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学位論文

Periostin and *Twist* mRNA Expression in the Hypofunctional Mouse Periodontal Ligament

Afanador Mendoza, Elaine Cecilia

Keywords：Periodontal ligament, Hypofunction, Periostin, Twist

Abstract：Periostin is a secreted protein preferentially expressed in the periodontal ligament (PDL) and the periosteum. In a previous study it has been demonstrated that periostin had putative roles during tooth movement. Recently, it was also reported that Twist, a basic helix-loop-helix transcription factor important for cell type determination and differentiation, could bind to the *periostin* promoter in undifferentiated preosteoblasts and promote *periostin* gene expression *in vitro*. Since occlusal force is thought to be important in the homeostasis of the PDL, the expression of *periostin* and *Twist* mRNA in the mouse periodontal tissue following removal of antagonizing teeth was investigated in this study. Unilateral maxillary tooth extraction was performed in 3-week-old male mice to produce occlusal hypofunction of the right mandibular molars. The expressions of *periostin* and *Twist* mRNA were examined by real-time PCR and *in situ* hybridization at 12, 24, 72 and 168 hours after the tooth extraction. *Periostin* and *Twist* mRNA expressions at 24 hours following removal of occlusal force were significantly decreased to 14.5% and 49.9% of the control level, respectively. A recovery was observed at 72 hours, and at 168 hours no significant difference was observed. As determined by *in situ* hybridization, the number of *periostin*- and *Twist*-mRNA expressing cells showed a remarkable decrease at 24 hours, although a gradual increase was observed from 72 hours until the distribution was almost identical to that of normal animals at 168 hours. These results suggested that occlusal force might have putative roles in *periostin* and *Twist* gene expression in the PDL.

INTRODUCTION

The periodontal ligament (PDL) is a complex, vascular and highly cellular soft connective tissue important in tooth support, proprioception and regulation of the alveolar bone volume[1]. In normal occlusal function, the PDL has a functional arrangement of dense, strong periodontal fibers that is a resistant structure against heavy mechanical stress. But, the changes of environmental conditions, for example, a loss of occlusal force or an application of orthodontic force, lead to an alteration in the structure of the PDL. The effects of occlusal hypofunction have been extensively studied in humans and animals. Experimental studies conducted in mice, rats[2-6] and monkeys[7, 8] indicate that occlusal hypofunction results in atrophic changes of the PDL, namely, narrowing of periodontal space, disorientation and decrease in the amount of periodontal fibers, and vascular occlusion.

Constitutive adaptation to applied forces is mediated in part by specific structural and regulatory proteins expressed by PDL cells. Periostin is a disulfide-linked 90 kDa secreted protein that is highly expressed in early osteoblastic cells *in vitro* and in periosteum and PDL tissues *in vivo*[9, 10]. It was originally isolated as an osteoblast-specific factor that functions as a cell adhesion molecule for preosteoblasts. Periostin has been regarded as a member of the Fasciclin

Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, The University of Tokushima

I family, which includes Big-h3, Algal-CAM, and midline fasciclin. Big-h3, a molecule induced by transforming growth factor-β (TGF-β), promotes fibroblasts attachment and spreading. The homology of periostin with these molecules suggests a function as a cell adhesion molecule[11, 12]. During tooth development, periostin is expressed at the sites of epithelial-mesenchymal interaction and it might be linked to deposition and organization of other extracellular matrix adhesion molecules during maintenance of the adult tooth[13]. Immunoelectronmicroscopic observation of the mature PDL verified the localization of periostin in the cell membrane of the cytoplasmic extensions of periodontal fibroblasts, indicating that periostin modulates the remodeling and metabolism of extracellular matrices by mediating a cell-to-matrix interaction[14].

Recently it was reported that overexpression of Twist, an evolutionarily conserved group of regulatory basic helix-loop-helix (bHLH) factors essential for cell type determination and differentiation, resulted in increased *periostin* expression *in vitro* and it was suggested that Twist could bind to the *periostin* promoter in undifferentiated preosteoblasts and up-regulate *periostin* gene expression. In addition, it has been found that *periostin* and *Twist* are co-expressed in the sutures of mouse calvarial bones[15], though the expression of *Twist* in periodontal tissue has not been studied yet. It is important to understand the physiological functions of periostin and Twist in periodontal tissue, and further clarify their changes in pathological conditions.

Previously it has been reported that orthodontic force induced divergent changes of *periostin* mRNA expression between the pressure and tension sites of rat PDL[16]. The expression of *periostin* was increased in the compressed side of the ligament by mechanical stress. These findings indicate that *periostin* is temporally and spatially expressed in bone cells and may play a potential role in maintenance of structure.

The present study was designed to accumulate further evidence on the role of periostin in the PDL against mechanical force. We investigated the effects of occlusal hypofunction of mouse mandibular molars on the expression level of *periostin* and *Twist* mRNA in the PDL.

