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ORIGINAL ARTICLE

Expression of Wnt3a, Wnt10b, β-catenin and DKK1 in periodontium during orthodontic tooth movement in rats

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Abstract

Objective: To investigate the expression of Wnt3a, Wnt10b, β-catenin and DKK1 in the periodontal ligament (PDL) during orthodontic tooth movement (OTM) in rats. *Materials and methods*: Nickel-titanium closed-coil springs were used to deliver an initial 50 g mesial force to the left maxillary first molars in 30 rats. The force was kept constant for 1, 3, 5, 7, 10 and 14 days until the animals were sacrificed. The right maxillary molars without force application served as control. Paraffin-embedded sections of the upper jaws were prepared for histological and immunohistochemical analyses to detect Wnt3a, Wnt10b, β-catenin and DKK1 expression in PDL. *Results*: Wnt3a, Wnt10b, β-catenin and DKK1 were expressed on both the ipsilateral and contralateral sides of PDL in each group. After the application of orthodontic force, the expression of β-catenin and DKK1 was initially increased and then decreased on both sides, with maximal levels of expression at day 7 and day 10, respectively. On the compression side, Wnt3a and Wnt10b levels started to increase at day 5, while on the tension side, these two molecules began to increase at day 1. Furthermore, the expression levels of Wnt3a, Wnt10b, and β-catenin were much stronger on the tension side than on the compression side at any of the observation points, while DKK1 level was much higher on the compression side. *Conclusion*: Wnt3a, Wnt10b, β-catenin and DKK1 expression may be related to the periodontal tissue remodeling following the application of an orthodontic force in rats. These observations suggest that the Wnt/β-catenin signaling pathway may play a crucial role in periodontal tissue remodeling during OTM.

Keywords: *Wnts*, *β-catenin*, *DKK1*, *orthodontic tooth movement*, *periodontal tissue remodeling*

Introduction

Orthodontic tooth movement (OTM) is achieved by the remodeling of both alveolar bone and periodontal ligament (PDL) in response to mechanical loading [1]. The production of biological mediators from PDL cells plays an important role in activating tissue remodeling, characterized by selective resorption and tissue deposition in the alveolar bone and PDL [2]. In bone remodeling, molecules such as type I collagen (COL-I) and alkaline phosphatase (ALP) are produced to promote bone formation [3], while the engagement of receptor activator of nuclear factor-κB (RANK) and its ligand (RANKL) facilitates bone resorption. Osteoprotegerin (OPG), a decoy receptor, can inhibit osteoclastogenesis and bone absorption by mediating RANK–RANKL interaction and recent studies have demonstrated the participation of the RANK system in OTM [4,5]. In the remodeling of PDL, matrix metalloproteases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) were reported to be involved [6]. Our previous study demonstrated that the calcium channel blocker nifedipinecan affect the expression of MMP-1, -10, -13, and COL-I during PDL remodeling [7]. Although much progress has been made in periodontal tissue remodeling, its exact mechanisms remain unclear.

Previous studies demonstrated that the Wnt/β-catenin signaling pathway plays an essential role in bone remodeling under mechanical loading [8,9]. Wnt3a and Wnt10b are canonical Wnt ligands which can be expressed in bone marrow. Wnt3a was shown to be a potent regulator of osteogenic differentiation and involved in the repair of the periapical bone destruction [10]. Wnt10b can increase post-natal bone formation by enhancing osteoblast differentiation [11]. The activity of β-catenin is essential for the

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differentiation of mature osteoblasts and, consequently, for bone formation. Eliminating an essential component of the Wnt/β-catenin network leads to a pathological widening of the PDL space [12]. Lim et al. [13] reported that elevation or repression of Wnt signaling alters the expression of osteogenic genes within the PDL space, which in turn affects the overall width of PDL and the turnover of alveolar bone. Furthermore, an *in vitro* study showed that canonical Wnt signaling leads to the differentiation of PDL fibroblasts into osteogenic lineage with the attendant stimulation of osteogenic transcription factors [14]. However, whether Wnt/β-catenin signaling plays a decisive role in periodontal tissue remodeling induced by orthodontic force remains unclear.

In this study, we established an OTM models in rats and tested whether Wnt/β-catenin signaling molecules such as Wnt3a, Wnt10b, β-catenin and DKK1 are involved in the remodeling of periodontal tissues during OTM.

Materials and methods

Animal model of OTM

Thirty 7-week-old male Sprague-Dawley rats (weighing 200–220 g) obtained from Harbin Medical University Animal Center were used in the present study. The animal protocol was approved by the Institutional Animal Care and Use Committee of Harbin Medical University. The experimental procedures have been described in detail by a previous report [15]. Briefly, the maxillary left first molar was moved mesially by a Ni-Ti closed-coil spring (Shinye Odontology Materials Corporation Company, Hangzhou, China) with an application force of 50 g. The maxillary incisors were used as the anchorage and were fixed with the spring by a 0.01 inch steel ligature (Grikin Advanced Materials, Beijing, China). To prevent slippage of the appliance, a 0.5 mm groove was prepared at the cervical line of the incisors, where the ligature wire was seated and covered with composite resin (Figure 1). The rats were sacrificed by cardiac perfusion with 4% paraformaldehyde at 1 day, 3 days, 5 days, 7 days, 10 days and 14 days after the application of the moving force (five rats for each time point). All procedures were performed under anesthesia, with intraperitoneal injections of 10% chloral hydrate (350∼400 mg/kg).

