Stretch-inducible Expression of Connective Tissue Growth Factor (CTGF) in Human Osteoblasts-like Cells is Mediated by PI3K-JNK Pathway

Li-Wei Xiao^{1*}, Min Yang^{2,5*}, Jing Dong³, Hui Xie², Guo-Liang Sui², Yu-Ling He², Jia-Xuan Lei¹, Er-Yuan Liao² and Xiao Yuan⁴

¹Stomatological Center, the Second Xiangya Hospital, Central South University, Changsha, ²Institute of Endocrinology and Metabolism, the Second Xiangya Hospital, Central South University, Changsha, ³Children's Hospital of Hunan Province, Changsha, ⁴Department of Orthodontics, the Affiliated Qingdao Municipal Hospital, Qingdao University, Qingdao, ⁵Department of Internal Medicine, Affiliated Hospital of National Research for Rehabilitation Technical Aids, Beijing *The authors contributed equally to this work

Key Words

Connective tissue growth factor (CTGF) • Cyclic stretch • Mitogen-activated protein kinase (MAP kinase) • JNK signaling pathway • Osteoblast

Abstract

To explore the possible role for connective tissue growth factor (CTGF) during tooth movement, we evaluated CTGF gene and protein expression in MG-63 cells subjected to cyclic stretch. Cyclic stretch caused a time-dependent increase in CTGF mRNA and protein levels. Inhibition of p38 MAP kinase or ERK activation did not affect cyclic stretch-induced CTGF expression. Specific inhibitors of PI3K suppressed stretch-induced CTGF expression in a time-dependent manner. Cyclic stretch activated JNK and ERK, but not p38 MAP kinase in osteoblast-like cells. PI3K inhibitors suppressed cyclic stretch-induced JNK, but not p38 MAP kinase activation. Finally, SP600125, a Specific Inhibitor of JNK, suppressed stretch-induced CTGF Expression. These results suggest that stretch-induced CTGF expression is mediated through the PI3K-JNK-dependent pathway, not by p38 MAP kinase and ERK pathways.

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Introduction

When a force is loaded onto a tooth in orthodontic treatment, the equilibrium that exists between bone formation and resorption is disrupted, which in turn results in more bone resorption than formation on the pressure side and more bone formation than resorption on the tension side in the alveolar bone, and the tooth moves in a specified direction [1-5]. Numerous important molecules in bone remodeling—such as osteopontin (OPN) [6], TNF- α [7], and connective tissue growth factor [8]—are involved with osteoclastogenesis.

Connective tissue growth factor (CTGF) is a potent and ubiquitous 38-kD protein that belongs to the CCN family, consisting of *cyr61/cefp10*, *ctgf/fisp12*, *nov*, *elm1/wisp-1*, *rcop-1/wisp-2*, and *wisp-3* [8]. CTGF was originally identified in conditioned medium from human umbilical vein endothelial cells [9], and is expressed in a variety of tissues, including bone and cartilage. In osteoblasts, CTGF expression is induced by BMP, transforming growth factor β (TGF- β), Wnt, and cortisol, suggesting a possible role in the activity of these agents in bone [10-13]. CTGF regulates different cellular functions, including adhesion, proliferation, migration, and differentiation. Previous study indicated that CTGF gene

increased after experimental tooth movement for 12 hrs in the rat alveolar bone, suggesting that CTGF was involved in the system that transmitted mechanical stimulation to the bone [14]. However, the role of CTGF in osteoblasts during the bone remodeling process triggered by mechanical stimulation has not been investigated.

The MAPK family of serine-threonine protein kinases is a highly conserved family consisting of the three primary members; extracellular signal-related kinases (ERK), c-Jun amino-terminal kinases (JNK), and p38. MAPKs have an important role in regulating cell growth, differentiation, apoptosis, and inflammation [15], and these effects are cell type and stimulus specific [16]. Cyclic stretch induces MAPK activation in various cells, including osteoblasts [17-19]. Thus, the MAP kinase superfamily regulates numerous cellular functions. However, the role of the MAP kinase superfamily in stretch-induced CTGF expression has not been determined.

The present study examines the roles of JNK, p38 MAP kinase, and ERK in stretch-induced CTGF mRNA expression in osteoblast-like cells; In addition, we investigated effects of wortmannin and LY294002, inhibitors of phosphatidylinositol 3-kinase (PI3K), on stretch-induced MAP kinase activation and CTGF expression.