MATERIALS AND METHODS

Animals and procedure for making occlusal hypofunctional teeth

3-week-old male C57BL/6NCrj mice (Nihon CREA Co. Osaka, Japan) were used in this study. The unilateral maxillar tooth extraction method was chosen to produce occlusal hypofunction of the contralateral teeth. The mice were randomly divided into two groups: untreated and operated. The first group included untreated mice of the same age that served as external control. The mice in the operated group were subjected to tooth extractions in order to eliminate occlusal force on the right mandibular molars. The molars were extracted with a fine-pointed forceps under intra-peritoneal anesthesia with ketamine-xylazine anesthesic solution (0.1 ml/30 g body weight). The animals recovered well and received water and solid diet freely after the surgery.

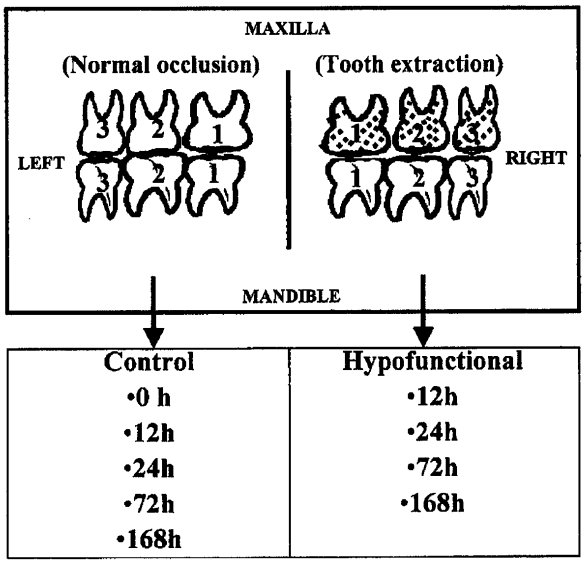

Fig. 1 Diagram to show the pattern of occlusion of the molars in mice.
Note that the extraction of the first, second, and third right maxillary molars (shaded) will produce a total loss of function of the first, second and third right mandibular molars.

Preparation of tissues

Mice were sacrificed and perfused transcardially under ether anesthesia with freshly prepared 4% paraformaldehyde in DEPC-treated 0.1 M Phosphate Buffer (PB), pH 7.4 at 0 (no extractions) and at 12, 24, 72, and 168 hours (after tooth extractions). Their mandibles were removed and divided into halves, right and left. The right halves of specimens were used as the experimental sides, and the left sides that were untreated served as internal controls. Mandibular specimens were also removed from untreated mice of the same age as external controls (Fig.1). The removed mandibular specimens were immediately immersed in the same fixative (4% paraformaldehyde) overnight at 4 ℃ . The tissues were decalcified with 19% EDTA (pH 7.4) at 4℃ for 3 weeks and then embedded in paraffin. The tissue blocks were cut into mesio-distal serial sections of 4μm thick and mounted on 3-(triethoxilosilyl)-propilamin-coated (Merck, Schucardt, Munich, Germany) glass slides. They were subjected to hematoxylin and eosin staining and *in situ* hybridization.

Experimental protocols concerning animal handling were approved by the ethical committee of the University of Tokushima.

In situ hybridization

A mouse *periostin* cDNA containing 819 bp fragment of four-repeated domain (2211-3030) was subcloned into pBluescript KS- (Stratagene, La Jolla, CA) and used to generate sense and anti-sense probes. A mouse *Twist in situ* probe was provided from Tokyo Institute of Technology. Digoxigenin UTP-labeled single-stranded RNA probes were prepared using a DIG RNA Labeling Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. *In situ* hybridization was performed according to a previous study[17].

Reverse-transcription-polymerase chain reaction (RT-PCR)

The total RNA for conventional and real-time PCR was extracted from periodontal tissues including mandibular molars at 0, 12, 24, 72 and 168 hours after tooth extraction with ISOGEN (Nippon Gene Co. Toyama, Japan) according to the manufacturer's instructions and stored at -80 ℃ . The RNA concentration was determined using spectrophotometry. *Periostin* and *Twist* mRNA were detected with the RT-PCR kit (RT-PCR kit, Takara, Japan) using the primer sets and conditions shown on Table 1. PCR products were run on 1% agarose gels along with a molecular size marker and the bands were visualized with ethidium bromide and photographed under transillumination. These RT-PCR assays were performed independently three times.

