Effect of Jagged-1 and Delta-Like-1 on the Proliferation of Primary Deciduous Pulp Cells

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Abstract

Objectives: The objective of this study was to compare the effect of Notch ligands, Jagged-1 and Delta-like-1 (Dll-1), on the proliferation of primary deciduous pulp cells.

Methods: Jagged-1 and Dll-1 ligands were immobilized to the tissue culture surface using an indirect affinity immobilization technique. At day 1, 3 and 7, the morphology of primary deciduous pulp cells was observed under an inverted transmitted light microscope. Cell proliferation was determined by an MTT assay and the mRNA expression levels of apoptosis related-genes were determined using reverse transcriptase polymerase chain reaction.

Results: Cells exhibited elongated spindle (fibroblast-like) morphology. There was no difference in morphology among different conditions. Cell proliferation was decreased in the Notch ligand treated groups. The Jagged-1 group exhibited the attenuated appearance of cell proliferation greater than Dll-1 and the control group. However, no statistical significant difference was observed. The increase of the *CASPASE3* and the *BAD* mRNA expression was noted in Notch ligand treated groups. Though, the significant difference was observed only for the *CASPASE3* mRNA expression in the Jagged-1 group compared to the control group ($p<0.05$). There was no marked difference in the *BCL-2* and the *BAX* mRNA expression.

Conclusion: Notch ligands, Jagged-1, may *attenuatethe* primary deciduous pulp cell proliferation via the activation of apoptosis pathway.

Keywords: Notch Signaling, Deciduous pulp cells, Proliferation, *CASPASE3*

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Introduction

Notch signaling is a conserved signaling pathway that plays important roles in several processes, such as cell fate determination, proliferation, differentiation, and cell death [1, 2]. Signal transmission in Notch pathway occurred upon the binding of ligands to receptors between adjacent cells [2]. Among various types of Notch ligands, Jagged-1 and Delta-like-1 (Dll-1) have been widely investigated [3-6], including their roles in dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs). In PDLSCs, Jagged-1 promoted osteogenic differentiation [3]. On the contrary, Jagged-1 overexpression in DPSCs resulted in the inhibition of the odontoblastic differentiation both *in vitro* and in vivo [4]. The study of Dll-1 ligands in DPSCs also suggested its role in cell proliferation and osteo/odontogenic differentiation [5, 6]. Taken together, the influence of Notch signaling on cell behaviors might vary depending on cell types and activating ligands. To date, the evidence of Notch signaling in controlling deciduous pulp cell functions is yet sparse. Therefore, the present study aimed to investigate the influence of Notch ligands, Jagged-1 and Dll-1, on human primary deciduous pulp cell proliferation *in vitro*.

Materials and methods

Isolation and culture of primary deciduous pulp cells

Primary deciduous pulp cells were isolated from the remnant of human dental pulp tissues of exfoliated deciduous teeth which had no caries or other pathology. The cell isolation procedure was approved by the Human Ethical Committee, Faculty of Dentistry, Chulalongkorn University. Explant 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. Medium was refreshed every 48 hours. Cells at passage 3-5 were used in the experiments.

Notch ligand immobilization

Surface-bounded Jagged-1/Fc and Dll-1/Fc (R&D systems, USA) were fabricated by an indirect affinity immobilization method as previously described [3, 7, 8]. Equal amount of Fc fragment (hFc, Jackson ImmunoResearch Laboratory, USA) was used as the control immobilization technique.

Cell morphology and cell proliferation assay

Cells were seeded at density of 25,000 cells/well in 24-well-plates and maintained in growth medium as described above. At day 1, 3, and 7, cell growth and morphology were investigated under the inverted transmitted light microscope (Nikon Eclipse TS100, Japan). Then, MTT assay was performed to determine cell viability. The protocol was performed according to Sukarawan W, et al 2013 [9].

