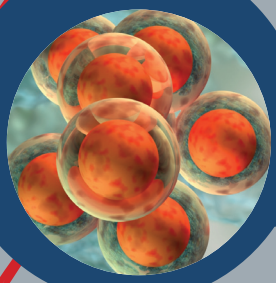

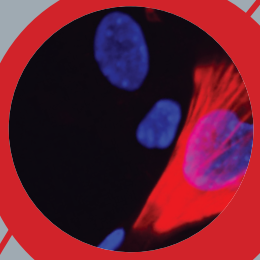

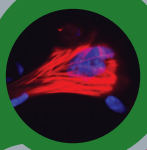

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Mouse neural crest stem cells image ©Deepak Srivastava

Friday Poster Abstracts

Poster Board Number: 1270

DIFFERENTIATION OF HUMAN PARTHENOGENETIC STEM CELLS INTO NEURAL STEM CELLS *IN VITRO*

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Unfertilized human oocytes can be artificially activated by appropriate chemical stimuli to develop into parthenogenetic blastocysts, the inner cell mass of which can be isolated and expanded as stem cell lines. Human parthenogenetic stem cells (hpSC) are similar to human embryonic stem cells (hESC) in their proliferation capacity and multilineage *in vitro* differentiation. The hpSC can be either heterozygous or homozygous depending on the way the genome forms from only the maternal chromosome set. Homozygous hpSC could be useful as a source of cells for use in transplantations as the set of HLA genes in hpSC may make differentiated derivatives less susceptible to immune rejection. Furthermore, if the HLA type is common, differentiated derivatives will match many millions of individuals. In addition to these immunogenetic advantages, as parthenogenesis does not involve the destruction of a viable human embryo, the use of hpSC does not raise the same ethical concerns as conventional hESC. Together these two advantages make hpSC a very promising and potentially unlimited source of numerous differentiated somatic cell lines including multipotent neural stem cells (NSC). In this study we derive NSC from hpSC using an approach based on the adherent model with modifications. Primary neural induction was provoked immediately in the colonies of hpSC grown in the absence of feeder cells for 5 days by replacing the culture medium with another one containing bFGF in higher concentration and no serum. After seven days under such conditions rosettes of neuroepithelial cells formed, recapitulating the neural tube of a developing embryo. The rosettes generated in this way expressed specific set of neuroepithelial markers including PAX6, SOX1, NES (Nestin), MSI1 (Musashi-1), and did not express pluripotency marker OCT4 (POU5F1). These rosettes were isolated, disaggregated into single cell suspension and then propagated as an adherent culture. These human parthenogenetic NSC (hpNSC) were passaged every 4-5 days enzymatically with a 1:2 split rate for more than 15 passages. Reverse transcriptase real-time quantitative PCR (qRT-PCR) revealed the expression of specific neural markers NES (Nestin), SOX2 and MSI1 (Musashi-1) in the hpNSC which was also confirmed by immunocytochemical staining of corresponding proteins. The expression of OCT4 was not detected at RNA and protein levels. The cells keep uniform morphology up to at least 15 passages and do not show constantly increasing levels of ectomesenchymal markers SNAI1 and FOXD3 as detected by qRT-PCR. In order to produce neurons and glia cells, we allowed the low density-seeded hpNSC to spontaneously differentiate in culture medium without growth factors for three weeks. After this time most of the differentiated cells have acquired specific neuron morphology and were positive for anti-Tuj1 (Tubulin beta III) labeled antibodies. The expression of neuronal markers TUBB3 (Tubulin beta III) and MAP2 as well as glial markers FOXO4 and GFAP was detected in these differentiated cells by qRT-PCR. Thus, in this study we demonstrated that human multipotent NSC are stable to long-term passaging can be derived from pluripotent hpSC followed by differentiation into neurons and glia.

Poster Board Number: 1272

INTRAARTERIAL DELIVERY OF HUMAN EMBRYONIC-DERIVED NEURAL STEM CELLS YIELDS FUNCTIONAL RECOVERY AND GREATER MYELINATION FOLLOWING HYPOXIC-ISCHEMIC STROKE IN NEONATAL RATS

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Perinatal hypoxic-ischemic insults are a significant cause of neonatal encephalopathy, developmental delays, epilepsy, and cerebral palsy. Studies have shown that demyelination and periventricular leukomalacia are common pathological changes seen in infants following hypoxia-ischemia (HI). While many studies have evaluated the effect of human embryonic-derived neural stem cells (hNSCs) on neurogenesis, angiogenesis and immunomodulation, the impact of hNSC treatment on myelination has not been adequately characterized. Using a neonatal rat model, we investigated the effect of hNSC treatment on remyelination and functional recovery following hypoxia-ischemia. Neonatal Wistar rat pups underwent left common carotid artery ligation followed by placement in a hypoxia chamber with 8% oxygen at 37C on post-natal day 7 (P7). Stroke size was evaluated on P9 using T2-weighted MRI. On P10, the neonates underwent intraarterial injection of either 500,000 fLuc/eGFP transduced hNSCs suspended in 0.9% saline or 0.9% saline only. *In vivo* bioluminescence images utilizing luciferin were obtained 1, 2, 3, 4, 7 and 10 days after injection. BrdU was administered intraperitoneally for 6 days following treatment to assess cell proliferation. Immunohistochemistry stains to evaluate hNSC survival and differentiation were done on stroke-size matched brains in addition to stains for Olig2, NG2 and CNP. Myelination was evaluated using luxol fast blue (LFB) and myelin basic protein (MBP) staining 10 days and 30 days post-treatment. RT-qPCR was performed on hNSCs and the stroked hemisphere of neonates from both treatment groups. Functional recovery was assessed using the elevated open-arm task and novel object recognition task at P30. Bioluminescence imaging demonstrated significant homing of hNSCs to the stroked hemisphere 1 day ($p=0.001$), 2 days ($p=0.002$), 3 days ($p=0.002$), 4 days ($p<0.0001$), and 7 days ($p=0.001$) following transplant. Iba-1-/GFAP-hNPCs were localized to the corpus callosum and cortex of cell-treated animals 3 days, 10 days, and 30 days after treatment. Counts of BrdU+ cells indicated a significant increase in cell proliferation around the lateral ventricle ($p=0.036$) and in the corpus callosum ($p=0.020$) of cell-treated animals compared to saline-treated animals. Cell-treated neonates also showed an increase in Olig2+ and NG2+ cells in the striatum 3 and 10 days post-treatment. LFB and MBP staining demonstrated greater myelination 10 days and 30 days after treatment in the corpus callosum ($p=0.022$, $p=0.049$) and striatum ($p=0.017$, $p=0.001$) of cell-treated animals. Increased expression of mRNA associated with cell proliferation (TGF1, p53, Stat3) and immunomodulation (IL-10, ApoE, Hsp1) were seen in the stroked hemisphere of cell-treated animals. Neonates transplanted with hNPCs demonstrated better performance on the elevated open arm task ($p=0.050$) and novel object recognition task ($p=0.016$). Intraarterial hNSC delivery following hypoxia-ischemia in neonatal rats resulted in early hNSC homing to the injured brain with engraftment seen up to 30 days following treatment. Behavioral performance on the elevated open arm task and novel object recognition task suggest cell-treated animals have reduced levels of anxiety and improved working memory compared to saline-treated animals. Functional recovery may be mediated by improved myelination and stimulation of oligodendrocyte-lineage cell proliferation in the corpus callosum and striatum.