Quantitative real-time PCR

The quantitative assessment of mRNA levels was performed using a detection system (Prism 7700 Sequence Detection System; Applied Biosystems Inc.) dedicated to the real-time monitoring of nucleic acid green dye fluorescence (SYBR Green I; Applied Biosystems Inc.). All reactions were contained 1μl of cDNA template along with 400 (*periostin*), 200 (*Twist*) or 100 (*GAPDH*) nM primers, 12.5 μl QuantiTect SYBR Green PCR (SYBR Green I; Applied Biosystems Inc.) and RNase free water, in a final reaction volume of 25μl. Preliminary experiments were performed to establish the amplification efficiency for each primer pairs. The primers used for real-time PCR, annealing temperatures, number of cycles, and expected length (bp) of the products are shown in Table 1. Standard curves were made using serial dilutions from pooled cDNA samples. *GAPDH* was used as a control gene to normalize each sample for RNA content. All experiments were repeated 3 times. The paired samples *t*-test was used to evaluate the significance of differences in expression in paired groups. The criterion of significance was taken to be a *P* value of less than 0.05 and 0.005.

RESULTS

Histological findings

In the control side of the mandibular PDL, the collagen fibers were arranged densely and regularly throughout the study period. Fibroblasts were generally arranged parallel to the fibers (Fig. 2 A-B).

In the experimental side of the mandible, 24 hours after the extraction of the antagonists the thickness of the PDL was almost normally preserved; however the well-ordered

Table 1 Specifications of the PCR primers used for conventional RT-PCR and quantitative real-time RT-PCR. Shown are the gene-specific primer sequences for the forward (f) and reverse (r) primers of *periostin*, *Twist*, and *GAPDH*, and their specific reaction conditions.

Gene	Primer sequence (5`→3`)	Annealing temperature (˚C)	Cycle used	Product size (bp)
Periostin	5`-GAACGAATCATTACAGGTCC-3` (f) 5`-TATTGCAAGAAGCTTATGACA-3` (r)	57˚C	30	819
Periostin (Real time PCR)	5`-GAACGAATCATTACAGGTCC-3` (f) 5`-GGAGACCTCTTTTTGCAAGA-3` (r)	57˚C	45	101
Twist	5`-ACGCAGTCGCTGAACGAGGC-3` (f) 5`-GTACAGGAAGTCGATGTACC-3` (r)	64˚C	30	119
Twist (Real time PCR)	5`-ACGCAGTCGCTGAACGAGGC-3` (f) 5`-GTACAGGAAGTCGATGTACC-3` (r)	64˚C	45	119
GAPDH	5`-GGCAAATTCAACGGCACAGTC-3` (f) 5`-AAGCAGTTGGTGGTGCAGGA-3` (r)	57˚C	30	310
GAPDH (Real time PCR)	5`-AAAAGGGTCATCATCTCCGCC-3` (f) 5`-AAGCAGTTGGTGGTGCAGGA-3 (r)	57˚C	45	121

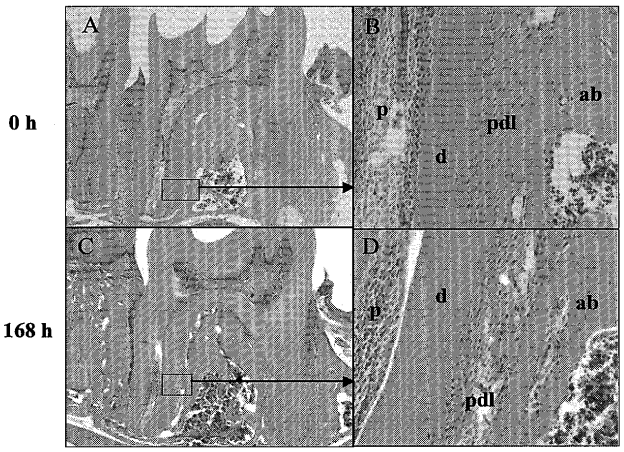

Fig. 2 Hematoxylin and eosin staining in the right mandibular molars of three week-old mice untreated and 168 hours after removal of the antagonizing teeth. ab: alveolar bone, pdl: periodontal ligament, d: dentin, p: pulp. Original magnification: A, C, x40; B, D, x200.

arrangement of fibers was lost.

In the 1-week experimental group, the PDL showed a decrease in thickness to about half of that of control side and the collagen fiber bundle arrangement was disturbed (Fig. 2 C-D).

Periostin and *Twist* in the PDL of normal animals

In control specimens, the expression of *periostin* mRNA was uniformly observed in PDL and it was present in spindle shaped cellular elements that were mainly fibroblastic cells (Fig. 3 A-B). In addition, a number of gingival fibroblasts between first and second molars and osteoblastic cells on alveolar bone surfaces also expressed *periostin* mRNA (Fig. 3 C-D). No positive reaction was observed in dentin, cementum, dental pulp, and alveolar bone. These distribution patterns agree with the findings in previous studies[10, 16]. The sections stained with sense probes showed no staining at all (Fig. 3 E). Although *Twist* mRNA was expressed by several cell types, its highest expression was detected in PDL cells which were uniformly distributed throughout the whole PDL (Fig. 3 F-G). Other *Twist*-mRNA expressing cells were a number of gingival fibroblasts between first and second molars, and osteoblasts neighboring the PDL (Fig. 3 H-I). The hybridization with the control sense probe was unable to produce any signal (Fig. 3 J).

