International Journal of Oral Biology, Vol. 34, No. 2, June 30 2009, p. 53~59
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International Journal of Oral Biology

Cyclic tensile stress inhibits Wnt/β-catenin signaling in human periodontal ligament cells

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(received March 27, 2009 ; revised April 10, 2009 ; accepted April 14, 2009)

Periodontal ligament (PDL) tissue is a connective tissue that is interposed between the roots of the teeth and the inner wall of the alveolar bone socket. PDL is always exposed to physiologic mechanical force such as masticatory force and PDL cells play important roles during orthodontic tooth movement by synthesizing and secreting different mediators involved in bone remodeling. The Wnt/β-catenin signaling pathway was recently shown to play a significant role in the control of bone formation. In the present study, we applied cyclic tensile stress of 20% elongation to cultured human PDL cells and assessed its impact after six days upon components of the Wnt/β-catenin signaling pathway. RT-PCR analysis showed that Wnt1a, Wnt3a, Wnt10b and the Wnt receptor LRP5 were down-regulated, whereas the Wnt inhibitor DKK1 was up-regulated in response to these stress conditions. In contrast, little change was detected in the mRNA expression of Wnt5a, Wnt7b, Fz1, and LRP6. By western blotting we found decreased expression of the β-catenin and p-GSK-3β proteins. Our results thus show that mechanical stress suppresses the canonical Wnt/β-catenin signaling pathway in PDL cells.

Key words : Cyclic tensile stress; Human periodontal ligament cell; Wnt; β-catenin

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Introduction

Periodontal ligament (PDL) is a specialized connective tissue located between the cementum covering the root of the tooth and the alveolar bone, both of which are hard tissue. PDL serves to anchor the tooth to the alveolus and functions as a cushion to disperse masticatory and orthodontic forces (Lekic et al., 1996). During the movement of teeth in the course of orthodontic treatment, it is generally agreed that bone resorption on the compression side and bone formation on the tension side change the position of the tooth within the alveolar bone. Mechanical stress from orthodontic appliances is considered to induce cells in the PDL to form biological mediators, such as cytokines and enzymes, which are responsible for remodeling of the connective tissue and bone (Davidovitch et al., 1988).

When the PDL is stretched, several cellular processes are apparently activated, along with an increase in the number of connective tissue cells. This initial phase is followed by deposition of osteoid tissue at the edge of the socket wall. PDL cells have osteoblastic properties, such as high alkaline phosphatase (ALP), and can express osteocalcin and form mineral-like nodules (Basdra et al., 1997). In response to the substance P known to be secreted during orthodontic tooth movement, PDL cells expressed the mineralization markers including ALP, osteonectin, and bone sialoprotein (Cho YM et al., 2008). In addition, it has been reported that cyclic tensile forces stimulate PDL cells and regulate the production of cytokines and chemical mediators such as interleukin-1, prostaglandin E_2 (Saito et al., 1991; Yamaguchi et al., 1994), and transforming growth factor-β (Bellows et al., 1982). Application of a tensile stress to human PDL cells

in vitro can up-regulate osteoblastic differentiation (Basdra et al., 1997) and the genes linked to the osteoblast phenotype, which include *BMP2*, *BMP6*, *ALP*, *SOX9*, *MSX1*, and *VEGF* (Wescott et al., 2007). These genes act in an autocrine/paracrine fashion to regulate the differentiation, proliferation, and function of osteoblasts, osteoclasts, mesenchymal cells, and other cell types. Recent studies have concluded that the PDL might be both the medium of force transfer (Choi et al., 2008) and the means by which alveolar bone remodels in response to applied forces (Verna et al., 2004), but the details of the gene expression pattern caused by mechanical stress are still unclear. During the bone formation that occurs in the alveolar bone on the tension side of orthodontic tooth movement, the PDL preserves a thin space and remains unmineralized under physiologic conditions. This function of the PDL is a remarkable process involving precisely controlled osteogenic resorption and deposition in the paradental tissues.

