

P-15

## Induction of neuron and glia cells from human amniotic mesenchymal cells in vitro and ex vivo

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**Purpose** : Mesenchymal stem cells(MSC) are one of the well-studied adult stem cells. Human MSC can differentiate into various cells including neurons. We investigated the ability of human amniotic mesenchymal (HAM) cells to differentiate into neural cells under experimental cell culture conditions. And we evaluated survival and neural projection of grafted HAM cells using organotypic slices of the middle cerebral artery occlusion (MCAO) induced rat ischemic brain model.

**Method** : To induce neuronal differentiation of HAM cells, dimethyl sulphoxide (DMSO), butylated hydroxyanisole (BHA) and N2 supplement in N2 medium were treated. Differentiation of cells to cholinergic neurons were induced by basic fibroblast growth factor (bFGF), retinoic acid (RA) and sonic hedgehog (Shh). Dopaminergic neuronal differentiation was induced by FGF8 and Shh. pKH 26 labelled HAM cells were seeded on the rat ischemic or pup brain organotypic slice. Combined cultures were maintained for 7 days in vitro. To confirm the neuro-glial characteristics of differentiated cells, immunocytochemistry stain for  $\beta$ -tubulin III, GFAP, Gal-c, ChAT, and TH were performed. RT-PCR was performed for detecting NeuroD1, GFAP and MBP mRNA.

Neurite outgrowth from grafted cells onto slice culture was visualized using immunohistochemistry.

**Result** : We showed in this experiment that neuro-glia markers ( $\beta$ -tubulin III, GFAP and Gal-c) were expressed. The rate was about 36% as neuron, 14% as astrocyte, 16% as oligodendrocyte. HAM cells treated with bFGF, RA and Shh were differentiated into cholinergic neurons that were immunopositive for ChAT antibody. HAM cells treated with FGF8 and SHH were differentiated into dopaminergic neurons that were immunopositive for TH antibody. In organotypic brain slice culture, transplanted cells were found to survive and to extend their fiber.

**Conclusion** : These results suggest that HAM cells were transdifferentiated into neuronal cells.

Moreover these cells were differentiated into dopaminergic or cholinergic neurons. That means HAM cells may be potential sources of treatment for neurodegenerative diseases.

**Keywords**: amniotic mesenchymal cell, cholinergic neuron, dopaminergic neuron, organotypic slice culture, ischemic brain.