Reverse transcription polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted with Trizol reagent (Roche Diagnostics, USA). RNA samples (1 μg) were converted to the complementary DNA using the ImPromII kit (Promega, UK). Polymerase-chain reaction (PCR) was performed using Taq polymerase (Invitrogen, Brazil). The amplified DNA was electrophoresed on a 1.8% agarose gel and visualized by ethidium bromide fluorostaining. Band density was measured using FusionSL4 (Vilber, Germany) and normalized against the density of 18S mRNA expression. The sequences of the primers were as followed; 18SForward: 5'-GTGATGCCCTTAGATGTCC-3', Reverse: 5'-CCATCCAATCGGTAGTAGC-3'; CASPASE-3 Forward:5'-CAAACTTTTTCAGAGGG GATCG-3', Reverse: 5'-GCATACTGTTTCAGCAT GGCAC-3';BCL-2 Forward: 5'-AGGAAGTGAACA TTTCGGTGAC-3', Reverse: 5'-GCTCAGTTCCAG GACCAGGC-3'; BAD Forward: 5'-GAGTGAGCA GGAAGACTCCAGC-3', Reverse: 5'-TCCACAAA CTCGTCACTCATCC-3'; and BAX Forward: 5'-TG CTTCAGGGTTTCATCCAG-3', Reverse: 5'-GGC-GGCAATCATCCTCTG-3'.

Statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey HSD. All experiments were repeated with isolated cells from at least three different subjects. Differences at $p < 0.05$ were considered as statistically significant.

Results

Cells isolated from human deciduous pulp exhibited an elongated and spindle-like morphology (Fig.1). Cells in Dll-1 and Jagged-1 groups maintained the regular morphology when cultured in the regular growth medium as compared to the control. The MTT assay results showed that cell proliferation had increased from day 1 to 7 in all conditions. Interestingly, the proliferation rate was highly decreased in Jagged-1 group as compared to Dll-1 and the control group (Fig.2). However, statistically significant difference ($p<0.05$) was not found at any time points (day 1, 3, and 7).

To investigate the potential mechanism of Jagged-1 decreasing human primary deciduous cell proliferation, the mRNA expression of apoptosis related-genes was determined. RT-PCR was done at day 7 after cells exposed to the treated surfaces. The results illustrated that mRNA expression of *CASPASE3* and *BAD* obviously increasedin both Jagged-1 and Dll-1 treated groups as compared to the control group. Though, the statistical significant difference was observed only for *CASPASE3* mRNA expression between Jagged-1 and the control ($p<0.05$). *No* marked difference was noted in *BCL-2* and *BAX* mRNA expressions (Fig.3).