Periostin and *Twist* in the PDL following removal of occlusal force

Twelve hours following removal of the antagonizing teeth, *periostin*-mRNA expressing cells showed a remarkable decrease in number compared with the control side (Fig. 4 A-B and 5 A-B). One day after the tooth extractions, almost all *periostin*-mRNA expressing cells disappeared from the entire ligament of the distal root (Fig.4 C and 5 C). Three days following the removal of occlusal forces, the number of *periostin*-mRNA expressing cells increased compared to those at the previous stage, but they were still lower compared to those of normal animals (Fig. 4 D and 5 D). Seven days following the extraction, the distribution of *periostin*-mRNA expressing cells was almost identical to those of normal animals (Fig. 4 E and 5 E).

The expression of *Twist* mRNA at 12 hours following removal of the antagonizing teeth showed a moderate decrease in number compared with the control side (Fig. 4 F-G and 5 F-G). At one day following the tooth extractions, the maximum changes were observed in *Twist* mRNA expression, which was still decreased compared with the control side (Fig. 4 H and 5 H).

After three days, *Twist*-mRNA expressing cells increased in number (Fig. 4 I and 5 I) and by one week the distribution was almost identical to those of normal animals (Fig. 4 J and 5 J).

Periostin and *Twist* mRNA expression during occlusal hypofunction detected by RT-PCR

Periostin mRNA expression detected by RT-PCR showed a considerable decrease at 12 and 24 hours after removal of the antagonizing teeth, and returned to levels similar to control after 1 week. In the left side of the mandible, where occlusal contact was kept during the length of the experiment, *periostin* mRNA expression remained the same throughout the experimental period.

Twist mRNA expression detected by RT-PCR seemed to slightly decrease after removal of occlusal force, but it was not as markedly as with *periostin* mRNA. In the left side of the mandible, which was control, *Twist* mRNA expression remained the same without any significant variations (Fig. 6).

Quantitative real-time PCR

The *periostin/GAPDH* mRNA ratio at 12 hours after the tooth extractions was 28.0±9.2 (mean ± SD), and there was a significant decrease in *periostin* mRNA expression during hypofunction compared to control ($p < 0.005$). One day following the tooth extractions, *periostin* mRNA significantly decreased to 14.5±1.8 ($p < 0.001$). At 72 hours after the tooth extractions, the expression of *periostin* mRNA increased to 39.0±5.4 ($p < 0.005$). One week after the tooth extractions, the *periostin/GAPDH* mRNA ratio increased to 126.7± 14.5, to a level almost identical to that of control group (Fig. 7 A). In the left side of the mandible, where occlusal contact was kept during the length of the experiment, no significant variations where observed in the expression of *periostin* mRNA (Fig. 7 B).

