Tissue and Cell 47 (2015) 382–388

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

The influence of the donor on dental apical papilla stem cell properties

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ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form 8 May 2015
Accepted 8 May 2015
Available online 16 May 2015

Keywords:
Dental apical papilla
Stem cells
Osteogenic differentiation
Adipogenic differentiation
Proliferation

ABSTRACT

Stem cells from the human dental apical papilla (SCAP) can be obtained from almost all extracted wisdom teeth with an immature tooth root. Although different stem cell lines are used for studies, it remains elusive whether specific characteristics of the dental stem cell cultures such as proliferation rates or the cell differentiation potential are related to the cell source, e.g. the donor tissue of the dental apical papilla. To answer this question, we compared two independent SCAP cell lines from the same donor and compared them with a third cell line from another donor. We investigated the expression of stem cell markers, the efficiency of colony forming units, cell proliferation and the differentiation potential. Results showed particular differences for typical stem cell attributes such as stem cell marker expression, cell proliferation and the adipogenic differentiation. These differences were regardless of the donor of the cell lines. In conclusion, we suppose that stem cell characteristics of SCAP cell cultures are independent from the donor.

1. Introduction

Somatic undifferentiated stem/progenitor cells have been isolated as plastic adherent and colony forming cells from different tissues and organs including human extracted teeth (Friedenstein and Kuralesova, 1971; Gronthos et al., 2000; Miura et al., 2003; Morsczeck et al., 2005; Pittenger et al., 2000; Seo et al., 2004; Sonoyama et al., 2006). One example for dental stem cells is the undifferentiated cells of dental apical papilla (SCAP), which are located apically to the developing dental pulp. These stem cells demonstrate high rates of cell proliferation, they express typical mesenchymal stem cell markers such as CD105 and STRO1 and they had the capability to differentiate into odontoblasts and adipocytes (Sonoyama et al., 2006). SCAP have been demonstrated to be a valuable source of stem cells in regenerative procedures (Huang et al., 2008; Lovelace et al., 2011) and were used in several studies to estimate diverse aspects of regenerative dentistry (Sonoyama et al., 2006; Bakopoulou et al., 2011; Trevino et al., 2011). The study of Sonoyama et al. for example showed that dentin and periodontal ligament were formed after co-transplanting SCAP (to form a root) and stem cells from the periodontal ligament (PDLSCs; to form a periodontal ligament) into tooth sockets of pigs.

Currently, the studies of stem/progenitor cells were encouraged by the advances in techniques related to stem cells characterization at molecular levels. These have provided new insights for our understanding of tooth tissue-derived stem cells. SCAP have the properties of mesenchymal stem cells including the marker gene expression and differentiation into mesenchymal cell lineages such as osteoblasts, chondrocytes and adipocytes (Sonoyama et al., 2006; Huang et al., 2008; Sonoyama et al., 2008; Bakopoulou et al., 2011). However, it remains elusive whether characteristics of cell cultures such as proliferation rates, the expression of marker genes or the cell differentiation potential are related to the donor of the dental tissue. We proposed that differences could be put down only to the donor. So properties of stem cell lines from the same donor should be more similar than properties of stem cell lines, which were isolated from different donors. While to prove this hypothesis is difficult to achieve, the motivation of our study was to refute this hypothesis. Therefore we isolated three SCAP cell lines from teeth with immature roots at a similar developmental stage. Two cell lines were isolated from the same donor and one cell line from a different one for comparison. We investigated the expression of stem cell markers, the efficiency of colony forming units, the cell proliferation and the differentiation potential. In contrast to our initial hypothesis, our results suggest that the influence of the donor is probably low on the properties of SCAP.

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http://dx.doi.org/10.1016/j.tice.2015.05.002
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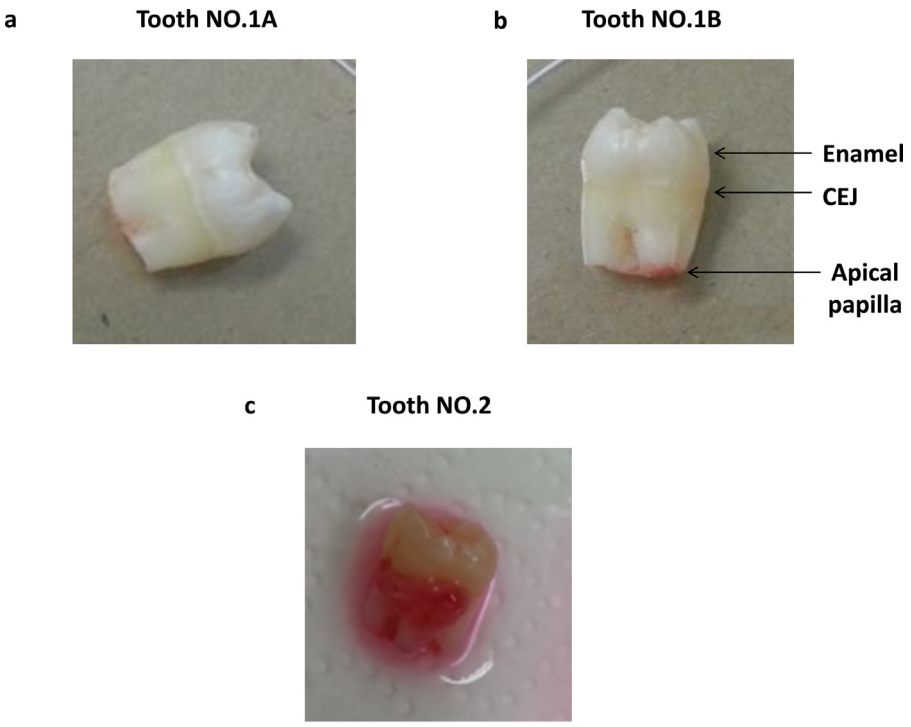

