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Identification of genes related to mechanical stress in human periodontal ligament cells using microarray analysis

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Background and Objective: Differential expression of genes in human periodontal ligament (PDL) under mechanical stress, such as orthodontic force, is thought to be involved in the remodeling of PDL cells and periodontal tissues. However, little is known about the genes expressed in PDL cells under mechanical stress.

Material and Methods: We employed microarray analysis to assess, in a comprehensive manner, the gene expression profiles in PDL cells compressed by a static force using an *in vitro* three-dimensional culture system. Six genes were selected and validated by quantitative real-time polymerase chain reaction analysis, consistent with the microarray data.

Results: The microarray data revealed that 108 of 30,000 genes tested were differentially expressed by mechanical force loading. Among them, 85 genes were up-regulated by mechanical stress, while 23 genes were down-regulated, judging by the thresholds of a two-fold increase/decrease compared with the controls. Thirty-two of the up-regulated and eight of the down-regulated genes, well-characterized in protein function, were involved in numerous biological processes including cell communication, cell signaling, cell cycle, stress response, and calcium release. However, several genes differentially expressed in our microarray data have not been well defined as stress-response molecules.

Conclusion: Our microarray is the first to show the gene profile in PDL cells caused by mechanical stress; however, further studies to clarify the physiological function of these molecules in PDL cells are required.

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The periodontal ligament (PDL) that exists between hard tissues, the cementum of the dental root and alveolar bone, receives mechanical stress such as occlusal pressure and orthodontic force (1). The biological response of the PDL to mechanical stress may have an effect on the homeostasis of the PDL itself as well as on other components of the periodontal tissues. PDL cells participate not only in the remodeling of the PDL itself, but also in the repair and regeneration of periodontal tissues. As ankylosed teeth directly connected with alveolar bone without intervention of PDL are unable to be moved by therapeutic mechanical stress, such as orthodontic forces (2), PDL cells are thought to play an important role in periodontal and osseous remodeling, including the resorption and formation of bone matrix during physiological and orthodontic tooth movement. Mechanical stress has been suggested to regulate the gene expression of biological mediators, such as interleukin-6

(3), interleukin-1β (4–6), matrix metalloproteinases and tissue inhibitor of metalloproteinases (7), plasminogen activator (8), cyclooxygenase (COX)-2 (9,10), alkaline phosphatase activity (11), type I collagen (12) and osteocalcin (13), in PDL cells. Kanzaki *et al.* (14) recently demonstrated that a static compressive force stimulated the expression of receptor activator nuclear factor kB ligand (RANKL), a potent osteoclastogenic factor, via the induction of prostaglandin E_2 (PGE_2) by COX-2 activation in a monolayer PDL cell culture system. Thus, PDL cells are recognized as a transducer of mechanical stress into the biological signaling in which a set of unique genes starts to be expressed under the regulation of their own mechanism, but the details of the gene expression pattern caused by mechanical stress is still unclear. Significant advances in gene expression analysis have been achieved by means of DNA microarray technology. A microarray is a powerful technique used to analyze the expression profiles of a large number of genes simultaneously (15). Recently, using this technology, the different gene expression patterns between human PDL cells and gingival fibroblasts have been displayed to examine intrinsic functional differences in the two cell populations (16). In the present study, to identify mechanical stress-related genes in human PDL cells, the gene profiles differentially expressed in PDL cells as a result of mechanical stress were examined by microarray analysis, using RNAs obtained from PDL cells compressed by mechanical forces in a three-dimensional collagen gel culture system.

Materials and methods

Cell culture

Human PDL cells were derived from the ligament tissues of periodontally healthy, noncarious human premolar teeth, extracted from donors for orthodontic reasons with informed consent, and this study was approved by the Institutional Review Boards of the University of Tokushima. The cells were isolated and maintained in alpha minimal essential medium (α-MEM) (Sigma, St Louis, MO, USA), containing 10% fetal bovine serum (FBS) and 100 U/ml penicillin, in a humidified atmosphere of 5% CO_2 at 37°C, as described previously (16) with slight modifications. In this study, we used PDL cells that had been cultured through three passages.