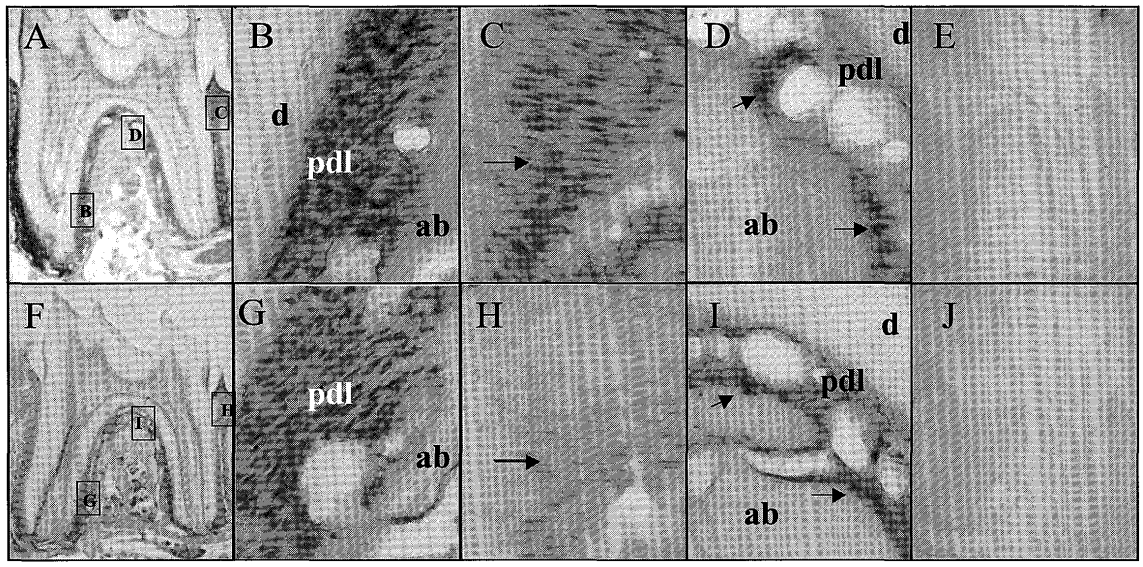

Fig. 3 *In situ* hybridization analysis of *periostin* and *Twist* mRNA in the left mandibular molars of three week-old control specimens.
Upper panels correspond to *periostin* (A-E); lower panels correspond to *Twist* (F-J). Small rectangles of B, C, D and G, H, I indicate areas enlarged in B, C, D, and G, H, I, respectively. (B)(G) Higher magnification of the PDL surrounding the mesial roots shows that fibroblastic cells within the PDL clearly expressed *periostin* and *Twist* mRNA, respectively. (C)(H) Higher magnification in the area of the alveolar crest revealed that a number of gingival fibroblasts (arrow) between the first and the second molars also expressed *periostin* and *Twist* mRNA. (D)(I) Higher magnification of the root bifurcation area showed that osteoblastic cells (arrow) on the alveolar bone surfaces also expressed *periostin* and *Twist* mRNA. (E)(J) The sections hybridized with sense probes showed no staining at all. ab: alveolar bone, pdl: periodontal ligament, d: dentin. Original magnification: A, F, E, J, x40; B-D, G-I x400.

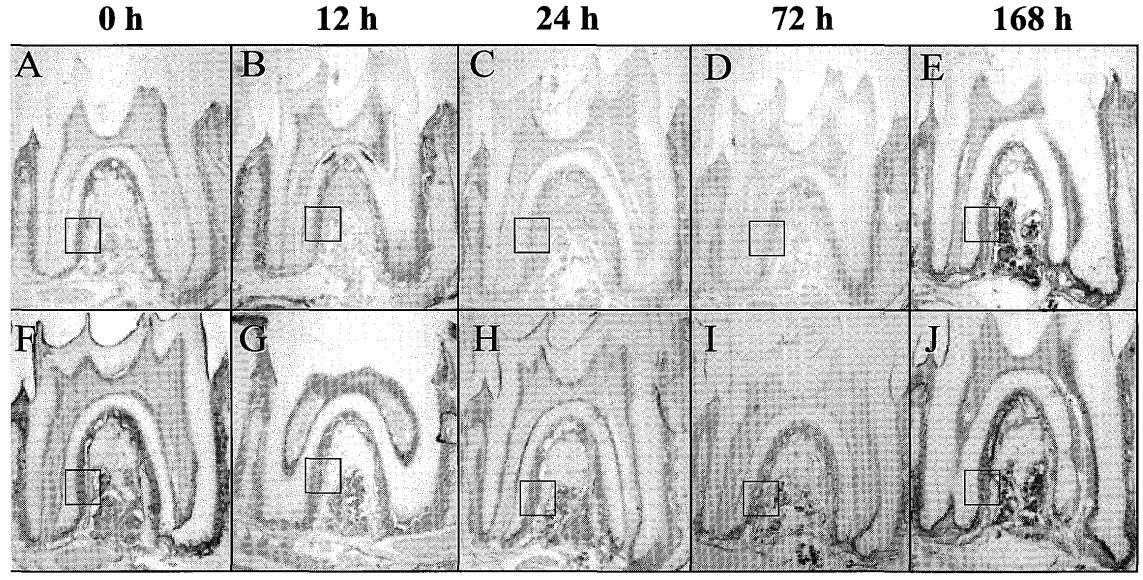

Fig. 4 Lower magnification showing *in situ* hybridization analysis of *periostin* and *Twist* mRNA in the right mandibular molars of three week-old mice following removal of occlusal force.
Small rectangles from A to J indicate areas enlarged in Figure 5, respectively. Upper panels correspond to *periostin* at 0 hours (A), 12 hours (B), 24 hours (C), 72 hours (D) and 168 hours (E) after tooth extraction; lower panels correspond to *Twist* at 0 hours (F), 12 hours (G), 24 hours (H), 72 hours (I) and 168 hours (J) after tooth extraction. Original magnification: A-J x40.

