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Expression and characterization of human homologue of UNC-50 (UNCL) in the periodontal ligament

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치의학과

배

최 치 원

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치주인대에서 사람 UNC-50 상동 유전자(UNCL)의
발현과 기능적 특성

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이 논문을 치의학 박사학위 신청 논문으로 제출함.

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최치원의 박사학위 논문을 인준함.

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초록

치주인대에서 사람 unc-50 상동유전자(UNCL)의 발현과 기능적 특성

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치주인대는 치아를 지지할 뿐만 아니라 인접 치조골 및 백악질의 수복과 재생에 관여하는 것으로 잘 알려져 있으나, 치주인대 세포의 분화나 발생에 선택적으로 관여하는 유전자에 관한 연구는 미미한 실정이다. 최근에 치은섬유모세포에는 존재하지 않으나 치주인대섬유모세포에 선택적으로 존재하는 치주인대-특이 유전자, PDLs (a *perio**d**ontal* *l*igament-*s*pecific)22가 보고되었다.

이 연구에서는 사람 치주인대섬유모세포 유전자 library 를 제작하여 *PDLs22* 유전자의 전체 염기서열을 동정하고 염기서열 분석과 homology 검색을 통하여 *PDLs22* 가 *unc*-50 관련 단백질(Genbank accession no. **AF077038**)의 사람 상동유전자임을 확인하였다. 777-bp 의 사람 PDLs22 cDNA 는 259 아미노산으로 되어 있는 단백질을 합성하는 것으로 치주인대섬유모세포 이외에, 뇌, 신장, 고환 및 태반에서도 발현되었다. PDLs22 는 치주인대섬유모세포의 핵막에 주로 위치하였으며 치주인대 세포의 분화과정 전반에 걸쳐 발현되었다. 또한, PDLs22 mRNA 와 단백질은 발육중인 치아의 치조골과 치근면을 따라서 분화중인 시멘트질모세포와 치주인대섬유모세포 그리고 골모세포에서 발현되었다. 교정력에 의한 치아 이동 후에 PDLs22 의 발현이 치주인대에서 증가하였다.

이상의 결과를 종합하여 볼 때 PDLs22는 unc-50의 사람 상동 유전자로서 시멘트질모세포와 치주인대섬유모세포 그리고 골모세포의 분화과정 뿐만 아니라 시멘트질, 치주인대 및 치조골의 항상성을 유지하는 데에도 중요한 역할을 할 것으로 사료된다.

핵심용어: PDLs22, 치주인대섬유모세포, 치주조직, unc-50 관련 단백질, UNCL, AF077038

I. Introduction

The human periodontal ligament (PDL) is a dense connective tissue located between the alveolar bone and cementum that is composed of cellular and extracellular matrix components. The PDL contains numerous fibroblasts, a small number of undifferentiated paravascular fibroblast precursors, epithelial cell rests of Malassez, endothelial cells, macrophages, cementoblastic cells and osteoblastic cells. PDL fibroblasts have the ability to differentiate into mineralized tissue-forming cells such as the cementoblasts responsible for cementum formation and osteoblasts for alveolar bone formation [1,2]. Therefore, PDL fibroblasts are also believed to play important roles not only in homeostasis of the periodontal tissue but also in bone remodeling, wound healing and tissue regeneration [3,4]. For the regeneration of the periodontal tissue, knowledge of the molecular mechanisms related to the differentiation of periodontal ligament fibroblasts with the capacity to support teeth and differentiated into various cells is essential [5,6,7].

Gingival fibroblasts and PDL fibroblasts are located close to each other in the periodontium and are of the same fibroblast group. However, they have different embryological origins. Gingival fibroblasts have a mesenchymal origin, whereas PDL fibroblasts have an ectomesenchymal origin [8]. Furthermore, PDL fibroblasts have different characteristics from gingival fibroblasts including high alkaline phostphatase activity, parathyroid hormone responsiveness, the production of bone-like matrix protein, and the expression of the osteoblast phenotypes such as the formation of mineralized nodules [9,10,11]. It was speculated that the genes uniquely expressed by human periodontal ligament fibroblasts are associated with the specific functions of the PDL. Although the biological functions of the PDL have been studied extensively [12,13], little is known about the genes or molecules uniquely expressed in this tissue.

