Large scale analysis of osteocyte lacunae in klotho hypomorphic mice using high-resolution micro-computed tomography.

(高解像度マイクロコンピュータ断層撮影による Klotho 低発現マウス骨小腔の大規模解析)

主指導教員：吉子 裕二教授

(医系科学研究科　硬組織代謝生物学)

副指導教員：津賀 一弘教授

(医系科学研究科　先端歯科補綴学)

副指導教員：加来 真人教授

(医系科学研究科　生体構造・機能修復学)

Faisal Ahmed

(医歯薬保健学研究科　医歯薬学専攻)

Background: Osteocytes, the most numerous cells in mature bone, interconnect via their dendritic processes to create the lacunocanalicular network in the mineralized bone matrix where they play a fundamental role in bone turnover and bone quality. Morphological anomalies in osteocytes and osteocyte lacunae

are known to be associated with skeletal impairment in mouse models of various conditions such as spontaneous type 1 diabetes, Schwartz-Jampel syndrome, and spinal cord injury. The analysis of fresh bovine tibiae using a digital image correlation strain measurement technique revealed that changes in lacunar geometry influence the transition of strains to the osteocyte microenvironment. However, the analysis areas in such studies are inevitably restricted, and there is always the question of whether the data reflect the results at an individual level. Micro-computed tomography (micro-CT) is a non-destructive technique to construct three-dimensional (3D) X-ray images of the internal structure of an object and applied to obtain bone morphometric parameters such as bone mineral density, bone volume, and bone mineral content in small laboratory animals. A digital image correlation strain measurement technique revealed that changes in lacunar geometry influence the transition of strains to the osteocyte microenvironment, and capturing these changes requires increasing the resolution levels of conventional micro-CT. However, new generation micro-CT systems have sub-micron resolution capabilities suitable for the analysis of osteocyte lacunae, and the analysis application CTAn allows discrimination of osteocyte lacunae from the vascular network in cortical bone using criteria for vesicular or tubular structures (closed or open pores). Mice with a homozygous hypomorphic mutation in the αKlotho gene (*kl/kl* mice) exhibit some characteristics resembling those seen in human aging, including osteopenia. Conventional microscopy and TEM revealed morphological anomalies in bone in *kl/kl* mice, e.g., pyknotic osteocytes and many lacunae lacking osteocyte.

Objective: With a goal to increase understanding of bone morphology and the anomalies in kl/kl mice, we applied high-resolution micro-CT to demonstrate and quantify multiple osteocyte lacunar parameters over an unprecedentedly large scale, i.e., within a thick (1.4 mm) slice, in a region of interest (ROI) in kl/kl mice versus wild type (WT) bone.

Materials and Methods: Klotho mutant (kl/+) and WT mice were purchased from CLEA Inc. Mice were housed and handled to minimize pain and discomfort according to protocols approved by the Institutional Animal Care and Use Committee at Hiroshima University. Tibias from 6-week-old male kl/kl and wild-type (WT) mice were isolated, fixed, trimmed, and embedded in epoxy resin. The cortical bone of the proximal metaphyseal tibias was scanned using the Skyscan 1272 high-resolution micro-CT system v1.1.9 (Bruker micro-CT). Data sets were reconstructed using NRecon, followed by an alignment using

DataViewer ROI was set at 2.5 mm away from the growth plate (total 2000 slices within 1.4 mm thickness), and osteocyte lacunar parameters (porosity parameters) were measured by a histogram-based global threshold using CTAn. Pores less than 50 μm3 and more than 2000 μm3 were excluded from the analysis. Osteocyte lacunae (closed pores) and blood vessels (open pores) in cortical bone were discriminated using CTAn, and visualized in CTVol software.

Results and conclusions: Scanning images of cortical bone at 700 nm resolution clearly demonstrated that osteocyte lacunae could be well visualized. Conventional bone parameters including bone volume (BV) and the number of osteocyte lacunae were not different between kl/kl and WT mice. However, the ratio of lacunar surface to lacunar volume and lacunar diameter (as a minor axis) were significantly smaller and greater, respectively, in kl/kl than WT mice, indicating that osteocyte lacunae in kl/kl mice are substantially different in shape, i.e., more spherical, compared to those in WT mice. The median frequency of low sphericity was significantly higher in WT than kl/kl mice, indicating that the oblateness of osteocyte lacunae was higher in WT than kl/kl mice. The median frequency of the major axis was significantly higher in WT than kl/kl mice, whereas the minor axis was higher in kl/kl mice rather WT mice. Differences between kl/kl mice and WT osteocyte lacunar diameter were more pronounced proximally than distally, suggesting that Klotho deficiency has a more profound effect on the tibial osteocyte lacunae on the proximal versus the distal side of the bone.

These data suggest that morphometric analysis of osteocyte lacunae by high-resolution micro-CT within optimized target ROIs may be useful in detecting pathological conditions. Thus, we successfully assessed osteocyte lacuna parameters on the largest scale ever in mice and demonstrated anomalies of osteocyte lacunae in kl/kl mice.