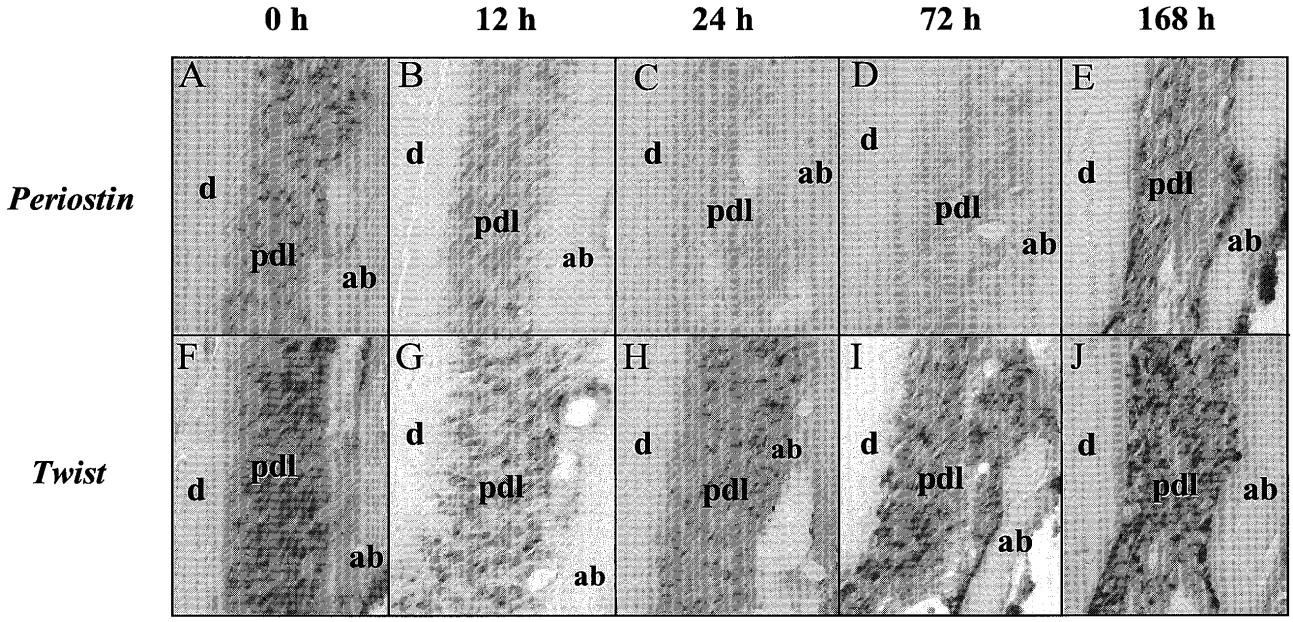

Fig. 5 Higher magnification of the PDL surrounding the distal roots of the right mandibular molars of three week-old mice showing *in situ* hybridization analysis of *periostin* and *Twist* mRNA following removal of occlusal force.
Upper panels correspond to *periostin* (A-E); lower panels correspond to *Twist* (F-J). The expression of *periostin* mRNA at 12 and 24 hours following the extraction (B-C), showed a marked decrease in number compared with the control side (A). At 72 hours after the extraction(D), the *periostin*-mRNA expressing cells gradually increased and at 168 hours the distribution was almost identical to those of normal animals (E). The expression of *Twist* mRNA at 12 and 24 hours following the extractions (G-H), showed a moderate decrease in number compared with the control side (F). At 72 hours after the extraction (I), the *Twist* mRNA-expressing cells gradually increased and at 168 hours the distribution was almost identical to those of normal animals (J).
p: pulp, d: dentin, pdl: periodontal ligament, ab: alveolar bone. Original magnification: A-H x400.

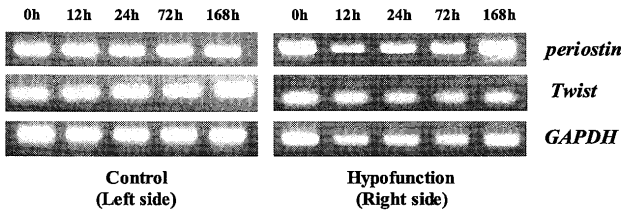

Fig. 6 Expression of *periostin* and *Twist* mRNA in mouse periodontal tissue following removal of occlusal force, analyzed by conventional RT-PCR. Total RNA was isolated from the mandibular periodontal tissues including the molars. RT-PCR to detect *periostin* and *Twist* mRNA was performed as described in Material and Methods.

Similarly, *Twist/GAPDH* mRNA ratio at 12 hours after the tooth extractions showed a considerable decrease compared to control group (51.6±10.5). At 24 hours it showed a maximum decrease of 49.9±1.4. From 72 hours it increased, reaching a similar level to control group at 168 hours. The *Twist/GAPDH* mRNA ratios for 72 hours and 168 hours were 56.0±2.5 and 121.8±17.7 respectively. The decrease observed in *Twist* mRNA expression during hypofunction compared to control at 12 hours was statistically significant at $P < 0.05$, and the ones observed at 24 and 72 hours were statistically significant at $P < 0.001$ (Fig. 7 A). In the left side of the mandible, which was control side, no significant variations were observed during the experimental period (Fig. 7 B).