Tissue preparation and immunohistochemistry

After perfusion, the maxillae were dissected. Molar-bearing segments of alveolar bone were cut from each site (left site as the experimental site and the right site as the control), fixed in 4% paraformaldehyde for 48 h at 4 °C and then decalcified in 15% ethylene-diaminetetraacetic acid (EDTA), PH 7.4, for 6–8 weeks. The decalcified specimens were dehydrated through ascending concentrations of ethanol, embedded in paraffin, and then the mesiodistal serial sections of 1–5 μm thickness were cut. The sections were used for hematoxylin–eosin (HE) and immunohistochemistry staining with the antibodies against Wnt3a, Wnt10b, β-catenin and DKK1.

These sections were deparaffinized by immersion in xylene and rehydrated through a graded ethanol series to double-distilled water. Endogenous peroxidase activity was blocked in 0.3% hydrogen peroxide for 15 min. Following antigen retrieval, the specimens were incubated with bovine serum at 37 °C for 20 min and then incubated with polyclonal the anti-Wnt3a antibody (bs-1700R; Beijing Biosynthesis Biotechnology Co Ltd, Beijing, China) at a 1:100 working dilution, the anti-Wnt10b (bs-3662R; Beijing Biosynthesis Biotechnology Co Ltd) at a 1:200 working dilution, the anti-β-catenin (bs-1165R; Beijing Biosynthesis Biotechnology Co Ltd) at a 1:100 working dilution and the anti-DKK1 (bs-2162R; Beijing Biosynthesis Biotechnology Co Ltd) at a 1:100 working dilution overnight at 4 °C. Negative controls were incubated with PBS instead of the primary antibody. Then, a secondary antibody (Boster Biotechnology, Wuhan, China) was incubated for 30 min. The positive expression was visualized by treating the sections with 3.3-diaminobenzidine (DAB; Boster Biotechnology) for 2 min; counterstaining was performed by hematoxylin for 60 s.

Image observation and statistical analysis

The mid-third root of the maxillary first molar on both the compression and tension sides was selected in the study area with Nikon E600 microscopy (Nikon, Japan). Expression levels of Wnt3a, Wnt10b, β-catenin and DKK1 proteins were assessed by the mean optical density (MOD) [16]. Both the area and the integrated optical density (IOD) of positive stains were measured by Image-Plus Pro 6.0

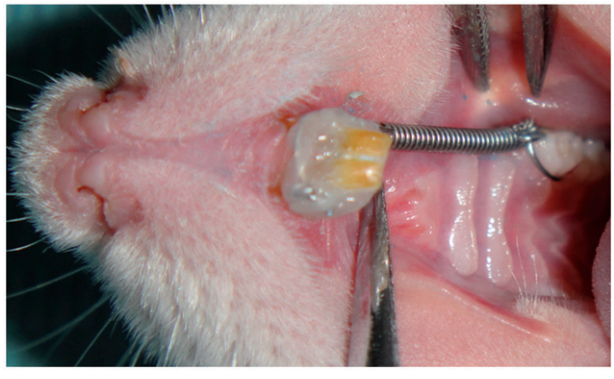

Figure 1. The orthodontic tooth movement model. A closed-coil spring was placed between the left first maxillary molar and the maxillary incisors.

(Media Cybernetics, Bethesda, MD). MOD was calculated as follows: MOD = IOD/study area. The statistical package SAS 9.1.3 (SAS Institute, NC) was employed to analyze the data. Mean values and standard deviations of MOD for Wnt3a, Wnt10b, β-catenin and DKK1 levels were calculated. One-way analysis of variance (ANOVA) was used to evaluate differences between the loaded and unloaded teeth; $p < 0.05$ was considered as the level of significance.

Results

The histological changes in periodontal tissue remodeling during OTM

In this study, the right maxillary first molar without loading served as the control. The unloaded teeth exhibited a smooth root surface without alteration in PDL (Figure 2A). As shown in Figure 2, the PDL space on the compression side was narrower than that in the control, while the space on the tension side was wider. The PDL fibers were disoriented on both sides (Figures 2B–G). Furthermore, osteoclasts and bone resorption pits were detected on the compression side, while osteoblasts and bone formation were observed on the tension side (Figure 2H).