One molecular pathway that has recently been implicated as an important inducer of bone formation is the Wnt/β-catenin signaling system. Wnts are a family of 19 secreted cysteine-rich glycoproteins that have been extensively studied in cancer and development, including embryogenesis and organogenesis (Cadigan et al., 1997). Wnt/β-catenin signaling is initiated when Wnt proteins bind to members of 2 distinct families of cell surface co-receptors, Frizzled (Fz) and either low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) or LRP6. Activation of these co-receptors ultimately leads to inhibition of glycogen synthase kinase (GSK)-3β and subsequent stabilization and accumulation of β-catenin in the nucleus. Once in the nucleus, β-catenin combines with a member of the lymphoid enhancing factor (LEF)/T-cell factor (TCF) family of transcription factors at specific DNA binding sites to activate Wnt-responsive genes (Wodarz et al., 1998). The Dickkopfs (DKKs) are secreted inhibitors of the Wnt/β-catenin signaling pathway that interfere with Wnt/β-catenin signaling by binding directly to LRP5/6 receptor.

Wnt/β-catenin signaling is active in several cell types, including chondrocytes, osteoblasts, and osteocytes (Chun et al., 2008; Bodine et al., 2006; Bonewald et al., 2008). Mechanical loading of bone initiates an anabolic response, and Wnt/β-catenin signaling induced by application of strain was recently recognized to promote bone anabolism (Armstrong et al., 2007; Norvell et al., 2004). The available evidence therefore suggests that the Wnt/β-catenin signaling pathway makes an important contribution to the adaptive responses of bone cells to mechanical stimulation. Considerable progress has been made in terms of our understanding of the role of Wnt/β-catenin signaling in osteogenesis, particularly in osteoblast biology. However, little is known about Wnt signaling in PDL cells under mechanical stress. In the present study, we investigated a possible involvement of the Wnt/β-catenin signaling pathway in human PDL cells in response to cyclic tensile stress.

Materials and Methods

Preparation of primary human PDL cells

Healthy human premolars extracted for orthodontic reasons were obtained with informed consent, and this study was approved by the Institutional Review Board of the Seoul National University Dental Hospital. After the teeth were extracted, the middle third of a root surface of the tooth was scraped with a surgical scalpel to exclude contamination from the gingiva and dental pulp. PDL explants were treated with 0.2% collagenase (Wako, Osaka, Japan) in α-minimal essential medium (α-MEM; Welgene, Daegu, Korea) and were incubated for 80 minutes at 37°C to digest the collagen matrix. Then the cells were cultured in α-MEM containing 20% fetal bovine serum (FBS) supplemented with five-fold-reinforced antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). After the first passage, PDL cells were cultured in 10% FBS α-MEM supplemented with 1X antibiotics at 37°C in a 5% CO_2 incubator. The medium was changed every 3 days throughout the experiment. The PDL cells used in these experiments underwent 2 to 3 passages.

Application of cyclic tensile stress to PDL cells

Cyclical tensile force was applied to PDL cells with a Flexercell FX-4000 Strain-Unit (Flexcell Corporation, Hillsborough, NC, USA). Human PDL cells were seeded at a density of 2.5×10^5 cells/well in 6-well, 35-mm flexible-bottomed culture plates and were pre-cultured for 1 day. Two plates (1 experimental and 1 control) were allocated to each of the 4 time intervals (6 hours, 1 day, 3 days, and 6 days). PDL cells were subjected to cyclic tensile stress (20% elongation, 5 sec elongation and 5 sec relaxation). Control cells were cultured under identical conditions but remained static. The experimental and control cells were then subjected to RNA and protein extraction.

Reverse transcription polymerase chain reaction

The mRNA expression of Wnt1, Wnt3a, Wnt5a, Wnt7b, Wnt10b, DKK1, LRP5, LRP6, β-catenin, GSK3β, ALP, and β-actin in PDL cells was analyzed by reverse transcription polymerase chain reaction (RT-PCR). Cultured cells were rinsed twice with phosphate-buffered saline (PBS). Total RNA was extracted from each culture with Trizol reagent (Invitrogen, Carlsbad, CA, USA) and was reverse-transcribed by using a Superscript II reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. First-strand complementary DNA (cDNA) was subjected to PCR amplification with gene-specific primers (Table 1). Each cycle consisted of a heat denaturation step at 94°C for 30 seconds, an annealing step at a temperature optimized for each primer pair for 30 seconds, and an extension step at 72°C for 60 seconds. The amplified cDNA products were electrophoresed on a 1% agarose gel containing ethidium bromide and were detected under ultraviolet light. The radioactive bands were quantified by

