Investigation of p75 neurotrophin receptor on human Dental Pulp Stem Cells (hDPSC)

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Declaration

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Abbreviations

Alpha- Modified eagles medium (a-MEM)

Analysis of variance (ANOVA)

β-mercaptoethanol (BME)

Brain derived neurotrophic factor (BDNF)

Basic fibroblast growth factor (bFGF)

Bovine serum albumin (BSA)

Butylated hydroxyanisole (BHA)

Calcium (Ca^{2+})

Central nervous system (CNS)

Dental pulp stem cells (DPSC)

Dulbecco's modified eagles medium (DMEM)

Dimethyl sulfoxide (DMSO)

Dorsal root ganglia (DRG)

4', 6-diamidino-2-phenylindole (DAPI)

Epidermal growth factor (EGF)

Embryonic stem cells (ESC)

Enzyme linked immunosorbent assay (ELIZA)

Ethylenediaminetetraacetic acid (EDTA)

Extracellular matrix (ECM)

Fluorescence activated cell sorting (FACS)

Foetal calf serum (FCS)

Glial-derived neurotrophic factor (GDNF)

Glial fibrillary acidic protein (GFAP)

Granulocyte-colony stimulating factor (G-CSF)

Ham's F-12 (F12)

Hanks balanced salt solution (HBSS)

Hematopoietic stem cells (HSC)

Human dental pulp stem cells (hDPSC)

Human immunodeficiency virus (HIV)

3-isobutyl-1- methylxanthine (IBMX)

Insulin, transferring & sodium selenite premix (ITS)

Melanoma cell adhesion molecule (MCAM),

Myenteric plexus (MP)

Mesenchymal stem cells (MSC)

Microelectrode arrays (MEA)

Middle cerebral artery occlusion (MCA-O)

Nerve growth factor (NGF)

Neural crest (NC)

Neural stem cells (NSC)

Neurofilament medium chain (NFM)

Neuronal nuclei (NeuN)

Nitric dioxide (NO)

Neurotrophin 3 (NT3)

Paraformaldehyde (PFA)

Penicillin-streptomycin-glutamine (PSG)

Peripheral nervous system (PNS)

Poly-l-lysine (PLL)

Propidium iodide (PI)

Polysialylated neural cell adhesion molecule (PSA-NCAM)

Recombinant tissue plasminogen activator (r-TPA)

Rostral migratory stream (RMS)

Standard error means (SEM)

Subventricular zone (SVZ)

Subgranular zone (SGZ)

Submucosal plexus (SP)

12-O-tetradecanoylphorbol 13-acetate (TPA)

Transcranial magnetic stimulation (TMS)

Tyrosine kinase receptors (Trk)

Abstract

p75 neurotrophin receptor has recently been suggested as a neural stem cell marker in cells of the brain subventricular zone and brain subgranular zone. Human adult dental pulp stem cells (hDPSC) with the ability to differentiate into neural, chondrocyte, osteocyte and adipocyte lineages contain heterogeneous stem cell populations. p75 is prototypically a neutrophin receptor. hDPSC expressing the neural precursor marker nestin are able to differentiate into functionally active neurons *in vitro* under differentiation protocols, though there is no definitive method established yet. p75 is thus likely to delineate a population of hDPSC that become neural cells. Therefore, this current project aims to characterize p75 expression on hDPSC by immunohistochemistry and flow cytometry (chapter 3); investigate the role of p75 on hDPSC neural potential *in vitro* (chapter 4)*;* and investigate hDPSC neural differentiation *in vitro* through neurosphere formation (chapter 5).

This current study demonstrated that p75 is a neural stem cell marker in hDPSC cultures and defines a cell population with the potential to give rise to neurons and glial cells. Immunohistochemistry showed that $p75^+$ hDPSC had higher expression of SOX1, SOX2, nestin, CD146 and SOX9 (nucleus) when compared to $p75^-$ hDPSC. Neurons generated from $p75^+$ hDPSC exhibited more neuronal-like properties in their morphology, and immunohistochemical expression pattern, in particular with neuronal marker neurofilament medium chain (NFM). This study has also shown that hDPSC are able to differentiate into a neural lineage via neurosphere formation *in vitro*.

The results indicated that $p75^+$ hDPSC are a functional cell population which could mediate the hDPSC neural protection and neural replacement seen in stem cell therapy for stroke brain repair. This study identified a molecular target that could be used to enrich populations through cell

sorting. In addition, the hDPSC neurosphere differentiation provides a superior analysis model to investigate the neural potential of hDPSC *in vitro*.