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Changes in the spatial distribution of sclerostin in the osteocytic lacuno-canalicular system in alveolar bone due to orthodontic forces, as detected on multimodal confocal fluorescence imaging analyses

Yuriko Nishiyama [a,1], Tsutomu Matsumoto [a,b,1], Ji-Won Lee [a,c], Takashi Saitou [d], Takeshi Imamura [c,d,e], Keiji Moriyama [b], Akira Yamaguchi [a,*], Tadahiro Iimura [a,c,e,**]

[a] Section of Oral Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, 113-8549, Japan

[b] Section of Maxillofacial Orthognathics, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, 113-8549, Japan

[c] Division of Bio-Imaging, Proteo-Science Center (PROS), Ehime University, Ehime, 791-0295, Japan

[d] Department of Molecular Medicine for Pathogenesis, Graduate School of Medicine, Ehime University, Ehime, 791-0295, Japan

[e] Translational Research Center and Artificial Joint Integrated Center, Ehime University Hospital, Ehime, 791-0295, Japan

ARTICLE INFO

Article history:
Accepted 23 August 2014

Keywords:
Bone remodelling
Osteocyte
Osteoblast
Osteoclast
Imaging
Mechanotransduction

ABSTRACT

Objective: Mechanical loading on the bone is sensed by osteocytes. Sclerostin is a molecule secreted by osteocytes that is downregulated by mechanical loading; therefore, its expression level is a potent sensor that indicates the spatial transduction of biomechanical properties in bone. This study applied macroconfocal microscopy to observe the spatial response of alveolar bone to orthodontic forces after immunofluorescence using anti-sclerostin antibodies.

Design: Orthodontic tooth movement with the Ni–Ti closed-coil spring was applied between the upper bilateral incisors and the left first molar of mice. Four days after this application, the animals were subjected to multimodal confocal fluorescence imaging analyses.

Results: Obvious downregulation of sclerotin in the osteocytic lacuna-canalicular system (LCS) was observed specifically in tensile sites of alveolar bone. Confocal-based three-dimensional fluorescence morphometry further quantitatively demonstrated that the distribution and expression of sclerostin in the tensile sites was significantly reduced compared to that observed in the corresponding control sites. Interestingly, the levels of sclerotin signals in the compression sites were significantly higher than those

* *Corresponding author at*: Section of Oral Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8549, Japan. Tel.: +81 3 5803 5451; fax: +81 3 5803 0188.

** *Corresponding author at*: Division of Bio-Imaging, Proteo-Science Center (PROS), Ehime University, Shitsukawa, Toon-City, Ehime, 791-0295, Japan. Tel.: +81 0 89 960 5507; fax: +81 0 89 960 5052.

E-mail addresses: akira.mpa@tmd.ac.jp (A. Yamaguchi), iimura@m.ehime-u.ac.jp (T. Iimura).

[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.archoralbio.2014.08.013

observed in the control sites, although the distribution of sclerotin was not significantly different.

Conclusions: Our observations suggest that spatial changes in the level and distribution of sclerostin in the alveolar LCS trigger successive bone remodelling due to orthodontic tooth movement. The multimodal confocal imaging analyses applied in this work will enhance comprehensive understanding regarding the spatial regulation of molecules of interest from the tissue to the cellular level.

1. Introduction

Osteocytes are cells buried in the mineralized bone matrix with extending numbers of dendritic cellular process that enable them to make contact with other osteocytes and bone cells on the bone surface, such as osteoblasts and osteoclasts [1]. A growing body of evidence obtained within the last decade has revealed the conceptual framework of osteocytes as master orchestrators of bone physiology, in which these cells are responsible for integrating the mechanical and biochemical signals that regulate the differentiation and function of osteoblasts and osteoclasts, thus governing bone modelling and remodelling [2,3].

Orthodontic tooth movement is achieved via a spatially well-balanced process of alveolar bone remodelling, with bone resorption and formation in compression and tensile sites, respectively [4]. Our research group recently documented the essential role of osteocytes in bone resorption during orthodontic tooth movement [5] using osteocyte-ablated mice in which the diphtheria toxin receptor-mediated cell knockout system was applied [6]. Orthodontic forces exert two distinct types of mechanical loading forces on alveolar bone: compression and tensile forces, which lead to bone resorption and formation, respectively. How osteocytes respond to these distinct forces remains to be explored.

Osteocytes are the major source of sclerostin, the protein product of the SOST gene [7–11]. Sclerostin antagonizes the canonical Wnt signaling pathway by binding to members of the Wnt coreceptor family, lipoprotein receptor-related proteins (LRP5/6), thereby interfering with Wnt ligands to interact with their receptor, Frizzled [12–14]. Since canonical Wnt signaling plays an essential role in early osteoblast differentiation, the sclerostin produced by osteocytes exerts an inhibitory effect on bone formation [14–16]. Mechanical loading decreases the expression of sclerostin; therefore, the sclerostin expression in osteocytes is proportional to the mechanical strain on bone [17].

In order to understand how osteocytes respond to the mechanical strain induced by orthodontic forces in a spatial manner at the tissue to cellular levels, we employed multimodal confocal imaging combining macroconfocal microscopy and confocal-based fluorescence morphometry to analyse spatial changes in the sclerostin expression in the osteocytic lacuna-canalicular system.

2. Materials and methods

2.1. Experimental animals

2.1.1. Animals and preparation of the skeletal tissues

Mice (laboratory strain C57BL6J) were purchased from a local distributor. Eight-week-old male mice were used in all experiments. All experimental procedures were approved by the Experimental Animal Committee of Tokyo Medical and Dental University (No. 0090252), and the experiments adhered to ARRIVE guidelines.

2.1.2. Orthodontic tooth movement

Orthodontic tooth movement with the Ni–Ti closed coil spring was achieved as described previously [5,18], and was applied continuously as a force of 10 gf between about 0.1 and 5.0 mm. In brief, we inserted this appliance between the upper bilateral incisors and the left first molar of mice. Four mice were treated with this orthodontic appliance; there were no cases of detachment of the appliance during the experiments. Successful bone remodelling was confirmed by the histological observation of Haematoxylin–Eosin staining and TRAP (tartrate-resistant acid phosphatase) staining on sections obtained 12 days after tooth movement, as described previously [5] (Supplementary Fig. 1). The right maxillary molars were used as the controls in each mouse.

2.1.3. Tissue preparation

Four days after the application of the orthodontic tooth movement, the maxillae were dissected from the mice under anesthesia with 0.1% ethylanthranilate, and then fixed in 4% paraformaldehyde at 4 °C. The specimens were decalcified in 20% ethylenediaminetetraaceticacid (EDTA) for three to four weeks at 4 °C. A total of 50 serial horizontal paraffin-embedded sections of the first molar region were cut from the first molar bifurcation to 300 μm toward the apex of the root [19]. The alveolar bone site encasing the distobuccal root of the upper first molar was focused [5] for the following confocal microscopy-based analyses, since this root was vertically enforced without rotation movement throughout the orthodontic tooth movement procedure [5]. We histologically evaluated 10 sections at 30-μm intervals in each animal, and analysed mid-horizontal sections of the whole distobuccal root.