Table 1. Primers used for RT-PCR

Target gene	Forward (5´-3´)	Reverse (5´-3´)	Product size (bp)	Annealing temperature (°C)
Wnt 1	FTCGCCCAACTTCTGCACGTA	TGGTTTGAGGAGTCCCC AGG	405	62
Wnt3a	GCTGCGAGGTGAAGACATGC	TACTGCCCCGTTTAGGTGGG	521	58
Wnt5a	GCCTGAAGACATGCTGGCTG	TGGCACCCACTACTTGCACA	410	58
Wnt7b	CTTCCTGCGCATCAAACAGC	CGCTTCTGCACCCGTCTATG	417	60
Wnt10b	CGCCTCTCAGGAGAGCTGGT	CCCTCCAATTGTTGGGGAGA	457	58
DKK1	CTTTCTCCCTCTTGAGTCCT	CTTTCAGTGATGGTTTCCTC	496	58
LRP5	GTACAGGCCCTACATCATTC	CTATTTACAGGGGCACAGAG	311	58
LRP6	TTCTCATCACCTCTACCCAC	ATATTCCTGGTCAGTGATGC	403	58
β-catenin	TGATGGAGTTGGACATGGCCATGG	CAGACACCATCTGAGGAGAACGCA	570	58
GSK3β	GACGCTCCCTGTGATTTATGT	TAGCCAGAGGTGGATTACTTG	710	58
ALP	CATGGTGGACTATGCTCACA	AGACTTTGGTTTCCTGGGTC	418	58
β-actin	ATGAGGATCCTCACCGAGCGCGGCTACAGC	ACACCACTGTGTTGGCGTACAGGTCTTTGC	327	58

using Image J 1.41o software (National Institutes of Health, Bethesda, MD, USA), and mRNA expression relative to β-actin mRNA expression was analyzed. The band intensity of the mRNA expression of the control at 6 h was assigned a value of 1.00.

Western blot analysis

To obtain whole cell extracts, PDL cells were rinsed twice with ice-cold PBS and were then lysed in 100 μL of lysis buffer containing 20 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, and protease and phosphatase inhibitors. A total of 40 μg of the cellular protein of each sample was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and was then transferred to a polyvinylidene difluoride membrane (Amersham Bioscience, Piscataway, NJ, USA). The membrane was blocked with 5% skim milk at room temperature for 2 h and was then incubated at 4°C overnight with appropriate primary antibodies followed by incubation at room temperature for 2 h with horseradish peroxidase (HRP)-conjugated secondary antibodies. The immunoreactive bands were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Buckinghamshire, UK). The changes in β-catenin, phospho-GSK-3β, and GSK-3β protein expression were quantified by densitometry as described above.

Results

Effect of cyclic tensile stress on the mRNA expression of Wnts and Wnt inhibitor DKK1

Because Wnt1, Wnt 3a, Wnt5a, Wnt7b, and Wnt10b have been reported to induce ALP activity or osteoblastogenesis (Hartmann, 2006; Liu et al., 2008), we selected these proteins for study and examined their mRNA levels after cyclic tensile stress by RT-PCR. The mRNA expression of Wnt1, Wnt3a, and Wnt10b increased at 1 day but decreased at 3 days and 6 days in response to cyclic tensile stress. By contrast, little change was detected in the mRNA expression of Wnt5a or Wnt7b at 3 days or 6 days in response to cyclic tensile stress (Fig. 1A). The mRNA expression of the inhibitor DKK1 decreased with time in the unstressed control condition but was up-regulated in response to cyclic tensile stress (Fig. 1B).

Effect of cyclic tensile stress on the mRNA expression of the cell membrane receptors of Wnt proteins

The mRNA expression of the receptors Fz1, LRP5, and LRP6 was analyzed by RT-PCR. The mRNA expression of Fz1 was not clear, nor reproducible. Whereas the mRNA expression of LRP6 did not change, the mRNA expression of LRP5 decreased during the culture period in response to cyclic tensile stress (Fig. 2).

