Involvement of JNK-AP-1 and ERK-NF-κB signaling in tension-stimulated expression of Type I collagen and MMP-1 in human periodontal ligament fibroblasts

Sung-Ho Kook,1 Yong-Suk Jang,2 and Jeong-Chae Lee3,3

1Division of Hematology and Oncology, Department of Medicine, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania; 2Research Center of Bioactive Materials, Chonbuk National University; 3Cluster for Craniofacial Development and Regeneration Research, Institute of Oral Biosciences and School of Dentistry (BK21 Program), Chonbuk National University, Jeonju, South Korea

Submitted 22 March 2011; accepted in final form 12 July 2011

Kook SH, Jang YS, Lee JC. Involvement of JNK-AP-1 and ERK-NF-κB signaling in tension-stimulated expression of Type I collagen and MMP-1 in human periodontal ligament fibroblasts. J Appl Physiol 111: 1575–1583, 2011. First published July 14, 2011; doi:10.1152/japplphysiol.00348.2011.—Type I collagen (COL I) and matrix metalloproteinase-1 (MMP-1) are the predominant matrix proteins in the extracellular matrix of the human periodontal ligament (PDL). The expression of these proteins in PDL fibroblasts (PLF) is sensitive to physiological and mechanical stress and is critical for PDL remodeling accompanied by alveolar bone remodeling. This study examined how dose tensile force regulates the expression of COL I and MMP-1 and explored the possible roles of mitogen-activated protein kinases (MAPKs) and transcription factors, such as activator protein-1 (AP-1) and nuclear factor-κB (NF-κB). Tensile force stimulated the mRNA expression of COL I and MMP-1 in the cells and also activated MAPKs including extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38 MAPK. A pharmacological inhibitor of ERK or JNK prevented the expression of matrix genes and the nuclear translocation of c-Jun proteins in the force-applied PLF. The knockdown of c-Jun by transfecting the cells with its antisense oligonucleotides reduced the force-induced increase in matrix gene expression. In particular, the ERK inhibitor but not JNK or p38 MAPK inhibitor attenuated the force-mediated stimulation of NF-κB-DNA binding and MMP-1 expression. Overall, these results highlight the mechanotransduction pathways involved in matrix gene expression in PLF, where the tension-stimulated expression of COL I and MMP-1 is controlled by the ERK/JNK-AP-1 and ERK-NF-κB signaling pathways.

The collagenous extracellular matrix (ECM) is remodeled in both the compression and tension sides of the PDL during orthodontic tooth movement (40). The PDL contains various types of collagen, with type I collagen (COL I) being the most common form (7, 63). The production of collagen molecules by PLF is an essential event in the process of alveolar bone remodeling (9, 53). In addition to collagens, matrix metalloproteinases (MMPs) play a key role in the degradation and remodeling of matrix proteins around and/or in the PDL. Among the MMPs, MMP-1 is a collagenase that degrades the native collagens initiating tissue remodeling (5).

As balanced regulation in the expression of matrix remodeling genes is critical for PDL homeostasis, many studies examined the role of PLF in the expression of collagen molecules and MMPs in response to mechanical forces. Accumulated evidence has shown that mechanical stress induces the upregulation of collagens and MMPs at the mRNA and protein levels in human periodontal fibroblasts (6, 18, 52). In particular, COL I is the highest collagen type synthesized in response to mechanical stimulation (9, 55). Our previous findings showed that the centrifugal force upregulated COL I expression in human gingival fibroblasts (25) and PLF (30). However, the expression patterns of COL I and MMP-1 by these fibroblasts in response to a mechanical force differed according to the origins of the cells examined as well as the modes of the mechanical stresses, such as shear, compression, and tension (17, 30, 44).

With regard to the mechanical stresses that can lead to cellular messages and outcomes through similar mechanoreceptors and signaling effectors in cells, it is believed that common signaling mechanisms are involved in the mechanotransduction pathways. With this regard, many studies and our previous findings suggested that the mitogen-activated protein kinases (MAPKs) are the most prominent kinase activated by mechanical stimuli (25, 30, 36). In general, MAPKs control a range of cellular events, such as embryogenesis, differentiation, proliferation, and death (10, 27, 62). MAPKs mainly consist of extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38 kinase. They regulate gene expression through the activation of transcription factors, such as activator protein-1 (AP-1) and nuclear factor-κB (NF-κB; 11, 43). The direct involvement of MAPKs on mechanotransduction has been largely suggested in muscle cells (35, 61) and in bone cells (16, 36). Previous studies showed that centrifugal force upregulated COL I expression in human periodontal fibroblasts through the activation of MAPK-AP-1.
signaling, supporting a central role of MAPKs on mechano-
transduction in cells (25, 30). Nevertheless, the mechanisms by
which MAPKs transmit mechanical signals into the nucleus
and regulate the expression of the matrix remodeling genes in
PLF are not completely understood.

