

SPONTANEOUS DIFFERENTIATION OF DENTAL PULP STEM CELLS TOWARDS NEURAL CELLS

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Introduction Dental pulp contains different populations of stem/progenitor cells that reside within the perivascular niche and are originated from migrating neural crest cells. In the past years, studies have demonstrated the self-renewal capacity, clonogenic efficiency and multi-lineage differentiation potential of human dental pulp stem cells (DPSC). However, only recently it was demonstrated that these cells have the ability to differentiate towards functional neurons after chemical induction of differentiation (Arthur *et al.*, 2008). Previously, we showed that immature DPSC were able to undergo neuronal differentiation spontaneously (Kerkis *et al.*, 2006). The goal of our study is to evaluate the capacity of human adult DPSC and immature DPSC to undergo spontaneous differentiation into different neural cells *in vitro*.

Methods Human adult and immature DPSC were characterized and maintained as previously described (Kerkis *et al.*, 2006). Differentiation towards neural cells was performed under culture conditions developed to neuronal cells in absence of known growth factors, such as: epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and retinoic acid (RA). Anti-human antibodies: mouse anti-beta-III-tubulin, goat anti-nestin and rabbit anti-gial fibrillary acidic protein (GFAP) and others were used after cell fixation in 4% paraformaldehyde. The expression of cell specific proteins was analyzed under confocal microscopy. Morphological studies were performed using hematoxylin/eosin, as well as neutral red staining and analyzed under light microscopy. Functional tests of these differentiated cells are being provided.

Results Human adult DPSC and immature DPSC present rapid proliferation and expansion *in vitro*. They can be maintained for a long period in culture, which indicates their self-renewal potential. These cells expressed mesenchymal stem cells markers, as well as reacted positively with human embryonic stem cells markers. Both undifferentiated DPSC cultured in basal medium already expressed neural progenitor markers, such as: nestin and GFAP. These cells were able to respond to culture conditions usually used to neuronal cells, even without the use of chemical inductors, presenting acquired neural cells-like morphology after eleven days of culturing. The decrease of nestin and GFAP proteins expression was evidenced during the process of neural differentiation. At the same time, the cells showed increasing of immature neural proteins expression. We observed that the cell populations, which undergo to neural differentiation present terminally differentiated neural cell types and at the same time showed neurosphere formation. Terminally differentiated neural cells survive during a long period in culture. Neurospheres also demonstrated continuous *in vitro* proliferation.

Conclusion We showed that undifferentiated human adult and immature DPSC are already committed to originate neuron and glial-like cells. We suggest that our model here demonstrated mimics neural stem cells growing and differentiation *in vitro* and possibly can be used to analyze the various stages of neural cell development. Dental pulp is a readily accessible source of stem cells, which has a potential use in cell therapy to treat neurological disease.