Mini Review

Role of mechanical stress in mandible bone metabolism

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Mechanical stress is an important factor in the regulation of bone metabolism. In addition, osteoblasts play a crucial role in bone metabolism. Osteoblasts produce bone matrix and osteotropic cytokines such as receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin (OPG). It was recently reported that mechanical stress loading affects the regulation of inflammatory cytokine, RANKL, and OPG expression by osteoblasts. The changes of the expression of these cytokines are thought to play a role in bone remodeling. The mandible is continuously exposed to mechanical stressors such as occlusal force. However, the mechanism by which occlusal force affects the mandible has not yet been determined at the molecular level. This article reviews the rapid progress made in the past few years to understand the role of mechanical stress in mandible bone metabolism.

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Introduction

Mechanical stress is known to be an important factor in the regulation of bone remodeling. Unsuitable mechanical conditions such as excessive mechanical stress or weightlessness may result in unbalanced bone remodeling[1-3].

It was recently reported that mechanical stress loading affects the regulation of inflammatory cytokine[4,5], receptor activator of nuclear factor κB ligand (RANKL), and osteoprotegerin (OPG)[6-8] expression by osteoblasts. We have also reported that mechanical stresses, such as hydrostatic pressure, induce cytokine production in human periodontal ligament cells[9,10]. The changes in the expression of these cytokines are believed to play a role in bone remodeling.

Mammalian bone has two distinct origins and two distinct processes of osteogenesis. The mandibula originates from the neural crest and induces osteogenesis in the in-

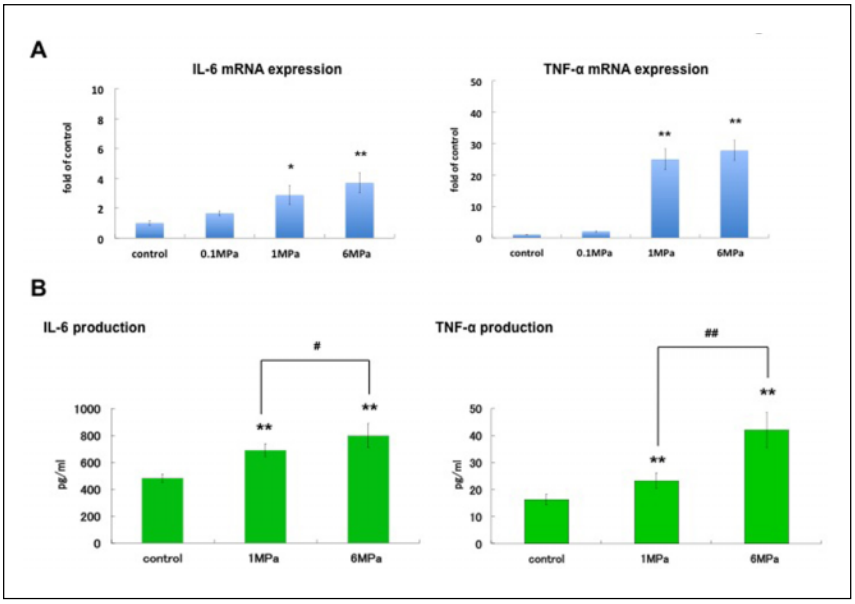

Fig.1 Effects of mechanical stress on inflammatory cytokine levels in mandible-derived osteoblasts (MDOB)
(A): Expression of IL-6 and TNF-α mRNAs. The mRNA levels of these cytokines were analyzed using Real-time RT-PCR.
(B): Production of IL-6 and TNF-α proteins. The levels of IL-6 and TNF-α proteins were measured using an ELISA kit.
MDOB were exposed to various pressures for 60 minutes.
*p<0.05; **p<0.01 vs. the control. p<0.05 and p<0.01 indicate significant differences between the experiments (n=4, values are expressed as mean ±SD).

tramembranous bone formation mode different from other main body bone that originate from mesoderm and osteogenize on endochondral bone formation mode[11]. In spite of the close resemblance of the end products, osteoblasts may have different signaling mechanisms and functions in each part of the processes to produce these differences. In the dental region, occlusal force is the representative mechanical stress, which can reach approximately 6 MPa[12]. The mandible is constantly exposed to occlusal force, and hence, it is one of the most important regulators of mandible homeostasis. However, there is little information available concerning the influence of mechanical stress similar to occlusal force on the mandible osteoblasts.