Wnt3a expression in PDL during OTM

Wnt3a was mainly localized in the cytoplasm and was expressed in the PDL of both the control and experimental group (Figure 3A). The expression of Wnt3a was much stronger on the tension side than on the compression side (Figures 3B–G). On the tension side, Wnt3a was predominantly distributed in the bone-formation region and its expression level began to increase from day 1 after the treatment and reached its maximal level at day 5. After day 5, the Wnt3a level started to decrease. Wnt3a expression levels on the tension side in the experimental group at each observation point were significantly higher than in the control group ($p < 0.05$). On the compression side, Wnt3a expression was decreased at days 1 and 3, but there was no significant difference when compared with the control ($p > 0.05$). From day 5, the Wnt3a level was higher on the compression side in the experimental group than in the control group and the increase at days 5, 7, 10 and 14 was statistically significant ($p < 0.01$ or $p < 0.05$). Furthermore, Wnt3a levels on the tension side were significantly higher than on the compression side at days 1 and 3 ($p < 0.05$) (Figure 3H).

Wnt10b expression in PDL during OTM in rats

Wnt10b was also expressed in the PDL of the control and experimental group (Figure 4A). The immunoreactivity of Wnt10b was observed in the osteoblasts, fibroblasts and osteoclasts and was mainly detected in the cytoplasm. Wnt10b was expressed more strongly on the tension side than on the compression side at any of the time points. On the compression side, Wnt10b was strongly positive in cells at the border between PDL and alveolar bone (Figures 4B–G). On the tension side, Wnt10b expression increased from day 1, reached the maximal level at day 5 and then started to decrease after day 5. On the compression side, Wnt10b expression was decreased at days 1 and 3, but there was no significant differences compared to the control ($p > 0.05$) (Figure 4H).

β-catenin expression in PDL during OTM in rats

β-catenin immunoreactivity was observed in the osteoblasts, fibroblasts and osteoclasts. While β-catenin was mainly seen in the cytoplasm, some signals for this molecule were also observed in the nuclei. β-catenin signals were stronger on the tension side than on the compression side at any time point; its signals were primarily observed in the bone-formation region (Figures 5B–G). β-catenin level elevated from day 1, peaked at day 7 and then decreased. Compared to the control group, β-catenin level was significantly higher on both sides at each time point ($p < 0.01$). Although β-catenin expression was higher on the tension side than on the compression side, the difference was not statistically significant ($p > 0.05$) (Figure 5H).

DKK1 expression in PDL during OTM in rats

DKK1 immunoreactivity was observed in the osteoblasts, fibroblasts and osteoclasts and was mainly detected in the cytoplasm. DKK1 expression was stronger on the compression side than on the tension side. On the compression side, DKK1 was heavily stained in cells at the border between PDL and alveolar bone (Figures 6B–G). DKK1 level reached a maximum at day 10, after which its level began to drop. In comparison with the control group, DKK1 expression was significantly higher on both sides at any of the observation points ($p < 0.01$). DKK1 level on the compression side was significantly higher than on the tension side at day 10 ($p < 0.05$) (Figure 6H).

Discussion

Mechanical stress from orthodontic appliances is considered to induce cells in the PDL to form biological mediators, which are responsible for remodeling of this non-mineralized connective tissue as well as for the mineralized bone [2–7]. Robinson et al. [8] reported that the Wnt/β-catenin signaling pathway is a normal response to mechanical loading in bone. Cells in the periodontal complex are Wnt responsive and the distribution of Wnt