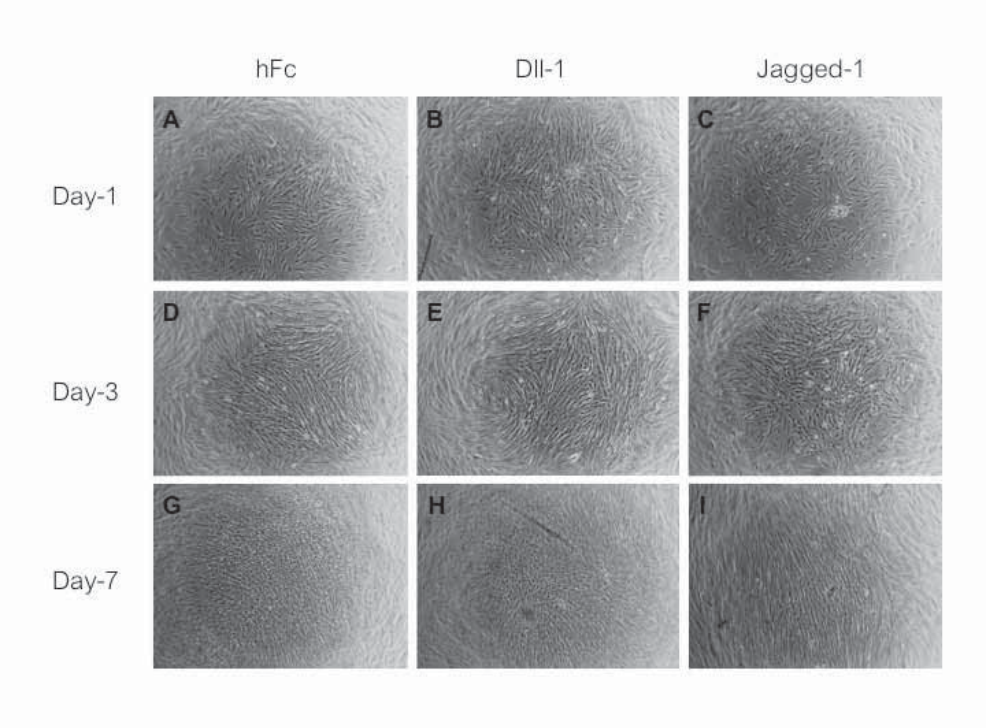

Figure 1. Cell morphology in Notch ligand treated and the control groups (magnification: 4X). (A) hFc control on day-1; (B) Dll-1 treated cells on day-1; (C) Jagged-1 treated cells on day-1; (D) hFc control on day-3; (E) DLL-1 treated cells on day-3; (F) Jagged-1 treated cells on day-3; (G) hFc control on day-7; (H) DLL-1 treated cells on day-7; (I) Jagged-1 treated cells on day-7.

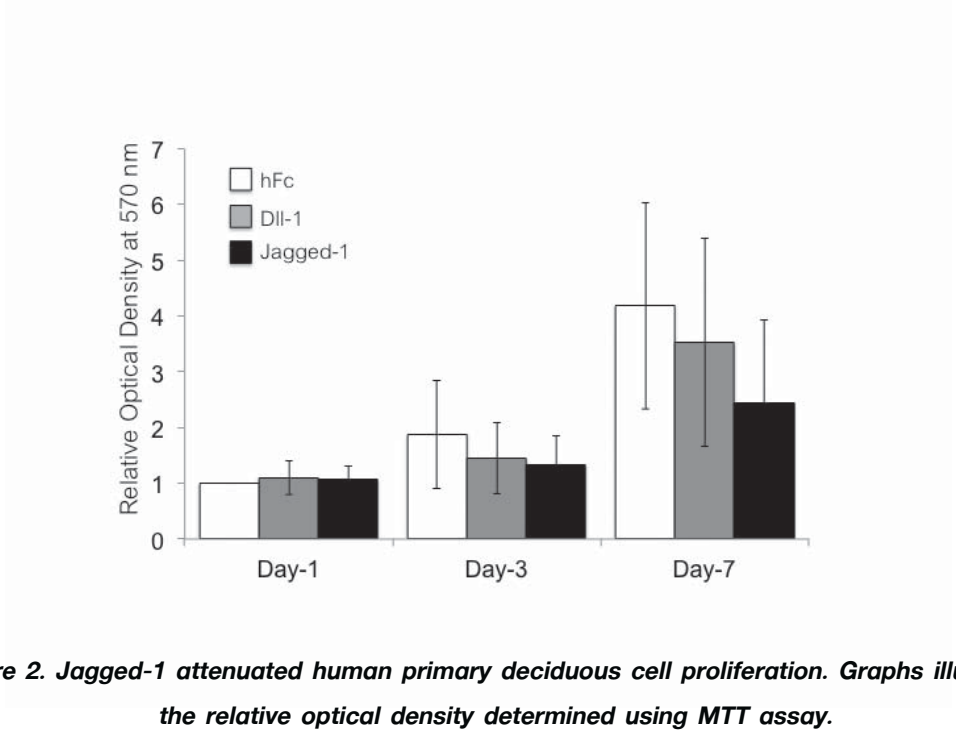

Figure 2. Jagged-1 attenuated human primary deciduous cell proliferation. Graphs illustrated the relative optical density determined using MTT assay.

