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Original Article

Expression of ATF4 and RUNX2 in periodontal tissue of pressure side during orthodontic tooth movement in rat

Jinyou Han[1], Xiaodong Xu[2], Bin Zhang[1], Baoxing Chen[1], Wangyan Hang[1]

[1]Department of Stomatology, Liaocheng People's Hospital, Shandong Province, Liaocheng 252000, China; [2]Hangzhou Normal University School of Medicine, Zhejiang Province, Hangzhou 310036, China

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Abstract: Objective: This study aims to explore the expression levels of ATF4 and RUNX and their interactions in periodontal tissue of the pressure side during orthodontic tooth movement. Methods: A total of 72 SPF level male Sprague Dawley rats were used in this study, they were divided into 9 groups randomly and 8 rats in each group. The expression changes of ATF4 and RUNX2 in periodontal tissue of pressure side at different straining time point were detected with RT-PCR and Western blotting methods. The morphological changes of cells in the tissue samples were observed by HE staining. The data were analyzed with SPSS 19.0 software. Results: The expression levels of ATF4 and RUNX2 increased during orthodontic tooth movement and were related with the movement time. They reached highest after straining for 24 h and began to decrease after straining for 12 d. Conclusions: The expression levels of ATF4 and RUNX2 in periodontal tissue can increase transiently induced by stress, which play a role in the process of osteogenesis and reconstruction of periodontal tissue during orthodontic tooth movement.

Keywords: Orthodontic tooth movement, ATF4, RUNX2, western blotting, HE staining

Introduction

External mechanical force can stimulate fibroblasts with differentiation potential in the periodontal tissue to differentiate into osteoblast like cells. In the process of differentiation, osteoblast specific transcription factors play a core role in regulation [1]. Recent studies showed that these transcription factors induced specific cell signal transduction pathway to respond to external mechanical force, which triggered a series of cellular reactions [2, 3].

Activation transcription factor 4 (ATF4) is a newly discovered transcription factor with the regulation function of osteoblast differentiation, however, there is no relevant report to confirm whether it is related with the mechanical signal transduction in the remodeling process of orthodontic periodontal tissue [4, 5]. Runt-related transcription factor-2 (RUNX2) is a multifunctional transcription factor and is the first confirmed osteoblast specific transcription factor [6]. It completed the regulation of skeletal development mainly by regulating the cell differentiation associated with the bone formation and the expression changes of extracellular matrix proteins [7]. In addition, RUNX2 is involved in the differentiation process of bone marrow mesenchymal stem cells into osteoblasts and play a key role in the mature process of osteoblast [8]. Studies also proved that the combined action of RUNX2 and OPG had inhibited effect on bone resorption and they regulated the biological processes of bone formation and bone resorption [9]. In this study, we investigated the expression levels and their correlation of RUNS2 and ATF4 in periodontal tissue during orthodontic tooth movement by constructing related animal pathological model.

Materials and methods

Experimental animals

A total of 72 SPF level male Sprague Dawley (SD) rats weighing 220 ± 10 g were obtained from the animal experimental center of Guangdong province. These SD rats were prefeeding for 2 days with free access to food and water to adapt to the environment. They were

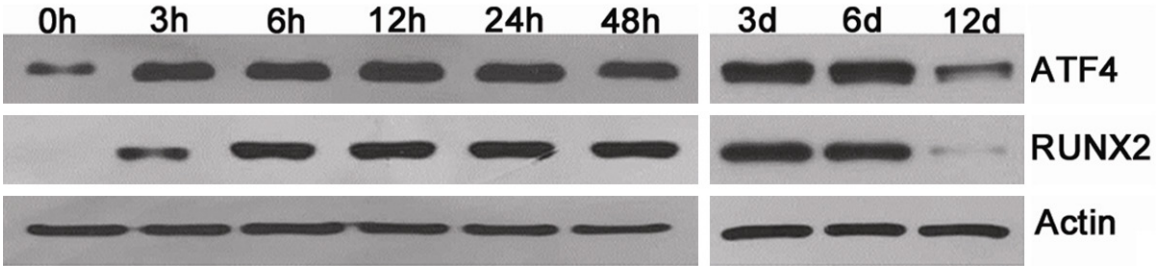

Figure 1. The expression changes of ATF4 and RUNX2 at different time point.

divided into 9 groups randomly (adding orthodontic force for 3 h, 6 h, 12 h, 24 h, 48 h, 3 d, 6 d and 12 d groups, control group) according to the random number table. Each group has 8 rats. Cages, food and water were regularly changed.

