Mini Review

Role of mechanical stress in mandible bone metabolism

Kenta Yamamoto¹,²,*), Toshiro Yamamoto¹), Narisato Kanamura¹) and Masakazu Kita²)
¹)Department of Dental Medicine, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan
²)Department of Microbiology and Immunology, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan

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.


*Correspondence should be addressed to: Kenta Yamamoto, Department of Dental Medicine, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kajii-cho 465, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan. Phone/Fax: +81-75-251-5641, E-mail: fiori30@koto.kpu-m.ac.jp

Keywords: bone remodeling, mechanical stress, osteoblast, osteoprotegerin, receptor activator of nuclear factor κB ligand

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 remodeling¹⁻³).

It was recently reported that mechanical stress loading affects the regulation of inflammatory cytokine⁴⁻⁶), receptor activator of nuclear factor κB ligand (RANKL), and osteoprotegerin (OPG)⁷⁻⁸) expression by osteoblasts. We have also reported that mechanical stresses, such as hydrostatic pressure, induce cytokine production in human periodontal ligament cells⁹,¹⁰). 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 mandible originates from the neural crest and induces osteogenesis in the in-
tramembranous bone formation mode different from other main body bone that originate from mesoderm and osteogenize on endochondral bone formation mode. 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. 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, T cells, and periodontal ligament cells. 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.

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). 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. 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. This RANK/RANKL/OPG axis controls the balance between bone formation and resorption. Under physiological conditions, bone is resorbed periodically by osteoclasts,
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 transducer and activator of transcription-3 (STAT3) and phosphoinositide 3-kinase (PI3K) pathway. In addition, there are several studies that MAPK perform the functional roles in regulating cytokine production in osteoblasts. 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...
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)\(^6\).

**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.*

**Acknowledgements**

We thank Dr. Kubo from the Department of Orthopaedics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, for supplying the hydrostatic pressure apparatus. Support for this research was provided by Kyoto Prefectural University of Medicine. The authors of this manuscript declare no conflict of interest.

**References**


---

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