Using subtractive hybridization between the cultured PDL fibroblasts and gingival fibroblasts, PDLs (a *perio**d**ontal **l**igament-**s**pecific) 22 was isolated as a periodontal ligament-specific cDNA, which is not expressed in the gingival fibroblasts [14]. Northern and in situ hybridizatioin analysis also confirmed that PDLs22 mRNA was differentially expressed in the periodontal ligament fibroblasts. PDLs22 was identified as a human homologue (GenBank accession no**. AF077038**) of the *C. elegans* gene *unc*-50 in the databases. However, the biological function of *unc*-50 is unknown with no experimental data available on this gene in the databases. UNCL, the human homologue of *unc*-50, was also reported to be an inner-nuclear membrane RNA-binding protein, even though the function of UNCL was not clearly described [15].

This paper describes for the first time the isolation of PDLs22, a human homologue of the unc-50 (UNCL), in the PDL and provides evidence of its relationship with the formation of cementum, PDL and alveolar bone during periodontium development and after mechanical

loading by orthodontic tooth movement.

II. Materials and methods

Preparation of cDNA library and isolation of cDNA clones. The mRNA was extracted from the PDL fibroblasts obtained from an explant culture of human PDL tissues. The PDL tissues were isolated from the premolars extracted for orthodontic treatment. The biopsies were obtained with the informed consent of the patients, and in accordance with the ethical standards of the Review Committee on the Use of Human Subjects at Chosun University (Gwang-ju, Korea). An oligo (dT)-primed cDNA library with 1×10^6 independent clones were prepared using a SMART PCR cDNA Library Construction Kit (Clontech) /Gigapack II Gold (Stratagene). The duplicate filters were probed with the ^{32}P-labeled subtracted cDNA fragments of PDLs22 (550 bp) [14] at 65 °C overnight in a hybridization buffer containing 5 × SSC (1 × SSC; 0.15M NaCl, 0.015 M sodium citrate, pH 6.8), 1% N-laurylsarcosine and 50 μg/ml salmon sperm DNA. The filters were washed at 65 °C twice in 2 × SSC, 0.1% SDS and the positive plaques re-screened until a pure clone had been obtained.

Cell culture. The human PDL tissues from the middle third of the root and gingival tissue were minced, plated and cultured in Dulbecco's modified Eagle medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Gibco) and antibiotics at 37℃ in humidified air containing 5% CO_2. When the cell had reached 80-90% confluence, they were collected by digestion with 0.15% trypsin and 0.5 mM EDTA in phosphate-buffered saline (PBS) and plated at a density of $5 \times 10^3/cm^2$ cells per dish. The confluent cells were treated with 50 μg/ml ascorbic acid (AA), 10 mM ß-glycerophosphate (GP) and 5 μM dexamethasone (Dex) for up to 2 weeks to induce PDL cell differentiation and mineralized nodule formation,

Northern analysis. The total RNAs from the PDL fibroblasts and gingival fibroblasts were isolated using a TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. A rat multi-tissue blot containing the total RNA (25 μg) from 14 different tissues was purchased from Seegene (Seoul, Korea). The blot was probed with ^{32}P-labeled cDNAs of human PDLs22, mouse secreted protein, acidic and rich in cysteine (SPARC), rat alkaline phosphatase (ALP), and rat bone sialoprotein (BSP), and rat glyceraldehyde-6-phosphate dehydrogenase (GAPDH) [11]. For rehybridization, the membranes were striped in a boiled 0.1% SDS solution.

Production of human PDLs22 antisera. Human PDLs22 antisera were produced using the peptide, ISNKYLVKRQSRD that was selected from the amino acid sequence of human PDLs22 (Fig. 1). The antisera were used for western and immunohistochemical studies.

Western analysis. Western blot analyses were carried out using 1:200 to 1: 1000 dilutions as previously reported [16].

Immunofluorescent staining of PDL cells. The PDL cells grown on glass cover slips were rinsed briefly in PBS (137mM NaCl, 2.7mM KCl, 4.3mM Na_2HPO_4, 1.4mM KH_2PO_4, pH 7.4)

and fixed for 15 min by immersion in 4% paraformaldehyde fixative, which was followed by permeabilization with 0.15% Triton X-100. The cells were blocked with 1.0% BSA in PBS, and incubated for 1 hr 30 min at RT with anti-PDLs22 as the primary antibody diluted in PBS containing 1.0% BSA. After washing, the cover slips were incubated with the affinity isolated secondary antibody (fluorescin-conjugated goat anti-rabbit IgG, Vector). The cover slips were mounted in a Fluorescent Mounting Medium with DAPI (Vector). The cells were observed for their epifluorescence using an Axioscope multiple microscope (Zeiss). The acquired images were manipulated with Axiovision 1.0 (Zeiss) and digitized using Adobe Photoshop software (Adobe Photosystems).