Housing and procedures involving experimental animals were in accordance with the Guide for the Care and Use of Laboratory Animals (eighth edition, published by the National Academies Press). All experimental procedures were approved by the Care of Experimental Animals Committee of our hospital.

Detection of the expression of ATF4 and RUNX2

RT-PCR: Total RNAs were extracted from periodontal tissue using Trizol RNA extraction kit. Total RNA was reverse transcribed and qRT-PCR carried out using SYBR Green master mix and primers specific for ATF4, RUNX2 and β-actin. The PCR primers were listed as follows: ATF4 primers: F: 5'-CCCTCCACCTTCTTACAACC-3'; R: 5'-TACGACTCTGGGCTCATAC-3'; RUNX2 primers: F: 5'-AGTGTGTGTGTCCGCATGAT-3'; R: 5'-CCACTTGGGGTCTAAGAACG-3'; β-actin primers: F: 5'-GAGACCTTCAACACCCCAGCC-3'; R: 5'-GGCCATCTCTTGCTCGAAGTC-3'. The amplification conditions are as follows: 94°C for 10 minutes followed by 40 cycles of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 30 seconds.

Western blotting: Total proteins were extracted from periodontal tissue and analyzed with SDS-PAGE electrophoresis. Then it was electrotransferred to the PVDF membrane. The membrane containing the proteins was used for immunoblotting with required antibodies for ATF4, RUNX2 and β-actin. They were blocked with 5% non-fat milk in TBST (10 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 0.1% Tween-20) for 2 h, then incubated with the primary antibodies at 4°C overnight. Then they were incubated with secondary antibodies conjugated with horseradish peroxidase at room temperature for 1 h. The images were analyzed using Alpha Imager 2200 system.

HE staining

The tissue samples were fixed in formalin and embedded in paraffin routinely. The paraffin blocks of specimen were cut into continuous sections with 5 μm respectively. The sections were dewaxed with xylene and washed with ethanol and water. They were stained with hematoxylin after that and then differentiated, washed and stained with eosin, then dehydrated, hyalinized and finally mounted on slides and observed under microscope, pictures were taken.

Detection of ATF4 and RUNX2 with immunohistochemical method

Briefly, the specimens fixed in 10% formaldehyde were taken out and washed, they were paraffin-embedded and were sliced at thickness of 4 μm. Following deparaffinization, dehydration, and antigen retrieval, the sections were blocked with 5% BSA and incubated at 37°C for 20 min, and then they were incubated with required antibodies at 4°C overnight. After that, they were washed with PBS and incubated at 37°C for 2 h after drop-adding the 2nd antibody and washed with PBS. After treated with the DAB solution, they were flushed completely, counterstained with hematoxylin and washed with water, treated with dehydration and transparency, then mounted on slides and observed under microscope.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Science

