Transforming Growth Factor-β1 and Runt-related Transcription Factor 2 as Markers of Osteogenesis in Stem Cells from Human Exfoliated Deciduous Teeth Enriched Bone Grafting

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Original Article

Transforming Growth Factor-β1 and Runt-related Transcription Factor 2 as Markers of Osteogenesis in Stem Cells from Human Exfoliated Deciduous Teeth Enriched Bone Grafting

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Abstract

Background: Stem cells from human exfoliated deciduous teeth (SHED) are one source of adult stem cells which can proliferate and differentiate into many types of tissues than any other stem cells. SHED represent potential stem cells for therapeutic therapy and tissue engineering. **Aims:** The aim of this study was to compare the expression of transforming growth factor-β1 (TGF-β1) and runt-related transcription factor 2 (RUNX2) in hydroxyapatite (HA) scaffold with SHED. **Subjects and Methods:** Eight experimental animals were divided into two groups. The first group was transplanted with HA and the second with HA and SHED. The expression of TGF-β1 and RUNX2 was seen 21 days later by means of immunohistochemical analysis. **Statistical Analysis Used:** Data were analyzed using an independent *t*-test with a significance level of 5%. **Results:** The analysis results of an independent *t*-test showed a significant difference between the two groups. The second group given HA with SHED showed a significantly higher expression of TGF-β1 and RUNX2 than that of the first group. **Conclusions:** Expression of TGF-β1 and RUNX2 occurs after the application of HA with SHED, while TGF-β1 and RUNX2 expression in the HA with SHED group was significantly higher than in the group without SHED.

Keywords: *Hydroxyapatite, runt-related transcription factor 2, stem cells from human exfoliated deciduous teeth, transforming growth factor-β1*

Introduction

Tissue engineering employing stem cells and scaffolds from natural biomaterial may be applied to bone regeneration in patients with alveolar bone defects. Hydroxyapatite (HA) from bovine bone, coral, or chemically synthesized material can be used as biomaterial for bone graft.[1,2] Research showed that the stem cells in teeth offer the potential for bone and periodontal ligament regeneration and may be beneficial for teeth regeneration.[3,4] Compared to dental pulp stem cells, stem cells from human exfoliated deciduous teeth (SHED) have the ability to differentiate into broader types of cells.[5,6]

Transforming growth factor-β1 (TGF-β1) and runt-related transcription factor 2 (RUNX2) exhibit important roles in bone tissue regeneration and remodeling, in addition to bone mass preservation.[7,8] is an important transcription factor in the osteoblast which plays a role in the physiological control of skeletal genes. RUNX2 has been identified as the master gene necessary for the osteoblastic differentiation process of mesenchymal precursor.[9,10]

This research aimed to determine the role of SHED-shedded HA in increasing TGF-β1 and RUNX2 expression in osteogenesis.

Subjects and Methods

This study incorporated an experimental laboratory with posttest-only control group design. Healthy, 3-month-old, male, Wistar strain *Rattus norvegicus* weighing approximately 200–300 g constituted the subjects of this study. Two groups participated in this study, each consisting of three subjects. Ethical clearance for the research was obtained from the Health Research Ethical Clearance Commission (No. 169/HRECC. FODM/VIII/2017).

Deciduous tooth pulp preparation was conducted at the Pedodonthic Clinic, Faculty of Dentistry, Airlangga University. Each extracted deciduous tooth was cut

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horizontally using a sterile fissure bur, before its pulp was carefully removed. Pulp tissue was placed into a medium contained in a 15 ml conical tube and transported to the tissue bank of Dr. Soetomo Hospital in a coolbox. Pulp tissue was cultured in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies/GIBCO BRL) having been enriched with 20% fetal bovine serum (Biochrom AG, Germany), 5 mM L-glutamine (Gibco Invitrogen 25 USA), 100 U/ml penicillin-G, 100 µg/ml streptomycin, and 100 µg/ml kanamycin.

After 3 days, the medium was disposed of in order to eliminate those cells which had failed to attach themselves to the dish and then replaced with fresh medium. At that point, fibroblast growth factor-2 was added to the culture. Passage by means of 0.05% trypsin-ethylenediaminetetraacetic acid was conducted after the cells were confluent. The cultures were then washed and recultured in 60- or 100-mm tissue culture dishes (Corning). When confluent, the cells were passaged and ready for use. These cells not used immediately were suspended in liquid N_2 if.

Alveolar bone defects in animal models were made by extracting their mandibular anterior teeth using a sterile needle holder. Twenty microliters SHED suspension from the third to fifth passage of 106 cell density was added to the HA scaffold (BioHydrox, Tissue Bank of Dr. Soetomo Hospital, Indonesia). The mixture was placed in a 24-well tissue culture plate. After 2 h, 980 µl DMEM was added to each well.