In this study, we examined how tensile force regulates the gene
expression of COL I and MMP-1 in PLF. In addition, the molec-
ular mechanisms by which tension stress regulates the expression
of these matrix components, particularly the regulatory roles of
MAPKs and transcription factors, such as AP-1 and NF-κB, were
investigated.

MATERIALS AND METHODS

Chemicals and laboratory wares. MAPK inhibitors including
SB20358, PD98059, and SP600125 were purchased from TOCRIS
(Bristol, UK). Fetal bovine serum (FBS) and pyrrolidinedithiocar-
bamic acid (PDTC) were obtained from Hyclone (Logan, UT) and
ALEXIS (Lausen, Switzerland), respectively. Flexible-bottomed six-
well plates (BioFlex plates) were purchased from Flexcell Interna-
tional (Hillsborough, NC). Unless specified otherwise, all other chem-
icals and laboratory wares were obtained from Sigma Chemical (St.
Louis, MO) and SPL Life Sciences (Pochun, South Korea), respec-
tively.

PLF culture. PLF were obtained from healthy men aged 20 to 30 yr and cultured using methods described elsewhere (21, 30, 31).
Written informed consent for tissue use was obtained from all donors.
This study was approved by the Ethics Committee of Chonbuk National University Hospital. All experiments were performed using the fibromodulin-positive PLF (>95%) at passages 4 through 7.

Application of tensile force. PLF suspensions (1×10⁶ cells/ml)
were spread onto flexible-bottomed six-well plates and cultured until
the cells reached 80% confluence. For the application of tension, the
flexible plates were subjected to static waves with 1.5% elongation for
1 h using a computer-controlled vacuum stretch apparatus (FX-4000
Tension Plus System, FlexCell International). The 1.5% elongation
belonged to the range of optimal force magnitude for orthodontic
tooth movement (12, 53). For the control experiments, PLF were
cultured in the same plates without a mechanical force. At various
times (0–12 h) after force application, the cells were processed for
analyses of COL I and MMP-1 mRNA expression and the activities of
MAPKs and transcription factors.

Fig. 1. Effects of tensile force (TF) on the expression of
Type I collagen (COL I) and matrix metalloproteinase-1 (MMP-1) in the expression of these proteins in peri-
odontal ligament fibroblasts (PLF). A: PLF were sub-
jected to 1.5% tension for 1 h and, at the indicated times
(0–12 h) after the force, the mRNA levels of COL I and
MMP-1 were determined by RT-PCR. B: mRNA in-
creases in these matrix genes were calculated from
triplicate experiments after normalizing the bands to
GAPDH. C: the expression patterns of these genes in
the tension-applied PLF were also analyzed by real-time
RT-PCR. D: PLF transfected with COL1A2 or MMP-1
promoter-reporter vector were subjected to 1.5% TF for
1 h and processed for the luciferase activity assay at the
indicated times after tension. *P < 0.05, **P < 0.01,
and ***P < 0.001 vs. the unloaded control cells (CTL).
RNA isolation and PCR analysis. Reverse transcription (RT) and PCR amplification for COL I and MMP-1 were performed using an Access RT-PCR System (Promega, Madison, WI) according to the manufacturer’s protocol, as described previously (30). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the positive control. Band intensities of COL I and MMP-1 were calculated using the Bradford method (8). Equal amounts of protein samples were placed into the microtiter plates of a p-p38 kinase assay kit (Assay Designs, Ann Arbor, MI), p-ERK enzyme assay kit (Assay Designs), and p-SAPK/JNK sandwich ELISA kit (Cell Signaling Technology, Beverly, MA). Finally, the absorbance per sample was measured using a microplate reader (Packard Instrument, Downers Grove, IL).