Tissue preparation. The embryonic and postnatal rat tissues were fixed in 4% paraformaldehyde, embedded in paraffin. Serial coronal sections of 5μm thickness were cut and subjected to immunohistochemistry and in situ hybridization analysis as described previously [14].

In situ hybridization. The plasmid pCRII-TOPO-PDLs22 was linearized with Xba I, and the cRNA probe was synthesized and labeled with digoxigenin-UTP using Sp6 RNA polymerase. In situ hybridization was carried out as described previously [14].

Immunohistochemistry of rat tissue. The tissue sections were pre-incubated with 1% BSA in PBS for 30 minutes, and incubated for 1 hr with the anti-PDLs22 antisera diluted 1:100 in 1% BSA in PBS. They were incubated for 1 hr at room temperature with the secondary antibody and reacted with an avidine-biotin-peroxidase complex (Vector) in PBS for 1 hr. After color development with 0.05% DAB (diaminobenzidine tetrahydrochloride), they were counterstained with hematoxylin.

Mechanical stress in rat periodontium. Mechanical stress was applied to the periodontal tissues using an orthodontic appliance. The orthodontic appliances, which consisted of a stain-less steel coil spring bonded to the left molar and the incisors, was inserted and activated under a dissecting microscope. A consistent and reproducible activation of the appliance was insured by determining the force/deflection characteristics of the spring, so that the level of force after activating the appliance was maintained between 30 and 50 g. Expression of the PDLs22 protein was observed by immunohistochemistry after 1, 6, and 12 days of orthodontic tooth movement.

III. Results

Cloning of human PDLs22 and homology search

In order to recover the full-length human clone the subtracted cDNA fragments of PDLs22 [14] were used as a probe to screen the human PDL fibroblast cDNA library. Following repeated screening of duplicate replica, a clone was identified and subcloned into the pBbluscript II KS (+) vector. The nucleotide sequences were determined using Autosequencer 377 (Perkin Elmer Applied Biosystems). The clone was approximately 1.1 kb in size and contained the entire reading frame of the human homologue of the *unc*-50 (Genbank accession no. **AF077038**) at the NCBI (http://www.ncbi.nlm.nih.gov/BLAST/). *PDLs22*, the human homologue of the *unc*-50 (UNCL) cDNA contains an open reading frame of 777 nucleotides, which codes for a 259 amino acid protein with a predicted molecular weight of 28 kDa. Although the putative protein sequence does not contain a recognizable signal sequence, a protein information analysis system (SOSUI WWW Server, http//sosui.proteome.bio.tuat.ac.jp) predicted 5 transmembrane domains, suggesting that PDLs22 may function as a transmembrane protein (Fig. 1).

Expression of PDLs22 mRNA in multiple tissues of rat

The subtracted cDNA fragments of PDLs22 were used as a probe in a Northern blot of the total RNAs from the human PDL fibroblasts, gingival fibroblasts, and 14 rat tissues. The PDLs22 cDNA probe detected a 2.2 kb mRNA fragment in the human PDL fibroblasts. However, PDLs22 expression could not be in the human gingival fibroblasts. PDLs22 mRNA was also expressed in the brain, kidney, testis, placenta, skin, heart, and skeletal muscle (Fig. 2). The level of PDLs22 mRNA was highest in the brain, PDL fibroblasts, kidney and testis and lowest in the skeletal muscle and all the tissues expressed a single sized species, suggesting that the transcript was not alternatively spliced.

Expression of PDLs22 mRNA and protein during PDL cell differentiation in vitro.

The general mRNA expression patterns for SPARC, ALP, and BSP during the PDL cell differentiation were investigated (Fig. 3). When the PDL cells are cultured in the differentiation and mineralization medium for approximately 2 weeks, the ALP activity and BSP in the PDL cells were gradually increased, whereas the SPARC mRNA expression level gradually decreased with cell differentiation. Although the expression of the PDLs22 mRNA and protein were slightly lower at the mineralization stage on day 14, the expression level remained relatively high

throughout the differentiation of the PDL cells (Fig. 3, 4).