Effect of cyclic tensile stress on the mRNA and protein expression of β-catenin and GSK-3β

Binding of the Wnt proteins to one of the Fz receptors inactivates GSK-3β and therefore prevents the phosphorylation and consecutive degradation of β-catenin. When GSK-3β is phosphorylated, it is inactivated and does not phosphorylate β-catenin, thereby resulting in a cytoplasmic pool of unphosphorylated β-catenin that translocates to the nucleus. The mRNA expression of cytoplasmic proteins including β-catenin and GSK-3β was determined by RT-PCR (Fig. 3A). No significant change in association with cyclic tensile stress was detected in the mRNA expression of β-catenin or GSK-3β.

The protein expression of cytoplasmic proteins including β-catenin, phopho-GSK-3β, and GSK-3β was analyzed by Western blot (Fig. 3B). β-Catenin protein decreased at 3

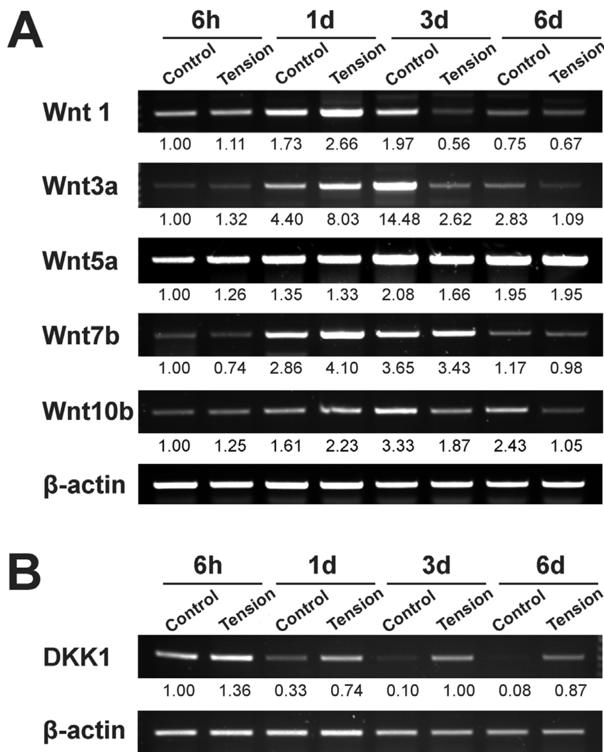

Fig. 1. Cyclic tensile stress of 20% elongation was applied to human periodontal ligament cells for up to 6 days, and the mRNA expression of Wnt1, Wnt3a, Wnt5a, Wnt7b, Wnt10b, and DKK1 was analyzed by RT-PCR. The band intensity of mRNA expression relative to β-actin expression in the control condition at 6 hours was assigned a value of 1.00. (A) At 3 days and 6 days, the mRNA expression of Wnt1, Wnt3a, and Wnt10b decreased in response to cyclic tensile force, whereas the mRNA expression of Wnt5a was unchanged. (B) The mRNA expression of the Wnt inhibitor DKK1 decreased with time in the control condition but was up-regulated in response to cyclic tensile stress.

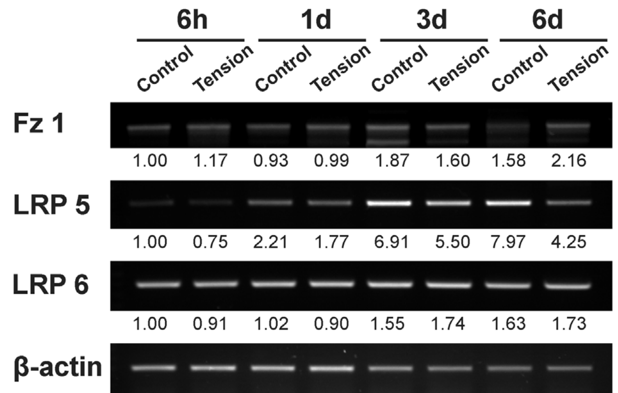

Fig. 2. Expression of cell membrane receptor mRNAs analyzed by RT-PCR in human periodontal ligament cells under cyclic tensile stress. Whereas the mRNA expression of LRP6 was little changed, the mRNA expression of LRP5 decreased during the culture period in response to cyclic tensile stress.