Fig. 1. Pictures of human third molars that have been surgically removed from 2 patients. (a and b) Teeth from the same person after the apical papilla and PDL tissue were dissected. (c) Tooth from the other person.

2. Materials and methods

2.1. Cell culture

In this experiment, stem cells from apical papilla (SCAP) were isolated with the isolation method as previously described (Gronthos et al., 2000; Sonoyama et al., 2006). The molar teeth were isolated from patients with informed consent. The local ethical committee approved the protocol for the isolation of dental stem cells (internal number 13-101-0063). The age of the two donors (19–23 years) and the stage of tooth root development were comparable for all teeth. Briefly, the apical papilla tissues were separated from the exterior of the root foramen area. The tissues were then minced into small pieces and digested enzymatically for 1 h at 37 °C. Cell suspensions were passed through a 70 μm strainer to get single suspension cells, then centrifuged at 400 × *g* for 10 min and then the cells were re-suspended by standard growth medium. Subsequently, the cell suspension was seeded into T25 flasks in cell culture medium. After 24 h, the cell culture medium was changed to remove non-adherent cells. Then, the remaining cells grew as a small cluster and formed colonies approximately 10–14 days after seeding cells. The standard cell culture medium for the cultivation of SCAP was DMEM supplemented with 10% FBS and 100 μg/ml Penicillin/Streptomycin.

2.2. Cell proliferation

For characterization cells were cultured in DMEM, supplemented with 10% FBS and 100 μg/mL Penicillin/Streptomycin (standard cell culture medium) in a 96-well plate with a cell seeding density of 5000 cells/cm^2. Cell growth/proliferation curves were estimated by cell counting kit-8 (CCK-8; Dojindo, Japan) following the instructions from the manufacturer. Cell cultures with CCK-8 were incubated for 2 h at 37 °C. After that, the optical density was measured at 450 nm. The cell proliferation was estimated at days 1, 2, 3, 5, 7, 10 and 14 of cell culture.

2.3. Flow cytometry

For characterization, cells were investigated for stem cell associated markers with flow cytometry. In short, SCAP were incubated with the following monoclonal antibodies; CD44-FITC, CD146-FITC, CD 105-APC, CD 90-PE (Miltenyi Biotec, Bergisch Gladbach, Germany), anti-human Nestin-Phycoerythrin monoclonal antibody (R&D Systems, Inc., MN, USA) and FITC-anti-human STRO-1 (BioLegend, San Diego, CA 92121, USA) for 45 min at 4 °C. Then, they were washed with PBS containing 0.5% bovine serum albumin (BSA) and 2 mM EDTA. Cells were permeabilized with 0.2% saponin and 0.1% BSA for 15 min and washed in PBS containing 0.1% BSA and 2 mM EDTA before intracellular staining. The following antibodies were used as negative controls; mouse IgG-FITC (Miltenyi Biotec), mouse IgG2b-APC isotype control antibody (Miltenyi Biotec), Mouse IgG1 isotype Control-PE (R&D Systems, Inc.) and FITC mouse IgM, λ isotype control (BioLegend). Flow cytometry analysis was performed using the FACS Canto II (Becton Dickinson).

2.4. Colony-forming efficiency

The assessment of colony forming unit fibroblast (CFU-F) was done with SCAP at cell passage 4. Cell suspensions were seeded into 6-well culture plates at 100 cells/well in a standard cell culture medium. Cultures were set up in quadruplicate and incubated at 37 °C in 5% CO2 for 2 weeks. For enumeration, colonies were fixed in 2% paraformaldehyde and stained with 0.2% Coomassie Brilliant Blue R250. Aggregates greater than 50 cells were scored as a colony.

2.5. Osteogenic differentiation

SCAP were cultivated in a standard cell culture medium until sub-confluence (>80%). Then, the culture medium was changed to osteogenic differentiation medium (ODM) or adipogenic