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Effect of Jagged-1 and Dll-1 on osteogenic differentiation by stem cells from human exfoliated deciduous teeth

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ABSTRACT

Objective: The aim of the present study was to determine the influence of Notch ligands, Jagged-1 and Dll-1, on osteogenic differentiation by stem cells from human exfoliated deciduous teeth.
Design: Notch ligands were immobilized on tissue culture surface using an indirect affinity immobilization technique. Cells from the remaining of dental pulp tissues from human deciduous teeth were isolated and characterized using flow cytometry and differentiation assay. Alkaline phosphatase (ALP) enzymatic activity, osteogenic marker gene expression, and mineralization were determined using ALP assay, real-time polymerase chain reaction, and alizarin red staining, respectively.
Results: The isolated cells exhibited CD44, CD90, and CD105 expression but lack of CD45 expression. Further, these cells were able to differentiate toward osteogenic lineage. The upregulation of *HES-1* and *HEY-1* was observed in those cells on Dll-1 and Jagged-1 coated surface. The significant increase of ALP activity and mineralization was noted in those cells seeded on Jagged-1 surface and these results were attenuated when cells were pretreated with gamma secretase inhibitor. The significant upregulation of ALP and collagen type I gene expression was also observed in those cells seeded on Jagged-1 surface. The inconsistent Dll-1 induced osteogenic differentiation was found and high Dll-1 immobilized dose (50 nM) slightly enhanced alkaline phosphatase enzymatic activity. However, the statistical significant difference was not obtained as compared to the hFc control.
Conclusion: The surface immobilization of Notch ligands, Jagged-1 and Dll-1, likely to enhance osteogenic differentiation of SHEDs. However, Jagged-1 had more ability in enhancing osteogenic differentiation than Dll-1 in our model.

1. Introduction

Mesenchymal stem cells (MSCs) are widely investigated in regenerative medicine as an alternative approach for potential tissue engineering due to their self-renewal and multi-differentiation properties (Machado, Fernandes, & Gomes Pde, 2012). One advantage of MSCs is their availability. These cells can be isolated from a variety of sources, such as bone marrow, peripheral blood, and adipose tissues (Fuchs & Segre, 2000). Among a variety of MSCs source, dental tissues-derived MSCs are of interest, especially MSCs from shedding primary teeth (Egusa, Sonoyama, Nishimura, Atsuta, & Akiyama, 2012). Dental pulp tissues from exfoliated deciduous teeth contain a population of undifferentiated ectomesenchymal cells, named as stem cells from human exfoliated deciduous teeth (SHEDs) (Miura et al., 2003). SHEDs are highly proliferative and able to differentiate into several cell types, including neurons, adipocytes, osteoblasts and odontoblasts-like cells (Miura et al., 2003; Sakai et al., 2010; Suchanek et al., 2010). In addition, SHEDs are easily obtained from normal shedding primary teeth, which make them less ethical concerns.

A conserved Notch signaling pathway has a critical role in various processes during development and organogenesis (Cai, Gong, Huang, & Lin, 2011; Mitsiadis, Feki, Papaccio, & Caton, 2011). Many studies demonstrated the essential of Notch signaling in cell fate determination, cell proliferation, differentiation, and apoptosis in various cell types (Bar & Efrat, 2014; Chen, Lee, & Bae, 2014; Mitsiadis, Henrique, Thesleff, & Lendahl, 1997). Signal transmission

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in Notch pathway occurs upon the binding of Notch ligand on one cell to the Notch receptor on the adjacent cell, resulting in the enzymatic cleavage of the receptor (Mitsiadis et al., 1997). Subsequently, the intracellular domain of Notch receptor is released from the membrane and translocated into the nucleus (Mitsiadis et al., 1997). Further, the binding of Notch intracellular domain with other co-activators induces the expression of the Notch target genes, particularly *HES* and *HEY* family (Mitsiadis et al., 1997).

Canonical Notch ligands in mammals can be divided into 5 types, Jagged-1, Jagged-2, Delta-like-1 (Dll-1), Dll-3, and Dll-4. In dental tissues, Notch signaling is one of the signals regulating tooth development. The expression of Jagged-1 and Dll-1 was detected during odontogenesis in the mouse and correlated with ameloblast and odontoblast differentiation (Mitsiadis et al., 1997; Mitsiadis, Hirsinger, Lendahl, & Goridis, 1998). Further, Notch signaling also participates in healing of injured dental tissues. In this respect, it was observed that Dll-1 and Jagged1 expression were detected in the cells near the pulp capping area (Lovschall, Tummers, Thesleff, Fuchtbauer, & Poulsen, 2005). Despite those reports, the contradict effects of Notch signaling on stem cells from dental tissues were noted. The activation of Notch signaling using indirect immobilized Jagged-1 promoted the osteogenic differentiation by human periodontal ligament stem cells (hPDLSCs) (Osathanon et al., 2013a). In contrast, several reports suggested that Notch signaling has inhibitory effect on odontogenic differentiation in dental pulp cells. Jagged-1 has been shown to inhibit the odontoblastic differentiation of human dental pulp stem cells (hDPSCs) (Zhang, Chang, Sonoyama, Shi, & Wang, 2008). In addition, Dll-1 promotes the self-renewal capacity of hDPSCs and reduces hDPSCs differentiation (He et al., 2009; Wang, He, Tan, Tian, & Qiu, 2011). However, the evidence of Notch signaling in controlling deciduous pulp cells' behaviors is yet sparse. Given that stem cell properties of SHEDs differ from hDPSCs in several aspects (Miura et al., 2003; Nakamura et al., 2009), the response to Notch signaling activation in deciduous pulp cell might vary from those observed in hDPSCs.