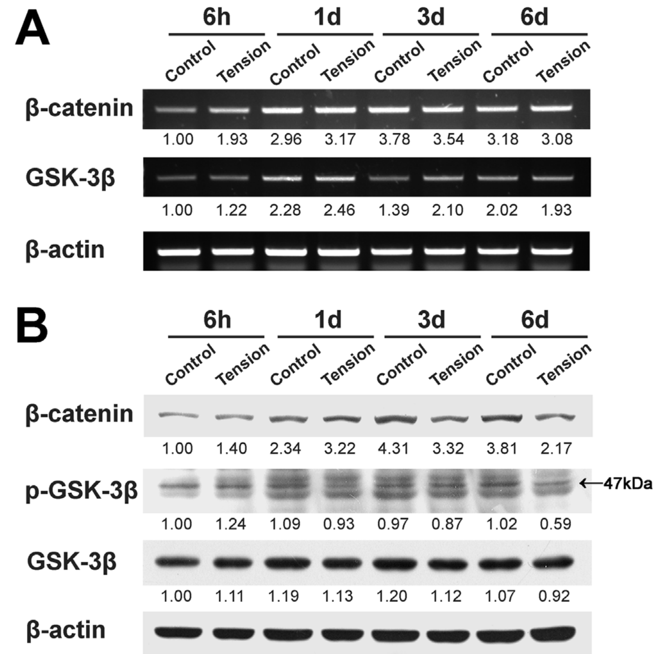

Fig. 3. Effect of cyclic tensile stress on the expression of the cytoplasmic proteins implicated in the Wnt/β-catenin signaling pathway in human periodontal ligament cells cultured up to 6 days. (A) The mRNA expression of β-catenin and GSK-3β was determined by RT-PCR. No significant changes in association with cyclic tensile stress were detected. (B) The expression of β-catenin, phopho-GSK-3β, and GSK-3β were analyzed by Western blot. The expression of β-actin is shown as a control for equal protein loading. β-Catenin protein decreased at 3 days and 6 days, and phospho-GSK-3β protein decreased at 6 days in response to the cyclic tensile stress.

days and 6 days and phospho-GSK-3β protein decreased at 6 days in response to the cyclic tensile stress.

Effect of cyclic tensile stress on the mRNA expression of alkaline phosphatase

Because it is known that β-catenin can induce ALP activity and enhance osteoblastogenesis (Liu et al., 2008) and we found that β-catenin protein decreased in response to cyclic tensile stress in this experiment, we investigated the changes in ALP mRNA expression in response to tensile stress with time. RT-PCR showed that cyclic tensile stress down-regulated the expression of ALP mRNA at 3 days and 6 days (Fig. 4).

Discussion

Healthy PDL is loaded with cyclical tensile force during occlusion, which maintains alveolar bone homeostasis. Mechanical force plays a major role during tooth movement by inducing the synthesis and secretion of different mediators involved in bone remodeling (Davidovitch et al., 1988). Recently, it was shown that PDL tissue possesses multipotential mesenchymal cells that can differentiate into mineralized tissue-forming cells such as osteoblasts and

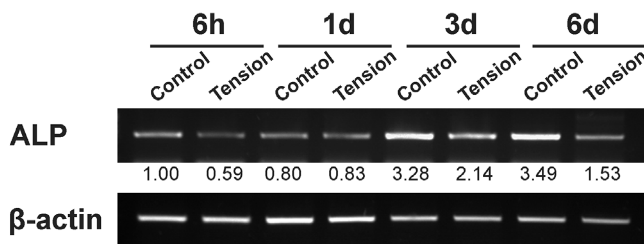

Fig. 4. Effect of cyclic tensile stress on the expression of alkaline phosphatase (ALP) mRNA in periodontal ligament cells as analyzed by RT-PCR. ALP mRNA expression was down-regulated in response to cyclic tensile stress at 3 days and 6 days.