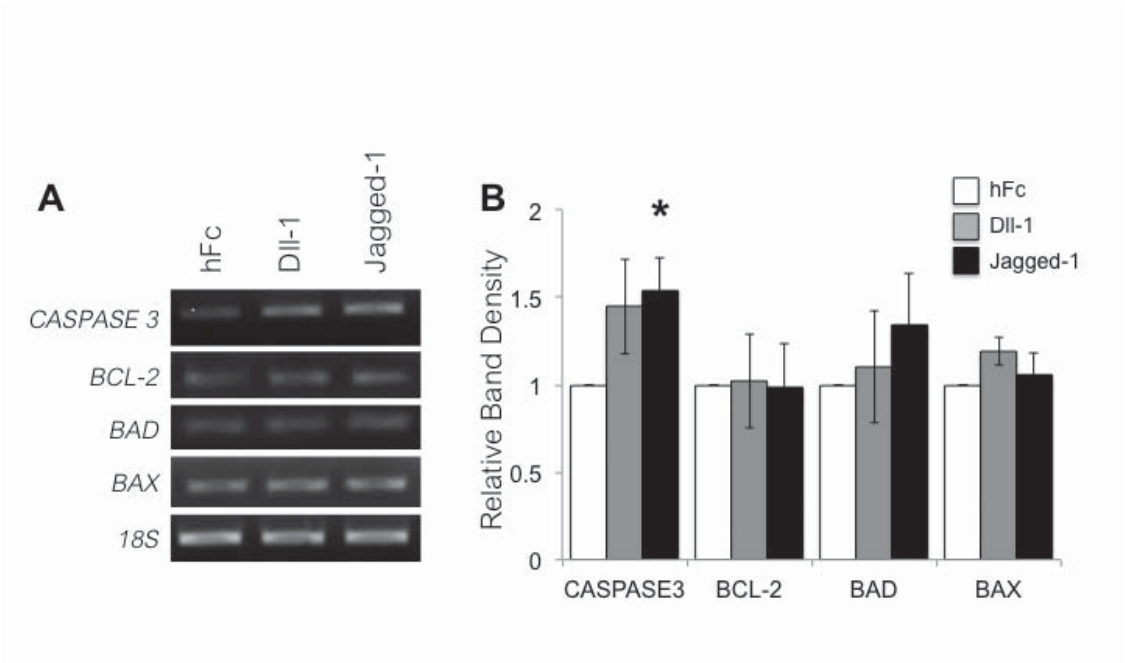

Figure 3. Jagged-1 enhanced the mRNA expression of apoptosis-related gene. The mRNA expression of apoptosis related-genes evaluating by semi-quantitative polymerase chain reaction after seeding cells for 7 days (A). Graph illustrated the relative density of apoptosis related-genes expression (B). Asterisk indicated the statistical significance ($p < 0.05$).

Discussion

In the present study, we described the influence of Notch ligands, Jagged-1 and Dll-1, on human primary deciduous cell proliferation. The activation of Notch signaling by Jagged-1 and Dll-1 attenuated cell proliferation, especially in the Jagged-1 treated group. Several studies were reported the role of Notch signaling in cell proliferation. Particularly on dental tissue-derived cells, Dll-1 overexpression promoted DPSCs proliferation [5, 6]. Further, the percentage of G0/G1 phase was significantly decreased, while the percentage of S phase was markedly increased in Dll-1 overexpressing DPSCs [5, 6]. Correspondingly, the endogeneous Dll-1 inhibition in DPSCs proliferation resulted in the significant decrease of cells and the percentage of S phase population [5, 6]. Inhibition of Notch signaling by γ-secretase inhibitor also attenuated human dental pulp cell proliferation [10]. However, Jagged-1 overexpressing DPSCs did not alter cell proliferation and cell survival [4]. The discrepancy among these studies may be due to several reasons. First, the response after Notch signaling activation may be specific to cell type. It has been suggested that the properties of human deciduous pulp cells deviated from DPSCs [11, 12]. Especially in cell proliferation, cells isolated from human deciduous pulp tissues exhibited a higher proliferation rate and expressed higher mRNA levels of proliferation-related genes [11, 12]. Thus, the influence of Notch activation

on cell proliferation may differ between cells from deciduous and permanent pulp tissues. Second, the different methods to activate Notch signaling can influence cell behaviors. In this regard, it was previously shown that soluble Jagged-1 slightly activated Notch signaling, while the surface immobilization of Jagged-1 was markedly enhanced Notch activation [13]. Moreover, the indirect affinity immobilization had more potent to activate Notch signaling than the direct immobilization [7, 8]. In the present study, the indirect affinity immobilization of Jagged-1 and Dll-1 was employed. This method could evaluate the direct effect of Notch signaling activation by particular ligand. It has been demonstrated that Notch signaling regulated cell apoptosis in several cell types. In the present study, Jagged-1 markedly enhanced *CASPASE3* mRNA expression, corresponded with the attenuation of cell proliferation. Similar to our study, it was previously reported that Notch 1 induced the *Caspase3* activity and *CASPASE3* gene expression in murine terminal-differentiated keratinocytes [14]. In addition, it was also demonstrated that Notch 1 activation resulted in the increase of *CASPASE3* promoter activity [14], indicated that *CASPASE3* was one of the targets of Notch signaling. Taken together, it is possible that Jagged-1 activates apoptosis in human primary deciduous pulp cells. However, further investigation is indeed needed to confirm this hypothesis.