Materials and Methods

Antibodies and Reagents

We already used the following antibodies and reagents in this study: Anti-connective tissue growth factor antibody, anti-extracellular signal-regulated kinase1/2 (ERK1/2), p-ERK1/2, p38, p-p38, c-jun N-terminal Kinase (JNK), p-JNK, AKT, and p-AKT antibodies, anti-mouse IgG peroxidase conjugate, anti-goat IgG peroxidase conjugate, and anti-rabbit IgG peroxidase conjugate antibodies (Santa Cruz Biotechnology Inc, Waltham, MA, USA), anti- β -actin polyclonal antibody (Sigma, St. Louis, MO, USA), SP600125, LY294002, PD98059 and SB203580 (Calbiochem Corp., San Diego, CA, USA), α -MEM (sigma, St. Louis, MO, USA), Trizol reagent, Fetal bovine serum (FBS) (Gibco-BRL Corp., Grand Island, NY); the reverse transcription system (Promega, Madison, WI, USA).

Cell Culture

The human osteosarcoma cell line MG-63 was purchased from ATCC (Rockville, MD, USA). MG-63 cells were cultured in α -MEM containing 10% FBS, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 50 μ g/ml ascorbic acid and maintained in a humidified 37°C incubator under 5% CO₂. After reaching confluence, they were incubated for 24 h in serum-free α -MEM, and then subjected to mechanical stretch.

Inhibition of MAP Kinases

To investigate the effects of some inhibitors, firstly, MG-63 cells were pretreated with 30 μ M SP600125, 25 μ M LY294002, 50 μ M PD98059 or 10 μ M SB203580 for 1 h prior to mechanical stimulation, then, the cells layers were harvested for Western Blot analysis.

Cyclic stretch

The Flexcell Strain Unit Fx-4000 (Flexcell Corp.) was used for the application of mechanical stretch to osteoblasts [20]. Cells were plated in six-well tissue culture dishes with type I collagen-coated, flexible silicone bottoms (Bioflex; Flexcell Corp.) at 1x10⁵ cells per well density. Seventy-two hours after seeding, cells were serum-starved overnight and then subjected to mechanical deformation. Mechanical deformation was induced with a Flexercell strain unit, which consists of a vacuum manifold regulated by solenoid valves that are controlled by a computer timer program. The Bioflex baseplate contain the Bioflex loading station that consists of six buttons per plate that insert into each plate, allowing a uniform magnitude of the strain across 85% of the surface of the flexible well. For these experiments a negative pressure of 80 kilopascals was applied through an air pump to the culture plate, bottoms were deformed to a known percentage of elongation, and then the membranes were released to their original conformation. The experimental regimens used in this study delivered 10% elongation for 1-6 hrs at a rate of 15 cycles/min (a 2-s deformation period followed by a 2-s neutral position). Cells remained adherent, and the deformation of the membrane is directly transmitted to cells. Unstretched cells grown on Bioflex plates were used as controls.

Detection of CTGF Gene Expression

In order to examine the expression of CTGF mRNA in MG-63 cells, RT-PCR detection was performed. The total RNA was isolated using Trizol reagent according to the manufacturer's recommended protocol. Reverse transcription (RT) was performed using 5.0 μ g total RNA and the RT system (Promega, Madison, WI, USA). For RT-PCR detection of CTGF (human), PCR primers were 5'-CCA AGG ACC AAA CCG TGG T-3' and 5'-TAC TCC ACA GAA TTT AGC T CG-3', yielding a 353 bp fragment. PCR was performed as follows: 94°C for 1 min, 56°C (human) or 58°C (mouse) for 45 s and 72°C for 1 min for 30 cycles followed by 10-min incubation at 72°C. β -actin was amplified as an internal control using the following primers: (human) 5'-CGGG AAA TCG TGC GTG AC-3' and 5'-CAG GCA GCT CGT AGC TCT T-3', yielding a fragment of 115 bp fragment. The identities of PCR products were confirmed by direct sequencing using an automatic DNA sequence (PE Applied Biosystems).

