Summary report of the ISS-Kibo utilization mission,
"Effect of microgravity on osteoclasts and the analysis of the gravity sensing system in medaka (Medaka Osteoclast)"

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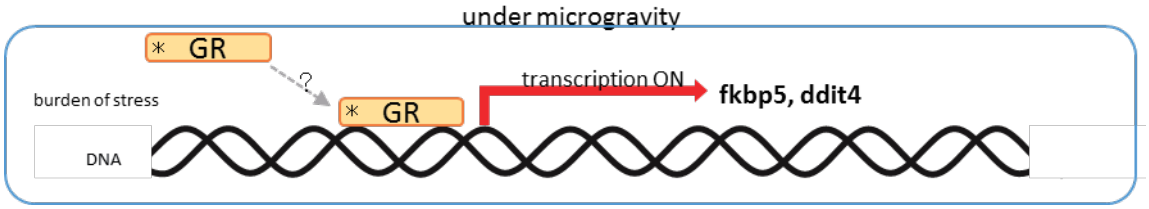
Bone density of astronauts decreases during space flight because of an imbalance between the activities of osteoclasts and osteoblasts. However, the molecular mechanism how these activities change in long-term space flight remains unclear.

Medaka fish serve as a model for research of microgravity environment on osteoclasts and osteoblasts. In particular, we have focused on the pharyngeal bone, which is the active bone remodeling tissue. To investigate the cellular activities related to bone formation and resorption under microgravity during animal growth, we examined the grown-bone from juvenile to adult fish for the first time in the space experiment. We established the TRAP promoter-GFP/Osterix promoter-DsRed double transgenic medaka line, in which osteoclasts and osteoblasts in the pharyngeal region are divided by two fluorescent signals. The 24 candidate fish selected from about 300 fish were transported to space, and reared for 2 months in AQH (Aquatic Habitat) in "Kibo". RNAs were isolated from 4 fish at day 60 after start of rearing in AQH, and PFA fixations were performed with 6 fish at day 14 and 6 fish at day 56. To study adaptation to the microgravity environment, medaka fish behaviors in AQH were filmed during 2 months, and the unique behaviors were observed, demonstrating that these behaviors were specified and adopted under microgravity. To examine the mineralization under microgravity, we performed the soft X-ray analysis. In the flight fish group at day 56, the calcified tissues in the pharyngeal bone region were decreased. Furthermore, BMD of the upper pharyngeal bone and the teeth region showed a significant decrease in the pQCT analysis. In addition, the μCT imaging showed the thinner bone in flight medaka. Regarding vertebral bones, BMD was slightly decreased. Furthermore, we found increase of GFP positive multi-nuclear osteoclasts, resulting that multi-nucleation was increased under microgravity. To study the molecular mechanism of bone loss in the spaceflight, RNAs were extracted from the medaka jaw, and the transcriptome analysis after cDNA sequencing was performed. Interestingly, some specific genes in the downstream of glucocorticoid receptor (GR) were strongly induced, suggesting that shear stress-like force caused by loosened tissues under microgravity activates GR, which probably enhances osteoclast activity (see the next page).



To investigate the mechanism of bone loss during spaceflight, it is important to study the initial response immediately after exposure to microgravity, because this response represents the trigger for bone loss. To examine the early effects of microgravity on bone cells, we embedded transgenic medaka larvae in a gel for a live-imaging study in space in 2014, and observed signals by fluorescence microscopy at the ISS via remote operation from Tsukuba Space Center. For this experiment, we utilized 4 different double medaka transgenic lines and, in particular, investigated up-regulation of fluorescent signals of osteoblasts and osteoclasts in these double transgenic lines as an important way to study osteoblast-osteoclast interaction under microgravity. In live-imaging for osteoblasts, intensity of *osterix*- and *osteocalcin*-DsRed in pharyngeal bones was significantly enhanced 1 day after launch, and this enhancement continued for 8 days and 5 days, respectively. In osteoclasts, the signals of *TRAP*-GFP and *MMP9*-DsRed were highly increased at day 4 and 6 after launch in flight. In addition, we examined the pattern of gene expression in these transgenic fish by transcriptome analysis. HiSeq analysis of the pharyngeal bones showed the enhanced expression of osteoblast- and osteoclast-related genes. Furthermore, Gene ontology analysis via RNA-Seq from the whole-body showed that transcription of “nucleus” was significantly enhanced; in particular, transcription-regulators were more up-regulated at day 2 than at day 6. Lastly, we identified 5 genes, *c-fos* and *jun-b*, *pai-1* and *ddit4*, and *tsc22d3*, which were up-regulated commonly in the whole-body at day 2 and 6, and in the pharyngeal bone at day 2.

Publications

1. Chatani, M., Mantoku, A., Takeyama, K., Abduweli,D., Sugamori, Y., Aoki, K., Ohya, K., Suzuki, H., Uchida, S., Sakimura, T., Kono, Y., Tanigaki, F., Shirakawa, M., Takano, Y. and Kudo, A. Microgravity promotes osteoclast activity in medaka fish reared at the international space station. *Sci. Rep*. 5: 14172 (2015)
2. Chatani, M., Morimoto, H., Takeyama, K., Mantoku, A., Tanigawa, N., Kubota, K., Suzuki, H., Uchida, S., Tanigaki, F., Shirakawa, M., Gusev, O., Sychev, V., Takano, Y., Itoh, T. and Kudo, A. Acute transcriptional regulation in osteoblasts/osteoclasts immediately after exposure to microgravity, uncovered by cell imaging in medaka. *Sci. Rep.* 6: 39545 (2016)