cementoblasts (Bartold et al., 2006; Seo et al., 2004). In fact, in vitro cultured PDL cells have various osteoblast-like properties, including the capacity to form mineralized nodules, expression of bone-associated markers, and response to bone-inductive factors such as bone morphogenic protein 2 (BMP2) (Cho et al., 1992; Shirai et al., 2009). Several genes involved in osteoblast differentiation and function, including *BMP2*, *BMP6*, *MSX1*, and *VEGF*, are up-regulated by mechanical deformation of PDL cells (Wescott et al., 2007). Interestingly, however, despite the ability of BMP to induce ectopic bone formation, PDL tissue is never ossified *in vivo* by BMP under normal circumstances. This suggests that some mechanisms exist to constitutively prevent the PDL from developing unorchestrated osteogenesis such as ankylosis. However, the regulatory mechanisms that control osteoblastic differentiation in PDL are not fully understood.

The Wnt signaling pathway plays an important role in the regulation of osteogenesis in the skeleton. In adults, Wnts function in homeostasis, and inappropriate activation of the Wnt pathway is implicated in various cancers (Miller, 2001). Reception and transduction of Wnt signals involves binding of Wnt proteins to cell-surface receptors. Wnt signals are relayed through several different mechanisms, but the canonical β-catenin-dependent Wnt pathway is the best described (Miller et al., 1999). Wnts also activate noncanonical pathways that are independent of β-catenin; for example, the Wnt5a class is known as a noncanonical Wnt (Bodine et al., 2006). In this study, we investigated the mRNA level of Wnt1, Wnt3a, Wnt5a, Wnt7b, and Wnt10b, all of which have been reported to induce ALP activity or osteoblastogenesis (Hartmann, 2006; Liu et al., 2008). All of the Wnts we studied were expressed by PDL cells. The mRNA expression of Wnt1, Wnt3a, and Wnt10b decreased in response to cyclic tensile stress, whereas the mRNA expression of the noncanonical Wnt5a was not affected. This finding suggests that tensile stress is not related to the noncanonical Wnt signaling pathway.

Among the several inhibitors of the Wnt signaling pathway, DKKs only suppress canonical signaling by binding directly to the LRP5/6 receptor (Kawano et al., 2003). We found that DKK1 increased in response to cyclic tensile stress, which suggests that up-regulation of these genes could create a suppressive environment in which the Wnt proteins that might be present are not activated by cyclic tensile stress. In the presence of DKK1, the cascade is disrupted, resulting in inhibition of osteogenesis. In light of recent work showing that Wnt/β-catenin signaling drives the differentiation of progenitor cell lines into osteoblasts (Bain et al., 2003) and that exogenous DKK1 can inhibit BMP-2 induced ALP activity (Tian et al., 2003), it seems reasonable that the increase in DKK1 in response to cyclic tensile stress may result in suppressing the Wnt/β-catenin signaling pathway, which in turn would decrease the level of ALP mRNA. In the present study, Western blotting of cytoplasmic proteins confirmed that cyclic tensile stress was associated with a decreased level of β-catenin and that the changes in the level of β-catenin were parallel to the inhibitory phosphorylation of GSK3β in PDL cells. β-Catenin is the molecular node of the canonical Wnt signaling pathway. Interestingly, these observations are consistent with the decreases in Wnt1, Wnt3a, and Wnt10b; the lack of decrease in the noncanonical Wnt5a; the decrease in the Wnt receptor LRP5; and the increase in the Wnt inhibitor DKK1 in response to cyclic tensile stress. Thus, our results suggest that tensile stress inhibits the Wnt/β-catenin signaling pathway.

It is generally accepted that ALP is involved in the process of calcification in various mineralizing tissues and is examined as marker of osteoblast differentiation. PDL has been shown to have intense ALP activity (Somerman et al., 1988). Concerning the relationship between tensile stress and ALP activity, cyclic tension force decreased the ALP activity of PDL cells in a manner that was both time- and magnitude-dependent (Yamaguchi et al., 1996). It has been reported that PDL cells produce prostaglandin E_2 and interleukin-1β in response to cyclic tension force, which decrease ALP activity in PDL cells (Yamaguchi et al., 1994). In other reports using real-time PCR microarray technology, however, ALP gene expression was up-regulated by mechanical deformation in PDL cells (Wescott et al., 2007). Here, we observed that when cyclic tensile force was applied to PDL cells, the level of ALP mRNA decreased in a pattern similar to the tensile stress-dependent decrease in β-catenin. These results suggest that Wnt/β-catenin signaling is mechano-sensitive and mediates the transmission of mechanical stimuli. Down-regulation of the Wnt/β-catenin signaling pathway in the PDL cells in response to cyclic tensile stress may be related to the fact that PDL is not calcified despite the tensile force applied during orthodontic treatment or in the physiologic condition.