In this mini-review, we discuss the role of mechanical stress on mandible bone remodeling and describe the findings of our recent study.

Effects of mechanical stress on inflammatory cytokine production in mandible-derived osteoblasts (MDOB)

We first analyzed the effects of mechanical stress on inflammatory cytokine expression and production. Inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are known to activate osteoclastogenesis and RANKL expression in osteoblasts[13], T cells[13], and periodontal ligament cells[14]. In addition, recent studies have shown that TNF-α directly stimulates the differentiation of osteoclast progenitors to osteoclasts, and this process is not dependent on the RANKL/RANK interaction[15].

Our study showed that mechanical stress loading induces the expression of IL-6 and TNF-α mRNA. In addition, the mRNA levels of IL-6 and TNF-α in MDOB increase in a magnitude-dependent manner (Fig.1A)[16]. Furthermore, IL-6 and TNF-α protein production from MDOB were also augmented in a magnitude-dependent manner after exposure to mechanical stress (Fig.1B).

Effects of mechanical stress on RANKL and OPG production and osteoclastogenesis

Next, we investigated the effects of mechanical stress on RANKL and OPG expression and production. After exposure to mechanical stress, we co-cultured MDOB with RAW 267.4 murine monocyte/macrophage cells and performed tartrate-resistant acid phosphate (TRAP) staining to examine whether MDOB induces osteoclast differentiation. RANKL is a member of the TNF ligand family and activates osteoclastogenesis by binding to its receptor RANK on osteoclast progenitors[17, 18]. In contrast, OPG, which is a member of the TNF receptor family, acts as a non-signaling decoy receptor that binds to RANKL and prevents osteoclast differentiation and activation[17, 19]. This RANK/RANKL/OPG axis controls the balance between bone formation and resorption[13, 20]. Under physiological conditions, bone is resorbed periodically by osteoclasts,

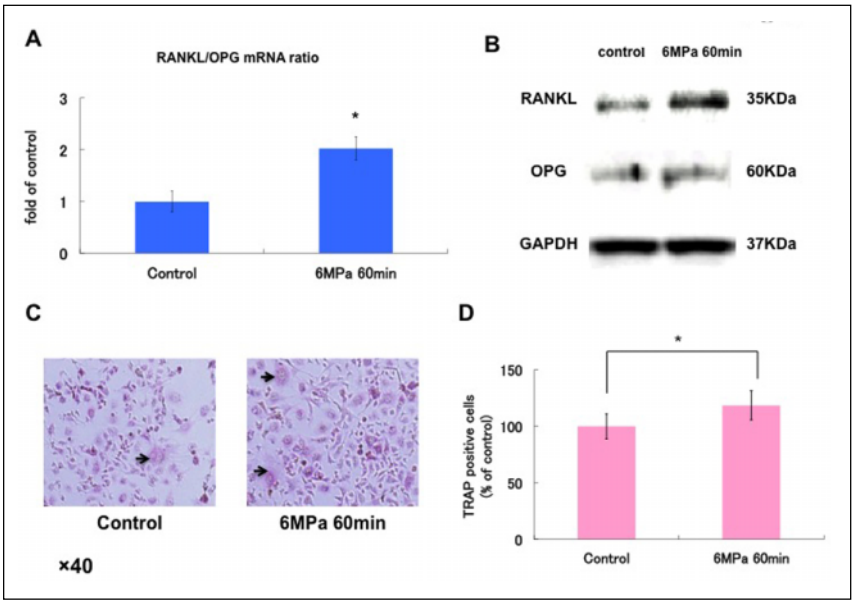

Fig.2 Effects of mechanical stress on RANKL and OPG expression in MDOB and on osteoclastogenesis

(A): RANKL/OPG mRNA ratio. The mRNA levels of the cytokines were analyzed using real-time RT-PCR.

