# Neurodifferentiation of Mesenchymal Like Stem Cells from Human Amnion and Umbilical Cord in vitro and in vivo

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Stem cells have been extensively studied by many researchers for the cell replacement therapy in various incurable diseases. Despite their pluripotency, usage of embryonic stem cells (ESC) and mesenchymal stem cells (MSC) are one of the well-studied adult stem cells as therapeutic and clinical applications. In recent years, great interest has been generated in MSC, due to their ability to self-renew, proliferate, and differentiate into a variety of cell types for the practical medicines. Since found in adult bone marrows, many studies have shown that under appropriate culture conditions in vitro, human MSC can differentiate into various cells including chondrocytes, osteocytes, adipocytes, hepatocytes, smooth muscle cells, astrocytes, glial cells, neurons and others (Barry and Murphy, 2004). Moreover, by transplanting into animals, human MSC derived from adult bone marrow have also been shown to differentiate into diverse tissues in vivo (Jiang et al., 2002). This and other results raised a possibility of the utilization of MSC as a universal cell source for cell-based therapy and a large amount of research have been carried out.

However, MSC are found not only in bone marrow but also in other tissues. They were isolated from the connective tissues of dermis and skeletal muscle derived from fetal, mature, and geriatric humans (Young et al., 2001), from lipoaspirate of human adipose tissue (Zuk et al., 2001), synovial membrane of knee joints (De Bari et al., 2001), from normal (Zvaifleret al., 2000) or growth factor-mobilized peripheral blood (Fernandez et al., 1997). Particularly extraembryonic tissues such as amniotic fluid (In't Anker et al., 2003), umbilical cord blood (Erices et al., 2000), placenta (Fukuchi et al., 2004) and umbilical cord (Covas et al., 2003: Romanov et al., 2003) have also been shown to contain MSC.

Whilst MSC appear to be derived mainly from mesoderm giving rise to the cells of the connective tissues during development, their differentiation is not restricted to the tissues of mesodermal origin. Several studies from different laboratories have recently reported that human or rodent MSC can be directed in vitro into a neuronal or astrocytic fate. Adult rat bone marrow cultured *in vitro* with epidermal growth factor (EGF) and basic fibroblast growth factor gave rise to cellular spheres which differentiated into neurons and glia (Kabos et al., 2002). Human bone marrow stromal cells also differentiated into a neuronal phenotype, under a simple culture condition (Woodbury et al., 2000). Adult rat ischemic brain that received MSC exhibited significantly improved functional performance and histological analyses revealed that transplanted human MSC expressed markers for astrocytes, oligodendroglia, and neurons (Zhao et al., 2002). These observations indicate that MSC may be potential sources of treatment for various neurodegenerative diseases.

In this study, we used four different population of MSC derived from human amnion and umbilical cord - umbilical cord vein, umbilical cord blood CD34-, Wharton's jelly, and amniotic membrane and investigated the neural differentiation of MSC as an optimum candidate for the cell therapy. Firstly, we showed that these cells could differentiate into neural cells under experimental cell culture conditions. Secondly, we tested whether intravenously infused human MSC enter brain, survive, differentiate, and improve neurological functional recovery in ischemic rat brain model.

To induce neuronal differentiation of MSC, dimethyl sulphoxide (DMSO), butylated hydroxyanisole (BHA) and N2 supplement in N2 medium were treated. Differentiation of MSC 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 cells were seeded on the rat ischemic or pup brain organotypic slice. Combined cultures were maintained for 5 days in vitro. To confirm the neuro-glial characteristics of differentiated cells, immunocytochemistry stain for NeuN, β-tubulin III, GFAP, Gal-C, ChAT, and TH were performed. RT-PCR was performed for detecting MSC and neuron marker. We showed in this experiment that neuro-glia markers (β-tubulin III, GFAP and Gal-c) were expressed. The expression rate was about 30%~40% as neuron, 15% as astrocyte, 10%~16% as oligodendrocyte. MSC treated with bFGF, RA and Shh were differentiatied into cholinergic neurons that were immunopositive for ChAT antibody. Also MSC treated with FGF8 and SHH were differentiatied into dopaminergic neurons that were immunopositive for TH antibody.

Adult SD ovariectomized rats were subjected to transient (1-hour) middle cerebral artery occlusion (MCAO) and then reperfusion. Experimental groups were as follows: group 1, MCAO alone; group 2, $3\times 10^6$ PKH26 labeled MSC injected into tail vein at 24 hours after MCAO. Rats were killed at 35 days after MCAO. Behavioral test (mNSS) were performed. Immunohistochemical staining was used to identify cells derived from MSC. Treatment at 24 hours after MCAO with MSC significantly improved functional recovery, as evidenced by the mNSS. A few MSC were reactive for the astrocyte marker gilial fibrillary acidic protein (GFAP), the neuronal marker β-tubulin III and oligodendrocyte marker Gal-C.

These results suggest that four different population of MSC derived from human amnion and umbilical cord were transdifferentiated into neuronal cells. Moreover these cells were differentiated into dopaminergic or cholinergic neurons. That means these MSC may be potential sources of treatment for various neuro-degenerative diseases.