Concerning the relationship between the mechanical stress and the response of osteoblasts, it was reported that mechanical strain in osteoblastic cells increases the level of active β-catenin, stimulates its accumulation in the nucleus, and increases the TCF/LEF transcriptional activity of the osteopontin promoter (Armstrong et al., 2007). Fluid shear

stress induces translocation of β-catenin and increases signaling through the TCF transcriptional pathway (Norvell et al., 2004). Under strained conditions, PDL cells can differentiate into osteoblasts (Basdra et al., 1997). Therefore, PDL cells were regarded as being "osteoblastic fibroblasts" (Yamaguchi et al., 1996). Our results, however, suggest that PDL cells express behaviors different from those of osteoblasts in response to mechanical stress. To date, most of the data point to a role of PDL cells in the bone formation on the tension side of tooth movement in the course of orthodontic treatment. It is nevertheless important to know how the PDL is not ossified despite osteogenic mediators. Whereas the Wnt ligands that might affect the Wnt signaling pathway of the other cell types by paracrine action were increased at 1 day or decreased at 3 days and 6 days, the receptor LRP5 that modulate the Wnt signaling pathway of the PDL cells showed tension-dependent decreases consistently during the culture period. The decrease of LRP5 mRNA, rather than the change of Wnts mRNA, could be related with the decrease of Wnt signaling or ALP mRNA expression of PDL cells in response to cyclic tensile stress. The underlying mechanism that is responsible for the inhibition of the osteogenic differentiation of PDL itself was not previously understood but might be related to the Wnt/β-catenin signaling pathway as shown in this study. There are large numbers of molecules involved in Wnt/β-catenin signaling pathway, only a small part of which was investigated in the present study. Further studies are needed to investigate the molecular mechanism and the role of Wnt/β-catenin signaling in PDL cells and to determine the relationship between ALP activity and the Wnt/β-catenin signaling pathway in PDL cells under mechanical stress.

In conclusion, cyclic tensile stress suppresses the Wnt/β-catenin signaling pathway in human PDL cells, probably by down-regulating Wnt1, Wnt3a, Wnt10b, and LRP5 and up-regulating DKK1. Our findings suggest that mechanical stress such as occlusal and orthodontic tensile force may modulate osteoblastogenesis in PDL cells via the Wnt/β-catenin signaling pathway.

Acknowledgements

This work was supported by a grant from the Center for Biological Modulators of the 21st Century Frontier R&D Program, and of the Korea Science and Engineering Foundation (KOSEF) Science Research Center through Bone Metabolism Research Center, the Korean Ministry of Education, Science and Technology.

References

Armstrong VJ, Muzylak M, Sunters A, Zaman G, Saxon LK, Price JS, Lanyon LE. Wnt/beta-catenin 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.

Bain G, Muller T, Wang X, Papkoff J. Activated beta-catenin induces osteoblast differentiation of C3H10T1/2 cells and participates in BMP2 mediated signal transduction. Biochem Biophys Res Commun. 2003;301:84-91.

Bartold PM, Shi S, Gronthos S. Stem cells in the periodontal ligament. Periodontol. 2000 2006;40:164-72.

Basdra EK, Komposch G. Osteoblast-like properties of human periodontal ligament cells: an in vitro analysis. Eur J Orthod. 1997;19:615-21.

Bellows CG, Melcher AH, Aubin JE. Association between tension and orientation of periodontal ligament fibroblasts and exogenous collagen fibers in collagen gels in vitro. J Cell Sci. 1982;58:125-38.

Bodine PN, Komm BS. Wnt signaling and osteoblastogenesis. Rev Endocr Metab Disord. 2006;7:33-9.