Application of compressive force

In order to determine the effect of static compressive force, PDL cells were embedded and cultured in a three-dimensional collagen gel system to mimic *in vivo* conditions, as illustrated in Fig. 1. The collagen gel cultures were assembled by mixing 7 volumes of 0.3% type I collagen solution (Nitta-gelatin, Osaka, Japan), 1 volume of 10× α-MEM, 1 volume of 20 mM HEPES (containing 2.2% sodium bicarbonate and 0.05% sodium hydroxide) and 1 volume of cell suspension to provide a final cell density of 1.25×10^6 cells/ml. The gel mixture (800 μl) was cast in 24-well plates and allowed to polymerize for 1 h. After polymerization, the gels were transferred to a six-well plate to promote nutrient diffusion from their surroundings. Three gels in each well were cultured with 2 ml of α-MEM containing 10% FBS and allowed to set for 24–36 h prior to force loading. Compressive forces were applied using a plastic cylinder placed over the gels, which was adjusted by adding lead granules to the cylinder (Fig. 1). The gels with cover plate alone served as controls. PDL cells were subjected to 3.6, 6.0, 7.1 or 9.5 g/cm^2 of compressive force for 6 h, or to a constant compressive force (6.0 g/cm^2) for 1, 3, 6, 12, 24 or 72 h.

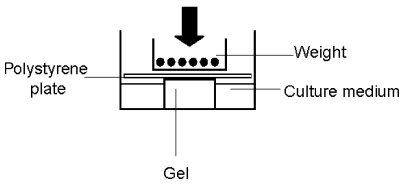

Fig. 1. Diagram of compression experiments using the three-dimensional collagen gel cell culture model. Static compressive force was loaded using a well insert bearing the weight of the plate cover and calculated load.

RNA isolation and reverse transcription–polymerase chain reaction (RT–PCR)

Total RNA from PDL cells embedded in the collagen gels was extracted using Isogen (Nippon Gene, Tokyo, Japan). Briefly, the collagen gels containing cells were minced in Isogen (1.5 ml/gel), and RNA from the lysed cells was then isolated according to the manufacturer's protocol. The RNA was reverse transcribed, and the first-strand cDNA was then subjected to PCR using a Takara RNA PCR kit (Takara Bio, Otsu, Japan), as reported previously (17). Each cycle consisted of a heat-denaturation step at 94°C for 30 s, an annealing step at a temperature optimized for each primer pair of COX-2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Table 1) for 30 s, and an extension step at 72°C for 1 min. The PCR products were electrophoresed and visualized by etidium bromide staining with ultraviolet (UV) light illumination.

PGE_2 measurement

PGE_2 production in the conditioned culture media was determined using a Prostaglandin E_2 EIA kit (Cayman Chemical, Ann Arbor, MI, USA).

Microarray experiments

Microarray analysis was performed using an AceGene human oligo chip 30 k (DNA Chip Research and Hitachi Software Engineering, Yokohama, Japan) spotted with 30,000 genes on the poly(lysine)-coated glass slides. Total RNA from PDL cells treated with compressive forces (6.0 g/cm^2) for 6–24 h was used as the compressive group. Total RNAs were individually extracted using Isogen (Nippon Gene), and then mixed and labeled using an Amino Allyl MeassageAmp aRNA kit (Ambion, Austin, TX, USA) for oligo-microarrays, according to the manufacturer's protocol. The control and compressive groups were labeled with

Table 1. Primers used for polymerase chain reaction (PCR)

Specificity	Oligonucleotide sequence (5′–3′)	Product (bp)	temperature (°C)	Annealing
COX-2	Sense:	5′-AACCCACTCCAAACACAG-3′	411	51
	Antisense:	5′-CTGGCCCTCGCTTATGATCT-3′		
BiP	Sense:	5′-GGTGAAAGACCCCTGACAAA-3′	200	58
	Antisense:	5′-GTCAGGCGATTCTGGTCATT-3′		
IL-6	Sense:	5′-AGGAGACTTGCCTGGTGAAA-3′	180	58
	Antisense:	5′-CAGGGGTGGTTATTGCATCT-3′		
RhoE	Sense:	5′-CACATGCCTAGCAGACCAGA-3′	176	58
	Antisense:	5′-GACTTTGGCTGTGCACTTCA-3′		
IL-1β	Sense:	5′-AATCTGTACCTGTCCTGCGTGTT-3′	79	52
	Antisense:	5′-TTGGGTAATTTTTGGGATCTACACT-3′		
DSCR1	Sense:	5′-CCCCAGGTATCACTGCACTT-3′	142	58
	Antisense:	5′-GGGGACTAACAGCCATCAAA-3′		
IP4P type I	Sense:	5′-CCACTTCGACTGAGGAGGAG-3′	177	60
	Antisense:	5′-TTGTCCACACGCTGAATGAT-3′		
GAPDH	Sense:	5′-GAGTCAACGGATTTGGTCGT-3′	185	58
	Antisense:	5′-GACAAGCTTCCCGTTCTCAG-3′		

