introduction of non-mammalian or mammalian enzymes. One of the earliest suicide gene/prodrug system is the herpes simplex virus thymidine kinase (HSVtk)/Ganciclovir (GCV) system (70, 71). In addition to HSVtk/GCV suicide gene/prodrug system, there have been several notable suicide enzyme-prodrug systems including cytosine deaminase (CD)/5-fluorocytosine (5-FC) (72, 73), and carboxylesterase (CE)/CPT-11 (74). In the CD/5-FC suicide gene system, CD deaminases the non-toxic pyrimidine 5-FC to the cytotoxic 5-fluorouracil (5-FU), and 5-FU is then processed to intermetabolites that inhibit RNA processing and DNA synthesis in the cancer cells (72).

When F3 human NSC line carrying CD enzyme gene (F3.CD) was transplanted intracranially at distant sites from the tumor, the F3.CD NSCs migrate through normal tissue and selectively 'home in' to the glioblastoma tumor mass and upon administration of prodrug 5-FC, 85~95% reduction in tumor volume was demonstrated (Kim, Unpublished).

Nude mice bearing human medulloblastoma were inoculated with F3.CD human NSC cells at the sites distant from tumor mass, and followed by systemic 5-FC treatment. Histological analyses showed that NSCs migrate to the tumor site leading to an 80% reduction of tumor volume (67). In metastatic leptomeningeal medulloblastoma lesions in spinal cord, F3.CD human NSCs were found to distribute diffusely to medulloblastoma tumor after injection in the cisterna magna, and CD gene in NSCs effectively catalyzed prodrug 5-FC into anticancer drug 5-FU and killed tumor cells by bystander effect (66).

PEX is a naturally occurring fragment of human metalloproteinase-2, and acts as an inhibitor of glioma proliferation, migration, angiogenesis and effectively inhibit tumor cell growth (75). In our study, F3 human NSCs carrying PEX gene, were found to "surround" the invading glioblastoma tumor cells, "chasing down" infiltrating tumor cells, and "attack and kill" tumor cells, causing a 90% reduction in tumor volume (68).

Patients diagnosed with metastatic cancer have almost uniformly poor prognoses. The treatments available for patients with metastatic cancer are usually not curative and have side effects that limit the therapy that can be given. The tumor-tropic property of NSCs could be utilized to selectively deliver a therapeutic gene to metastatic solid tumors, and that expression of an appropriate transgene at tumor loci might mediate cures of metastatic disease. In a recent study, F3 human NSCs transduced to express carbonyl esterase enzyme (CE) that efficiently activates the anti-cancer prodrug CPT-11 were injected intravenously into mice bearing disseminated neuroblastoma tumors, and F3.CE NSCs migrated selectively to tumor sites, then treated systemically with CPT-11, and the effi-
cacy of treatment monitored. Mice treated with the combination of F3 NSCs expressing the CPT-11-activating enzyme CE and prodrug CPT-11 produced tumor-free survival of 100% of the mice for more than 6 months (64, 65).

Cancer cells are immunogenic in nature with cancer-specific antigens being intracellular molecules, thus T-cell mediated immunity is more obvious than B-cell mediated immunity (76). Thus genetic immunotherapy aims to boost T-cell mediated immune response against cancer cells. One major immunotherapeutic approach involves the gene transfer of immune-stimulating cytokines including IL-4, IL-12, TRAIL and IFN-α/β. IFN-α/β has been known to have multiple antitumor effects including direct inhibition of tumor cell proliferation through both cell cycle arrest and induction of apoptosis (77, 78) as well as indirect antitumor activity through immunomodulation (79) and inhibition of angiogenesis (78). In the past, clinical trials with IFN-α/β in tumor therapy did not achieve expected outcome because of its extremely short half-life upon administration and their systemic toxicity. Recently we have generated F3 human NSCs overexpressing human IFN-β (F3. IFN-β) by adenoviral transduction and then intravenously injected in SCID mice bearing metastatic neuroblastoma. F3 human NSCs expressing IFN-β displayed a high tropism for metastatic neuroblastoma in liver and kidney and targeted delivery of antitumor IFN-β, resulting in significant reduction in tumor growth (69).

Treatment for brain cancers involves surgical resection followed by chemotherapy and radiotherapy. Previously numerous gene therapy trials for brain cancers particularly for malignant glioblastoma have been conducted but the most of these trials achieved only limited success. Recently (December 5, 2007), NIH Recombinant cDNA Advisory Committee (NIH RAC) has approved an application of the City of Hope Medical Center (Duarte, California outside of LA) to conduct a clinical trial in patients with recurrent high grade glioma using F3.CD immortalized human NSCs that have been retrovirally transduced to express CD therapeutic transgene (80); the human NSC cell line has been generated at my laboratory at the University of British Columbia, Vancouver, Canada. In animal models, the safety, feasibility, and efficacy of F3.CD human NSCs to track invasive tumor cells and distant micro-tumor foci and to deliver therapeutic gene products to tumor cells, have been demonstrated. Thus F3 human NSCs could provide an effective anti-tumor response overcoming obstacles facing current gene therapy strategies. In this pilot study with ten patients with recurrent glioma to determine the safety and feasibility of F3.CD immortalized human NSC line that expresses the suicide enzyme CD. Human NSCs injected in tumor resection sites will distribute throughout the primary tumor site and will co-localize with infiltrating tumor cells within in 5 days. An oral prodrug (5-FC), administered on the fifth day and continued for 7 days, will be converted to chemotherapeutic agent 5-FU by NSCs expressing CD, which will then be secreted at the tumor site to produce an anti-tumor effect (80).

The F3 human NSC line to be used in the glioblastoma clinical trial (F3.CD) at the City of Hope Medical Center (US) has been generated at my UBC laboratory (SUK), and is readily available for clinical trials in Korea. I am currently working continuously to prepare and carry out the clinical trial of NSC-based cancer gene therapy for glioblastoma multiforme in collaboration with clinicians in major medical centers in Seoul, Busan and Gwangju.

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Potential Conflict of Interest

The authors have no conflicting financial interest.

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