Intracellular sub-localization of PDLs22 protein

In order to determine the intracellular sub-localization of PDLs22, cultured human PDL cells were immunostained with a polyclonal anti-PDLs22 antibody. Based on the PDLs22 protein analysis data from the SOSUI WWW Server (http//sosui.proteome.bio.tuat.ac.jp), it was expected that the protein would be localized to the cell membrane or the nuclear membrane (Fig. 1). As expected, the immunofluorescent staining of the cultured human PDL cells revealed its localization on the nuclear membrane. However, a small amount of fluorescence was also observed in the cytoplasm. The distribution was in a linear form and concentrated near the nucleus in addition to a small amount of scattered staining in the cytoplasm (Fig. 5).

Expression of PDLs22 mRNA and protein in vivo

In situ hybridization analysis of a coronal section in 3-week old rat showed that PDLs22 mRNA was strongly expressed in the developing PDL cells and reduced dental epithelium containing the internal and external dental epithelium (Fig. 6A, 6B). However, PDLs22 mRNA was not detected in the odontoblasts of the developing tooth and gingival fiboblasts of the tooth supporting tissues. Immunohistochemical localization of the PDLs22 protein in a 3-week old rat showed that the PDLs22 protein was localized in differentiating cementoblasts, and also in PDL fibroblasts close to the developing root surface and alveolar bone (Fig. 6C, 6D). In the 5-week old rat, PDLs22 protein expression was detected in the PDL fibroblasts, specifically in the developing cementoblasts and osteoblasts (Fig. 6E, 6F).

Expression of PDLs22 protein after mechanical loading by orthodontic appliance in rat teeth

The tooth was moved with an orthodontic coil spring bonded to the maxillary first molar and incisors, and the contralateral left molar was used as the control. The manner of loading produced a favorable paradental tissue remodeling response with no significant damage of the hyalilization of the periodontium, which is consistent with the rapid onset of tooth movement. The initial tooth movement resulted in an osteoinductive signal on the alveolar bone in the tension side of the periodontium. In the control tooth, PDLs22 protein expression was weakly detected in the PDL fibroblasts along the alveolar bone surface (Fig. 7a). However, a strong and distinct PDLs22 protein expression was observed in the PDL fibroblasts and the osteoblasts at 1 day after tooth movement (Fig. 7B). Expression of the PDLs22 protein remained relatively high

throughout the orthodontic tooth movement at 6 and 12 days.

IV. Discussion

PDL fibroblasts have an ectomesenchymal origin and are known to participate not only in formation of PDL but also in the repair and regeneration of the adjacent alveolar bone and cementum *in vivo* [3,4,8]. After examining the factors related to the development and differentiation of the PDL fibroblasts, many researchers have attempted to understand the functional characteristics of the PDL using morphological observations [9,10,11,17]. Recent studies also have focused on determining the function of the specific genes such as the epidermal growth factor, the type XII collagen, the S100 calcium-binding protein family, and the osteoclasts specific factor-2 (OSF-2, periostin) of the PDL [18,19,20]. However, little is known about the markers for PDL fibroblasts expressed during their differentiation. In order to identify the marker molecules expressed specifically in the human PDL fibroblasts, a method of mRNA subtraction between gingival fibroblasts and PDL fibroblasts was employed and the PDLs22 cDNA was obtained [14].

After screening the human PDL fibroblasts cDNA library the PDLs22 cDNA clone was isolated and identified as a human homologue of the *unc*-50 (Genbank accession no. **AF077038**) (Fig. 1). The sequence showed 99% homology between *PDLs22* and the human homologue of *unc*-50. The human homologue of *unc*-50, UNCL, bound RNA and was involved in the surface expression of some combinations of neuronal nAChR subunits [15,21]. However, it was also suggested that UNCL has a highly conserved and fundamental biochemical role involved in, but not restricted to, the surface expression of nAChRs. In this study, PDLs22 was cloned in the PDL fibroblasts that do not express the neuronal or muscle-type nicotinic receptors. This suggests that the role of the UNCL was not restricted to the nicotinic receptor assembly.

In this study, it was found that PDLs22 mRNA was expressed in the PDL fibroblasts, brain, kidney, testis, placenta, skin, heart, and skeletal muscle (Fig. 2). The strongest expression of PDLs22 mRNA was found in the PDL fibroblasts, brain, kidney and testis, even though there was weak expression in the placenta and skeletal muscle. The widespread expression of PDLs22 mRNA is also supported by the origin of the PDLs22 clones in the EST database, which demonstrates that PDLs22 is expressed in many such as the brain, kidney, testis, placenta, aorta, and mammary glands. The brain has a high concentration of neuronal nicotinic receptors but it is not known why PDLs22 is expressed in the kidney, testis, and other tissues [15].