Conclusion

Surface immobilization of Notch ligands slightly reduced the proliferation of human primary deciduous pulp cells. However, Jagged-1 enhanced mRNA levels of apoptosis related-gene. Therefore, Notch ligands, Jagged-1, may attenuate human primary deciduous pulp cell proliferation via the activation of apoptosis pathway. However, further study is necessary to confirm these results. In addition, the influence of Notch signaling in other aspects of human primary deciduous pulp cell behaviors such as self renewal and differentiation should be examined.

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References

1. Cai X, Gong P, Huang Y, Lin Y. Notch signalling pathway in tooth development and adult dental cells. Cell Prolif 2011; 44: 495-507.

2. Mitsiadis TA, Feki A, Papaccio G, Caton J. Dental pulp stem cells, niches, and notch signaling in tooth injury. Adv Dent Res 2011; 23: 275-279.

3. Osathanon T, Ritprajak P, Nowwarote N, Manokawinchoke J, Giachelli C, Pavasant P. Surface-bound orientated Jagged-1 enhances osteogenic differentiation of human periodontal ligament-derived mesenchymal stem cells. J Biomed Mater Res A 2013; 101: 358-367.

4. Zhang C, Chang J, Sonoyama W, Shi S, Wang CY. Inhibition of Human Dental Pulp Stem Cell Differentiation by Notch Signaling. J Dent Res 2008; 87: 250-255.

5. He F, Yang Z, Tan Y, Yu N, Wang X, Yao N, et al. Effects of Notch ligand Delta1 on the proliferation and differentiation of human dental pulp stem cells in vitro. Arch Oral Biol 2009; 54: 216-222.

6. Wang X, He F, Tan Y, Tian W, Qiu S. Inhibition of Delta1 promotes differentiation of odontoblasts and inhibits proliferation of human dental pulp stem cell in vitro. Arch Oral Biol 2011; 56: 837-845.

7. Beckstead BL SD, Giachelli CM. Mimicking cell-cell interactions at the biomaterial-cell interface for control of stem cell differentiation. J Biomed Mater Res A 2006; 79: 94-103.

8. Beckstead BL TJ, Liang KJ, Tavakkol Z, Usui ML, Olerud JE, Giachelli CM. Methods to promote Notch signaling at the biomaterial interface and evaluation in a rafted organ culture model. J Biomed Mater Res A 2009; 91: 436-446.

9. Sukarawan W, Nowwarote N, Kerdpon P, Pavasant P, Osathanon T. Effect of basic fibroblast growth factor on pluripotent marker expression and colony forming unit capacity of stem cells isolated from human exfoliated deciduous teeth. Odontology / the Society of the Nippon Dental University 2013. E-pub ahead of print July 20, 2013; In press.

10. Zou XY, Zhuang H, Yue L, XJ. G. Involvement of Notch signalling pathway in senescence of human dental pulp cells. Chin J Dent Res 2010; 13: 45-49.

11. Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M. Stem Cell Proliferation Pathways Comparison between Human Exfoliated Deciduous Teeth and Dental Pulp Stem Cells by Gene Expression Profile from Promising Dental Pulp. J Endod 2009; 35: 1536-1542.

12. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 2003; 100: 5807-5812.

13. Osathanon T, Ritprajak P, Nowwarote N, Manokawinchoke J, Giachelli C, Pavasant P. Surface-bound orientated Jagged-1 enhances osteogenic differentiation of human periodontal ligament-derived mesenchymal stem cells. J Biomed Mater Res A 2013; 101A: 358-367.

14. Okuyama R, Nguyen BC, Talora C, Ogawa E, Tommasi di Vignano A, Lioumi M, et al. High Commitment of Embryonic Keratinocytes to Terminal Differentiation through a Notch1-caspase 3 Regulatory Mechanism. Dev Cell 2004; 6: 551-562.

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