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EXPERIMENTAL STUDY

An efficient induction protocol for deriving mature oligodendrocytes from human dental stem cells

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ABSTRACT

INTRODUCTION: This study examined the process of deriving mature oligodendrocytes from human dental pulp stem cells (hDPSCs) via addition of cerebrospinal fluid (CSF).

METHODS: The hDPSCs were cultured in the presence of retinoic acid and CSF. The oligodendrocytes were confirmed using immunocytochemistry for specific glial markers, namely Olig2 and MBP markers.

RESULTS: The differentiated oligodendrocytes were immunopositive for Olig2 and MBP markers at the end of induction phase.

CONCLUSION: It is concluded that this study indicated the glial differentiation of hDPSCs in the presence of CSF and appropriate inducers, which is a usable therapeutic technique in neuroregenerative medicine *(Fig. 3, Ref. 24)*. Text in PDF *www.elis.sk.*

KEY WORDS: human dental pulp stem cells, oligodendrocyte, cerebrospinal fluid, retinoic acid.

Introduction

Oligodendrocytes form the myelin sheet which surrounds the axons. The impairment of these cells disrupts the myelin structure and leads to severe neurological symptoms (Grade et al, 2013; Czepiel et al, 2015). Cell therapy is considered an efficient method to improve remyelination in demyelination diseases such as multiple sclerosis (MS) (Chang et al, 2014; Bojnordi et al, 2017). Among various types of stem cells, human dental pulp stem cells are recognized as a novel noninvasive source with the property of neuroglial differentiation under *in vitro* culture conditions (Sloan et al, 2007; Gronthos et al, 2002). However, *in vitro* culture derivation of mature oligodendrocytes is accompanied with some limitations. Cerebrospinal fluid (CSF) can promote the neuroglial differentiation of mesenchymal stem cells (Johanson et al, 2008; Miyan et al, 2003). Nevertheless, the effect of CSF on *in vitro* differentiation of hDPSC to glial differentiation has not been investigated so far. Therefore, the aim of our study was to evaluate the differentiation potential of hDPSC into oligodendrocytes in the presence of glial inducers and CSF.

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Based on the fact that the enrichment plexus of CSF consists of various growth elements and essential factors, we used CSF and designed an efficient culture system by adding CSF to a cocktail of inducers. Our culture system is usable as an alternative procedure leading to significant enhancement in the process of generating oligodendrocytes from BMSC.

Material and methods

CSF Collection

Rat embryos were used for collecting CSF in each experiment. Cerebrospinal fluid was isolated from *cisterna magna* (Lee et al, 2012).

Morphological characterization of cultured hDPCs

The hDPSCs were collected from human dental pulp of molar teeth in Mazandaran University of Medical Sciences. After mechanical and enzymatic digestion, the cells were cultured and cell proliferation and morphological changes of hDPSCs were monitored daily via phase contrast microscopy. The morphological changes of differentiated glial cells were evaluated during the differentiation period.

Flow cytometry of mesenchymal surface markers

At the fourth passage, the hDPSCs were immunostained for mesenchymal surface markers antibodies, namely CD90, CD44 and CD73.

Differentiation of hDPSCs in to oligodendrocyte

The hDPSCs were cultured in DMEM/F12 medium, 10 ng/ml epidermal growth factor (EGF), 1 μM retinoic acid (RA), 20 ng/ml

basic fibroblast growth factor (bFGF) for 4–6 days. This procedure was followed by adding CSF. Addition of CSF to medium was done daily for 8–10 days (Kaka et al, 2012).

Immunocytochemical evaluation with fluorescence microscopy

Immunocytochemistry technique was performed to confirm the differentiated oligodendrocytes. The specific oligodendrocyte markers, e.g. Olig2 and MBP were investigated. The stained cells were evaluated via fluorescent microscope.

Statistical analysis

Dates were analyzed using one-way analysis of variance test (ANOVA) and (SPSS 13.0 software, while $p < 0.05$ was considered significant.

Results

Characterization of hDPSc

The hDPSCs appeared to gain the spindle fibroblastic morphology typical for mesenchymal stem cells (Fig. 1). The flow cytometry data proved hDPSCs to be immunopositive for mesenchymal markers, namely CD 44, CD 90, and CD 105 (Moayeri et al, 2017).