PDL cells differentiate and form mineralized nodules when cultured in the presence of Dex (5 μM), GP (10 mM) and AA (50 μg/ml) for up to 2 weeks [22]. The expression of PDLs22 mRNA and protein was increased with PDL cell differentiation from the confluent to multilayer stage but decreased slightly with mineralized nodule formation (Fig. 3, 4). This suggests that

PDLs22 might play important roles in PDL fibroblast proliferation and differentiation, even though it is also related to mineralized nodule formation of the cells.

PDLs22 was mainly localized in the nuclear membrane of the cultured PDL fibroblasts and was also observed in the cytoplasm (Fig. 5). The distribution was either in the spots or a linear form. In particular, the linear form was observed in the region toward the peripheral cytoplasm. The PDLs22 was scattered in the cytoplasm and concentrated near nucleus. Interestingly, it was also found in the peripheral cell membrane and cell process. These observations are in agreement with the result from the sub-localization of UNCL [15].

In this study, PDLs22 mRNA was strongly expressed in the internal and external dental epithelial cells (Fig. 6A, 6B). During tooth development, after the completion of crown formation, the apical ectomesenchyme continued to proliferate to form a developing periodontium while the internal and external dental epithelium fused below the level of the crown cervical enamel to produce a bilayered epithelial sheath known as the Hertwig's epithelial root sheath (HERS) [13]. It is well known that HERS cells involve in the formation of acellular cementum during tooth development [4]. PDLs22 was also expressed in the differentiating cementoblasts, PDL fibroblasts and preosteoblasts along the developing root surface and alveolar bone (Fig. 6C, 6D, 6E, 6F). This suggests that the function of PDLs22 is associated with bone or cementum formation for homeostasis and regeneration in the periodontal ligament. It has been known that the periodontal ligament fibroblasts are essential for osteogenesis and cementogenesis in periodontal tissues can differentiate into osteoblasts or cementoblasts [3]. It has recently been reported that growth and differentiation factors regulate the cellular function in the periodontal ligament during regeneration and homeostasis [10].

Unc (uncoordinated) and *uncl* (uncoordinated-like) were previously identified in the course of an intensive mutagenesis *C. elegans* and mapping of genes based on the X-chromosome of *Drosophila melanogaster* [23,24]. A mutation in the two genes, *unc* and *uncl*, was found to eliminate the mechanotransduction and showed uncoordination, touch-insensitive larva, adult behavioral defects and a failure to elicit a scratch reflex of *Drosophila* [25]. Most *unc* and *uncl* hemizygous animals survive through the larval and pupal stages if they are reared in the absence of competing wild-type siblings. However, immediately upon eclosion, all adult *unc* flies show uncoordination so severe that they can neither stand nor walk, leading to an early death by adhesion, which is a similar phenotype to that of the bristleless mutant. Only semilethal alleles are unable to walk because of a lack of coordination in their leg movements. The lethal phase of all three occurs during pupariation or shortly after emergence, and no defects were detected in either the CNS or PNS of the mutant embryos derived from heterozygous females. These studies imply that mechanical and functional stress may be related to *PDLs22*, which is the human homologue of *unc* and *uncl*.

Mechanical forces play an important role in the homeostasis of tissues during normal development [26]. In addition, mechanical forces are involved in the generation of specific conditions, such as tooth movement and mastication [27]. During tooth movement and mastication, the PDL cells must be able to sense the mechanical stimuli and respond to these changes by altering the metabolism of the biomolecules and by adapting the surrounding extracellular matrix [28]. Mechanical stress is also an important regulatory factor in bone homeostasis and a determinant of the skeletal morphology from the early developmental stages and throughout lifespan [29]. Because of its influence on and interactions with all the other regulators of the bone metabolism, the effect of mechanical loading on proliferation and phenotype expression in osteoblasts has been extensively studied [30,31]. The differential expression of the genes in the PDL cells under physiological stress such as an occlusal force is believed to be orchestrated not only for the remodeling of PDL itself but also for the repair and regeneration of periodontal tissues. However little is known about the genes expressed in the PDL cells under mechanical stress.

In this study, expression of PDLs22 protein was weakly detected in the PDL fibroblasts along the alveolar bone surface of the control tooth (Fig. 7a). However, strong and distinct PDLs22 protein expression was present in the PDL cells and osteoblasts especially at 1 day after tooth movement (Fig. 7B). This suggests that the mechanical stimulation of PDL cells results in an increased pattern of PDLs22 expression in developing osteoblasts.

