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ORIGINAL ARTICLE

Neurogenic differentiation of dental pulp stem cells to neuron-like cells in dopaminergic and motor neuronal inductive media

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Background/Purpose: Dental pulp stem cells (DPSCs) have been proposed as a promising source of stem cells in nerve regeneration due to their close embryonic origin and ease of harvest. The aim of this study was to evaluate the efficacy of dopaminergic and motor neuronal inductive media on transdifferentiation of human DPSCs (hDPSCs) into neuron-like cells.
Methods: Isolation, cultivation, and identification of hDPSCs were performed with morphological analyses and flow cytometry. The proliferation potential of DPSCs was evaluated with an XTT [(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide)] assay. Media for the induction of dopaminergic and spinal motor neuronal differentiation were prepared. The efficacy of neural induction was evaluated by detecting the expression of neuron cell-specific cell markers in DPSCs by immunocytochemistry and quantitative real-time reverse transcription polymerase chain reaction (RT-PCR).
Results: In the XTT assay, there was a 2.6- or 2-fold decrease in DPSCs cultured in dopaminergic or motor neuronal inductive media, respectively. The proportions of βIII-tubulin (βIII-tub), glial fibrillary acidic protein (GFAP), and oligodendrocyte (O1)-positive cells were significantly higher in DPSCs cultured in both neuronal inductive media compared with those cultured in control media. Furthermore, hDPSC-derived dopaminergic and spinal motor neuron cells after induction expressed a higher density of neuron cell markers than those before induction.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Conclusion: These findings suggest that in response to the neuronal inductive stimuli, a greater proportion of DPSCs stop proliferation and acquire a phenotype resembling mature neurons. Such neural crest-derived adult DPSCs may provide an alternative stem cell source for therapy-based treatments of neuronal disorders and injury.

Introduction

Nerve degeneration or damage may cause severe complications leading to severe disability in patients. The use of stem cell therapy for nerve regeneration may be an important strategy for treatment. Mesenchymal stem cells (MSCs) or bone marrow stem cells (BMSCs) may be used as the source of stem cells for nerve regeneration treatment. Several studies have demonstrated the ability of MSCs to transdifferentiate into functional cells of the nervous system.[1–6] MSCs cultured in neural stem cell (NSC) culture conditions can further differentiate into astrocytes, neurons, and oligodendrocytes.[7–9] However, the transdifferentiating efficacy of MSCs and BMSCs is low and unpredictable.

Recently, dental pulp cells (DPCs) and dental pulp stem cells (DPSCs) have been proposed as a promising source of stem cells in nerve regeneration due to their close embryonic origin and ease of harvest.[10,11] DPSCs are a source of progenitor/stem cells that can proliferate and differentiate into multiple cell lineages *in vitro*.[10,11] Interestingly, DPSCs originate from the cranial neural crest and have neural characteristics.[12] The dental pulp tissue is termed ''ectomesenchyme'', because it derives from ectodermal cells growing on the periphery of the neural tube during embryonic development, migrating into the oral region, and transdifferentiating into a mesenchymal phenotype. MSCs are immune-privileged and their propagation is not challenged by ethical concerns.[13] Therefore, DPSCs may represent a promising source in cell therapy for neurological disorders.

DPCs can significantly promote survival of embryonic tyrosine hydroxylase (TH)-positive neurons in culture, because DPCs can release some neurotropic factors.[14] In animal studies, grafting of dental pulp tissue into hemisected spinal cord increases the number of surviving motor neurons. Neurotropic factors secreted from the implanted dental pulp tissue are considered to be the main contributor towards the rescuing effect on motor neurons.[15] Even under non-neuronal inductive conditions, human adult DPCs and DPSCs can express neural progenitor marker nestin and glial marker glial fibrillary acidic protein (GFAP) at both the gene and protein levels.[16] DPSCs can differentiate into a variety of cell lineages, including adipocytes, odontoblasts,[10] neural cells,[17] and glial cells.[11,18] Following *ex vivo* expansion, such multipotential DPSCs still retain their neural crest properties.[19–21] Under neural inductive conditions, DPSCs can express the postmitotic neuron-specific marker neuronal nuclei (NeuN), which indicates their potential in neural differentiation.[11]

Although DPSCs in media supplemented with differentiation factors can differentiate into neuron-like cells, their differentiations are incomplete.[22] Previous studies compared murine DPCs with murine retinal progenitor cells to evaluate their potential for differentiation into neuron-like cells. The differentiation of the murine DPCs into glial- and neuron-like cells was incomplete. After differentiation, 37% of murine retinal progenitor cells, but only 5% of murine DPCs, expressed the astroglia cell-marker GFAP.[23] We hypothesized that DPSCs exposed to the appropriate environmental conditions would differentiate into functionally active neurons. Moreover, neural crest-derived adult DPSCs might provide an alternative stem cell source for therapy-based treatments for neuronal disorders and injury.

This study examined the neuronal differentiation potential of adult human DPSCs (hDPSCs) *in vitro*. The aim of this study was to evaluate the neuronal differentiation potential of hDPSCs into neuron-like cells in both dopaminergic and motor neuronal inductive media containing growth factors such as brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF), and retinoic acid.[24–27] The expression of neuron cell-specific cell markers in hDPSCs cultured in neuronal induction media was assessed by immunocytochemistry and quantitative real-time reverse transcription polymerase chain reaction (RT-PCR).

Methods

Materials

Dulbecco's modified Eagle's medium (DMEM) and F12 media (DMEM/F12), fetal calf serum (FCS), N2 supplements, penicillin, and streptomycin were obtained from Invitrogen (Carlsbad, CA, USA); insulin-like growth factor (IGF)-I and transforming growth factor (TGF)-βIII from Prospec Protein Specialists (Rehovot, Israel); and retinoic acid and cyclic adenosine monophosphate (cAMP) from Sigma-Aldrich Corp. (St. Louis, MO, USA). Other cytokines were purchased from PeproTech Systems (Rocky Hill, NJ, USA). Goat polyclonal anti-choline acetyltransferase antibody (anti-ChAT), rabbit polyclonal anti-GFAP, rabbit polyclonal anti-dopa decarboxylase (anti-DDC), mouse monoclonal anti-tyrosine hydroxylase (anti-TH), mouse monoclonal anti-neuronal nuclei (anti-NeuN), mouse monoclonal anti-βIII-tubulin (anti-βIII-tub), and mouse monoclonal anti-oligodendrocyte (anti-O1) were purchased from Chemicon-Millipore (Billerica, MA, USA); poly-L-lysine (PLL) was obtained from ScienCell Research Laboratories (Carlsbad, CA, USA). GAPDH was purchased from Mission Biotech Co. (Taipei, Taiwan), collagenase type I, dispase, L-glutamine, nonessential amino acids were purchased from Invitrogen (Carlsbad, CA, USA). Sonic hedgehog, FGF-8b were purchased from PeproTech Systems (Rocky Hill, NJ, USA). Ascorbic acid, heparin, paraformaldehyde, NaCl, Tris-HCl,