Immunofluorescence. Tension force-applied PLF were fixed with 3.5% paraformaldehyde, washed three times with PBS, and incubated in PBS containing 0.2% Triton X-100 and 1% FBS at room temperature for 10 min. The cells were blocked with 0.5% bovine serum albumin and probed overnight with primary antibody specific to c-Jun (sc-822, 1:50), RelA (sc-109, 1:50), or p50 (sc-7178, 1:50) at 4°C before staining with FITC-bound secondary antibody. The cells were counterstained with 0.5 µg/ml 4′-6-diamidino-2-phenylindole (DAPI) for 15 min in a blocking solution. After being washed with PBS, the cell-adherent bottom of the flexible plates was cut and placed on microscope slides followed by fluorescence microscopy observation (Axiovision 3.1, Carl Zeiss, Oberkochen, Germany).

Cell fractionation. Nuclear and cytosolic extracts were prepared according to the method described elsewhere (22). Briefly, the cells were resuspended in buffer A [20 mM Tris (pH 7.5), 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS] and the protein contents were quantified using the Bradford method (8). Equal amounts of protein samples were placed into the microtiter plates of a p-p38 kinase assay kit (Assay Designs, Ann Arbor, MI), p-ERK enzyme assay kit (Assay Designs), and p-SAPK/JNK sandwich ELISA kit (Cell Signaling Technology, Beverly, MA). Finally, the absorbance per sample was measured using a microplate reader (Packard Instrument, Downers Grove, IL).

Enzyme immunometric assay. The activities of ERK, JNK, and p38 MAPK were determined using immunometric assay kits according to the manufacturer’s instructions. In brief, PLF were exposed to tension with 1.5% elongation for 1 h and at various times, cell lysates were prepared in a RIPA cell lysis buffer (50 mM Tris-Cl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS) and the protein contents were quantified using the Bradford method (8). Equal amounts of protein samples were placed into the microtiter plates of a p-p38 kinase assay kit (Assay Designs, Ann Arbor, MI), p-ERK enzyme assay kit (Assay Designs), and p-SAPK/JNK sandwich ELISA kit (Cell Signaling Technology, Beverly, MA). Finally, the absorbance per sample was measured using a microplate reader (Packard Instrument, Downers Grove, IL).

Western blot analysis. Cell lysates were made in a NP-40 lysis buffer as described elsewhere (30). Equal amounts (25 µg/sample) of protein extracts were separated by 12–15% SDS-PAGE and blotted onto polyvinyl difluoride membranes. The blots were probed with primary antibodies, incubated with a horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG secondary antibody, blotted with 0.5 µg/ml 4′-6-diamidino-2-phenylindole (DAPI) for 15 min in a blocking solution. After being washed with PBS, the cell-adherent bottom of the flexible plates was cut and placed on microscope slides followed by fluorescence microscopy observation (Axiovision 3.1, Carl Zeiss, Oberkochen, Germany).
gated anti-IgG, and then exposed to X-ray film (Eastman-Kodak, Rochester, NY) immediately after treatment with enhanced chemiluminescence (Santa Cruz Biotechnology, Santa Cruz, CA). In this study, polyclonal antibodies specific to JNK, ERK, p38, JunB, c-Jun, JunD, RelA, RelB, p50, and IkBα and monoclonal antibodies specific to p52, p-ERK, p-JNK, p-p38, p-c-Jun, and Ref-1 were obtained from Santa Cruz Biotechnology. Monoclonal antibody against α-tubulin was purchased from BD Bioscience Pharmingen (San Diego, CA).

Transfection with nonsense or antisense c-Jun oligonucleotides. PLF were seeded at 5×10^5 cells/well in six-well plates and transfected with 1 μM nonsense (5′-ATG ACT GCA AAG ATG-3′) or c-Jun antisense oligonucleotides (5′-CAT TCT CGT AGT CAT-3′). The 15-mer antisense sequences for c-Jun were short enough to penetrate the cells with the longevity for their specificity (51). Each transfection was performed using Lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. At 24 h thereafter, the cells were subjected to 1.5% tension for 1 h followed by Western blot and RT-PCR analyses.