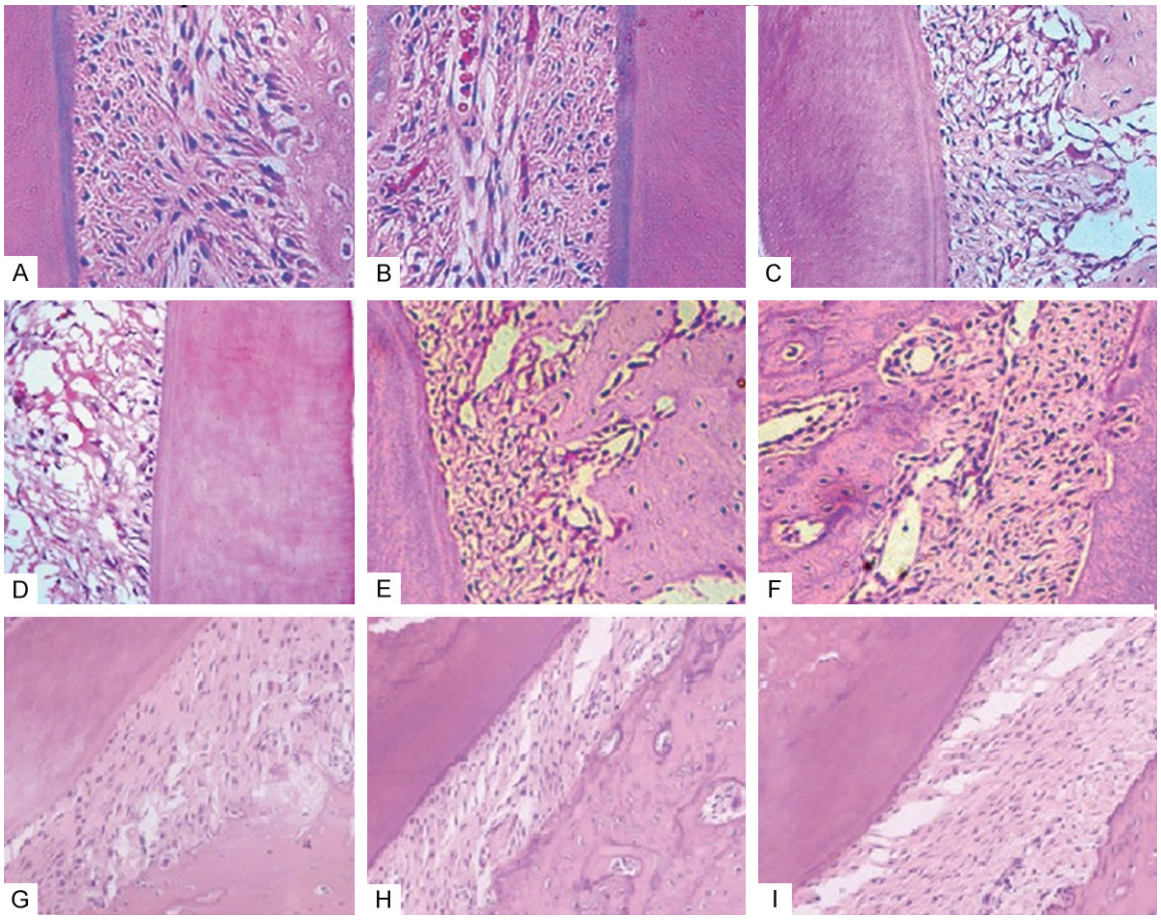

Figure 2. HE staining results at different time point. A. Control; B. Straining for 3 h; C. Straining for 6 h; D. Straining for 12 h; E. Straining for 24 h; F. Straining for 48 h; G. Straining for 3 d; H. Straining for 6 d; I. Straining for 12 d.

(SPSS, version 19.0). Differences between experimental groups were analyzed by One-way ANOVA and t-test. $P < 0.05$ was considered to be significant.

Results

Expression changes of ATF4 and RUNX2

The results of RT-PCR and Western blotting showed that the expression levels of ATF4 and RUNX2 increased during orthodontic tooth movement and were related with the movement time. They increased after straining for 3 h and reached highest after straining for 24 h ($P < 0.05$). We found that they began to decrease after straining for 12 d ($P < 0.05$). Western blotting result was shown in **Figure 1**.

HE staining results

In control group, the tissue space of periodontal membrane was uniform, the fibers arranged neatly and the fibroblast distributed evenly in main fiber. The tissue space of periodontal membrane became narrow after straining for 3 hours, it became narrower along with the formation of bone resorption lacunae after straining for 6 h, 12 h and 1 day. After straining for 3 d, 6 d and 12 d, it appeared a plurality of osteoclasts and showed obvious bone resorption. The formation of new alveolar bone appeared in the periodontal fibers after straining for 12 d (**Figure 2**).

Immunohistochemical results

The results of immunohistochemical detection showed that ATF4 and RUNX2 mainly distributed in nucleus. The ATF4 and RUNX2 was weakly positive in control group, the positive cells with brown yellow granule increased after straining and were the most after straining for 24 h. They decreased after straining for 48 h but still more than that of control group after straining for 12 d (**Figure 3**).