Determination CTGF Protein Expression

In order to evaluate the effect of cyclic stretch on CTGF protein expression, Western blot analysis was performed. Cells were lysed with Triton lysis buffer (50 mM Tris-HCl, pH 8.0, containing 150 mM NaCl, 1% Triton X-100, 0.02% sodium azide, 10 mM EDTA, 10 μ g/ml aprotinin, and 1 μ g/ml aminoethylbenzenesulfonyl fluoride (ABSF)). Lysates were

Fig. 1. CTGF mRNA and protein expression induced by cyclic stretch in human osteoblasts, MG63 cells. (A) Time-dependent induction of CTGF mRNA after continuously stretched for 0,1,3,6h. Total RNA was extracted and hybridized with human CTGF cRNA probes. Loading control was β-actin probe. (B) Time-dependent induction of CTGF protein after continuously stretched for 0,1,3,6h. Total cellular protein isolated from MG63 cells was subjected to Western Blot determination using anti-CTGF and anti-β-actin antibodies (Fig. 1B). The fold change of CTGF expression as a ratio of β-actin obtained by densitometric analysis is shown in MG63 cells. The values are the means ± SD. * $p < 0.05$ compared with the stretched cells in the absence of inhibitors.

centrifuged for 15 min at 12000g to remove debris. Protein concentrations were determined using the Bradford protein assay. Forty micrograms protein were mixed with 2 x SDS gel-loading buffer (100 mM Tris-HCl, pH 6.8, and 200 mM dithiothreitol (DTT), 4% SDS, 0.2% bromophenol blue, and 20% glycerol), then heated to 95°C for 5 min. Samples were loaded onto a 10% polyacrylamide gel, electrophoresed, then transferred to a PVDF membrane (Amersham Pharmacia Biotech, Arlington Heights, IL, USA). Membranes were blocked with 5% non-fat milk in phosphate-buffered saline (PBS), and then incubated with specific antibodies against CTGF (diluted at 1:500) in PBS at 4°C overnight. After extensive washing with

Fig. 2. Effect of cyclic stretch on JNK, p38 MAP kinase and ERK activation in human osteoblasts. MG63 cells were continuously stretched for indicated periods. Activities of JNK, p38 MAP kinase, and ERK were determined by Western blotting. Whole cell lysates using antibodies specific for phosphorylated, activated forms of JNK (A, top), ERK (B, top), and p38 MAP kinase (C, top). Corresponding bottom panels show Western blottings using antibodies to total JNK (A, bottom), ERK (B, bottom) and p38 MAP kinase (C, bottom) to show amounts of each precipitated enzyme. Four identical experiments independently performed yielded similar results.

PBS, membranes were reprobed with anti-goat IgG peroxidase conjugated secondary antibodies diluted 1:5000 in PBS for 1h at room temperature. Blots were processed using an ECL kit, exposed to film, then analyzed by densitometry. Membranes were stripped and reprobed with an anti-β-actin polyclonal antibody to control for loading. Densitometric data were normalized using β-actin values.

Levels of phosphorylated ERK, JNK and p38 were examined by Western Blot. MG63 cells were stimulated by cyclic stretch for indicated times, then the cells were washed quickly with cold PBS containing 5 mM EDTA and 0.1 mM Na_3VO_4 , lysed with lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl,

Fig. 3. Effects of PD98059 and SB203580 on cyclic stretch-induced CTGF expression. (A, E) MG63 cells were cultured with 0.1% DMSO (control vehicle), PD98059 (specific inhibitor of ERK; A) ,or SB203580 (specific inhibitor of p38 MAP kinase; E) for 60 min before stimulation with cyclic stretch. (A). ERK activity and p38 MAP kase was determined by Western blotting as described in the legend to Figure 3. (A, E) . The values are the means $\pm$ SD.* $p < 0.05$ compared with the stretched cells in the absence of inhibitors. The fold change of CTGF expression as a ratio of β -actin obtained by densitometric analysis is shown in Figure 3 (C, G). The values are the means $\pm$ SEM of four experiments.

1% Triton X-100, 10 mM NaH_2PO_4 , 10% glycerol 2 mM Na_3VO_4 , 10 mM NaF, 1 mM ABSF, 10 $\mu\text{g/ml}$ leupeptin, and 10 $\mu\text{g/ml}$ aprotinin). Western blots were performed as above with anti-p-JNK, JNK, p-ERK, ERK, p-p38 and p38 antibodies.

