ARC '16

مؤتمر مؤسسة قطر
السنوي للبحوث
QATAR FOUNDATION
ANNUAL RESEARCH
CONFERENCE

Towards World-class
Research and Innovation

مؤسسة قطر
Qatar Foundation
لإطلاق قدرات الإنسان
Unlocking human potential

Health and Biomedical Pillar

http://dx.doi.org/10.5339/qfarc.2016.HBPP1046

# Human Olfactory Bulb Neural Stem Cell Based Therapy for CNS Traumatic and Neurodegenerative Diseases

**Hany Marei[1], Asmaa Althani[2]**

[1]Professor, Project Manager, Biomedical Research Center, New Research Complex (NRC), Zone 5, Room D130, Qatar University, P.O.Box: 2713, Doha, Qatar. Tel. No.: 00974-4403-6817, QA
[2]Department of Health Sciences, College of Arts and Science, Qatar University, Doha, 2731, QA

Email: hmady@qu.edu.qa

Neural stem cells (NSC) have self-renewal and multipotent properties and may serve as an ideal cell source for transplantation to treat neurodegenerative insults such as Parkinson's, Alzheimer's, and spinal cord injury (SCI). Obtaining NSCs from adult human olfactory bulb (OB) would avoid ethical issues associated with the use of embryonic tissue, and provide an easily accessible cell source that would preclude the need for invasive brain surgery.

We used Agilent and Illumina Whole Human Genome Oligonucleotide Microarray to compare the genomic profiles of human embryonic NSC at a single time point in culture; and a multicellular tissue from postmortem adult substantia nigra (SN), an area rich in dopaminergic (DA) neurons. We identified 13525 up-regulated genes in both cell types of which 3737 (27.6%) genes were up-regulated in the hENSC, 4116 (30.4%) genes were up-regulated in the human substantia nigra dopaminergic cells, and 5672 (41.93%) were significantly up-regulated in both cell populations. Careful analysis of the data using DAVID has permitted us to distinguish several genes and pathways involved in dopaminergic (DA) differentiation, and to identify the crucial signaling pathways that direct this process. The set of genes expressed more highly at hENSC is enriched in molecules known or predicted to be involved in the M phase of the mitotic cell cycle. On the other hand, the genes expressed in SN cells include a different set of functional categories, namely synaptic transmission, central nervous system development, structural constituents of the myelin sheath, the internode region of axons, myelination, cell projection, cell somata, ion transport, and the voltage-gated ion channel complex. These data were compared with data from various databases, and between different types of arrays, Agilent versus Illumina. This approach has allowed us to confirm the consistency of our results for a large number of genes that delineate the phenotypical differences of embryonic NSCs, and SN cells.

**Cite this article as:** Marei H, Althani A. (2016). Human Olfactory Bulb Neural Stem Cell Based Therapy for CNS Traumatic and Neurodegenerative Diseases. Qatar Foundation Annual Research Conference Proceedings 2016: HBPP1046 http://dx.doi.org/10.5339/qfarc.2016.HBPP1046.

Next we studied global gene expression profiling using RNA from human embryonic neural stem cells (hENSC), and adult human olfactory bulb-derived neural stem cells (OBNSCs), to define the gene expression pattern and signaling pathways specific for each cell lineage. We demonstrated large differences in the gene expression profile of human embryonic NSC, and adult human OBNSCs, but less variability between parallel cultures. Transcripts of genes involved in neural tube development and patterning (ALDH1A2, FOXA2), progenitor marker genes (LMX1a, ALDH1A1, SOX10), proliferation of neural progenitors (WNT1 and WNT3a), neuroplastin (NPTN), POU3F1 (OCT6), neuroligin (NLGN4X), MEIS2, and NPAS1 were up-regulated in both cell populations. Using Gene Ontology, 325 out of 3875 investigated gene sets were different. 41 out of the 307 investigated Cellular Component (CC) categories, 45 out of the 620 investigated Molecular Function (MF) categories, and 239 out of the 2948 investigated Biological Process (BP) categories were significant. KEGG Pathway Class Comparison revealed that 75 out of 171 investigated gene sets passed the 0.005 significance threshold. Levels of gene expression were explored in three signaling pathways, Notch, Wnt, and mTOR, all known to be involved in NS cell fate determination. The transcriptional signature also clarifies the role of genes involved in epigenetic modifications. The SWI/SNF DNA chromatin remodeling complex family, including SMARCC1 and SMARCE1, was specifically up-regulated in OBNSC but not in hENSC. Differences in the gene expression profile of transcripts controlling epigenetic modifications and signaling pathways might indicate differences in the therapeutic potential of the two cell populations with respect to potential cell survival, proliferation, migration, and differentiation following engraftments in different CNS insults.

Next, the transcriptional factors (TF) and genomic markers associated with neurogenesis, proliferation, differentiation, and epigenetic control in human embryonic neural stem cells (hENSC), and adult human olfactory bulb neural stem cells (OBNSC) were studied using immunohistochemistry (IHC) and DNA microarrays. The biological impact of TF gene changes in the examined cell types was estimated using DAVID to specify a different GO class and signaling pathway based on KEGG database. Eleven, and twenty eight TF genes were up-regulated (fold change≤2–39) in OBNSC, and hENSC respectively. KEGG pathway analysis for the up-regulated TF genes revealed significant enrichments for the basal transcription factor pathway, and Notch signaling pathway in OBNSCs, and hENSCs, respectively. Immunofluorescence analysis revealed a significantly greater expression of β-tubulin III (TUBB3),MAP, glial fibrillary acidic protein (GFAP), and O4 in hENSC compared to OBNSC. Furthermore, the expression of epigenetic-related TF-genes SMARCC1, TAF12, and UHRF1 increased significantly in OBNSC when compared with hENSC.