COX-2, cyclooxygenase-2; DSCR1, Down syndrome critical region protein 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL, interleukin; IP4Ptype I, inositol polyphosphate-4-phosphatase type I.

Cy3 and Cy5, respectively, mixed, and hybridized on a microarray according to the manufacturer's instructions. For each sample, the experiment was repeated once, wherein the dye was reversed between the experimental and reference sample to account for dye-incorporation bias. The microarrays were then scanned using Scan Array Lite, and the signal values were calculated using QUANTARRAY (Perkin-Elmer, Boston, MA, USA). Following subtraction of the background, the gene-spot signals were adjusted to compensate for excitation differences between the two dyes. Genes showing highly inconsistent data between the dye-swap experiments were discarded, and the expression levels of the remaining genes were represented as the average for each gene spot. The data were analyzed using DNASIS software (Hitachi Software Engineering) according to the methods of Quackenbush (18). The results were expressed as the gene expression ratio (i.e. the ratio of the intensities of Cy5–Cy3). Using a two-fold balanced differential expression as the appropriate 'cut-off' value, we identified those genes showing greater than a two-fold change in this experiment.

Quantitative real-time PCR analysis

PCR amplification was performed using the QuantiTect SYBR Green PCR kit (Qiagen, Tokyo, Japan) in a 25-μl reaction mixture containing 0.3 μM of each primer, 2.5 mM $MgCl_2$ and 1 μl of cDNA. Primers are listed in Table 1. TaqStart antibody was systematically added to the amplification reaction mixture to block *Taq* DNA polymerase activity during the set-up of the PCRs at room temperature. DNA amplification and detection were carried out in the ABI Prism 770 (Perkin Elmer Applied Biosystems, Foster City, CA, USA) as follows: the reaction mixture was initially incubated at 95°C for 15 min to inactivate the TaqStart antibody and to denature DNA. Amplification was performed for 45 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min. The ramp rate was 20°C/s. The cumulative fluorescence for each amplicon was normalized to that seen with GAPDH amplification using the ABI sequence detector software (Perkin Elmer Applied Biosystems). Results were expressed as the fold increase, at each time point, over the respective GAPDH controls. Amplified products were identified as distinct single bands on agarose gel electrophoresis.

Statistical analysis

Results are reported as the mean ± SD for a typical experiment and compared by the Student *t*-test. Results were considered to be significantly different for *p*-values less than 0.05.

Results

Mechanical stress up-regulated COX-2 mRNA and PGE_2 production

Because previous reports have demonstrated the induction of COX-2 and PGE_2 in PDL cells in response to mechanical stress (19,20), COX-2 gene expression and PGE_2 production were analysed in order to evaluate the effect of compressive force in this three-dimensional culture system. Under compression, the expression of COX-2 mRNA in PDL cells was increased in a force-dependent manner up to 7.1 g/cm^2, but the expression was slightly reduced at 9.5 g/cm^2 of loading force (Fig. 2A). The time-course experiment showed that the expression of COX-2 mRNA was increased up to 24 h compared with the control, and a similar expression level was revealed after 48 h (Fig. 2B). Because COX-2 is an inducible enzyme that catalyzes prostaglandin production, to determine if COX-2 induced by mechanical stress synthesizes prostaglandin, we performed an enzyme immunoassay (EIA) for PGE_2 secreted in the conditioned culture media. PGE_2 in the conditioned culture media was significantly increased in a time-dependent

