

## Globo-series glycosphingolipids deficiency in mice resulted in the attenuation of bone formation through decrease of osteoblasts

Kazunori Hamamura<sup>1</sup>, Kosuke Hamajima<sup>1,2</sup>, Yoshitaka Mishima<sup>1,2</sup>, Koichi Furukawa<sup>3</sup>, Ken Miyazawa<sup>2</sup>, Shigemi Goto<sup>2</sup>, Akifumi Togari<sup>1</sup>

<sup>1</sup>Dept. Pharmacol., Sch. Dent., Aichi Gakuin Univ., <sup>2</sup>Dept. Orthodontics, Sch. Dent., Aichi Gakuin Univ., <sup>3</sup>Dept. Biomed. Sci., Chubu Univ.

**Purpose:** Globo-series glycosphingolipids have not only been used as markers for stem cells and tumors but are also considered to regulate maintain stemness and immune system. However, there have no reports on the involvement of globo-series glycosphingolipids in bone metabolism. In this study, we investigated the effects of genetic deletion of Gb3 synthase, which initiates the synthesis of globo-series glycosphingolipids on bone metabolism.

**Material & Methods:** We examined expression levels of globo-series glycosphingolipids (Gb3, Gb4, and Gb5) in MC3T3 E1 mouse osteoblast-like cells and SaM-1 human osteoblast cells using flow cytometry. To determine whether globo-series glycosphingolipids are involved in bone metabolism, we analyzed bone phenotype of Gb3 synthase-knockout (Gb3S KO) mice using  $\mu$ CT. Furthermore, we conducted a calcein double labeling method to evaluate bone formation.

**Results & Conclusion:** Among Gb3, Gb4, and Gb5, only Gb4 was expressed in osteoblasts.  $\mu$ CT analysis revealed that femoral cancellous bone mass in Gb3S KO mice was lower than that in wild type (WT) mice. Calcein double labeling also revealed that bone formation in Gb3S KO mice was lower than that in WT mice. We demonstrated that Gb4 is expressed in osteoblasts, and globo-series glycosphingolipids regulate bone mass through bone formation.