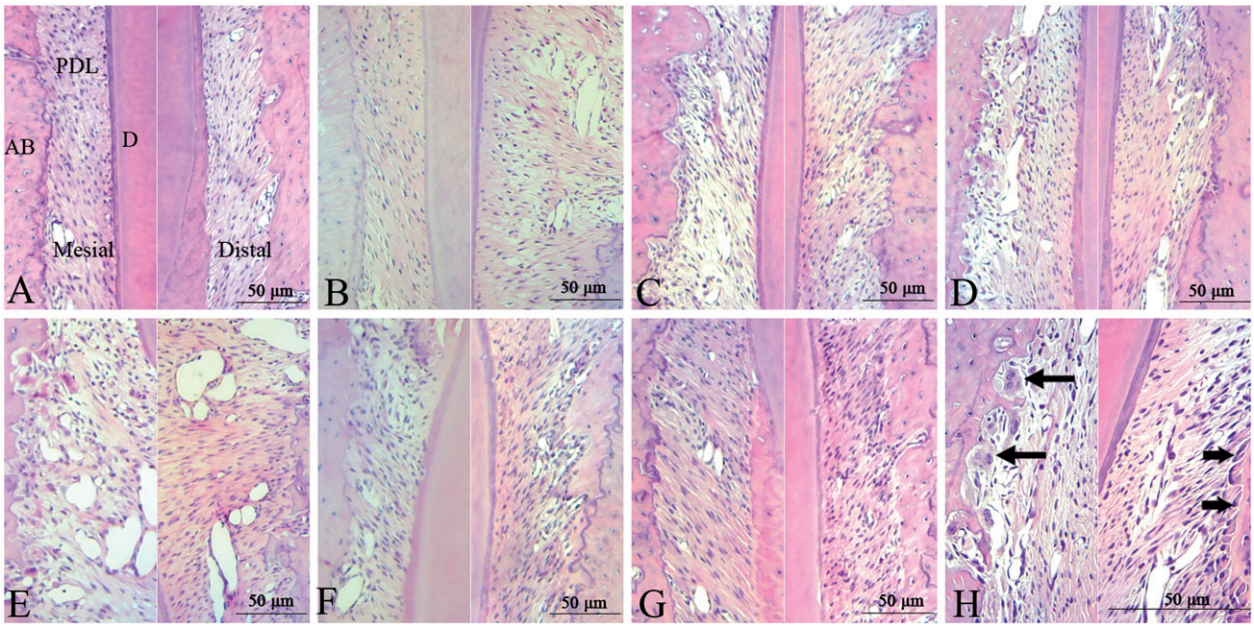

Figure 2. H&E staining of the unloaded teeth (A) and the loaded teeth (B–G) at days 1, 3, 5, 7, 10 and 14 after the application of force. Higher magnification views of the loaded teeth at day 5 (H). Mesial: the compression side; Distal: the tension side; Thick arrows: osteoclasts; thin arrows: osteoblasts. AB, alveolar bone; D, dentin; P, periodontal ligament. Bar = 50 μm.

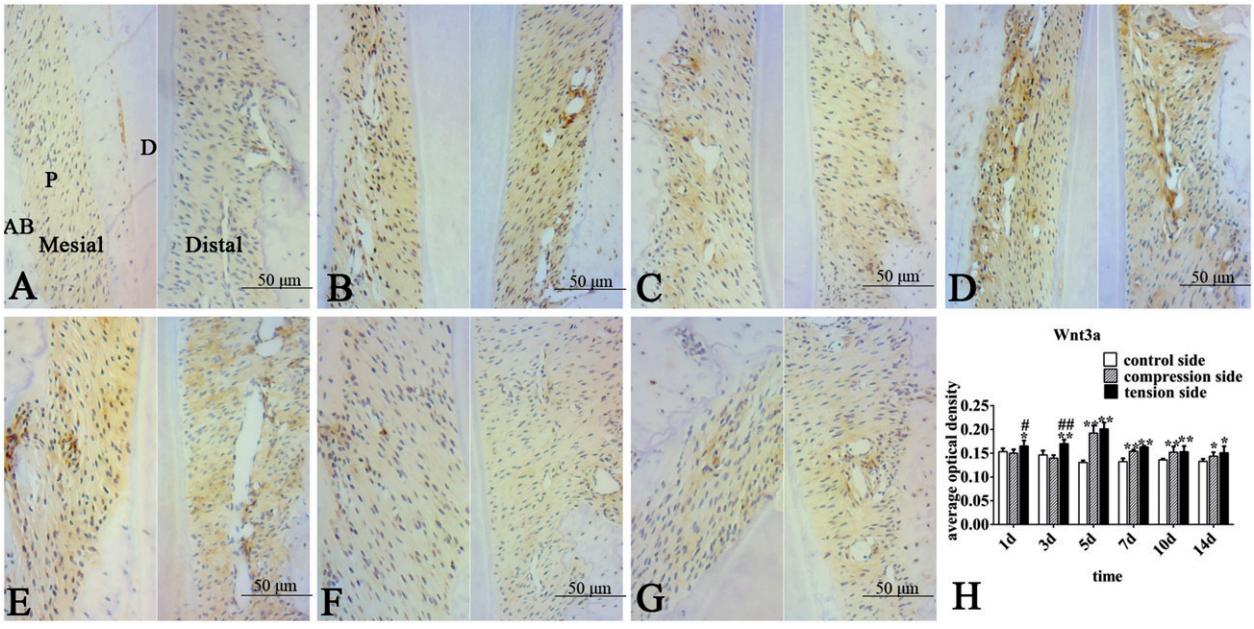

Figure 3. Wnt3a immunohistochemistry staining of the unloaded teeth (A) and the loaded teeth (B–G) at days 1, 3, 5, 7, 10 and 14 after force application. The quantitative analyses for the mean optical density of Wnt3a in the PDL (mean ± SD) (H). # and ## indicate comparisons between the tension and compression side; * and ** comparisons between the control and experimental group; # and *, $p < 0.05$; ## and **, $p < 0.01$.

responsive cells was reported to coincide with regions of cell proliferation in the PDL of the continuously erupting incisors [17]. Furthermore, it was demonstrated that the components of the Wnt/β-catenin signaling pathway (such as wnt3a, wnt10b, β-catenin, DKK1) can be detected in the PDL fibroblasts, osteoblasts and osteoclasts [18]. The Wnt/β-catenin signaling pathway enhances osteoblastogenesis and differentiation of human PDL fibroblasts [13]. In the present investigation, the staining of Wnt3a, Wnt10b, β-catenin and DKK1 was observed in the normal periodontal tissues without the application of any orthodontic force, which was consistent with previous studies. Besides, we found that the expression of Wnt3a, Wnt10b, β-catenin and DKK1 was higher on the loaded teeth than that on the unloaded

