Int J Clin Exp Med 2016;9(8):16206-16211
www.ijcem.com /ISSN:1940-5901/IJCEM0021013

Original Article

Expression and function of PLAP-1 and caveolin-1 in periodontal tissues and cells of rat orthodontic tooth movement models

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Received December 2, 2015; Accepted March 2, 2016; Epub August 15, 2016; Published August 30, 2016

Abstract: Objective: To investigate the expression of periodontal ligament-associated protein 1 (PLAP-1) and caveolin-1 in orthodontic tooth movement in rats and their roles in periodontal tissue reconstruction. Methods: Forty SD rats were randomly assigned to 4 groups of orthodontic tooth movements for 0, 3, 5, and 7 days, respectively. The controls and treatments were the first right and left maxillary molars without and with orthodontic pressure, respectively. After the treatments, the animals were sacrificed and periodontal tissues were collected to analyze gene expression using qRT-PCR and Western blot. The tissues were digested and cells were isolated to culture primary teeth cells. The cultured cells were induced to mineralize and assayed for expression of PLAP-1 and Caveolin-1 using Western blot. Results: Within 3 days after the orthodontic tooth movement, there was no significant difference in expression of PLAP-1 and Caveolin-1 between in the pressure side and control side ($P > 0.05$). On day 5, PLAP-1 was significantly upregulated in the pressure side as compared to the control side; on day 7, expression of PLAP-1 was downregulated and was significantly lower than that on day 3 ($P < 0.05$). For Caveolin-1, the expression was significantly up-regulated from day 3 as compared with the control ($P < 0.01$). In mineralizing cells, the expression of Caveolin-1 increased with the induction time; and the expression of PLAP-1 trended to increase two weeks after the induction and decreased afterward. In un-induced cells, the expression of the two genes did not changed with the culture time. Conclusion: In early orthodontic tooth movement, PLAP-1 was significantly up-regulated and then down-regulated with the increase in stimulation time, while the expression of Caveolin-1 constantly increased with treatment time, which is similar to what happen during the mineralization of cultured cells.

Keywords: Orthodontic force, periodontal tissue, periodontal tissue, PLAP-1, Caveolin-1

Introduction

Orthodontic treatment applies orthodontic force to the tooth through a variety of appliances to treat wrong jaw deformity and to achieve periodontal tissue reconstruction. During orthodontic tooth movement, mechanic orthodontic force exerted on the periodontal tissue can activate osteoblasts and osteoclasts, resulting in their resorption and deposition in the alveolar bone, and stimulate the growth of cells and periodontal tissue remodeling [1, 2]. Orthodontic tooth movement is a complex biological and mechanical movement involving in a variety of molecules. How mechanical stimulation is converted to biochemical signals has been the hot spot of research in related fields. Studies have shown that external stimulus can activate signaling pathways, thereby affecting the proliferation and differentiation of periodontal cells [3, 4]. In this process, expression of relevant proteins is crucial. Therefore, better understanding of the regulation mechanism of signaling molecules is of great significance for elucidating the molecular mechanism of orthodontic tooth movement.

Teeth periodontal ligament cells (PDLCs) are a group of heterogeneous cells [5], which are mainly originated from the mesenchymal cells. After mineralization, they differentiate into osteoblasts and cementoblasts to form a new primary fibers, cementum, and to reconstruct alveolar bone. Therefore, PDLCs are very important for periodontal tissue repair. Studies have shown that when stimulated by mechanical

Table 1. Primer for qRT-PCR

Gene	GenBank Accession	Primer (5'-3')
PLAP-1	NM_001172481	For: AACAAGCTAACGAAGATTCACCC
		Rev: CCCCTGGCTCTATCCCATTATT
Caveolin-1	NM_010493.2	For: ATGTCTGGGGGCAAATACGTG
		Rev: CGCGTCATACACTTGCTTCT
GAPDH	NM_008085	For: AATGGATTTGGACGCATTGGT
		Rev: TTTGCACTGGTACGTGTTGAT

For, forward; Rev, reverse.

force, the mechanical signals are converted to biological signals in PDLCs [6, 7], resulting in a cascade of biological reactions such as alternation of metabolism in periodontal tissue and alveolar bone remodeling. In this process, a number of regulators are involved, such as insulin-like growth factors, transforming growth factors, fibroblast growth factors, bone morphogenetic proteins, in the orthodontic tooth movement [8, 9].

Periodontal ligament associated protein-1 (PLAP-1) is a protein over-expressed in periodontal ligament [10]. It is shown to be involved in the inhibition of mineralization process, and closely related to the differentiation and mineralization of periodontal tissue. As a negative regulator, PLAP-1 inhibits the production of osteoblasts and maintains a balance with other growth factors to stabilize dynamic microenvironment in the periodontal tissue [11, 12]. Caveolin-1 gene is involved in regulating the cellular membrane composition during cell migration and cell surface expansion [13]. However, it is unclear if it is involved in the reconstruction of periodontal tissue during orthodontic tooth movement.