(B):RANKL, OPG, and GAPDH protein expression. The protein levels of RANKL and OPG were investigated using western blot analysis.

(C):Photomicrographs of TRAP staining of RAW 264.7 cells co-cultured with MDOB after exposure to mechanical stress (magnification, x40).

(D):The percentage of TRAP-positive multinucleate cells. TRAP-positive multinucleate cells with ≥3 nuclei were counted.

MDOB were exposed to a pressure of 6 MPa for 60 minutes.

*p<0.05 vs. the control (n=4-5, values are presented as mean ±SD).

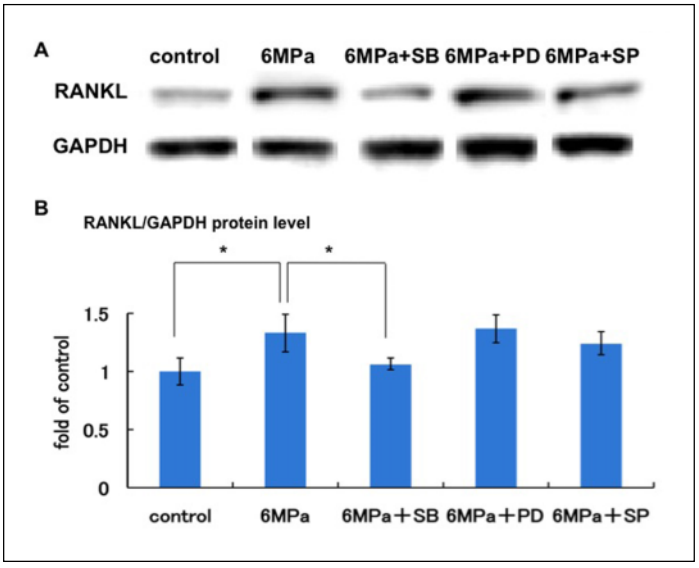

Fig.3 Effects of MAPK inhibition on mechanical stress-induced RANKL upregulation

(A): RANKL and GAPDH protein levels after exposure to 6 MPa and 6 MPa + each MAPK inhibitor.

(B): The ratio of RANKL/GAPDH production after exposure to 6 MPa and 6 MPa + each MAPK inhibitor.

*p<0.05 vs. the 6 MPa loading group. n=5, values are presented as mean ±SD.

SB: SB202190, a p-38 pathway-specific inhibitor; PD: PD98059, a ERK1/2 pathway-specific inhibitor; and SP: SP600125, a JNK pathway-specific inhibitor.

whereas new bone is formed by osteoblasts. Thus, the bone remodeling process is initiated by osteoclast activation, after which old bone resorption occurs[20]. Subsequently, osteoblasts are activated, and new bone formation ensues[21].

Mechanical stress loading changed the RANKL/OPG ratio in favor of RANKL at the mRNA and protein levels (Fig.2A, B)[16]. In addition, MDOB loaded with mechanical stress showed higher numbers of TRAP-positive cells than unloaded MDOB (Fig.2C, D). These results indicate that in the mandible, mechanical stress loading could stimulate the activation of bone remodeling via osteoclastogenesis.

Identification of intracellular signaling for RANKL upregulation-induced mechanical stress loading

The current manuscript reported that RANKL is induced via mitogen activated protein kinase (MAPK), NF-κB, signal tranducer and activator of transcription-3 (STAT3) and phosphoinositide 3-kinase (PI3K) pathway[22-24]. In addition, there are several studies that MAPK perform the functional roles in regulating cytokine production in osteoblasts[25-27]. However, the role of MAPK on RANKL expression in MDOB loaded mechanical stress similar to occlusal force has not yet been estimated. To confirm the signal transduction