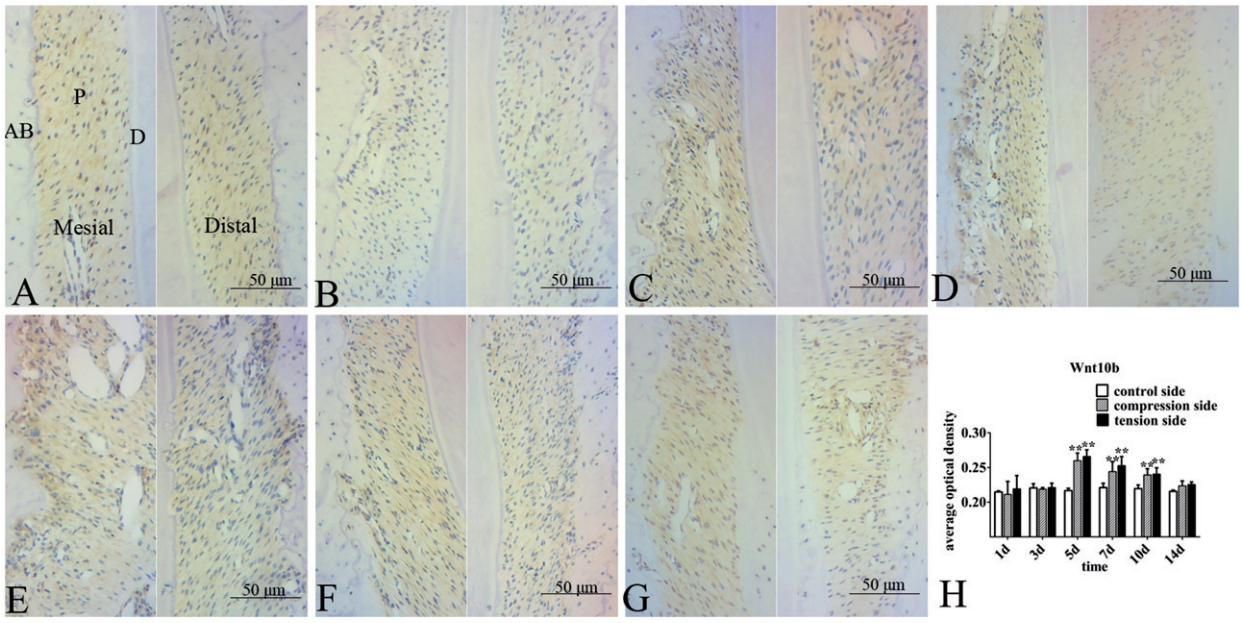

Figure 4. Wnt10b immunohistochemistry staining of the unloaded teeth (A) and the loaded teeth (B–G) at days 1, 3, 5, 7, 10 and 14 after force application. The quantitative analyses for the mean optical density of Wnt10b in the PDL (mean ± SD) (H).

teeth, suggesting Wnt3a, Wnt10b, β-catenin and DKK1 may be associated with periodontal tissue remodeling during OTM. Moreover, the expression of Wnt3a, Wnt10b and β-catenin was much more on the tension side than on the compression side, while the expression level of DKK1 was much higher on the compression side.

Although the exact mechanism of periodontal tissue remodeling is not fully understood, there is evidence that many bioactive molecules such as ALP, OPG, IL-1 and transforming growth factor-β1 (TGF-β1) are produced to evoke complicated response during OTM. Previous studies showed that the levels of OPG, ALP and TGF-β1 were up-regulated after mechanical loading during OTM [4,19,20]. Wnt ligands such as Wnt1, Wnt3a and Wnt10b can regulate bone mass and mineralization by inducing ALP activity and osteoblastogenesis [11,21]. Previous studies also implicated the elevation of TGF-β1 level can stimulate Wnt10b production in osteoclasts, which may enhance restoration of the bone loss during the resorptive phase of bone turnover [22]. Furthermore, a cluster of Wnt/β-catenin target genes (e.g. Wnt10b, sFrp1, DKK3) was proven to increase after mechanical loading *in vivo* [23]. β-catenin is the molecular node of Wnt/β-catenin signaling pathway. Canonical Wnt ligands inhibit the degradation of β-catenin in the cytoplasm and lead to translocation of β-catenin into the nucleus, where it interacts with transcription factors of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family to affect the transcription of target genes. Wnt3a and Wnt10b can up-regulate β-catenin expression to promote bone formation [24]. In the absence of β-catenin, the expression of early osteoblast markers such as COL-1, Osterix and OCN were greatly diminished [25]. In addition, Premaraj et al. [26] reported that mechanical loading stimulates the production of β-catenin and it can be served as an effector of mechanical signals in PDL cells. Therefore, the up-regulation of Wnt3a, Wnt10b and β-catenin in periodontal tissue, observed in our present study, may be induced by the orthodontic force, which seems to be consistent with the previous reports [26]. Our results suggested that Wnt3a, Wnt10b and β-catenin were involved in periodontal tissue remodeling induced by orthodontic force.