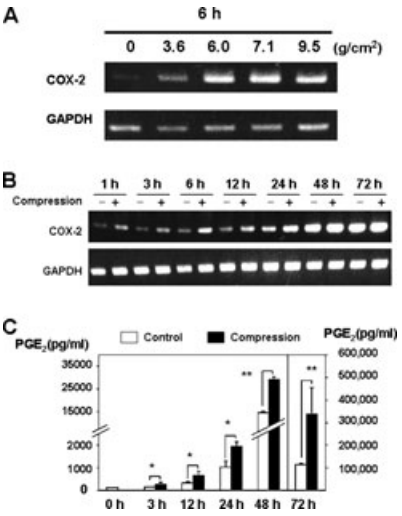

Fig. 2. Mechanical stress induced the expression of cyclooxygenase (COX)-2 mRNA and prostaglandin E_2 (PGE_2). Reverse transcription–polymerase chain reaction (RT–PCR) analyses were performed using the RNAs isolated from periodontal ligament (PDL) cells stimulated by compressive forces for the indicated force (A) and time (B). COX-2 mRNA was detectable at 28 cycles of PCR, while only 20 cycles of PCR were required to detect glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Conditioned culture media from PDL cells stimulated by compressive force (6.0 g/cm^2) were collected at the indicated time points and then subjected to enzyme immunoassay (EIA) assay for PGE_2 (C). Results represent the mean ± standard deviation (SD) of quadruplicate determinations for a typical experiment. Similar results were seen in three independent experiments ($*p < 0.05$, $**p < 0.01$).

manner compared with controls (Fig. 2C).

Identification of genes in response to mechanical stress

In the present microarray analysis, 108 independent genes related to mechanical stress were identified. Among them, 85 were up-regulated by mechanical stress, while 23 were down-regulated. As shown in Table 2, 32 up-regulated and eight down-regulated genes were found, which were already biologically well characterized in terms of protein function, including cell communication, cell signaling, cell cycle, stress response and calcium release. Regarding these biological processes, we identified interesting molecules, such as cytokines (interleukin-6, interleukin-1β), a transcriptional factor [transforming growth factor-β-stimulated clone (TSC)-22], a glycoprotein [tumor necrosis factor-α-stimulated gene (TSG)-6], stress-related proteins (BiP/glucose-regulated protein 78), a G-protein (RhoE), a GTPase-activating protein [regulator of G-protein signaling (RGS)] (2) and calcium channel-related proteins. The prominent induction of interleukin-6, RhoE, Down syndrome critical region protein 1 (DSCR1), and heat shock protein, BiP, was observed. On the other hand, inositol polyphosphate-4-phosphatase type I (IP4P I) and zinc finger protein (ZFP) 2 were markedly down-regulated.

To confirm the expression level of the genes indicated in the microarray data, we selected five genes (interleukin-6, interleukin-1β, RhoE, DSCR1 and BiP) from the up-regulated group and IP4P1 from the down-regulated group, as having various levels of differential expression, for quantitative real-time PCR analysis. As shown in Fig. 3, all genes demonstrated an alteration of their expression level as a result of mechanical stress during a 24-h time period; therefore, these results well supported the present microarray data.

Discussion

We first developed an *in vitro* three-dimensional cell culture system, using collagen gels, that resembles the *in vivo* condition for analyzing the gene profiles regulated by a static compressive force in PDL cells. We then demonstrated that upon mechanical stress application, the compressed PDL cells exerted, in a time-dependent manner, an increase in PGE_2 production in the culture media, in parallel with an induction of COX-2, which is a key enzyme in prostaglandin synthesis. It has previously been reported that PGE_2, an important chemical mediator of bone resorption, is synthesized in human PDL cells in response to mechanical stress (3,9,14,19,20). Therefore, this *in vitro* culture system is thought to be applicable for using to evaluate the effect of mechanical stress on the cell response of PDL under the three-dimensional conditions. Using this cell culture system, we collected the RNA samples and then applied them to the microarray for comprehensively analyzing gene-expression profiles in response to mechanical stress. The microarray results displayed 32 up-regulated and eight down-regulated genes that have already been biologically well characterized in protein function. Our microarray data showed several interesting molecules that might be involved in biological processes in PDL cells and periodontal tissues, consisting of cytokines, signal transduction proteins, transcription factors, stress-related, and extracellular matrix-related, cytoskeleton-related proteins. However, with the exception of cytokines, none of these molecules in the present microarray data have yet been well defined as a mechanical stress-related regulator in PDL cells or periodontal tissues. Orthodontic tooth movement consists of the processes of periodontal tissue remodeling, including bone formation on the tension side and bone resorption on the compression side, mediated by PDL cells. Uematsu *et al.* (21) previously demonstrated that certain cytokines, such as interleukin-1β, interleukin-6 and TNF-α, were implicated in bone remodeling during orthodontic tooth movement. Each of these cytokines has multiple activities, which include bone remodeling, bone resorption and new bone formation (22–25). It is well documented that compressive forces induce the expression of pro-inflammatory mediators and that interleukin-1β has been implicated as one of the major cytokines synthesized in response to compressive forces applied on the PDL (6,26–28). Interleukin-1β stimulates PGE_2 synthesis, which is essential to induce an osteogenic response during bone resorption and formation (14,29).