DISCUSSION

Various procedures have been used to study the effects of occlusal hypofunction on animal PDL such as masticatory and muscle resection[18], soft diet[19, 20, 21], reduction in height by resection of the crown of the opposite teeth[5, 22-26], and antagonist extraction[2-4, 27, 28-35]. In the present study, the unilateral maxillary tooth extraction was the method chosen to successfully create occlusal hypofunction of the opposing mandibular molars. The reason why the antagonist extraction method was chosen is because it often resembles clinical situations.

Periostin mRNA was present in spindle-shaped cellular elements within the PDL and its distribution was relatively intense in the PDL surrounding root of molars compared to the PDL of the root bifurcation area. The expression level

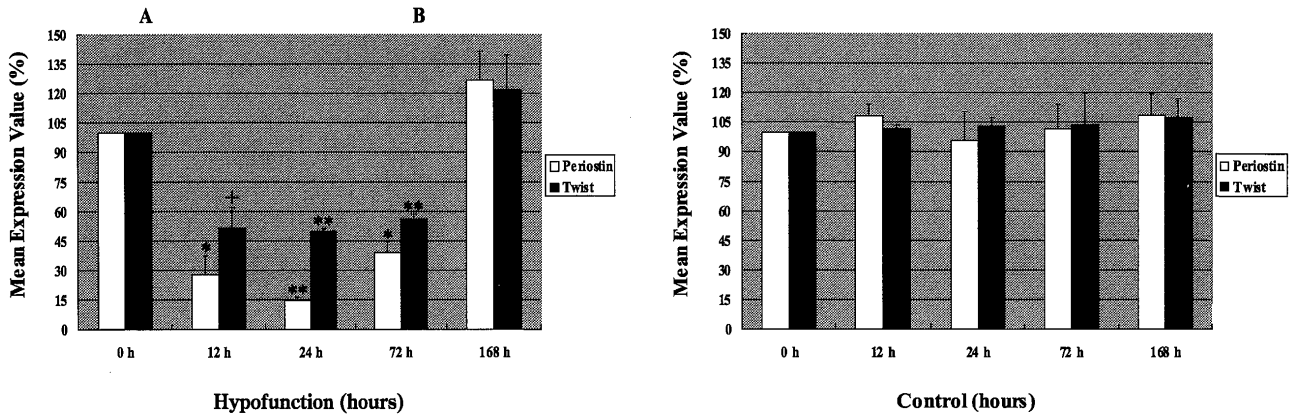

Fig. 7A Comparative expression of *periostin* and *Twist* following removal of occlusal force, analyzed by real-time PCR. Quantitative PCR analysis of mouse periodontal tissue was carried out as described in Materials and Methods. The level of expression in control group (untreated mice) was set to 100 (relative expression). Data shown represent mean ± S.D. values for n=3 individual mice per group after normalization to *GAPDH*. Statistical differences (*t*-test) are reported as follows: untreated control group versus 12-24-72-168 hours hypofunction groups (+, P <0.05; *, P <0.005; **, P <0.001).

Fig. 7B Comparative expression of periostin and Twist in the control group, analyzed by real-time PCR. Quantitative PCR analysis of mouse periodontal tissue was carried out as described in Materials and Methods. The level of expression in control group (untreated mice) was set to 100 (relative expression). Data shown represent mean ± S.D. values for n=3 individual mice per group after normalization to *GAPDH*. Statistical differences (*t*-test) are reported as follows: untreated control group versus 12-24-72-168 hours non-hypofunction groups (+, P <0.05; *, P <0.005; **, P <0.001).

of *Twist* mRNA in mouse PDL was remarkably high when compared to that of other oral tissues suggesting some physiological role for *Twist* in the PDL. The examination of adjacent sections of *in situ* hybridization revealed similarities in the pattern of distribution of *periostin* and *Twist* mRNA expression. They appeared to be co-localized within the same cells of the periodontal tissue of adult mice. Although the function of periostin in the periodontal tissues is still unknown, periostin has been found to support the attachment and spreading of fibroblasts and MC3T3-E1 cells[10, 36], and this effect was impaired by anti-periostin antiserum, suggesting that periostin is involved in cell adhesion recruitment. TGF-β1 induces periostin gene expression *in vitro*, and it seems that TGF-β1 might have a putative role in the expression of *periostin* mRNA in PDL during physiological movement. Big-h3, a molecule highly homologous to periostin, which is also induced by TGF-β1, promotes fibroblast attachment and spreading, and inhibits bone nodule formation of osteoblasts *in vitro*[37]. It has also been shown that MLO-A5 (a preosteocyte-like cell line), expresses *periostin* mRNA but not MLO-Y4 (an osteocyte cell line)[38]. Periostin appears to be a specific marker for the preosteoblasts. *Periostin* expression in osteocytes is nearly undetectable, which suggests that osteoblasts cease production of periostin during the process of differentiation. PDL cells express genes thought to be specific to osteoblasts including *periostin* and Runx2/Osf2, which is known to be a transcription factor essential for osteoblast differentiation; but they do not express bone sialoprotein and osteocalcin, suggesting that PDL cells may be at a stage a little before the middle stage of osteoblast differentiation. However, PDL cells do not mineralize without BMP-2 treatment[39] and even then the response is minimal, which suggests that they do not yet appear to be committed towards osteoblastic cells and there might be a regulatory mechanism preventing differentiation of PDL cells toward osteoblasts. Recent findings indicate that Msx2, a homeobox gene implicated in bone development, plays a central role in preventing osteoblastic differentiation and mineralization of ligament fibroblasts by repressing Runx2/Osf2 transcriptional activity[40]. In addition, Bialek et al.[41] revealed that Twist-1 and Twist-2 suppress the activity of Runx2 and thereby regulate bone formation. Their study further supports that osteoblast differentiation is a negatively regulated process early during skeletogenesis, despite a normal expression of Runx2. It has already been reported that overexpression of *Twist* results in increased *periostin* expression *in vitro*[15], and it is also known that Twist negatively regulates osteoblast differentiation. Since specific localization and gene expression of *periostin* was observed in periosteum and PDL which is non-mineralized tissue adjacent to bone, and the present study is the first to reveal that *periostin* and *Twist* gene expression are co-localized in the fibroblastic cells of the PDL, it is conceivable that the function of periostin in