DKK1 is thought to inhibit the Wnt/β-catenin signaling pathway and decrease bone formation by binding to LRP5/6 and another high-affinity receptor Kremen1/2 [27]. The receptor activator of the NFκB ligand, RANKL, is one of the key regulatory molecules in osteoclast formation. Obvious up-regulation of RANKL level in periodontal tissue on the compression side was detected during experimental movement of rat molars [28]. DKK1 increased the expression of RANKL through inhibiting Wnt3a signaling, which indirectly promoted differentiation of osteoclasts and bone resorption [29]. Moreover, a previous study showed that the expression of DKK1 increased in the periodontal tissues of chronic periodontitis patients, suggesting that it may be involved in the alveolar bone resorption of periodontal diseases [30]. More importantly, DKK1 has also been confirmed to be a novel target gene of TCF and involved in mechano-transduction [31]. In agreement with findings in the previous studies, the elevation of DKK1 observed in the present study is likely to be due to the application of the orthodontic force.

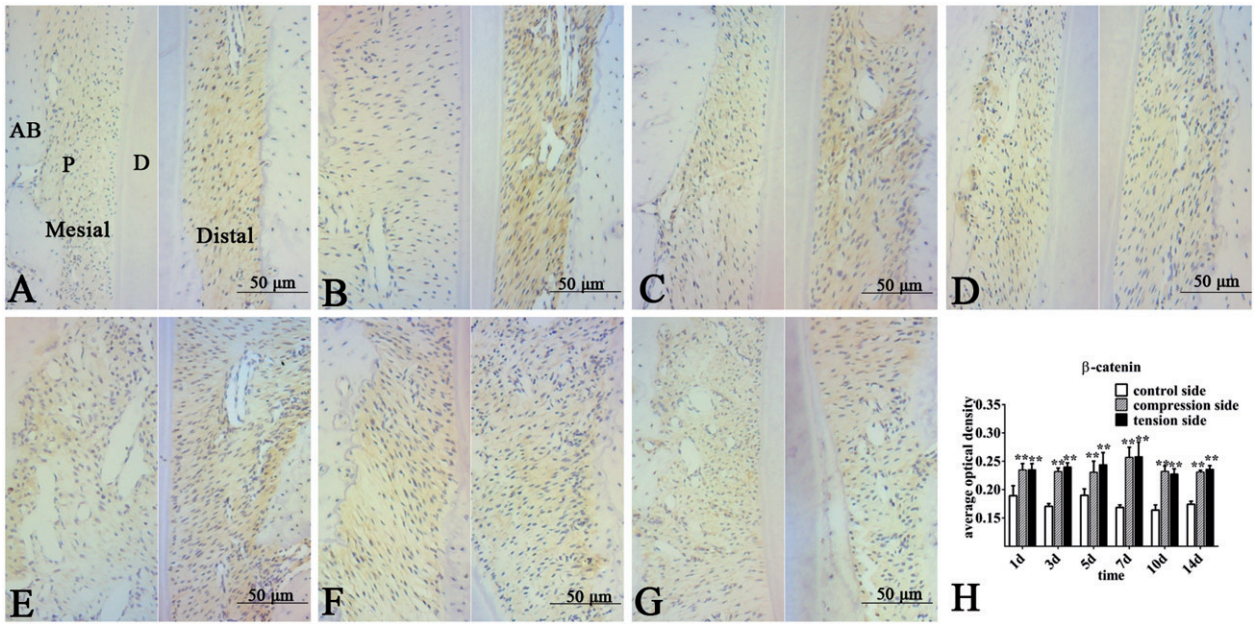

Figure 5. β-catenin immunohistochemistry staining of the unloaded teeth (A) and the loaded teeth (B–G) at days 1, 3, 5, 7, 10 and 14 after force application. The quantitative analyses for the mean optical density of β-catenin in the PDL (mean ± SD) (H).