In this study, we analyzed the expression of PLAP-1 and Caveolin-1 in periodontal tissue using rat model, and in cultured periodontal cells after induced mineralization. The findings provide new insights into the molecular regulation and mechanism of orthodontic tooth movement.

Materials and methods

Animal model and orthodontic treatment

Male Wistar rats (SPF, weighing 200 ± 50 g), purchased from Silaike Experimental Animal Co., Ltd. (Shanghai, China), were to use for the study. The rats were fed with standard pellet feed in animal cages (5 per cage) with free access to drinking water and feed during the experiments. After 7 days of adaptive feeding, the rats were randomly divided to 4 groups to establish orthodontic tooth movement model as described [14]. Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (3 mL/kg), and shallow concaves of about 0.5-1.0 mm deep were cut between the axial plane and angle in the first molars of upper jaw. The shallow concaves were used to fasten Ni-Ti coil springs (Grikin, Beijing) using stainless steel wire of 0.25 mm in diameter. One end of the wire was tied to the neck of the first molar and the other end was attached to the Ni-Ti coil springs to provide orthodontic force of 0.4 N on the pressure side for 0, 3, and 5 and 7 days, while in control, the same orthodontic appliance was used without applying the orthodontic pressure. Ten rats were used in each treatment. The experimental protocols were approved by the Ethics Committee of the 5th Affiliated Hospital of Zhengzhou University.

Periodontal tissue

Rats were sacrificed by cervical dislocation at the end of experiments, and tissues surrounding the first molars were taken, washed in pre-chilled PBS buffer and snap-frozen in liquid nitrogen.

Isolation and culture of primary periodontal ligament cells

Periodontal tissues were isolated immediately from the sacrificed rats, washed repeatedly in PBS, cut into small pieces with ophthalmic scissors. The tissue pieces were added with 10 volume of type I collagenase, digested at 37°C for 40 min and centrifuged at 1000 rpm for 4 min. The pellet was re-suspended in DMEM medium and inoculated into the culture bottles and cultured at 37°C and 5% CO_2. The cells were subcultured when reaching 80% confluence for three passages and then were induced for mineralization using an induction medium containing 50 g/mL ascorbic acid, 10 nM dexamethasone and l0 mM β-glycerol phosphate. Aliquots of the cells were taken to determine the growth rate by measuring the OD_{570} at a wavelength of 570 nm, and cells were collected at 0, 7, 14 and 21 days after induction for expression analysis.

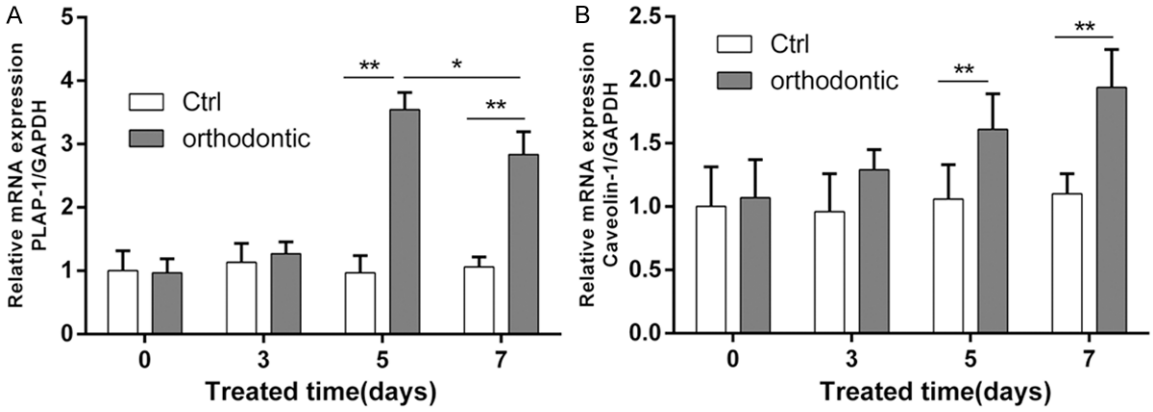

Figure 1. Relative mRNA levels of PLAP-1 (A) and Caveolin-1 (B) genes in periodontal tissues after orthodontic movement treatment. ** and * denote highly significant or significant difference between the bars underneath the lines.

