IMMORTALIZED HUMAN NEURAL STEM CELL LINE GENETICALLY MODIFIED FOR BRAIN REPAIR IN NEUROLOGICAL DISEASES

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Cell replacement and gene transfer to the diseased or injured CNS 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), amyotrophic lateral sclerosis (ALS), Alzheimer disease (AD), stroke and spinal cord injury. In PD and HD, previous studies have documented improvements in motor and cognition performance in patients following fetal human brain cell transplantation. However ethical, religious and logistic difficulties associated with the use of fetal tissues limit wide adoption of this approach. An ideal source of cell transplantation in neurological diseases is human neural stem cells (NSCs) that could integrate into host brain tissue and differentiate into neurons or glial cells in response to environmental factors. Successful application of in vivo gene transfer to the CNS will depend on the identification of suitable cells that can serve as carriers for a wide range of potentially therapeutic transgenes and provide platforms for functionally efficient expression and secretion of transgene products. Immortalized CNS-derived NSCs have recently been introduced as potentially interesting candidates for this purpose.

Stable cell lines of human NSCs were generated from primary cultures of human fetal telencephalon (14 weeks gestation) via transfection with a retroviral vector encoding v-myc gene and one of the cell lines, HB1.F3, expresses nestin and ABCG2, both cell type-specific markers for neural stem cells and normal human karyotype of 46XX without any chromosomal abnormality. F3 NSC cell line has the ability to self-renew, differentiate into cells of neuronal and glail lineage (Cho et al., Neuroreport 13, 1447, 01), and integrate into the damaged CNS loci upon transplantation into the brain of animal models of stroke (Jeong et al., Stroke 34, 2258, 03) and lysosomal storage disease (Meng et al., J Neurosci Res 74, 266, 03).

For brain transplantation studies in animal models of PD, F3 NSC cell line was transduced to carry human genes, tyrosine hydroxylase (TH) and GTP cyclohydrolase I (GTPCHI), via retrovirally mediated procedures. The latter is an enzyme responsible for production of tetrahydrobiopterine, a co-factor essentail for catecholamine biosynthesis. HPLC analyses of L-DOPA produced by the cell lines were 62 ng/hr/10^6 cells in F3.TH.GTPCH and 30 pg/hr/10^6 cells in control F3.TH cell line (more than 2000-fold increase). L-DOPA producing F3.TH.GTPCH NSCs were transplanted into the striatum of rat model of PD (6-hydroxydopamine-induced substantia nigra pathology) to replace missing dopaminergic neuron population, and the results indicate that there was a marked improvement in amphetamine-induced turning behavior in these animals 4-12 weeks post-operation and a good survival of TH-positive cells in the grafted sites in striatum.

For brain transplantation studies for HD, HB1.F3 human NSC line was transduced with a retroviral vector encoding BDNF gene, and F3.BDNFcells actively produced BDNF at 62 ng/ 6 hr/ 10^6 cells. Proactive transplantation of BDNF-producing human NSCs protected striatal neurons from cell death caused by excitotoxic application of quinolinic acid 1 week later. Proactive transplantation of BDNF-producing human NSCs resulted in increased survival of NeuN+, GAD+ and ChAT+ neurons (Neurobiol Disease, in press) and also in improved behavioral performance.

The results of the present study have particular relevance to cell therapy in neurodegenrative diseases including PD, HD, ALS and AD and also for stroke and spinal cord injury. NSC transplantation has the great potential to prevent or restore anatomic of functional deficits associated with injury or disease in the CNS via cell replacement, the release of specific neurotransmitters, and the production of factors that promote neuronal growth and regeneration.

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