The cells were incubated in 5% CO_2 at 37°C. After 3 days, the SHED culture in 26-hydroxyapatite was transplanted to the alveolar bone defect. Termination and alveolar bone resection were conducted after day 21. A slide was then made from the resection.

Expression of TGF-β1 and RUNX2 was determined by immunostaining. Data were analyzed using an independent *t*-test with a significance level of 5%.

Results

A quantitative count of TGF-β1 and RUNX2 expression was based on color intensity. Observation was conducted by means of a light microscope at a magnification of ×1000. A histological image of each group can be seen in Figure 1. The results of a statistical analysis using an independent *t*-test can be seen in Table 1.

Discussion

RUNX2 is the first identified marker of osteoblast differentiation. The results of this research showed a statistically significant difference (P = 0.0108) in RUNX2 expression between the two experimental groups. The HA with SHED group showed higher RUNX2 expression when compared to the control group. RUNX2 plays a role in controlling osteoblast differentiation. The results of this research are consistent with the findings of Xu *et al.* which showed that RUNX2 expression is linear to osteoblast differentiation from BM-MSCs.[11]

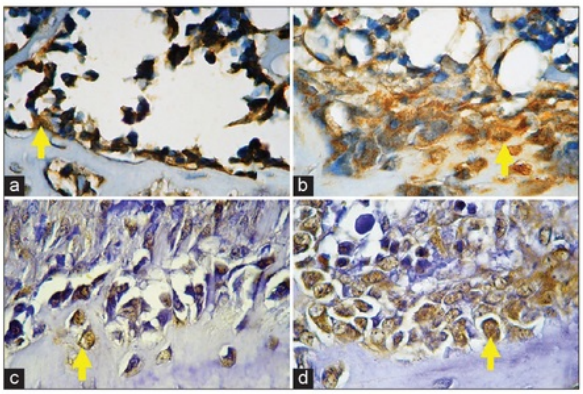

Figure 1: Immunohistochemistry examination of transforming growth factor-β1 and runt-related transcription factor 2 expression using light microscope at a magnification of ×1000. Transforming growth factor-β1 expression (yellow arrow) on osteoblast on hydroxyapatite group (a) and hydroxyapatite + stem cells from human exfoliated deciduous teeth group (b). Runt-related transcription factor 2 expression (yellow arrow) on osteoblast on hydroxyapatite group (c) and hydroxyapatite + stem cells from human exfoliated deciduous teeth group (d)

Table 1: Expression of transforming growth factor-β1and runt-related transcription factor 2 between group

Group	Mean±SD	*P*
Osteoblast		
HA	3±1.000	0.001*
HA + SHED	11.2±1.527	
RUNX2		
HA	8.33±2.081	0.0108*
HA + SHED	14.8±2.588	

*Significantly difference (P<0.05). SHED: Stem cells from human exfoliated deciduous teeth; HA: Hydroxyapatite; SD: Standard deviation; RUNX2: Runt-related transcription factor 2

SHED has the ability to control differentiation and release paracrine factors such as growth factor and cytokine. Transforming growth factor-β (TGF-β), a multifunctional cytokine, plays a role in the proliferation, migration, differentiation, and apoptosis of cells, in addition to the deposition of extracellular matrix.[12] The results showed a statistically significant increase of TGF-β in HA with SHED (P = 0.001) which is consistent with those of the study by Galatz *et al.*[13] showing TGF-β expression in the wound healing site.

The application of bone graft in this study aimed to regenerate bone by indicating that the increase of bone regeneration is linear to RUNX2 and TGF-β expression. Bone regeneration is regulated by TGF-β and bone morphogenetic proteins (BMPs).[8] TGF-β is a potential stimulator of bone regeneration which induces osteoblast

differentiation and osteoid matric synthesis, while also inhibiting protease synthesis, especially metalloprotease matrix (MMP).

Zimmermann *et al.* reported a significant increase of TGF-β1 in patients suffering fractures compared to normal patients. A decrease in TGF-β1 prolongs wound healing.[14] TGF-β1 and BMP play an important role in regulation and differentiation which induce RUNX2.[12] A study by Lee *et al.* confirmed RUNX2 to be the main target of TGF-β1 and BMP-2, whereas RUNX2 is responsible for stimulating extracellular matrix component. However, it is insufficient to stimulate the osteoblast gene.[15]

RUNX2 and TGF-β1 affect each other. RUNX2, which is regulated by TGF-β, is a DNA-binding transcription factor. RUNX2 plays an important role in the identification of osteogenic derivatives and transcription factor involved in bone formation.[7] This research showed that increasing expression of RUNX2 and TGF-β1 as the marker of bone regeneration may be useful for future SHED application in alveolar bone regeneration.

Conclusion

Expression of TGF-β1 and RUNX2 occurs after the application of HA with SHED, and TGF-β1 and RUNX2 expression in HA in the SHED group was significantly higher than in the group without SHED.

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Conflicts of interest

There are no conflicts of interest.

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