

**P-25**      **Differentiation of Cholinergic Neuron from Human Umbilical Cord Blood Derived Mesenchymal Stem Cells**

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**Object:** We studied transdifferentiation-promoting conditions and cholinergic neuron induction of Human umbilical cord blood (HUCB) derived mesenchymal stem cells (MSCs). And survival and neural projection of grafted MSCs were investigated using the middle cerebral artery occlusion (MCAO) induced rat brain organotypic slice cultures.

**Method:** MSCs was confirmed by immunophenotyping. To induce neuronal differentiation of cells, dimethyl sulphoxide (DMSO) and butylated hydroxyanisole (BHA) in N2 medium and N2 supplement were treated. Differentiation of cells to cholinergic neurons were induced by basic fibroblast growth factor (bFGF), retinoic acid (RA) and sonic hedgehog (Shh). And PKH26 labeled MSCs were seeded onto the MCAO induced ischemic rat brain organotypic slices. Combined cultures were maintained for 7days in vitro. To confirm the neuro-glial characteristics of differentiated cells, immunocytochemistry stain for  $\beta$ -tubulin III, GFAP, Gal-C and ChAT were performed. RT-PCR was performed for detecting NeuroD1, GFAP and MBP mRNA. The grafted cells onto slice culture were visualized under fluorescence microscopy.

**Result:** We showed in this experiment that neuro-glia markers ( $\beta$ -tubulin III, GFAP and Gal-C) were expressed. The rate was about 30% as Neuron, 15% as Astrocyte, 10% as Oligodendrocyte. HUCB derived MSCs treated bFGF, RA and Shh were differentiated into cholinergic neurons that were immunopositive for ChAT antibody. In organotypic brain slice culture, transplanted cells were found to survive and to extend their fiber.

**Conclusion:** These results suggest that HUCB derived MSCs may be potential sources of treatment for neurodegenerative diseases such as Alzheimer's disease.

**Key Words:** Human umbilical cord blood mesenchymal stem cell, Cholinergic neuron differentiation, Organotypic slice culture, Ischemic brain