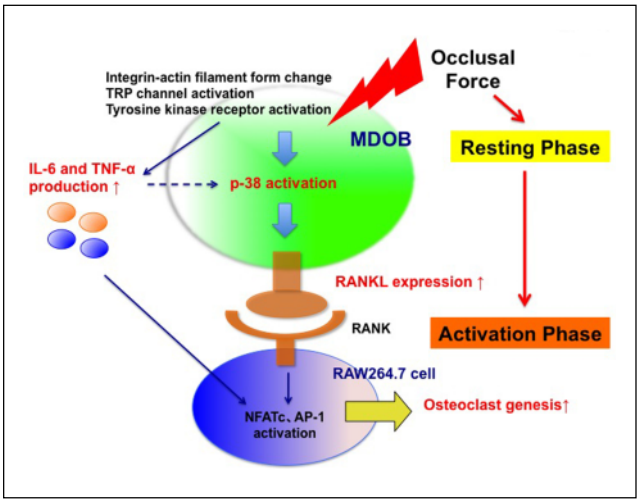

Fig.4 Schematic representation of the effects of mechanical stress on MDOB

pathway of mechanical stress-induced RANKL production in MDOB, we used MAPK inhibitors of extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p-38. MAPK cascades are among the most well-studied and well-established signal transduction systems.

We demonstrated that the upregulation of RANKL production induced by mechanical stress was significantly suppressed by the addition of a p-38-specific inhibitor (SB203580) but not by the addition of ERK1/2- and JNK-specific inhibitors (PD98059 and SP600125, respectively) (Fig.3)[16].

Conclusion

Mechanical stress is a key regulator of bone metabolism. Moreover, the mandible is constantly exposed to occlusal force, which is a type of mechanical stress.

We demonstrated that in MDOB, mechanical stress loading augmented the production of inflammatory cytokines and changed the RANKL/OPG ratio in favor of RANKL. In addition, MDOB exposed to mechanical stress induced osteoclastogenesis in RAW 264.7 cells. Moreover, in MDOB, mechanical stress upregulates RANKL expression via the p-38 pathway (Fig.4).

These results suggest that MDOB play a role in cytokine production in response to mechanical stress and that occlusal force may support the maintenance of mandible bone homeostasis by activating bone remodeling by osteoclastogenesis *in vivo*.

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References

1) Huiskes R, Ruimerman R, van Lenthe GH, Jassen JD: Effects of mechanical force on maintenance and adaptation of form in trabecular bone. Nature. 2000; 405: 704-706.
2) Garmeliet G, Vico L, Bouillon R: Space flight: a challenge for normal bone homeostasis. Crit Rev Eukaryot Gene Expr. 2001; 11: 131-144.
3) Ehrlich PJ, Lanyon LE: Mechanical strain and bone function: a review. Osteoporos Int. 2002; 13: 688-700.
4) Koyama Y, 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 Sao-2 cells. Arch Oral Biol. 2008; 53: 488-496.
5) Sakao K, Takahashi KA, Arai Y, Saito M, Honjo K, Hiraoka N, Asada H, Shin-Ya M, Imanishi J, Mazda O, Kubo T: Osteoblasts derived from osteophytes produce interleukin-6, interleukin-8, and matrix metalloprotease-13 in osteoarthritis. J Bone Miner Metab. 2009; 27: 412-423.
6) Tang L, Lin Z, Li YM: Effects of different magnitude of mechanical strain on osteoblasts in vitro. Biochem Biophys Res Commun. 2006; 344: 122-128.
7) Kreja L, Liedert A, Hasni S, Claes L, Ignatius A: Mechanical regulation of osteoclastic gene in human osteoblasts. Biochem Biophy Res Commun. 2008; 368: 582-587.
8) Nakai T, Yoshimura Y, Deyama Y, Suzuki K, Iida J: Mechanical stress up-regulates RANKL expression via the VEGF autocrine pathway in osteoblastic MC3T3-E1 cells. Mol Med Report. 2009; 2: 229-234.
9) Yamamoto T, Kita M, Kimura I, Oseko F, Terauchi R, Takahashi K, Kubo T, Kanamura N: Mechanical stress induces expression of cytokines in human periodontal ligament cells. Oral Dis. 2006; 12: 171-175.
10) Yamamoto T, Kita M, Yamamoto K, Akamatsu Y, Oseko F, Kanamura N: Mechanical stress enhances production of cytokines in human periodontal ligament