It was observed that signal transduction proteins were preferentially expressed in compressed PDL cells. RhoE protein constitutes a subfamily within the Rho GTPase family. Rho GTPases are major regulators of cytoskeleton dynamics, including microtubules and

Table 2. Differentially expressed genes in periodontal ligament (PDL) cells under compressive forces

Name	Ratio	Description	Accession no.
Up-regulated gene			
Signal transduction			
ARHE	3.6	ras homolog gene family member E, RhoE	NM_005168
DSCR1	3.2	Down's syndrome critical region protein 1	XM_048638
GABD	2.2	gamma-aminobutyric acid (gaba) a receptor; delta	NM_000815
CCR9	2.0	chemokine (cc motif) receptor 9	NM_031200
BDKRB1	2.0	bradykinin receptor B1	NM_000710
RGS2	2.0	regulator of G protein signaling 2	NM_002923
DRD1IP	2.0	dopamine receptor D1 interacting protein, calcyon	NM_015722
Cytokine/growth factors			
IL-6	5.0	interleukin-6 (interferon, beta 2)	NM_000600
PTX3	2.2	pentaxin-related gene, rapidly induced by IL-1β	NM_002852
IL-1BEATA	2.0	interleukin-1beta, IL-1β	NM_00057
Transcription factor			
DDIT3	2.5	DNA damage-inducible transcropt 3	NM_004083
FUS-CHOP	2.3	myxoid liposarcoma specimens	S75763
FUS-CHOP	2.2	partial fuschop chiramic fusion protein type8 transcript variant	AJ301611
TSC22	2.0	transforming growth factor-beta-stimulated protein	NM_006022
Stress related			
BiP	2.6	heat shock 70 kDa protein 5, GRP78, HSPA5	AF188611
HIG2	2.0	hypoxia-inducible protein 2	BC001863
Cell-cycle regulation			
CDK7	2.0	cyclin-dependent kinase 7	NM_001799
INHBA	2.0	inhibin beta a subunit precursor	NM_002192
Calcium channel related			
CACNA1G	2.0	calcium channel, voltage-dependent, alpha 1G subunit	NM_018896
VADC1	2.0	voltage-dependent anion channel 1	NM_003374
Metabolism related			
ENO1	2.2	enolase1	NM_001428
ERP70	2.0	protein disulfide isomerase related protein	NM_004911
ENO2	2.0	enolase 2	NM_001975
GA	2.0	breast cell glutaminase	NM_013267
Cytoskeleton related			
PBEF	2.1	pre-B cell colony-enhancing factor	NM_005746
Dystrophin	2.0	dystrophin	S71486
CETN1	2.0	centrin 1	NM_004066
Extracellular matrix related			
PLOD2	2.0	procollagen-lysine, 2-oxoglutarate 5-dioxygenase(lysine hydroxylase)2	NM_000935
TSG-6	2.0	hyaluronate-binding protein	NM_007115
Protein transporter			
ARF1	2.0	ADP-ribosylation factor 1	NM_000710
Apoptosis related			
GBP	2.0	lectin, galactoside-binding, soluble, 1, galectin-1	BC001693
Protease inhibitor			
CAST	2.0	calpastatin	NM_00175
Down-regulated gene			
Signal transduction			
INP4A	3.9	inositol polyphosphate-4-phosphatase type 1, isoform B	NM_001566
HSPC121	2.6	butyrate-induced transcript 1	NM_016395
Cytokine/growth factor			
BDNF	2.0	brain-derived neutrophic factor	NM_001709
Transcription factor			
ZFP2	3.5	zinc finger protein 2	AF067164
Metabolism related			
ETF1	2.0	eukaryotic translation termination factor 1	NM_004730
Transporter			
DBI	2.0	diazepam binding inhibitor	NM_020548
NBC2B	2.0	sodium bicarbonate cotransporter 2b	AF089726
Cell adhesion related			
PCDH13	2.0	protocadherin 13	F169693