Electrophoretic mobility shift assay. Nuclear proteins from PLF were prepared at various times after the application of tension, and the binding activities of AP-1 and NF-κB to their specific DNA were determined by electrophoretic mobility shift assay (EMSA), as described elsewhere (32). The samples were separated on 6% polyacrylamide gels, dried, and exposed to X-ray film (Eastman Kodak) for 12–48 h at −70°C. The following oligonucleotide primer sequences were used for EMSA: 5′-AAG GGA TCC TGC TGA CTC ATC ACT AG-3′ and 5′-AAG GCT AGT GAT GAG TCA GCC GGA TC-3′ for AP-1; and 5′-AAG GCC TGT GCT CGG CTA GCC GTG A-3′ and 5′-AAG GTC GAG GCC AGG GAA ATG CCG GGA CAG G-3′ for NF-κB. In addition, an antibody-clearance assay was carried out by incubating the antibodies to Jun proteins with a reaction mixture for 2 h at room temperature before performing DNA-protein binding reactions between the nuclear proteins and 32P-labeled oligonucleotides.

Luciferase activity assay. PLF were transfected with pLuc-human α2(I) collagen (COL1A2) promoter and pCI-neo plasmid containing neomycin phosphotransferase gene for Geneticin resistance using Superfect transfection reagent (Invitrogen) according to the manufacturer’s protocol. At 24 h thereafter, stable transfecants were selected, isolated, and maintained as described elsewhere (23). MMP-1 gene was also amplified from human genomic DNA (Promega) and its promoter luciferase construct was transfected into PLF according to the methods previously described (13). When the transfected cells reached 80–90% confluence, the cells were applied to 1.5% tensile force for 1 h. The cells were lysed in a lysis buffer (Promega) and luciferase activity was assayed with a luciferase assay kit (Promega), according to the manufacturer’s instructions.

Statistical analysis. Unless specified otherwise, all the data are expressed as the mean ± SD. A one-way ANOVA (SPSS version 18.0 software) followed by a Scheffe’s test was used for multiple comparisons. A value of P < 0.05 was considered significant.

RESULTS

Tensile force upregulates COL I and MMP-1 expression in PLF. We initially examined the effects of tensile force on mRNA expression of COL I and MMP-1 by RT-PCR, where PLF were subjected to 1.5% elongation for 1 h (Fig. 1, A and B). The mRNA levels of COL I increased from 1 h after the force and were maintained for up to 6 h, whereas a rapid and transient increase in MMP-1 expression was observed in the force-applied PLF. To confirm the tension-mediated increases in the matrix remodeling genes, the mRNA expression of COL I and MMP-1 was evaluated by real-time RT-PCR. As shown in Fig. 1C, mRNA levels of these genes were significantly increased by tension. Approximately four- and threefold increases in the COL I and MMP-1 mRNA levels, respectively, were observed at 1 h after tension application. These results were further confirmed by luciferase activity assay, where tensile force increased both COL1A2 and MMP-1 promoter-reporter activities (Fig. 1D). The most prominent increases in the luciferase activity were shown between 0 and 1 h after

![Fig. 3. Involvement of MAPKs in the nuclear increases in c-Jun protein and the mRNA expression of COL I and MMP-1 in the tension-applied PLF. A: PLF were exposed to 1.5% tension for 1 h and at various times (0–6 h) after the force, the cellular activities of MAPKs were determined by enzyme immunoassay. B: PLF were subjected to tension for 1 h in the presence of 20 μM of each inhibitor of MAPKs and at the time 0 after the force, nuclear levels of c-Jun and p-c-Jun were evaluated by immunoblotting. C: the cells were also exposed to the tensile force under the same conditions as described in B, and after 1 h of the force, the cells were processed for real-time RT-PCR. *P < 0.05 and +++P < 0.001 vs. the unloaded control values. #P < 0.05 and ##P < 0.01 vs. the force treatment alone.](http://jap.physiology.org/)
tension and were not detected 6 h after the tension application. These findings clearly support the stimulating effect of tensile force on COL I and MMP-1 expression in PLF.

Tensile force activates DNA binding of AP-1, especially of c-Jun. AP-1 regulates a wide range of cellular events in response to mechanical stimuli by binding to the promoter of mechanosensitive genes. Therefore, we examined the effects of tension on the nuclear translocation of Jun subfamily proteins and AP-1 binding to its specific target DNA. Applied tension resulted in the nuclear increase in c-Jun and JunD proteins but not JunB (Fig. 2A). In parallel, the force-mediated increase in c-Jun protein was observed by immunostaining (Fig. 2B). Applied tension also activated AP-1 binding to its specific DNA in a transient manner, where the activity peaked at time 0 h after the force and decreased to the basal levels by 1 h after tension (Fig. 2C). Antibody clearance assay showed that the bands specific to the AP-1-DNA complex had disappeared by adding the primary antibodies of Jun proteins in a dose-dependent manner (Fig. 2D). The most prominent clearance was detected when the anti-c-Jun antibody was added.