This suggests that exogenous application of NGF may be a promising therapeutic strategy for traumatic and neurodegenerative diseases. However, effective delivery of NGF into the CNS parenchyma is still challenging due to its limited ability to cross the blood–brain barrier, and the intolerable side effects if administered into the brain ventricular system. An effective method to ensure delivery of NGF into the parenchyma of CNS might be genetic modification of NSC to overexpress the NGF gene. Overexpression of NGF in adult human OBNSC is expected to alter their proliferation and differentiation, possibly enhancing their therapeutic potential. We genetically modified adult human OBNS/PC to overexpress human NGF (hNGF) and green fluorescent protein (GFP) genes, hoping to provide insight about the effects of hNGF and GFP gene overexpression in adult human OBNS/PC and on their in vitro multipotentiality using DNA microarray, immunophenotyping, and Western blot (WB) protocols. Our analysis revealed that OBNS/PC-GFP and OBNS/PCGFP- hNGF differentiation is a multifaceted process involving changes in major biological processes as reflected in alteration of the gene expression levels of crucial markers such as cell cycle and survival markers, stemness markers, and differentiation markers. The differentiation of both cell classes was also associated with modulations of key signaling pathways such MAPK signaling pathway, ErbB signaling pathway, and neuroactive ligand-receptor interaction pathway for OBNS/PC-GFP, and axon guidance, calcium channel, voltage-dependent, gamma subunit 7 for OBNS/PC-GFP-hNGF, as revealed by GO and KEGG. Differentiated OBNS/PC-GFP-hNGF displayed extensively branched cytoplasmic processes, a significantly faster growth rate and up modulated the expression of oligodendroglia precursor cells markers (PDGFRα, NG2 and CNPase) respect to OBNS/PC-GFP counterparts. These findings suggest an enhanced proliferation and oligodendrocytic differentiation potential for OBNS/PC-GFP-hNGF as compared to OBNS/PC-GFP.

To assess the therapeutic potential of OBNSCs, we studied the fate of allogenic adult human olfactory bulb neural stem/progenitor cells (OBNSC/NPCs) transplanted into the rat hippocampus treated with ibotenic acid (IBO), a neurotoxicant specific to hippocampal cholinergic neurons that are lost in Alzheimer's disease. We assessed their possible ability to survive, integrate, proliferate, and differentiate into different neuronal and glial elements: we also evaluated their possible therapeutic potential, and the mechanism(s) relevant to neuroprotection following their engraftment into the CNS milieu. The isolated OBNSC/NPCs-hNGF were stereotaxically transplanted into the hippocampus of IBO-treated animals and controls. Stereological analysis of engrafted OBNSCs eight weeks post transplantation revealed a 1.89 fold increase with respect to the initial cell population, indicating a marked ability for survival and proliferation. In addition, 54.71_11.38%, 30.18_6.00%, and 15.09_5.38% of engrafted OBNSCs were identified by morphological criteria suggestive of mature neurons, oligodendrocytes and astrocytes respectively. Taken together, this work demonstrated that human OBNSCs expressing NGF ameliorate the cognitive deficiencies associated with IBO-induced lesions in AD model rats, and the improvement can probably be attributed primarily to neuronal and glial cell replacement as well as the trophic influence exerted by the secreted NGF.

Next, the hNFG-GFP-OBNSCs were transplanted into the striatum of 6-OHDA Parkinsonian rats. The grafted cells survived in the lesion environment for more than eight weeks after implantation with no tumor formation. The grafted cells differentiated in vivo into oligodendrocyte-like (25 ±2.88%), neuron-like (52.63 ±4.16%), and astrocytic-like (22.36 ±1.56%) lineages based on morphological criteria. Transplanted rats exhibited a significant partial correction in stepping and placing non-pharmacological behavioral tests, using a pole and rotarod. Taken together, our data encourage further investigations for the possible use of OBNSCs as a promising cell-based therapeutic strategy for Parkinson's disease.

Finally, hNGF-GFP-OBNSCs were engrafted in a rat model of SCI at day 7 post injury. All transplanted animals exhibited successful engraftment. The survival rate was about 30% relevant to initially transplanted cells. 27% of the engrafted cells differentiated along the oligodendrocyte, nearly as many (16%) differentiated into neurons, and about 56% of the cells displayed astrocyte morphology. The study revealed that hNGF-GFP-OBNSCs were able to survive in the lesion environment for more than eight weeks after implantation; this was supported by transgenic overexpression of hNGF on engrafted cells. We didn't observe locomotor recovery by BBB test, footprint analysis and grid walk tests three months post-treatment. No sign of immunorejections were recorded during the 8 week time window of the study. Engrafted cells were distributed throughout gray and white matter of the cord with no evidence of abnormal morphology or any mass formation indicative of tumorigenesis.