Differentiation of hDPSCs into mature oligodendrocytes

The differentiation of hDPSCs to oligodendrocytes was confirmed by glial structure. The differentiated cells exposed to CSF showed the specific morphological changes as shown whith phase contrast microscopy (Fig. 2). Also, the glial differentiation into oligodendrocytes was confirmed by immunostaining the specific glial markers, namely Olig2 and MBP (Fig. 2).

After counting, the mean percentages of cells immunopositive for Olig2 and MBP were 47.25±0.15 % and 45.12±1.75 %, respectively.

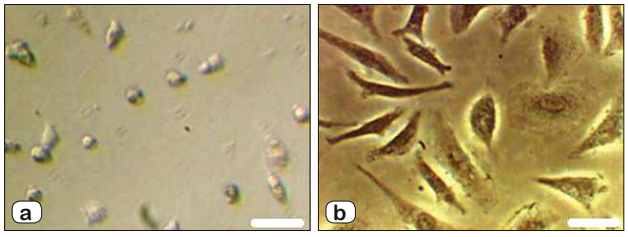

Fig. 1. The morphology of human dental pulp stem cells cultured in vitro. a: Primary culture, b: 4th passage culture. Scale bars 40 µm.

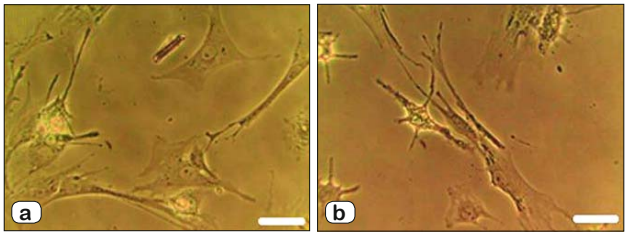

Fig. 2. Differentiation of human Dental pulp stem cells to Oligodendrocyte in vitro. a: Oligoprogenitor cells' morphology, b: Glial differentiation in the presence of CSF. Scale bars 40 µm.

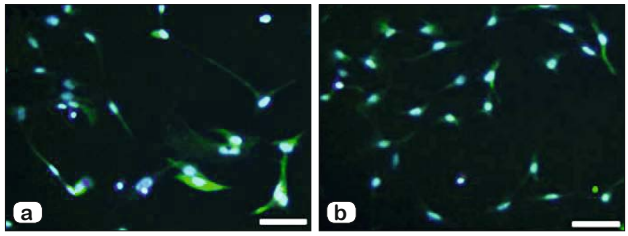

Fig. 3. Immunostaining for differentiated oligodendrocyte. Fluorescence photos of olig2 (a) and MBP (b) in treated group. Scale bars 10 µm.

Discussion

The hDPSCs have a therapeutic potential in neural tissue engineering and neuroregenerative medicine based on their ability in neuroglial differentiation (Gronthos et al, 2000, Huang et al, 2008). The hDPSCs are multipotent stem cells that arise from neural crest and have the ability to differentiate into neurons and oligodendrocytes.

These characteristics predispose them to become an applicable cell source in cell therapy for neurodegenerative diseases (Alizadeh et al, 2017, Chun et al, 2016). *In vitro* differentiation of oligodendrocytes from hDPSCs depends on various differentiation procedures with different inducers. In our study, we have designed an efficient culture condition for oligodendrocytes differentiation by adding CSF.

CSF contains a variety of growth factors and natural nutrients, which provides a niche that is similar to extracellular matrix of neural networks. These properties improve the maturation and differentiation processes of stem cells or progenitor cells (Yalvac et al, 2009, Haratizadeh et al, 2016, Yang et al, 2009; Ye al, 2012,).

Our results showed that hDPSCs appeared to gain fibroblastic morphology and high adherent potential, which is in agreement with previous researches (Martens et al, 2014; Song et al, 2002; Mattei et al, 2015; Gervois et al, 2015).

We observed that hDPSCs can be differentiated into mature oligodendrocytes in the presence of CSF, the fact of which can be used in promoting remyelination in frame of therapeutic strategies applied in neuroregenerative medicine.

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