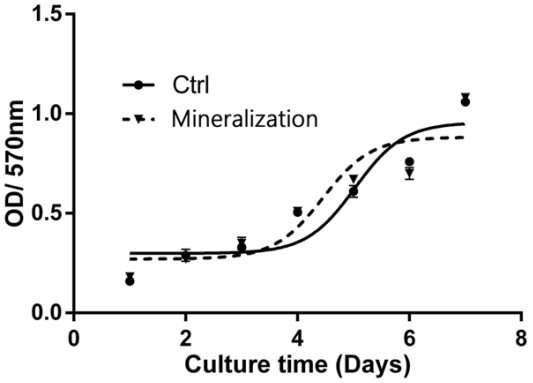

Figure 2. Growth curve of *cultured periodontal cells* after induction of mineralization.

qRT-PCR

Total RNA was extracted from the frozen samples using a RNA extract kit (Qiagen, USA) according to the manufacturer's instructions and reversely transcripted into cDNA using a reverse transcription kit (Applied Biosystems, USA) according to the manufacturer's instructions. mRNA was quantified using qRT-PCR with gene-specific primers (**Table 1**) and the 2(-Delta Delta C(T)) method [15]. Glyceraldehyde phosphate dehydrogenase (GAPDH) gene was used as internal reference for the quantification. All determinations were repeated three times. The Reverse-transcription reaction contained 4 μL 5 × iScript reaction mix, 1 μL iScript reverse transcriptase (Bio-Rad, USA) and 1 μg RNA template. PCR was run with 2 μL SoAdvanced SYBR Green Super mix (Bio-Rad, USA), 0.5 μL each of primers (300-450 nM), 100 ng cDNA template and nuclease-free water to fill up to a total reaction volume of 10 μL, and performed according to the manufacturer's protocols (Invitrogen, USA).

Western blot analysis

Total protein was extracted using a cell lysis buffer (ComWin Biotech Co., Ltd., Beijing) supplemented with a protease inhibitor cocktail (Biotool, USA) according to manufacturer's instruction. The protein content was determined using a Protein Assay Kit (Beyotime Biotechnology, Beijing). The proteins were size-fractionated by SDS-polyacrylamide gel electrophoresis, and transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories Inc., Hercules, CA, USA). After blocking, the membranes were incubated with the primary monoclonal antibodies against PLAP-1 and Caveolin-1 (Abcam, UK) and rabbit anti-GAPDH antibody (Boster, Wuhan) at 4°C overnight. Protein was detected using horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibodies using enhanced chemiluminescence (EMD Millipore, USA). Band intensity was measured using a JS-1035 image analysis scanning system (Shanghai Peiqing Science & Technology, Co., Ltd., Shanghai, China), and quantitatively analyzed using Quantity one v4.62.

Statistical analysis

Experimental data were analyzed using SPSS16.0 statistical software for statistical analysis. The measurement data were expressed as mean ± SD. Means between the two groups were compared using Student's *t*-test and value

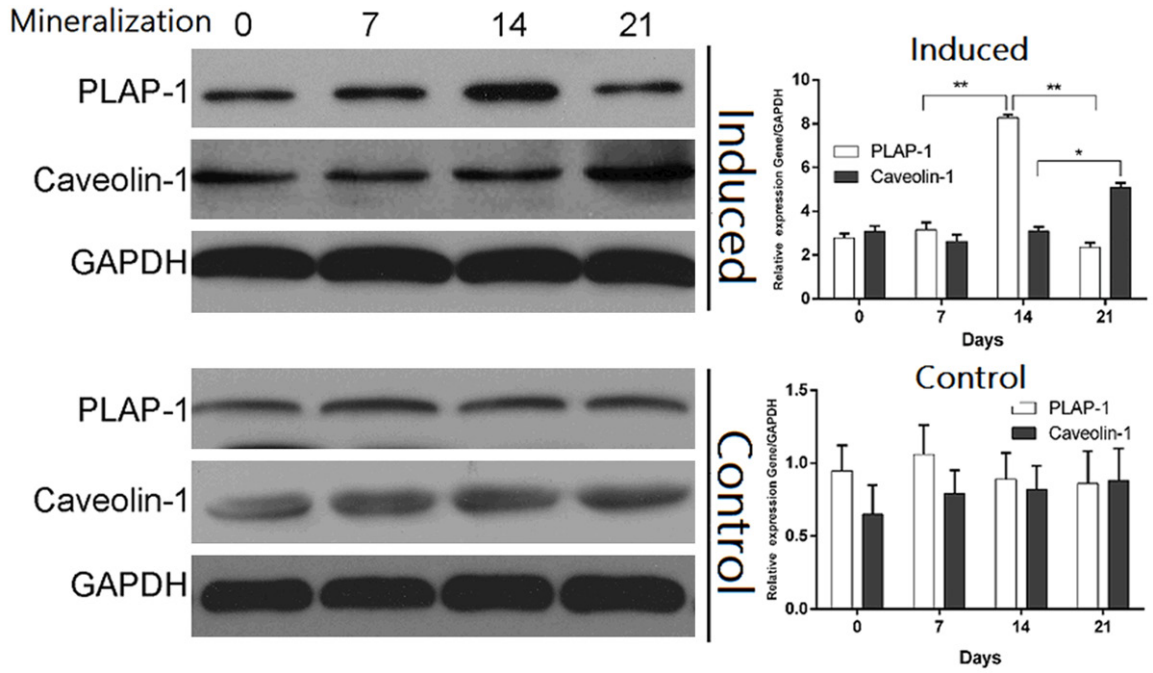

Figure 3. Relative protein levels of PLAP-1 and Caveolin-1 genes in *cultured periodontal cells* after induction of mineralization. ** and * denote highly significant or significant difference between the bars underneath the lines.

was considered as significant or highly significant if $P < 0.05$, or < 0.01.