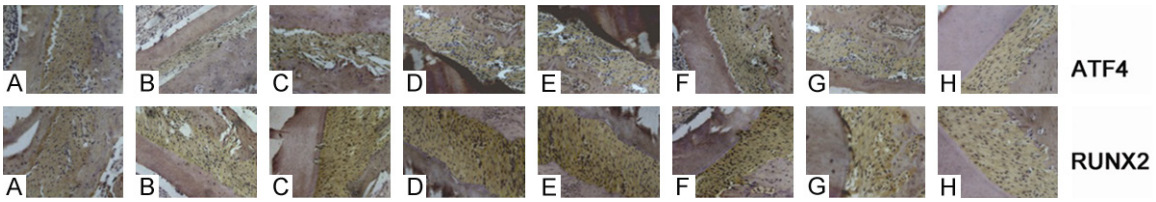

Figure 3. Immunohistochemical results at different time point. A. Control; B. Straining for 3 h; C. Straining for 12 h; D. Straining for 24 h; E. Straining for 48 h; F. Straining for 3 d; G. Straining for 6 d; H. Straining for 12 d.

Discussion

During orthodontic treatment, mechanical force caused bone resorption in the pressure side and bone formation in the tension side, which led to the tooth movement. Mechanical stimulation can induce the change of extracellular matrix and also can produce a series of biological reaction, then induce rebuilding and restructuring of periodontal tissue. There are many kinds of cells in the periodontal membrane. It is possible for these cells to differentiate to cementocyte and osteoblast only in the specific mechanical signal transduction pathway. The implementation of orthodontic pressure on periodontal tissue which inducing differentiation of periodontal ligament cells into osteoblasts to be involved in bone resorption and bone formation is the key of periodontal tissue remodeling of orthodontic tooth [10]. Recent researches showed that orthodontic mechanical stimulation can induce periodontal ligament cells to differentiate into osteoblast in vitro [11, 12]. However, it is complex to study the clinical application of tissue remodeling and mechanical signal transduction, which involving many cytokines, extracellular matrix proteins and signal channel [13, 14].

Studies in vitro found that RUNX2 played an important regulation role in the differentiation of mesenchymal stem cells to osteoblast [15]. Baumert found that the expression of transcription factor closely associated with the process of osteoblast differentiation and bone formation up-regulated under the condition of external pressure, which confirmed that these differentially expressed genes play a key role in mature process of osteoblasts terminal differentiation [16]. Some researchers thought that the ion channel played a role in orthodontic process, which mediated polymerization of actin and made the biological response to stress [17]. Other studies found that cytokines such as prostaglandin and IL-1 can participate in the mechanical signal transduction, then interact with various extracellular and intracellular proteins and cytokines to form more complex network pathway and transform the external mechanical signals into tissue reconstruction process [18, 19].

In this study, we constructed related animal model and simulated the process of clinical treatment of patients. We found that the expression of RUNX2 and ATF4 increased gradually with time prolonging in the early stage of straining. They reached the highest levels after straining for 24 h and began to decrease after straining for 12 d. These maybe because RUNX2 and ATF4 proteins have the similar biological functions, they play a cooperative role in the formation of periodontal tissue. After their transcriptions were completed, the MAPK signal pathway induced by mechanical force made phosphorylation of RUNX2, RUNX2 was activated and entered the nucleus to regulate the downstream effect elements and involved in the transcriptional regulation. However, the specific mechanisms are not yet elucidated which need further studies.

In conclusion, during the orthodontic tooth movement, we speculated that the reaction of periodontal tissue was transferred by orthodontic force of teeth. Expression changes of RUNX2 and ATF4 protein caused reconstruction of periodontal tissue and made the displacement of teeth position. RUNX2 and ATF4 play an important role in remodeling of periodontal tissue, but the specific mechanism needs further research and confirmation.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiaodong Xu, Hangzhou Normal University School of Medicine, Zhejiang province, China. Tel: +86-571-88854954; E-mail: xiaodongxux@126.com

References

[1] Rawlinson S, Pitsillides A and Lanyon L. Involvement of different ion channels in osteoblasts' and osteocytes' early responses to mechanical strain. Bone 1996; 19: 609-614.

[2] Moustafa A, Sugiyama T, Prasad J, Zaman G, Gross T, Lanyon L and Price J. Mechanical loading-related changes in osteocyte sclerostin expression in mice are more closely associated with the subsequent osteogenic response than the peak strains engendered. Osteoporos Int 2012; 23: 1225-1234.