**Tensile force increases the nuclear translocation of c-Jun proteins and the mRNA expression of COL I and MMP-1 through activation of ERK and JNK-mediated signaling.** Since MAPKs play critical roles in mechanosignal transduction by acting as upstream effectors of transcription factors, we subsequently investigated the roles of MAPKs on the tension-mediated increases in the matrix genes. Applied tensile force induced the rapid and temporal phosphorylation of p38 MAPK, whereas the force-mediated increases in p-ERK1/2 and p-JNK levels were maintained for 3 and 6 h after the force, respectively (Fig. 3A). Similarly, Western blot analysis revealed the increases in all three MAPKs in tension-applied PLF (data not shown). Pretreatment of PLF with the inhibitor of ERK or JNK but not p38 MAPK 2 h before tension application reduced the nuclear levels of c-Jun and p-c-Jun proteins (Fig. 3B). Pharmacological inhibitor of ERK or JNK prevented the expression of both COL I and MMP-1 mRNAs (Fig. 3C). On the other hand, SB203580, a p38 MAPK inhibitor, did not reduce the expression of the genes increased in the tension-applied PLF.

**Knockdown of c-Jun suppresses the tension-mediated increase of COL I and MMP-1 expression.** To further understand the role of c-Jun in the tension-mediated expression of COL I and MMP-1, we transfected the cells with c-Jun antisense oligonucleotides. The transfection itself did not induce cytotoxicity to a significant level during the experimental periods (data not shown). The cellular levels of c-Jun protein were diminished after the transfection in a time-dependent manner (Fig. 4A). Applied tensile force increased markedly the nuclear levels of c-Jun in PLF, which was completely attenuated by the knockdown of c-Jun (Fig. 4B). Figure 4C shows that the blockage of c-Jun prevents the tension-induced increases in COL I and MMP-1 expression to the basal levels. This is supported by the results from real-time RT-PCR showing that c-Jun knockdown inhibits the tension-stimulated mRNA expression of COL I and MMP-1 in PLF (Fig. 4D).

**NF-κB signaling is associated with tension-mediated expression of MMP-1.** We next examined the role of NF-κB in tension-induced stimulation of COL I and MMP-1 expression.
Among the subproteins of NF-κB, RelA/p65 and p50 proteins were translocated to the nucleus after tension in a rapid and transient manner (Fig. 5A). Tension-mediated nuclear relocation of the proteins was correlated with the decrease in the cellular IκBα levels. The immunofluorescence assay also revealed the tension-stimulated increases in RelA and p50 proteins in the nucleus (Fig. 5B). In parallel with these results, NF-κB binding to its specific DNA was increased dramatically at time 0 after application to tension but was reduced to its basal level at 1 h after tension (Fig. 5C). Treatment of the cells with a NF-κB inhibitor, PDTC, decreased the tension-stimulated expression of MMP-1 in a dose-dependent manner, but not COL I (Fig. 5D). PDTC-mediated suppression of MMP-1 expression in the tension-applied PLF was confirmed by real time RT-PCR analysis, where the addition of 50 μM PDTC mostly inhibited MMP-1 expression (Fig. 5E).

**ERK acts as an upstream effector of NF-κB signaling in tension-stimulated MMP-1 expression.** To examine the roles of MAPKs as the upstream effectors of NF-κB signaling, PLF were exposed to 1.5% tension for 1 h in the presence of MAPK inhibitors. Applied tensile force decreased the cellular IκBα levels, but this decrease was prevented by the pharmacological inhibitor of ERK. On the other hand, the pharmacological inhibitor of JNK or p38 MAPK had no effect (Fig. 6A). Pretreatment of PLF with 20 μM PD98059 also reduced the nuclear translocation of RelA and p50 proteins (Fig. 6B), the DNA-binding activation of NF-κB (Fig. 6C), and MMP-1 expression (Fig. 6D).