Each gene is represented with a common name and a differential expression ratio. A brief description has also been added for each gene to indicate possible functions.

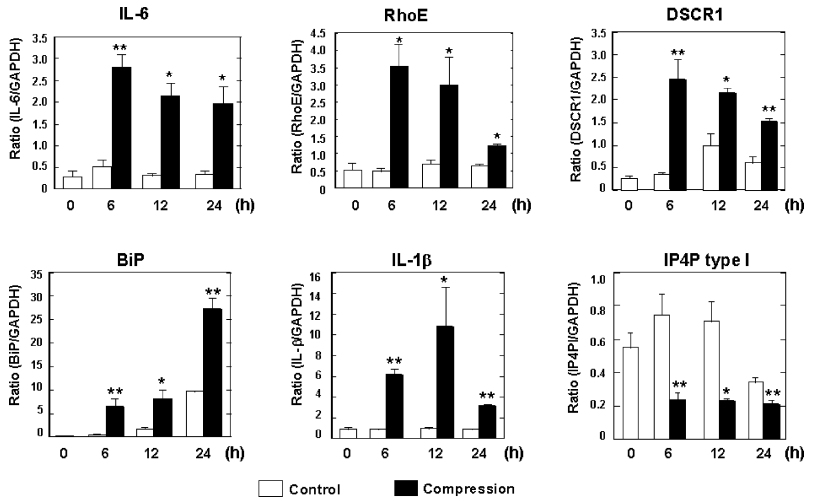

Fig. 3. Confirmation of differentially expressed genes observed in the microarray results. The expression level of six genes [interleukin (IL)-6, G-protein (RhoE), Down syndrome critical region protein 1 (DSCR1), stress-related protein (BiP), IL-1β and inositol polyphosphate-4-phosphatase type I (IP4P1)], selected from the array results, was quantitatively examined by real-time polymerase chain reaction (PCR) analysis. Real-time PCR analyses were performed using the RNAs obtained from periodontal ligament (PDL) cells stimulated by compressive force (6.0 g/cm^2) for 6, 12 and 24 h. Results represent the mean ± standard deviation (SD) of quadruplicate determinations for a typical experiment. (*$p < 0.05$, **$p < 0.01$). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

F-actin filaments, and consequently play a crucial role in biological processes involving the cytoskeleton, such as the control of cell shape and mobility (30,31). Like other small GTPases, Rho family members serve as molecular switches that cycle between an active GTP-bound state and an inactive GDP-bound state. The hydrolysis of GTP to GDP is catalyzed by the RGS protein family, such as RGS2 (32), which was expressed at a 2.0-fold ratio in our microarray data. Once activated, they bind to and activate many downstream effectors. RhoE can inhibit cell proliferation and transformation in addition to its known effects on the actin cytoskeleton and can contribute to the balanced co-ordination of cell proliferation and migration (30,31). These results demonstrate that RhoE affects stress fiber dynamics in response to mechanical stress. DSCR1 (33,34) was identified from a chromosomal region (21q22.1–q22.2) associated with the clinical phenotypic features of Down syndrome. DSCR1 functions as a small cytoplasmic signaling molecule, regulated by calcineurin A, and plays a crucial role during the cellular response to various extracellular signals and stresses, and is important in the regulation of apoptosis, and skeletal and cardiac muscle growth and differentiation (34). Recent studies (35,36) have revealed that DSCR1 is a novel vascular endothelial growth factor (VEGF) target gene that regulates the expression of inflammatory markers, such as COX-2, PGE_2 and thromboxane, on activated endothelial cells in angiogenesis. As a depressed gene in the microarray data, IP4P type I was observed. IP4P type I has recently been identified and implicated in the regulation of phosphoinositol 3-kinase (PI3K) signaling in human platelets (37). IP4P types I and II are encoded by different genes and have 37% amino acid identity (38). These second messengers are involved in the regulation of numerous cellular events, including growth, differentiation, vesicular sorting and signal transduction mediating the small G protein (39). The inhibitory mechanisms of this PI3K signaling molecule in the PDL cells under mechanical stress have yet to be clarified.