Results

PLAP-1 and Caveolin-1 mRNA levels in periodontal tissues

Expression of the PLAP-1 and Caveolin-1 genes was measured using RT-PCR in the periodontal tissues after different days of orthodontic movement treatment. The results showed that there was no significant difference in the mRNA levels of the two genes between pressure and control sides within 3 days of treatment ($P > 0.05$, **Figure 1A**); compared to control side, the expression of PLAP-1 in the pressure side was significantly higher on day 5 and then reduced to the levels significantly less than that on day 3 ($P < 0.05$). The expression of Caveolin-1 in pressure side was up-regulated from day 3 as compared to that in control side ($P < 0.01$, **Figure 1B**).

PLAP-1 and Caveolin-1 mRNA levels in cultured periodontal cells

Growth of primary periodontal cells cultured in DMEM medium was determined and no difference in growth rate at different time points was found between the cells induced and uninduced for mineralization ($P > 0.05$, **Figure 2**). The expression of the PLAP-1 and Caveolin-1 genes was measured in the cultured cells after induction of mineralization using Western blot. The results showed that the expression of Caveolin-1 increased as the induction time increased (**Figure 3**) while the expression of PLAP-1 trended to increase within two weeks of induction and then decreased afterward (**Figure 3**); however, in the cells without induction, the expression of the two genes remained unchanged over the culture period (**Figure 3**).

Discussion

Mechanical pressure in orthodontic tooth movement has been shown to generate a number of biological activities in bone cells through signal transduction, resulting in alveolar bone resorption and deposition, and causing the remodeling of periodontal tissues [6, 16, 17]. Osteoclasts and cementoblasts are involved in these processes, which are regulated by many signal molecules and growth factors such as fibroblast growth factors, bone morphogenetic protein growth factors [18-20].

PLAP-1 is an extracellular matrix protein, a member of the SLRP family. It is highly expressed in the periodontal ligament [10]. Since

mature PLAP-1 has a repetitive sequence of asparagic acid at the N terminal, it is also called Asporin. The studies have showed that the gene is highly and specifically expressed in periodontal ligament, and the expression increases with the development of teeth, and has potential roles in maintaining and promoting the stability and regeneration of periodontal tissues [18-20]. In this study, we found that the expression of PLAP-1 in the periodontal tissues under orthodontic pressure increased only after day 3. Since orthodontic tooth movement is a slow and gradual action, it is likely that the tissues and cells will response to external stimulus after certain time, when relevant genes are motivated and expressed to response. We did detect significant up-regulation of the gene on day 5 in the periodontal tissues subjected to the orthodontic pressure, although the increase was reduced on day 7, but still higher than that before day 3. These results are similar to early works [21, 22]. Since PLAP-1 is a negative regulator of osteoblasts, such change in PLAP-1 expression is in line with its function, which is to maintain the tissue stability in local microenvironment of periodontal tissue and to activate and enhance relevant signal pathways to response to external signals such as orthodontic pressure and to inhibit the differentiation of osteoblasts.

Caveolin-1 gene is important molecule located in a central position of cell signal transduction that recruits signal molecules in the signal transduction pathways [23]. In this experiment, caveolin-1 was constantly up-regulated after 3 days of orthodontic pressure and increased with the treatment time.

To further investigate the role of the two genes in tooth tissue reconstruction, we used primary periodontal cell culture to mimic tooth differentiation, in which cell mineralization is a key process. Our data showed that the growth of periodontal cells were not affected by the induction of mineralization but expression of Caveolin-1 and PLAP-1 showed similar trends as in the periodontal tissues under orthodontic pressure. These results demonstrate that both Caveolin-1 and PLAP-1 play role in maintaining the stability of micro environment in periodontal tissues, and can quickly response to external signals such as orthodontic force by regulating their expression and transmitting the signals downstream. Both of them are likely involved in periodontal tissue reconstruction. However, as negative regulators, their regulations are likely to be complex and temporal- and spatial-specific, rather than simple signal transduction. More works are needed to further elucidate the molecular mechanisms underlying the regulation.

Disclosure of conflict of interest

None.

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