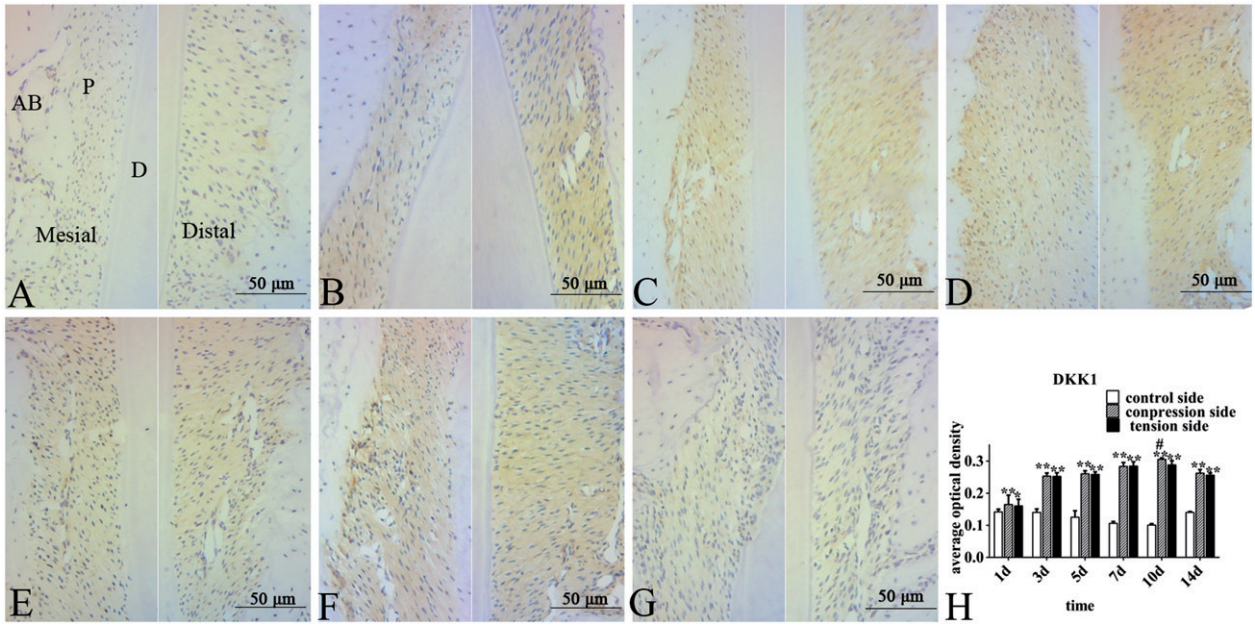

Figure 6. DKK1 immunohistochemistry staining of the unloaded teeth (A) and the loaded teeth (B–G) at days 1, 3, 5, 7, 10 and 14 after force application. The quantitative analyses for the mean optical density of DKK1 in the PDL (mean ± SD) (H).

The study also showed that the expression of Wnt3a, Wnt10b and β-catenin was much stronger on the tension side than on the compression side, while the expression of DKK1 was much higher on the compression side. Orthodontic force is a stimulus to either the compression region or the tension region of PDL. Although bone formation as well as bone resorption can concur on both sides, the main character on the pressure side is bone resorption, while on the tension side it is bone formation [32]. A big difference between tension and compression sides for DKK1 at day 10 may suggest that this molecule may play a more important role in bone resorption than in bone formation, while the difference of Wnt3a at days 1 and 3 between the two sides suggests that Wnt3a may be more vital in bone formation than in bone resorption during the initial stage of periodontal remodeling.

In conclusion, Wnt3a, Wnt10b, β-catenin and Dkk1 were expressed in PDL and showed a certain