**DISCUSSION**

Alveolar bone remodeling induced by mechanical strain is accompanied by remodeling of the PDL (9). The PDL is a fibrous tissue with a high rate of turnover through a balanced interaction between collagen molecules and MMPs (2, 48). The PDL is exposed to continuous mechanical loading derived from bite forces, where the PLF play important roles by receiving mechanical loads and by producing various components that are essential for bone and tissue remodeling (9, 28, 58). Therefore, it is important to understand the precise mechanisms of the signal transduction pathways involved in mechanotransduction in PLF. MMPs also play critical roles in the turnover of connective tissue in both physiological and pathological conditions and the altered regulation of MMP activity might cause the abnormal destruction of periodontal tissue (65).

The present findings show that tensile force induces the upregulation of COL I and MMP-1 in PLF. This is consistent with the findings that mechanical stress can stimulate the expression of bone formation-related factors (15, 38). There are also considerable findings supporting our current results in that MMP-1 and COL I expression are significantly increased in tension and/or compression side, compared with the unloaded control tissues (17, 40). In addition, it was reported that in human PDL cells, tension application with 10% cyclic equibiaxial force increased total COL I protein level (18). Similarly, the expression of MMP-1 and COL I was increased in a magnitude-dependent manner, when human PDL cells were subjected to 5% and 10% tension (49). We recently reported that a centrifugal force stimulates the expression of bone and tissue components, such as COL I and osteopontin in human periodontal fibroblasts (20, 25, 30). Therefore, PLF are quite sensitive to mechanical stimuli and stimulate the expression of matrix remodeling genes, which is related to the capacity of the PDL to respond to the physiological and...
mechanical loading. In addition, it is believed that tension-mediated upregulation of COL I and MMP-1 is closely related to the nature of PLF with osteogenic property. Indeed, PLF can differentiate into osteoblastic cells and the expression of osteoblast-specific genes in the cells is quite sensitive to mechanical stimulation (55, 56, 64).

The major significance of this study is the understanding of mechanotransduction involved in the tension-stimulated expression of COL I and MMP-1 in PLF. Indeed, many previous studies and our recent findings demonstrated the mechanically stimulated expression of matrix remodeling genes, suggesting the cellular signaling mechanisms involved in mechanotransduction in PLF. Mechanosignal transduction pathways are believed to be controlled by common signaling molecules, although the cells are exposed to different modes of mechanical stress (36). Nevertheless, the interaction between intracellular signaling molecules and the signal transition cascades can differ according to the origins of the cells and the conditions of the stresses exposed. Moreover, the mechanotransduction pathways by which tensile force controls the expression of COL I and MMP-1 in PLF are still unclear. In the present study, we demonstrate that tensile force upregulates the expression of these matrix proteins by activating the JNK/ERK and AP-1/NF-κB signaling pathways in PLF.

The most important kinases involved in mechanotransduction are the MAPK, because most mechanical signals are transmitted to the nucleus through the activation of these kinases (36, 57). AP-1 subfamily proteins are sensitive to mechanical stimuli and control a range of cellular events as the downstream effector of MAPKs (19, 29). Our current findings revealed that applied tensile force induced the rapid translocation of c-Jun with the attendant activation of AP-1-DNA binding in PLF. Tensile force also increased the phosphorylated levels of all three MAPKs, whereas a pharmacological inhibitor of ERK or JNK, but not p38 MAPK, prevented c-Jun activation and COL I and MMP-1 expression in the tension-applied PLF. Application of cyclic strain to vascular smooth muscle cells was reported to cause a rapid and transient activation of MAPKs (34). The current results are also consistent with the previous findings where centrifugal force activated the DNA-binding capacity of AP-1 rapidly and transiently through ERK and JNK-mediated signaling, leading to COL I expression in PLF (30). These findings suggest the involvement of ERK/JNK- and AP-1-mediated signaling in the tension-stimulated expression of COL I and MMP-1 in PLF.

AP-1 is a key transcription factor that regulates the gene promotion of a variety of molecules for inflammatory mediators, oncogenes, growth factors, proliferation, differentiation, and apoptosis in cells (45, 46, 60). MAPKs regulate the phosphorylation of Jun and Fos proteins; Jun proteins are directly phosphorylated at the NH2-terminal domain of c-Jun by p-JNK, whereas ERK phosphorylation activates the Fos family at COOH-terminal serine (2, 14, 59). A critical step in AP-1 activation is ERK/JNK-mediated c-Jun activation followed by phosphorylation (26, 29). We previously reported that centrifugal force upregulated COL I in human gingival fibroblasts by activating ERK-c-Fos and JNK-c-Jun pathways

![Fig. 7. Proposed model of mechanosignal transduction pathways involved in the upregulation of COL I and MMP-1 in tension-applied PLF.](http://jap.physiology.org/)