Statistical Analyses

All data are expressed as means $\pm$ SE. One-way ANOVA was performed when more than two groups were compared with a single control, and then differences between individual groups within the set were assessed by a multiple-comparison test (Tukey) when the F statistic was < 0.05 . An unpaired Student's t-test was used to assess the difference between two groups. A P value of < 0.05 was considered significant. All experiments were repeated at least three times with reproducible results.

Results

Cyclic stretch upregulated CTGF mRNA and protein expression in human osteoblasts in a time-dependent way

We examined the expression of CTGF by cyclic stretch in the human osteoblast-like cell line, MG63. Figure 1A shows that cyclic stretch time-dependently increased CTGF mRNA levels after continuously stretch for 1h, and reached a maximal 3- to 4-fold response after continuously stretch for 3 h in MG63 cells. The response of CTGF protein expression to cyclic stretch was also studied. As shown in Figure 1B, cyclic stretch caused a

Fig. 4. Effects of SP600125 on cyclic stretch-induced CTGF expression. MG63 cells were cultured with 0.1% DMSO (control vehicle), SP600125 (specific inhibitor of JNK) for 60 min before stimulation with cyclic stretch. Activities of JNK were determined by Western blotting. Whole cell lysates using antibodies specific for phosphorylated, activated forms of JNK. Corresponding bottom panels show Western blottings using antibodies to total JNK. Four identical experiments independently performed yielded similar results. The fold change of CTGF expression as a ratio of β -actin obtained by densitometric analysis is shown in Figure 4. (C, D). The values are the means $\pm$ SEM of four experiments.

significant increase in CTGF protein expression in MG63 cells. The steady-state level of CTGF protein was maximally up regulated nearly 3-fold within 12 hr as compared with the cells cultured under the unstretched condition.

Cyclic stretch-induced JNK, ERK Kinase, and p38 MAP activation in human osteoblasts

We determined the roles of the three MAP kinases in mechanical stretch signal transduction by detecting their dually phosphorylated (Thr/Tyr) forms by Western blotting against specific anti-phospho kinase antibodies. Amounts of phosphorylated threonine and tyrosine of JNK significantly increased, reached a maximum at 10 min, and remained elevated for up to 30 min after stretch (Fig. 2A). Levels of phosphorylated threonine and tyrosine of ERK similarly increased, reaching a maximum at 30 min and remaining elevated for up to 60 min after stretch (Fig. 2B). Cyclic stretch did not affect the amounts of phosphorylated of P38 MAPK (Fig. 2C).

Inhibition of ERK activation or p38 MAP Kinase did not affect stretch-induced CTGF protein expression

To further understand the mechanism of cyclic stretch-induced CTGF expression, we investigated the roles of MAP kinases using selective inhibitors of MAP kinases. We blocked the ERK pathway by incubating human osteoblast-like cells with PD98059, a specific inhibitor of ERK activity, for 60 min before stretch. Figure 3A shows that 50 μ M PD98059 markedly suppressed stretch-induced ERK phosphophate. However, PD98059

did not affect stretch-induced CTGF expression (Figs. 3C and 3D). We blocked the p38 MAP kinase pathway by incubating human osteoblast-like cells with SB203580, a specific inhibitor of p38 MAP kinase activity. Figure 3E shows that 10 μ M SB203580 markedly suppressed stretch-induced p38 MAP kinase activity. However, SB203580 did not affect stretch-induced CTGF protein expression (Figs. 3G and 3H).

Inhibition of JNK activation suppressed stretch-induced CTGF protein expression

To further confirm the role of JNK in stretch-induced CTGF expression, we investigated the roles of JNK using selective inhibitors of JNK pathway. We blocked the JNK pathway by incubating human osteoblast-like cells with 30 μ M SP600125, a specific inhibitor of JNK pathway, for 60 min before stretch. Figure 4A shows that 30 μ M SP600125 markedly suppressed stretch-induced JNK phosphophate. Then, we examined susceptibility to stretch-induced CTGF expression under these conditions. Figure 4C shows that the expression of CTGF protein induced by cyclic stretch was blocked by 30 μ M SP600125. Densitometry showed that PD98059 significantly ($P < 0.05$) inhibited cyclic stretch-induced CTGF expression (Fig. 4D).