We also observed that genes encoding transcription factors, stress-related proteins, cytoskeleton-related proteins and extracellular matrix-related proteins tended to be highly expressed in compressed PDL cells. TSC-22 is a transcription factor which is a direct target of TGF-β. TSC-22 is detected in the mesenchymal component of many tissues and organs, including the tooth buds to be regulated by TGF-β (40). However, the regulatory mechanisms of TSC-22 in PDL cells have not been identified. It has been demonstrated that TGF-β stimulates the expression of periostin (41), which is preferentially expressed in the periosteum and PDL, and is thought to be one of the mechanical stress mediators in PDL cells during tooth movement in rats (42). BiP is an endoplasmic reticulum (ER)-resident molecular chaperone that plays a role in the protection against cytotoxity and apoptosis induced by an environmental attack and stress (43,44). Hori *et al*. (45) reported that BiP is implicated in the secretion of the cytokine, interleukin-6, from astrocytes. Dystrophin is a protein of the membrane cytoskeleton that associates with a complex of integral and membrane-associated proteins, such as dystroglycans (46). The loss of dystrophin from skeletal muscle leads to the clinical features of Duchenne muscular dystrophy (47). In muscle, dystrophin is thought to participate in maintaining the stability of the membrane during repeated cycles of contraction and relaxation of muscle fiber by binding to actin filaments. It has been reported that short C-terminal dystrophin isoforms, ranging in size from 71 to 260 kDa, are found in many different tissues (48,49). However, little is known about the functions of dystrophin or dystrophin isoforms in non-muscle tissues, including PDL. Collagens fibrils mediate mechanical forces between teeth and the surrounding tissues and cells. In addition, collagens play an important role in cell adhesion and migration (50). Procollagen-lysine, 2-oxogulutarate, 5-dioxygenase 2 (PLOD2) is a lysyl hydroxylase gene which catalyses the hydroxylation of lysine residues during the post-translational modification of collagenous proteins (51,52). The

hydroxylation of specific peptidyl lysine residues is a modification critical for glycosylation and cross-linking of the collagen molecule. Maturation of procollagen also requires the hydroxylation of lysine residues mediated by PLOD. The turnover rate of collagen in PDL under mechanical stress may become faster, indicating a need for rapid remodeling for changing functional demands (53). We hypothesize that the induction of PLOD2 in PDL cells under mechanical stress may promote collagen cross-linking, eventually the accumulation of extracellular matrix. TSG-6 is a 35-kDa-secreted glycoprotein that binds to hyaluronan and is up-regulated by various cytokines, including interleukin-1β and TNF-α (54). It is strongly believed that TSG-6 may play a crucial role in extracellular matrix remodeling, inflammatory cell migration and developmental processes (55). Microarray analysis by Han *et al.* (16) showed that the constitutive expression of TSG-6 mRNA was 5.3-fold higher in human gingival fibroblasts than in PDL fibroblasts, suggesting that gingival and PDL fibroblasts display distinct activities during the maintenance of tissue integrity and in an inflammatory response as a result of TSG-6. Similarly, in the PDL cells under compressive forces, up-regulated TSG-6 may affect the regulation of cell activities. Thus, our microarray results provided many interesting molecules that should be worth focusing on in the research on the regulatory mechanisms in PDL cells.

In summary, our microarray study is the first to identify the profiles of genes using the *in vitro* three-dimensional culture system, possibly indicating involvement in the cellular response to mechanical stress in the PDL tissues. Novel and interesting genes related to mechanical stress (in many of which the function is unclear) were differentially expressed. Further studies on their physiological roles are necessary and may contribute to clarifying the molecular function of periodontal ligament tissue and the remodeling mechanisms of periodontal tissues in response to mechanical stress.

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