![Fig. 6. Effects of MAPK inhibitors on NF-κB-DNA binding and MMP-1 expression in tension-applied PLF.](http://jap.physiology.org/)

**Fig. 6.** Effects of MAPK inhibitors on NF-κB-DNA binding and MMP-1 expression in tension-applied PLF. PLF were pretreated with 20 μM of each MAPK inhibitor 2 h before application of 1.5% tension for 1 h, and the cytosolic and nuclear fractions were prepared immediately after the force. These fractions were evaluated by immunoblotting to determine the cellular levels of IκBα (A) and RelA and p50 (B). C: NF-κB-DNA binding was also estimated by EMSA using the same nuclear fractions. D: PLF were exposed to 1.5% tension for 1 h in the presence of 20 μM PD98059, and at 1 h after the force the cells were processed for RT-PCR.
The current study also showed that in addition to JNK inhibitor, ERK inhibitor attenuated the tension-mediated increases in c-Jun and p-c-Jun levels and deceased the mRNA levels of COL I and MMP-1 significantly. Furthermore, knockdown of c-Jun inhibited the expression of these matrix genes in tension-exposed PLF. This suggests that tension-mediated stimulation of c-Jun induction and its phosphorylation is essential for the expression of COL I and MMP-1 and this is controlled by JNK and ERK signaling. Furthermore, the nuclear induction of Fos proteins in tension-applied PLF was not affected by ERK inhibitor (data not shown). Collectively, these results suggest that the mechanotransduction pathways involved in the expression of matrix remodeling genes in PLF are, in part, different from that of gingival fibroblasts and also from the modes of the mechanical stimulus, i.e., centrifugation and tensile force, although MAPKs and AP-1 are involved in these processes as the common signaling molecules.

In general, NF-κB functions in gene promotion for proinflammatory mediators as well as proliferation, differentiation, and apoptosis (47). Mechanical strain regulates anti-inflammatory and pro-inflammatory action through NF-κB transcription pathway (1). Many studies have suggested the rapid and transient activation of NF-κB in PLF and in other cells exposed to mechanical stimuli (24, 37, 41). The present study demonstrated that in addition to JNK/ERK-AP-1 signaling, tensile force stimulates MMP-1 expression through the ERK-NF-κB-mediated pathways. The inhibitor of ERK but not p38 and JNK prevented the force-induced degradation of IkBα and reduced nuclear levels of NF-κB subproteins. A mechanical stimulation can lead to a range of cellular messages and outcomes through similar mechanoreceptors and intracellular signaling effectors in cells, whereas different signaling molecules cause a similar outcome in the mechanotransduction pathways. Overall, our present findings suggest the involvement of ERK and NF-κB signaling pathways in MMP-1 expression in the tension-subjected PLF, where the RelA and p50 complex is mainly formed.

In addition to ERK and JNK, p38 MAPK regulates a range of cellular processes including inflammation, apoptosis, survival, proliferation, and differentiation (42, 62). Especially p38 MAPK has been implicated in the regulation of cell proliferation by modulating expression and activity of cell cycle regulatory factors (39, 50). This study showed that tension force activated p38 MAPK, although this kinase did not affect the expression of COL I and MMP-1 in tension-applied PLF. We currently investigated the precise roles of p38 MAPK in tensile force-exposed PLF and found a significant increase in DNA synthesis by the cells after treatment with SB203580, a p38 MAPK inhibitor (31). Ras inhibitor also blocked the tension-mediated increases in p-p38 MAPK and p-p21. These findings suggest that p38 MAPK signaling is closely involved in the regulation of proliferation in cells, and applied tensile force leads to Ras-p38 MAPK activation with the attendant cell cycling inhibition followed by p21 upregulation in tension-subjected PLF.

In summary, this study demonstrates that tensile force upregulates the expression of COL I and MMP-1 in PLF and provides mechanotransduction pathways involved in the expression of these matrix genes. In particular, our present findings show that applied tensile force stimulates the expression of COL I and MMP-1 in PLF and this is controlled by ERK/JNK-AP-1 and ERK-NF-κB signaling pathways (Fig. 7).

**REFERENCES**


20. Hong SY, Jeon YM, Lee HJ, Kim JG, Baek JA, Lee JC. Activation of RhoA and FAK induces ERK-mediated osteopontin expression in me-