This study demonstrated that PDLs22, a human homologue of the unc-50 related protein, is related to the formation of cementum, PDL and alveolar bone during periodontium development and mechanical loading. In order to better understand the molecular mechanisms by which PDLs22 regulates both the development and regeneration of the periodontium, future studies will be aimed at clarifying the mode of action of PDLs22 using methods such as overexpression, inactivation of this gene and identification of the related proteins *in vitro* along with the intracellular signaling pathway of this specific gene.

V. Conclusion

The periodontal ligament (PDL) anchors the roots of teeth to the alveolar bone and keeps teeth in position by resisting to mechanical strain like an occlusal force. Recently, we reported the expression of PDLs (a *p*erio*d*ontal *l*igament-specific) 22 mRNA specifically in PDL fibroblasts, but not in gingival fibroblasts. In this study, a full-length human clone of PDLs22 was obtained from the human PDL fibroblast cDNA library and was identified as a human homologue of the *unc*-50 (Genbank accession no. **AF077038**). The 777-kb human PDLs22 cDNA encodes a 259 amino acid protein that is expressed in the PDL fibroblasts, brain, kidney, testis and placenta. PDLs22 was localized on the nuclear membrane and expressed throughout the differentiation of PDL cells *in vitro*. In addition, the PDLs22 mRNA and protein were expressed in the differentiating cementoblasts, PDL fibroblasts and osteoblasts along the root surface and alveolar bone of the developing rat teeth. After mechanical loading by orthodontic appliance, the PDLs22 expression level was increased in the PDL. Taken together, these results indicate that the PDLs22, human homologue of the *unc*-50 (UNCL), plays an important role in the differentiation of cementoblasts and osteoblasts and thus homeostasis of cementum, PDL ligament and alveolar bone.

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Legend for figures

Fig. 1. Predicted amino acid sequence of the human PDLs22 (human homologue of *unc*-50) that codes a 259 amino acid protein with a molecular weight of 28 kDa. Five transmembrane domains predicted by the protein information analysis system (SOSUI WWW Server, http//sosui.proteome.bio.tuat.ac.jp) are underlined. The peptide used for the antibody production is denoted in *italic* characters.

Fig. 2. Northern blot analysis of the mRNAs for PDLs22 in human PDL fibroblasts, gingival fibroblasts, and multiple tissues of rat. Total RNAs were isolated from both cultured human PDL and gingival fibroblasts. A rat multi-tissue blot was purchased from Seegene (Seoul, Korea).

Fig. 3. Northern blot analysis of the mRNAs for PDLs22 and the matrix components (SPARC; secreted protein acidic and rich in cysteine, ALP; alkaline phosphatase, BSP; bone sialoprotein) in the PDL fibroblasts cultured for up to 14 days.

Fig. 4. Western blot analysis of the PDLs22 protein in PDL fibroblasts cultured for up to 14 days. Nitrocellulose membranes were revealed, after incubations with the antibody ant-PDLs22.

Fig. 5. Immunofluorescent localization of PDLs22. PDL fibroblasts grown on coverslips were fixed with paraformaldehyde and incubated with polyclonal anti-PDLs22 antibody (*green*). After incubation the cells were mounted in DAPI (*blue*) mounting medium. *Panel A*, localization of PDLs22; *panel B*, nuclear staining with DAPI; *panel C*, the composite of *A* and *B*. Bar = 20 μm.

Fig. 6. Expression of PDLs22 mRNA and protein in developing rat periodontium. *Panel A and B*, 3-week old rat. PDLs22 mRNA was localized in the PDL cells. *Panel C and D*, 3- week old rat. PDLs22 protein expression was observed in the developing cementoblasts (*arrows*) and also in PDL fibroblasts (*arrowheads*) close to the alveolar bone surface. *Panel E*, interdental area of 5- week old rat; *panel F*, root apex area of 5- week old rat. The PDLs22 protein was expressed in developing cementoblasts (*arrows*), osteoblasts (*arrowheads*), and PDL fibroblasts. Bar = 50 μm.

Fig. 7. Expression of PDLs22 protein in tension side of the periodontium after orthodontic

tooth movement in rat. *Panel A*, normal periodontium of 5- week old rat. In the control tooth, PDLs22 protein expression was weakly detected in the PDL fibroblasts along the alveolar bone surface. *Panel B*, tension side of the periodontium of 5- week old rat. After 1 day of orthodontic tooth movement, a strong and distinct PDLs22 protein expression (*arrows*) was observed in the PDL fibroblasts and the osteoblasts. IDS; interdental septum. Bar = 50 μm.