LY294002, specific inhibitors of PI3K, suppressed stretch-induced CTGF protein Expression

Next, we examined effects of LY294002, specific inhibitor of PI3K activity, on stretch-induced CTGF expression in human osteoblast-like cells. In contrast to

Fig. 5. Effects of LY294002 on stretch -induced CTGF expression in MG63 cells. MG63 cells cultured in 0.1% DMSO (control vehicle), or LY294002 for 60 min before continuously stretched for 60 min. Total cellular protein isolated from MG63 cells was subjected to Western Blot determination using anti-CTGF and anti-β-actin antibodies (Fig. 5A). The fold change of CTGF expression as a ratio of β-actin obtained by densitometric analysis is shown in MG63 cells. The values are the means ± SEM of four experiments.

Fig. 6. Effects of LY294002 on stretch -induced Phospho-JNK in MG63 cells. MG63 cells cultured in 0.1% DMSO (control vehicle), or LY294002 for 60 min before continuously stretched for 30 min. Total cellular protein isolated from MG63 cells was subjected to Western Blot determination using anti-P-JNK and anti-total-JNK antibodies (Fig. 6A). The fold change of Phospho-JNK, as a ratio of total-JNK obtained by densitometric analysis is shown in MG63 cells (Fig. 6B). The values are the means ± SEM of four experiments.

SB203580 and PD98059, LY294002 dose-dependently suppressed CTGF protein expression in osteoblasts cells stimulated by cyclic stretch (Fig. 5A). Densitometry showed that 100 nM LY294002 significantly inhibited stretch-induced CTGF expression (Fig. 5B).

LY294002 suppressed stretch-induced JNK, but not p38 MAP Kinase or ERK activation

We investigated whether or not LY294002 affect stretch-induced MAP kinase activities in human osteoblast-like cells. Figure 6A shows that LY294002 notably suppressed stretch-induced JNK phosphorylation (Fig. 6B). On the other hand, these PI3K inhibitors did not affect stretch-induced ERK kinase and p38 MAP

kinase activity in osteoblast-like cells (Data not shown). These results suggest that the JNK pathway, but not p38 MAP kinase or ERK, is downstream of PI3K in mechanical signaling transduction, which regulates cyclic stretch-induced CTGF expression.

Discussion

The main finding of the present study is that cyclic stretch induces CTGF protein expression in human osteoblast-like cells through the PI3K-JNK-dependent pathway, but not through the ERK- ,or p38- dependent pathways. This is the first report to describe the role of

JNK in regulating cyclic stretch-induced CTGF expression in human osteoblast-like cells.

The signaling pathways that mediate cyclic stretch induced expression of CTGF have been shown to vary depending on the cell type being examined [21] and to date there have been no studies in osteoblasts. In general, cyclic stretch increases production and release of various vasoactive substances and growth factors in cultured cells, including TGF- β , which in turn up-regulates CTGF mRNA expression [22-27]. However, several other downstream signaling pathways are also activated by cyclic stretch, including increased actin polymerization and NF-kappaB activation [28], RhoA [29], and PI3K [30]. Furthermore, cyclic stretch has been reported to activate the MAP kinase pathway [31]. In the present study, we tested what kind of MAP kinase was involved in stretch-induced CTGF expression. we found that exposing osteoblast-like cells to cyclic stretch resulted in the activation of JNK (Fig. 2A) and ERK (Fig. 2B), but not of p38 MAP kinase (Fig. 2C). In addition, the specific pathway was clarified using specific inhibitors of the MAPK pathway. Our studies showed that an inhibitor of JNK (SP600125) potently inhibited the induction of CTGF prtein expression by cyclic stretch (Fig. 4). In contrast, blocking the p38 MAP kinase signaling pathway by SB203580 and the ERK signaling pathway by PD98059 did not obviously affect the induction of CTGF expression by cyclic stretch (Fig. 3), indicating that activation of the p38 MAP kinase and the ERK pathway is not essential for the enhancement of CTGF expression in osteoblast-like cells. Furthermore, we demonstrated that LY294002, as specific inhibitors of PI3K, suppressed the activation of JNK (Fig. 6), but have no effect on the activation of p38 MAP kinase and ERK

(Data not shown). LY294002 also suppressed the induction of CTGF protein expression by cyclic stretch (Fig. 5). These results indicate that stretch-induced CTGF prtein expression is mediated through the JNK-dependent pathway, and that PI3K may act as upstream of JNK. We are the first to show that the PI3K-JNK pathway plays a crucial role in the expression of CTGF induced by cyclic stretch in human osteoblast like cells.