the PDL tissue might be the negative regulation of osteoblast differentiation.

Following the removal of occlusal force, *periostin* and *Twist* mRNA expression patterns behaved in a similar fashion under hypofunction conditions over a short-term period. A marked decrease in the expression levels was observed in the early stage of the present study, but they returned to normal levels in later stages (Fig. 7 A). These results indicate that *periostin* and *Twist* mRNA expressions are regulated by changes in the occlusal force. Other occlusal force-responsive proteins that have been reported to decrease in absence of occlusal stimuli using a similar experimental model include decorin, a proteoglycan that has been proposed as an inhibitor of mineralization in the soft connective tissues; heparan sulfate, a proteoglycan that might be related to the bone resorption mechanism; chondroitin sulfate, a proteoglycan that provides cartilage tissues with the property of resisting compressional forces[42]; and calbindin, a calcium-binding protein that might play important roles in the cytoprotection of PDL against mechanical force[43]. Interestingly, calbindin not only was found to almost disappear after 24 hours following removal of the antagonizing teeth, but also gradually returned to normal levels after 1 week. The authors attributed the reappearance to weak occlusal forces due to the impact of the food.

The process of alveolar bone formation can be observed in the hypofunctional model as well as in the tension side of the experimental tooth movement model, and the transient decrease in the expression of *periostin* and *Twist* mRNA in mouse PDL as indicated in this study might be involved in the process of acute remodeling of the alveolar bone. Regarding the subsequent increase of the expression levels of *periostin* and *Twist* mRNA, weak occlusal forces that could have been exerted on the mandibular molars due to impact of the food were considered but the elongation of a tooth that has lost its antagonist has been proved to be a long process where it takes more than 3 months before occlusal contact can be regained[3, 5, 22, 23, 44]. Thus, the most likely explanation might be that changes in environmental conditions cause living tissues to undergo transient acute changes, and then to adapt to the change. Throughout this study, histological changes observed in the PDL on the experimental side suggested that occlusal hypofunction persisted throughout the study period (one week). Therefore, changes in thickness of the PDL as well as the expression level of the bone-matrix proteins periostin and Twist may be considered a form of adaptation to environmental changes. The functional significance of the altered expression level of *periostin* and *Twist* mRNA in the PDL during hypofunction remains unclear, and further studies are necessary to elucidate the mechanisms by which periostin and Twist might be acting in response to altered occlusal force.

CONCLUSIONS

1. This is the first study to show *Twist* expression in tooth and its supporting tissues. *Periostin* and *Twist* mRNA are co-localized in fibroblastic cells of the PDL and osteoblastic cells on the alveolar surface.
2. The PDL shows constant adaptative remodeling of the fetal-like soft tissue, and is poorly mineralized. Our results suggest that periostin might be one of the regulators of mineralization and calcification of PDL and maintain the homeostasis of PDL *in vivo* by being a target gene of *Twist*.
3. The present study revealed the altered expression level of *periostin* and *Twist* in the PDL in response to lack of occlusal force. Experimentally induced occlusal hypofunction resulted in a marked decrease in the level of *periostin* and *Twist* expressions. Although the physiological significance remains unclear, these results indicate that occlusal force might have putative roles in *periostin* and *Twist* gene expression in the PDL.
4. The transient decrease in the expression of *periostin* and *Twist* mRNA in mouse PDL as indicated in this study might be involved in the process of acute remodeling of the alveolar bone.

Further studies are necessary to elucidate the mechanisms by which periostin and Twist might be acting in response to altered occlusal force.

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