[3] Bivi N, Pacheco-Costa R, Brun LR, Murphy TR, Farlow NR, Robling AG, Bellido T and Plotkin LI. Absence of Cx43 selectively from osteocytes enhances responsiveness to mechanical force in mice. J Orthop Res 2013; 31: 1075-1081.

[4] Wang W, Lian N, Ma Y, Li L, Gallant RC, Elefteriou F and Yang X. Chondrocytic Atf4 regulates osteoblast differentiation and function via Ihh. Development 2012; 139: 601-611.

[5] Danciu TE, Li Y, Koh A, Xiao G, McCauley LK and Franceschi RT. The basic helix loop helix transcription factor Twist1 is a novel regulator of ATF4 in osteoblasts. J Cell Biochem 2012; 113: 70-79.

[6] Ducy P, Zhang R, Geoffroy V, Ridall AL and Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 1997; 89: 747-754.

[7] Byun MR, Kim A, Hwang JH, Sung MK, Lee YK, Hwang BS, Rho JR, Hwang ES and Hong JH. Phorbaketal A stimulates osteoblast differentiation through TAZ mediated Runx2 activation. FEBS Lett 2012; 586: 1086-1092.

[8] Baniwal S, Shah P, Shi Y, Haduong J, Declerck Y, Gabet Y and Frenkel B. Runx2 promotes both osteoblastogenesis and novel osteoclastogenic signals in ST2 mesenchymal progenitor cells. Osteoporos Int 2012; 23: 1399-1413.

[9] Liu TM and Lee EH. Transcriptional regulatory cascades in runx2-dependent bone development. Tissue Eng Part B Rev 2012; 19: 254-263.

[10] Kitaura H, Kimura K, Ishida M, Sugisawa H, Kohara H, Yoshimatsu M and Takano-Yamamoto T. Effect of cytokines on osteoclast formation and bone resorption during mechanical force loading of the periodontal membrane. Sci World J 2014; 2014: 617032.

[11] Howard PS, Kucich U, Taliwal R and Korostoff JM. Mechanical forces alter extracellular matrix synthesis by human periodontal ligament fibroblasts. J Periodontal Res 1998; 33: 500-508.

[12] Pavlin D, Dove SB, Zadro R and Gluhak-Heinrich J. Mechanical loading stimulates differentiation of periodontal osteoblasts in a mouse osteoinduction model: effect on type I collagen and alkaline phosphatase genes. Calcif Tissue Int 2000; 67: 163-172.

[13] Weyts F, Bosmans B, Niesing R, Leeuwen J and Weinans H. Mechanical control of human osteoblast apoptosis and proliferation in relation to differentiation. Calcif Tissue Int 2003; 72: 505-512.

[14] Hatton JP, Pooran M, Li CF, Luzzio C and Hughes-Fulford M. A Short Pulse of Mechanical Force Induces Gene Expression and Growth in MC3T3-E1 Osteoblasts via an ERK 1/2 Pathway. J Bone Miner Res 2003; 18: 58-66.

[15] Huang J, Zhao L, Xing L and Chen D. MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation. Stem Cells 2010; 28: 357-364.

[16] Baumert U, Golan I, Redlich M, Aknin JJ and Muessig D. Cleidocranial dysplasia: Molecular genetic analysis and phenotypic-based description of a Middle European patient group. Am J Med Genet A 2005; 139: 78-85.

[17] Zayzafoon M. Calcium/calmodulin signaling controls osteoblast growth and differentiation. J Cell Biochem 2006; 97: 56-70.

[18] Ding J, Ghali O, Lencel P, Broux O, Chauveau C, Devedjian JC, Hardouin P and Magne D. TNF-alpha and IL-1beta inhibit RUNX2 and collagen expression but increase alkaline phosphatase activity and mineralization in human mesenchymal stem cells. Life Sci 2009; 84: 499-504.

[19] Yoshida K, Oida H, Kobayashi T, Maruyama T, Tanaka M, Katayama T, Yamaguchi K, Segi E, Tsuboyama T, Matsushita M, Ito K, Ito Y, Sugimoto Y, Ushikubi F, Ohuchida S, Kondo K, Nakamura T and Narumiya S. Stimulation of bone formation and prevention of bone loss by prostaglandin E EP4 receptor activation. Proc Natl Acad Sci U S A 2002; 99: 4580-4585.