cells induced by Porphyromonas gingivalis. Arch Oral Biol. 2011; 56: 251-257.
11) Chung UI: Essential role of hypertrophic chondrocytes in endochondral bone development. Endocr J. 2004; 51: 19-24.
12) Nagai I, Tanaka N, Noguchi M, Suda Y, Sonoda T, Kohama G: Changes in occlusal state of patients with mandibular prognasthism after orthognathic surgery: a pilot study. Br J Oral Maxillofac Surg. 2001; 39: 429-433.
13) Takayanagi H: Mechanistic insight into osteoclast differentiation in osteoimmunology. J Mol Med. 2005; 83: 170-179.
14) Yamaguchi M: RANK/RANKL/OPG during orthodontics tooth movement. Orthod Craniofac Res. 2009; 12: 113-119.
15) Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Morinaga T, Higashio K, Martin TJ, Suda T: Tumor necrosis alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med. 2000; 17: 275-286.
16) Yamamoto K, Yamamoto T, Ichioka H, Akamatsu Y, Oseko F, Mazda O, Imanishi J, Kanamura N, Kita M: Effects of mechanical stress on cytokine production in mandible-derived osteoblasts. Oral Dis. 2011; 17: 712-719.
17) Takayanagi H: Inflammatory bone destruction and osteoimmunology. J Priodont Res. 2005; 40: 287-293.
18) Wada T, Nakashima T, Hiroshi N, Penninger LM: RANKL-RANK signaling in osteoclastgenesis and bone disease. Trends Mol Med. 2006; 12: 17-25.
19) Khosla S: Minireview: the OPG/RANKL/RANK system. Endocrinology. 2001; 142: 5050-5055.
20) Sims NA, Gooi JH: Bone remodeling: Multiple cellular interaction required for coupling of bone formation and resorption. Semin Cell Dev Biol. 2008;19: 444-451.
21) Boyce BF, Xing L: Function of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys. 2008; 15: 139-146.
22) Chen JR, Shankar K, Nagarajan S, Badger TM, Ronis MJ: Protective effects of estradiol on ethanol-induced bone loss involve inhibition of reactive oxygen species generation in osteoblasts and downstream activation of the extracellular signal-regulated kinase/signal transducer and activator of transcription 3/receptor activator of nuclear factor-kappaB ligand signaling cascade. J Pharmacol Exp Ther. 2007; 324: 50-59.
23) Tsubaki M, Kato C, Manno M, Ogaki M, Satou T, Itoh T, Kusunoki T, Tanimori Y, Fujiwara K, Matsuoka H, Nishida S: Macrophage inflammatory protein-1alpha (MIP-1alpha) enhances a receptor activator of nuclear factor kappaB ligand (RANKL) expression in mouse bone marrow stromal cells and osteoblasts through MAPK and PI3K/Akt pathways. Mol Cell Biochem. 2007; 304: 53-60.
24) Li X, Kim KW, Cho ML, Ju JH, Kang CM, Oh HJ, Min JK, Lee SH, Park SH, Kim HY: IL-23 induces receptor activator of NF-kappaB ligand expression in fibroblast-like synoviocytes via STAT3 and NF-kappaB signal pathways. Immunol Lett. 2010; 127: 100-107.
25) Kim YH, Kim JM, Kim SN, Kim GS, Baek JH: p44/42 MAPK activation is necessary for receptor activator of nuclear factor-kappaB ligand induction by high extracellular calcium. Biochem Biophys Res Commun. 2003; 304: 729-735.
26) Luo XH, Guo LJ, Xie H, Yuan LQ, Wu XP, Zhou HD, Liao EY: Adiponectin stimulates RANKL and inhibits OPG expression in human osteoblasts through the MAPK signaling pathway. J Bone Miner Res. 2006; 21: 1648-1656.
27) Eda H, Shimada H, Beidler DR, Monahan JB: Proinflammatory cytokines, IL-1β and TNF-α, induce expression of interleukin-34 mRNA via JNK- and p44/42 MAPK-NF-κB pathway but not p38 pathway in osteoblasts. Rheumatol Int. 2010; 31: 1525-1530.