APPLICATION NOTE

A1R MP+ Multiphoton Confocal Microscope

Development of Technology to Visualize Interaction of Osteoblasts and Osteoclasts in Living Bone Tissue

A research group led by Professor Masaru Ishii (Immune cell biology), Graduate School of Medicine, Osaka University, has developed a technology to observe the inside of living bones, and to simultaneously visualize osteoblasts, which make new bone, and osteoclasts, which dissolve old bone, using the A1R MP+ multi-photon confocal microscope, which is capable of deep tissue imaging. This application note introduces an example of the world's first successful imaging of the moment of direct contact and communication between osteoblasts and osteoclasts in living bone tissue.

Background of Experiment

Bones are dynamic organs that are constantly being regenerated. Bone remodeling is the combined interaction of osteoclasts and osteoblasts, resulting in a densely formed bone structure. However, when the balance between osteoclasts and osteoblasts is changed by aging or inflammation, it can cause bone and joint deformation due to osteoporosis, rheumatoid arthritis, etc. Correct understanding of the relationship between osteoblasts and osteoclasts is very important in treating these diseases. Several factors involved in bone remodeling have been reported so far, but the mode of interaction between osteoblasts and osteoclasts has not been elucidated (Fig. 1).

Therefore, the research group of Professor Ishii et al. set about verifying whether the behavior of osteoblasts and osteoclasts can be observed temporally and spatially in living bone tissue at high resolutions, using a multi-photon microscope as an evaluation system for the live imaging of bone tissue.

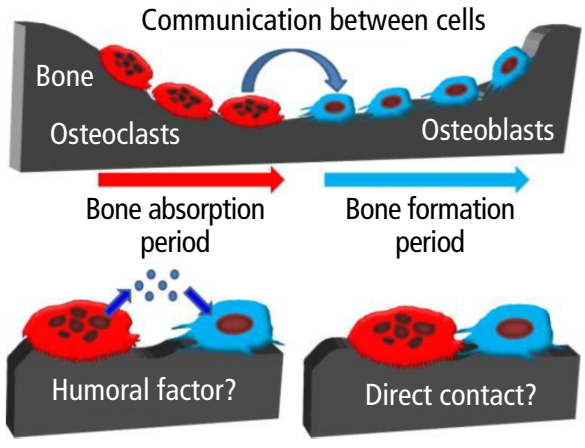

Fig. 1: Conceptual diagram of bone remodeling

Results

Professor Ishii et al. succeeded in simultaneously visualizing osteoblasts and osteoclasts in living bone tissue using a unique bioimaging technology, and capturing the moment when the two interact.

As a result, they were able to clarify that osteoblasts and osteoclasts, each forming a small population of dozens of cells, come into direct contact and communicate with each other in some areas, although no mutual contact between them is observed along most of their boundaries. In much of this cell-to-cell communication, it was observed that osteoclast protrusions form nerve synaptic-like structures that extend to osteoblasts, and that cell morphology changes over time (Fig. 2).

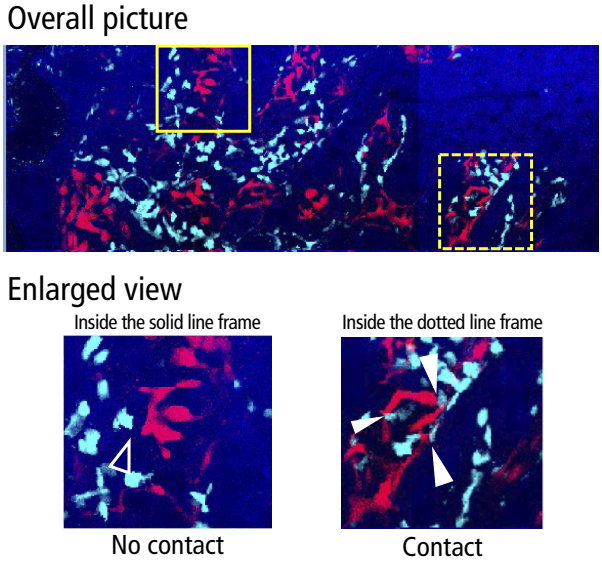

Fig. 2: Imaging of the inside of a living bone

The moment of direct contact and communication between osteoblasts expressing Col2.3-ECFP (light blue) and osteoclasts expressing TRAP-tdTomato (red) were captured. Blue is visualized bone tissue with SHG.
Objective: CFI75 Apochromat 25XC W 1300 (NA 1.10)

Additionally, live imaging was performed, utilizing a pH-responsive fluorescent probe that emits fluorescence by sensing the acid produced by osteoclasts, to shed light on the biological significance of communication between osteoblasts and osteoclasts. The results clarified that osteoclasts in contact with osteoblasts had a reduced ability to dissolve bone compared to those not in contact with osteoblasts. This consequently confirmed that physical contact between osteoblasts and osteoclasts plays an important role in the adjustment of bone remodeling.