In conclusion, we demonstrated that cyclic stretch up-regulates CTGF expression in cultured human osteoblast like cells, and cyclic stretch -induced CTGF activation is mediated through the JNK-dependent pathway, whereas p38 MAP kinase and ERK pathways contribute only minimally in human osteoblast like cells. Based on our study on CTGF gene regulation, we have identified that cyclic stretch regulates CTGF expression through PI3K-JNK pathway. These findings suggest that CTGF expression in response to mechanical stimulation, might play a significant role in triggering bone remodeling during tooth movement. Understanding the molecular mechanisms of CTGF in bone remodeling stimulated by mechanical stress may provide new options for regulating proliferation and differentiation of osteoblast during tooth movement.

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References

- Vignery A, Baron R: Dynamic histomorphometry of alveolar bone remodeling in the adult rat. *Anat Rec* 1980;196:191-200.
- Takano-Yamamoto T, Takemura T, Kitamura Y, Nomura S: Site-specific expression of mRNAs for osteonectin, osteocalcin, and osteopontin revealed by in situ hybridization in rat periodontal ligament during physiological tooth movement. *J Histochem Cytochem* 1994;42:885-896.
- Terai K, Takano-Yamamoto T, Ohba Y, Hiura K, Sugimoto M, Sato M, Kawahata H, Inaguma N, Kitamura Y, Nomura S: Role of osteopontin in bone remodeling caused by mechanical stress. *J Bone Miner Res* 1999;14:839-849.
- Wise GE, Yao S: Expression of tumor necrosis factor-alpha in the rat dental follicle. *Arch Oral Biol* 2003;48:47-54.
- Nayak BN, Wiltshire WA, Ganss B, Tenenbaum H, McCulloch CA, Lekic C: Healing of periodontal tissues following transplantation of cells in a rat orthodontic tooth movement model. *Angle Orthod* 2008;78:826-831.
- Koyama Y, Mitsui N, Suzuki N, Yanagisawa M, Sanuki R, Isokawa K, Shimizu N, Maeno M: Effect of compressive force on the expression of inflammatory cytokines and their receptors in osteoblastic Saos-2 cells. *Arch Oral Biol* 2008;53:488-496.
- Yamashiro T, Fukunaga T, Kobashi N, Kamioka H, Nakanishi T, Takigawa M, Takano-Yamamoto T: Mechanical stimulation induces CTGF expression in rat osteocytes. *J Dent Res* 2001;80:461-465.
- Lau LF, Lam SC: The CCN family of angiogenic regulators: the integrin connection. *Exp Cell Res* 1999;248:44-57.
- Bradham DM, Igarashi A, Potter RL, Grotendorst GR: Connective tissue growth factor: a cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. *J Cell Biol* 1991;114:1285-1294.