tendency in the time course of OTM, which was in accordance with the periodontal tissue remodeling during this orthodontic treatment. However, much more work is needed to clarify the roles of the Wnt/β-catenin signaling pathway in periodontal tissue remodeling during OTM.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Melsen B. Biological reaction of alveolar bone to orthodontic tooth movement. Angle Orthod 1999;69:151–8.
[2] Garlet TP, Coelho U, Silva JS, Garlet GP. Cytokine expression pattern in compression and tension sites of the periodontal ligament during orthodontic tooth movement in humans. Eur J Oral Sci 2007;115:355–62.
[3] Pavlin D, Dove SB, Zadro R, 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;67:163–72.
[4] Kanzaki H, Chiba M, Takahashi I, Haruyama N, Nishimura M, Mitani H. Local OPG gene transfer to periodontal tissue inhibits orthodontic tooth movement. J Dent Res 2004;83:920–5.
[5] Kanzaki H, Chiba M, Arai K, Takahashi I, Haruyama N, Nishimura M, et al. Local RANKL gene transfer to the periodontal tissue accelerates orthodontic tooth movement. Gene Ther 2006;13:678–85.
[6] Grant M, Wilson J, Rock P, Chapple I. Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement. Eur J Orthod 2013;35:644–51.
[7] Zhang MM, Feng DF, Shao P, Sun TT, Wang L, Yang BF. Nifedipine regulated periodontal ligament remodeling: an experimental study in rats. Acta Odontol Scand 2008;66:327–33.
[8] Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, Cullen DM, Zhao W, Li C, et al. Wnt/beta-catenin signaling is a normal physiological response to mechanical loading in bone. J Biol Chem 2006;281:31720–8.
[9] Armstrong VJ, Muzylak M, Sunters A, Zaman G, Saxon LK, Price JS, et al. Wnt/betacatenin signaling is a component of osteoblastic bone cell early responses to load-bearing and requires estrogen receptor alpha. J Biol Chem 2007;282:20715–27.
[10] Tang Y, Zhou X, Gao B, Xu X, Sun J, Cheng L, et al. Modulation of Wnt/β-catenin signaling attenuates periapical bone lesions. J Dent Res 2014;93:175–82.
[11] Bennett CN, Ouyang H, Ma YL, Zeng Q, Gerin I, Sousa KM, et al. Wnt10b increases postnatal bone formation by enhancing osteoblast differentiation. J Bone Miner Res 2007;22:1924–32.
[12] Lim WH, Liu B, Cheng D, Williams BO, Mah SJ, Helms JA. Wnt signaling regulates homeostasis of the periodontal ligament. J. Periodont. Res. 2014;49:751–9.
[13] Lim WH, Liu B, Mah SJ, Yin X, Helms JA. Alveolar Bone Turnover and Periodontal Ligament Width are Controlled by Wnt. J Periodontol 2015;86:319–26.
[14] Heo JS, Lee SY, Lee JC. Wnt/beta-catenin signaling enhances osteoblastogenic differentiation from human periodontal ligament fibroblasts. Mol Cells 2010;30:449–54.
[15] Alhashimi N, Frithiof L, Brudvik P, Bakhiet M. CD40-CD40L expression during orthodontic tooth movement in rats. Angle Orthod 2004;74:100–5.
[16] Tan L, Ren Y, Wang J, Jiang L, Cheng H, Sandham A, et al. Osteoprotegerin and ligand of receptor activator of nuclear factor kappaB expression in ovariectomized rats during tooth movement. Angle Orthod 2009;79:292–8.
[17] Rooker SM, Liu B, Helms JA. Role of Wnt signaling in the biology of the periodontium. Dev Dyn 2010;239:140–7.
[18] Glass DA, 2nd Karsenty G. Canonical Wnt signaling in osteoblasts is required for osteoclast differentiation. Ann N Y Acad Sci 2006;1068:117–30.
[19] Megat Abdul Wahab R, Md Dasor M, Senafi S, Abang Abdullah AA, Yamamoto Z, Jemain AA, et al. Crevicular Alkaline Phosphatase Activity and Rate of Tooth Movement of Female Orthodontic Subjects under Different Continuous Force Applications. Int J Dent. 2013;2013:245818
[20] Nagai M, Yoshida A, Sato N, Wong DT. Messenger RNA level and protein localization of transforming growth factor-beta1 in experimental tooth movement in rats. Eur J Oral Sci 1999;107:475–81.
[21] Boland GM, Perkins G, Hall DJ, Tuan RS. Wnt 3a promotes proliferation and suppresses osteogenic differentiation of adult human mesenchymal stem cells. J Cell Biochem 2004;93:1210–30.
[22] Ota K, Quint P, Ruan M, Pederson L, Westendorf JJ, Khosla S, et al. TGF-β induces Wnt10b in osteoclasts from female mice to enhance coupling to osteoblasts. Endocrinology 2013;154:3745–52.
[23] Kim-Weroha NA, Ferris A, Holladay B, Kotha SP, Kamel MA, Johnson ML. In vivo load activated propagation of β-catenin signaling in osteocytes through coordinated downregulation of inhibitors of Lrp5. J Bone Miner Res 2008;23:S13
[24] Vinyoles M, Del Valle-Pérez B, Curto J, Viñas-Castells R, Alba-Castellón L, García de Herreros A. Multivesicular GSK3 sequestration upon Wnt signaling is controlled by p120-catenin/cadherin interaction with LRP5/6. Mol Cell 2014;53:444–57.
[25] Hu H, Hilton MJ, Tu X, Yu K, Ornitz DM, Long F. Sequential roles of Hedgehog and Wnt signaling in osteoblast development. Development 2005;132:49–60.
[26] Premaraj S, Souza I, Premaraj T. Mechanical loading activates β-catenin signaling in periodontal ligament cells. Angle Orthod 2011;81:592–9.
[27] Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA. (2001). Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nat Cell Biol 2001;3:683–6.
[28] Shiotani A, Shibasaki Y, Sasaki T. Localization of receptor activator of NFkappaB ligand, RANKL, in periodontal tissues during experimental movement of rat molars. J Electron Microsc (Tokyo) 2001;50:365–9.
[29] Fujita K, Janz S. Attenuation of WNT signaling by DKK-1 and -2 regulates BMP2-induced osteoblast differentiation and expression of OPG, RANKL and M-CSF. Mol Cancer 2007;6:71
[30] Napimoga MH, Nametala C, da Silva FL, Miranda TS, Bossonaro JP, Demasi AP, et al. Involvement of the Wnt-β-catenin signalling antagonists, sclerostin and dickkopf-related protein 1, in chronic periodontitis. J Clin Periodontol 2014;41:550–7.
[31] Niida A, Hiroko T, Kasai M, Furukawa Y, Nakamura Y, Suzuki Y, et al. DKK1, a negative regulator of Wnt signaling, is a target of the beta-catenin/TCF pathway. Oncogene 2004;23:8520–6.
[32] Kalajzic Z, Peluso EB, Utreja A, Dyment N, Nihara J, Xu M, et al. Effect of cyclical forces on the periodontal ligament and alveolar bone remodeling during orthodontic tooth movement. Angle Orthod 2014;84:297–303.