Lastly, Professor Ishii et al. investigated utilizing the live imaging technology of this research as a drug efficacy evaluation system. They focused on a parathyroid hormone (PTH) analog*, which is clinically applied as an osteoporosis drug but whose mechanism of action is not well understood, and analyzed its effect on osteoblasts and osteoclasts in living bone tissue. A comparative study was conducted with groups that were treated with PTH analog for one week, three weeks, and six weeks respectively, in order to analyze the time course of the therapeutic effect of the PTH analog. As a result, an increase in direct cell-to-cell contact between osteoblasts and osteoclasts was observed in groups that were treated with the PTH analog for three weeks or more (Fig. 3a and Fig. 3b). These results revealed the in vivo mechanism of the PTH analog, which promotes interaction between osteoblasts and osteoclasts and increases bone mass by suppressing the function of osteoclasts.

* Human parathyroid hormone 1-34 (teriparatide) was approved for manufacture and sale in 2010 as a treatment for osteoporosis. It achieved sufficient bone mass gain due to strong bone formation and a proven fracture prevention effect, and is the first drug to be approved for the promotion of bone formation.

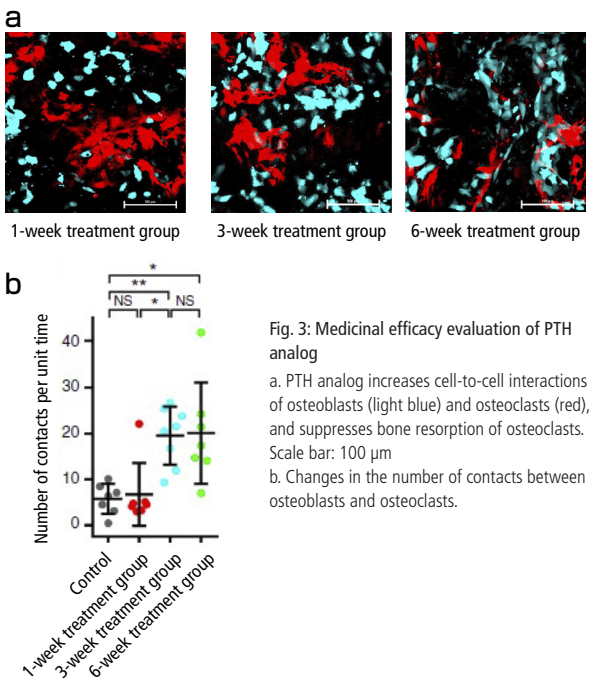

Fig. 3: Medicinal efficacy evaluation of PTH analog

a. PTH analog increases cell-to-cell interactions of osteoblasts (light blue) and osteoclasts (red), and suppresses bone resorption of osteoclasts. Scale bar: 100 μm

b. Changes in the number of contacts between osteoblasts and osteoclasts.

Summary

It has been difficult to prove the presence of cell-to-cell communication between osteoblasts and osteoclasts and biological significance thereof using the conventional analysis method of extracting fixed bone tissue and observing it with a microscope. However, detailed four-dimensional visualization of the surface of live bone was made possible in this study, verifying that physical contact between osteoblasts and osteoclasts controls the bone-dissolving function of osteoclasts. This can lead to the development of new treatments that control the balance between osteoblasts and osteoclasts for diseases in which bone structure is disrupted, such as osteoporosis and bone metastasis due to cancer.

Reference

Direct cell-to-cell contact between mature osteoblasts and osteoclasts dynamically controls their functions *in vivo*
NATURE COMMUNICATIONS | DOI: 10.1038/s41467-017-02541-w

Product Information

A1R MP+ multiphoton confocal microscope

This microscope achieves high-speed and high-resolution imaging because it is equipped with both resonant and Galvano scanners. It supports simultaneous excitation imaging using a 1300 nm-compatible dual beam IR laser as well as single-photon confocal imaging, and can be mounted on both upright and inverted microscopes.

- High speed: up to 720 fps (512 x 16 pixels)
- High resolution: up to 4096 x 4096 pixels