- 10 Igarashi A, Okochi H, Bradham DM, Grotendorst GR: Regulation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair. *Mol Biol Cell* 1993;4:637-645.
- 11 Parisi MS, Gazzerro E, Rydziel S, Canalis E: Expression and regulation of CCN genes in murine osteoblasts. *Bone* 2006;38:671-677.
- 12 Luo Q, Kang Q, Si W, Jiang W, Park JK, Peng Y, Li X, Luu HH, Luo J, Montag AG, Haydon RC, He TC: Connective tissue growth factor (CTGF) is regulated by Wnt and bone morphogenetic proteins signaling in osteoblast differentiation of mesenchymal stem cells. *J Biol Chem* 2004;279:55958-55968.
- 13 Pereira RC, Durant D, Canalis E: Transcriptional regulation of connective tissue growth factor by cortisol in osteoblasts. *Am J Physiol Endocrinol Metab* 2000;279:E570-576.
- 14 Sakai Y, Balam TA, Kuroda S, Tamamura N, Fukunaga T, Takigawa M, Takano-Yamamoto T: CTGF and apoptosis in mouse osteocytes induced by tooth movement. *J Dent Res* 2009;88:345-50.
- 15 Cobb MH, Goldsmith EJ: How MAP kinases are regulated. *J Biol Chem* 1995;270:14843-14846.
- 16 Pardo OE, Arcaro A, Salerno G, Raguz S, Downward J, Seckl MJ: Fibroblast growth factor-2 induces translational regulation of Bcl-XL and Bcl-2 via a MEK-dependent pathway: correlation with resistance to etoposide-induced apoptosis. *J Biol Chem* 2002;277:12040-12046.
- 17 Hsu HJ, Lee CF, Locke A, Vanderzyl SQ, Kaunas R: Stretch-induced stress fiber remodeling and the activations of JNK and ERK depend on mechanical strain rate, but not FAK. *PLoS One* 2010;5:e12470.
- 18 Ji G, Liu D, Liu J, Gao H, Yuan X, Shen G: p38 mitogen-activated protein kinase up-regulates NF-kappaB transcriptional activation through RelA phosphorylation during stretch-induced myogenesis. *Biochem Biophys Res Commun* 2010;391:547-551.
- 19 Li JL, Cui B, Qi L, Li XY, Deng LF, Ning G, Liu JM: NMDA enhances stretching-induced differentiation of osteoblasts through the ERK1/2 signaling pathway. *Bone* 2008;43:469-475.
- 20 Boutahar N, Guignandon A, Vico L, Lafage-Proust MH: Mechanical Strain on Osteoblasts Activates Autophosphorylation of Focal Adhesion Kinase and Proline-rich Tyrosine Kinase 2 Tyrosine Sites Involved in ERK Activation. *J Biol Chem* 2004;279:30588-30599.
- 21 Chaqour B, Goppelt-Strube M: Mechanical regulation of the Cyr61/CCN1 and CTGF/CCN2 proteins. *FEBS J* 2006;273:3639-3649.
- 22 Davis MJ, Meininger GA, Zawieja DC: Stretch-induced increases in intracellular calcium of isolated vascular smooth muscle cells. *Am J Physiol* 1992;263:H1292-1299.
- 23 Awolesi MA, Sessa WC, Sumpio BE: Cyclic strain upregulates nitric oxide synthase in cultured bovine aortic endothelial cells. *J Clin Invest* 1995;96:1449-1454.
- 24 Sumpio BE, Widmann MD, Ricotta J, Awolesi MA, Watase M: Increased ambient pressure stimulates proliferation and morphologic changes in cultured endothelial cells. *J Cell Physiol* 1994;158:133-139.
- 25 Hishikawa K, Lüscher TF: Pulsatile stretch stimulates superoxide production in human aortic endothelial. *Circulation* 1997;96:3610-3616.
- 26 Hishikawa K, Oemar BS, Yang Z, Lüscher TF: Pulsatile stretch stimulates superoxide production and activates nuclear factor-kappa B in human coronary smooth muscle. *Circ Res* 1997;81:797-803.
- 27 Riser BL, Denichilo M, Cortes P, Baker C, Grondin JM, Yee J, Narins RG: Regulation of connective tissue growth factor activity in cultured rat mesangial cells and its expression in experimental diabetic glomerulosclerosis. *J Am Soc Nephrol* 2000;11:25-38.
- 28 Chaqour B, Yang R, Sha Q: Mechanical stretch modulates the promoter activity of the profibrotic factor CCN2 through increased actin polymerization and NF-kappaB activation. *J Biol Chem* 2006;281:20608-20622.
- 29 Chowdhury I, Chaqour B: Regulation of connective tissue growth factor (CTGF/CCN2) gene transcription and mRNA stability in smooth muscle cells. Involvement of RhoA GTPase and p38 MAP kinase and sensitivity to actin dynamics. *Eur J Biochem* 2004;271:4436-4450.
- 30 Rosales OR, Isales CM, Barrett PQ, Brophy C, Sumpio BE: Exposure of endothelial cells to cyclic strain induces elevations of cytosolic Ca^{2+} concentration through mobilization of intracellular and extracellular pools. *Biochem J* 1997;326:385-392.
- 31 Matsuda N, Morita N, Matsuda K, Watanabe M: Proliferation and differentiation of human osteoblastic cells associated with differential activation of MAP kinases in response to epidermal growth factor, hypoxia, and mechanical stress in vitro. *Biochem Biophys Res Commun* 1998;249:350-354.