In this study, the influence of Notch ligands, Jagged-1 and Dll-1, on osteogenic differentiation by SHEDs was investigated using the indirect affinity immobilization. Our results showed that Jagged-1 was more potent in enhancing osteogenic differentiation compared to Dll-1 in this Notch signaling activation model.

2. Materials and methods

2.1. Cell isolation and characterization

The study protocol was approved by the Human Ethical Committee, Faculty of Dentistry, Chulalongkorn University. Cells were isolated from the remnant of dental pulp tissues of exfoliated deciduous teeth from healthy children patients. The collected teeth were extracted according to normal treatment plan i.e. prolong retention of primary teeth and shedding. Remaining pulp tissues were separated from remnant crowns by barbed broach and washed with sterile PBS. Tissue explant was employed to isolate cells. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco®, USA), supplemented with 100 unit/mL penicillin, 100 μg/mL streptomycin, 5 μg/mL amphotericin B, 2 mM L-glutamine (Glutamax®, USA) and 10% fetal bovine serum (FBS, Gibco®, USA) at 37 °C, humidified atmosphere with 5% CO_2. The medium was changed every 48 h. After confluence, cells were passaged at a 1:3 ratio using 0.25% trypsin-EDTA. Cells at passage 3–5 were used in the experiments.

For characterization, the surface marker expression was evaluated by flow cytometry. Cells were stained with FITC-conjugated anti-CD44 antibody (BD Biosciences Pharmingen), PerCP-conjugated anti-CD45 antibody (BD Biosciences Pharmingen), PerCP-CyTM5.5-conjugated anti-CD90 antibody (BD Biosciences Pharmingen), and PE-conjugated anti-CD105 antibody (BD Biosciences Pharmingen). The expression was analyzed using FACSCalibur (BD Bioscience). The values were illustrated as mean fluorescence intensity (MFI).

The differentiation ability toward osteogenic lineage was determined using alkaline phosphatase activity assay and alizarin red S staining, as described below.

2.2. Alkaline phosphatase activity assay

Cells were seeded at a density of 25,000 cells per wells in a 24-well plate and maintained in growth medium supplemented with ascorbic acid (50 μg/mL), dexamethasone (100 nM), and β-glycerophosphate (10 mM). Cells were harvested and lysed in alkaline lysis buffer. Cell lysis was incubated with the substrate solution, containing 2 mg/mL *p*-nitrophenol phosphate, 0.1 M 2-amino-2-methyl-1-propanol and 2 mM$MgCl_2$. The presence of *p*-nitrophenol was measured at an absorbance of 410 nm. Total cellular protein was determined using BCA assay. The enzyme activity was further normalized to total cellular protein concentration.

2.3. Mineralization assay

Cells were fixed with cold methanol for 10 min, washed with deionized water and stained with 1% Alizarin Red S solution for 3 min at room temperature on a shaker. For quantitative determination of calcium deposition, the samples were destained with 10% cetylpyridinium chloride in 10 mM sodium phosphate for 15 min at room temperature. The concentration was determined by absorbance measurement at 570 nm and further normalized as the relative fold change to the control.

2.4. Real-time quantitative polymerase chain reaction

Total cellular RNA was extracted with Trizol reagent (Roche Diagnostics, USA). RNA was quantified using a Nanodrop® and RNA samples (1 μg) were converted to complementary DNA using the ImPromII kit (Promega, UK). For real-time quantitative PCR, the amplification of the cDNA template was performed using the LightCycler® 480 SYBR Green I Master kit (Roche Diagnostic) on MiniOpticon™ Real-Time PCR Detection System (Bio-Rad). PCR conditions was at 95 °C for 1 min followed by 40 cycles of amplification consisting of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 20 min. The expression value was normalized against 18S mRNA expression. The fold change was calculated compared to the control. The sequences of the primers are shown in Table 1 (Aoi, Nakahama, Morita, & Safronova, 2011; Chuenjitkuntaworn et al., 2010; Isenmann et al., 2009; Muller, Kietz, Gustafsson, & Strom, 2002; MacKenzie et al., 2004; Wongkhantee, Yongchaitrakul, & Pavasant, 2008; Xu et al., 2004). The melting curve analysis was performed in all experiments. In some experiments, the conventional PCR was performed using thermocycling machine and the products were electrophoresis in 1.8% agarose gel and visualized the bands under UV light.

2.5. Indirect affinity immobilization of Jagged-1 and Dll-1 ligands

The protocol was performed according to previous reports (Beckstead, Santosa, & Giachelli, 2006; Osathanon et al., 2013a). Tissue culture polystylene plates were incubated overnight at room temperature with 250 μL of recombinant protein G (50 μg/mL, Invitrogen, USA). The surfaces were washed three times with sterile PBS, and blocked with 10 mg/mL bovine serum albumin (BSA, Sigma, USA) in PBS for 2 h at room temperature. The surfaces