Bonewald LF, Johnson ML. Osteocytes, mechanosensing and Wnt signaling. Bone. 2008;42:606-15.

Cadigan KM, Nusse R. Wnt signaling: A common theme in animal development. Genes Dev. 1997;11:3286-305.

Cho MI, Lin WL, Moshier A, Ramakrishnan PR. In vitro formation of mineralized nodules by periodontal ligament cells from the rat. Calcif Tissue Int. 1992;50:459-67.

Cho YM, Suh CH, Chun SW, Kim EC, Kang KH. Effects of substance P on mineralization markers and heme oxygenase-1 expression in human immortalized periodontal ligament cells. Int J Oral Biol. 2008;33:131-5.

Choi YS, Jang HS, Lee DS, Kim HJ, Park JT, Bae HS, Park JC. The effecto of *UNCL* inactivation on the expression of mechanical stress related genes in cultured human PDL fibroblasts. Int J Oral Biol. 2008;33:51-8.

Chun JS, Oh H, Yang S, Park M. Wnt signaling in cartilage development and degeneration. BMB Rep. 2008;41:485-94.

Davidovitch Z, Nicolay OF, Ngan PW, Shanfeld JL. Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. Dent Clin North Am. 1988; 32:411-35.

Hartmann C. A Wnt canon orchestrating osteoblastogenesis. Trends Cell Biol. 2006;16:151-8.

Kawano Y, Kypta R. Secreted antagonists of the Wnt signaling pathway. J. Cell Sci. 2003;116:2627-34.

Lekic P, McCulloch CA. Periodontal ligament cell population: the central role of fibroblasts in creating a unique tissue. Anat Rec. 1996;245:327-41.

Liu F, Kohlmeier S, Wang CY. Wnt signaling and skeletal development. Cell Signal 2008;20:999-1009.

Miller JR. The Wnts. Genome Biol. 2002;3:3001.1-15.

Miller JR, Hocking AM, Brown JD, Moon RT. Mechanism and function of signal transduction by the Wnt/beta-catenin and Wnt/Ca2+ pathways. Oncogene. 1999;18:7860-72.

Norvell SM, Alvarez M, Bidwell JP, Pavalko FM. Fluid shear stress induces beta-catenin signaling in osteoblast. Calcif Tissue Int. 2004;75:396-404.

Saito M, Saito S, Ngan PW, Shanfeld J, Davidovictch Z. Interleukine-1 beta and prostaglandin E are involved in the response of periodontal cells to mechanical stress in vivo

and in vitro. Am J Orthod Dent Orthop. 1991;99:226-40.
Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 2004;364:149-55.
Shirai K, Ishisaki A, Kaku T, Tamura M, Furuichi Y. Multipotency of clonal cells derived from swine periodontal ligament and differential regulation by fibroblast growth factor and bone morphogenic protein. J Periodont Res. 2009;44:238-47.
Somerman MJ, Archer SY, Imm GR, Foster RA. A comparative study of human periodontal ligament cells and gingival fibroblasts in vitro. J Dent Res. 1988;67:66-70.
Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy Jr JD. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med. 2003;349:2483-94.
Verna C, Dlastra M, Lee TC, Cattaneo PM, Melsen B. Microcracks in the alveolar bone following orthodontic tooth movement: a morphological and morphometric study. Eur J Orthod. 2004;26:459-67.
Wescott DC, Pinkerton MN, Gaffery BJ, Beggs KT, Milne TJ, Meikle MC. Osteogenic gene expression by human periodontal ligament cells under cyclic tension. J Dent Res. 2007; 86:1212-6.
Wodarz A, Nusse R. Mechanisms of Wnt signaling in developments. Annu Rev Cell Dev Biol. 1998;14:59-88.
Yamaguchi M, Shimizu N, Goseki T, Shibata Y, Takiguchi H, Iwasawa T, Abiko Y. Effect of different magnitudes of tension force on prostaglandin E_2 production by human periodontal ligament cells. Arch Oral Biol. 1994;39:877-84.
Yamaguchi M, Shimizu N, Shibata Y, Abiko Y. Effects of different magnitudes of tension-force on alkaline phosphatase activity in periodontal ligament cells. J Dent Res. 1996;75: 889-94.