Real-time imaging and transcriptome analysis of medaka fish aboard space station

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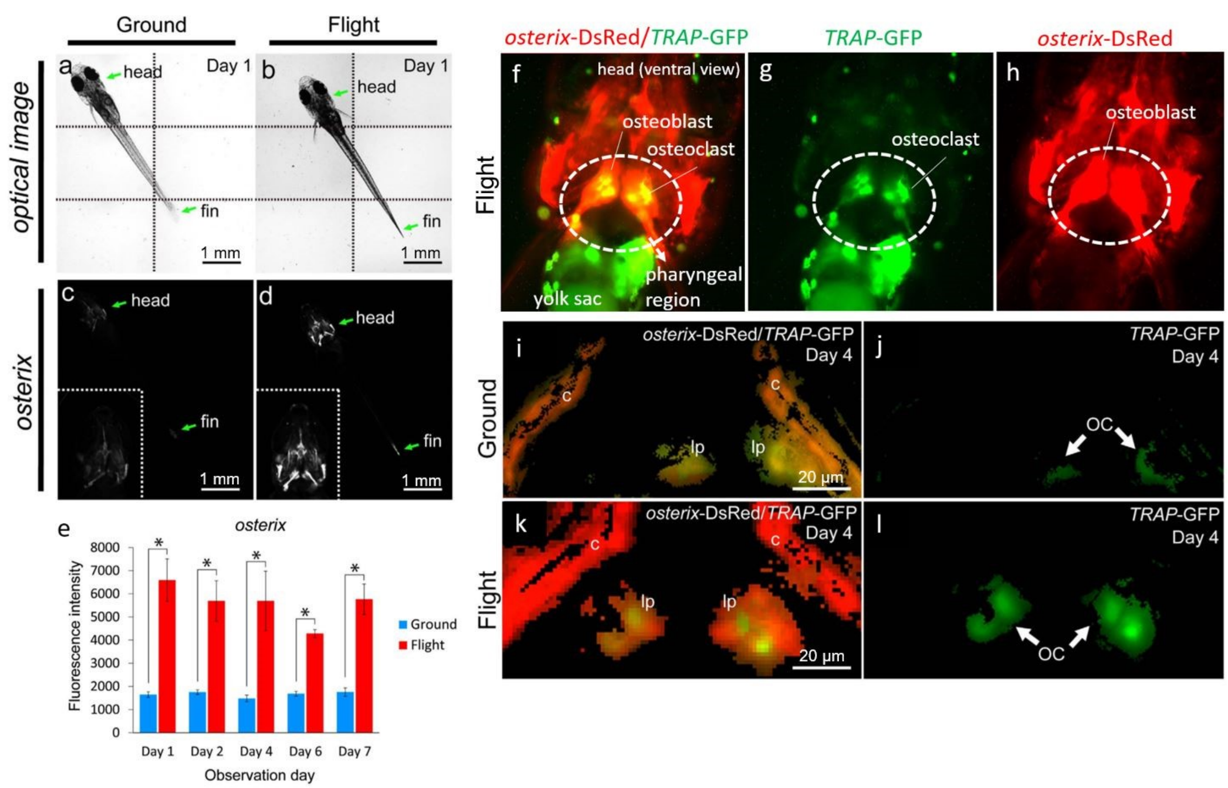


(a-d) Whole-body imaging of the osterix-DsRed transgenic line. The left-side images show the same ground control at day 1; and the right-side images, the same flight medaka at day 1. Arrows point to the head and fin region. All images show ventral views. Montage images were made from 6 captured optical images, divided by dotted lines (a,b). The white region shows an osterix-DsRed fluorescent signal. Embedded views show the enlarged head region (c,d). (e) The fluorescent intensity from day 1 to 7 of observation day constantly increased in the flight group. (f-h) The representative visualizing data for osterix-DsRed/TRAP-GFP in the flight group. All images show ventral views in the head region. (i-l) The merged images were captured by 3D views for osterix-DsRed and TRAP-GFP in the pharyngeal bone region of the double transgenic line. The pharyngeal bone region in the ground control (i) or the flight (k) group at day 4. The image for TRAP-GFP in the pharyngeal bone region of "i" (j) or "k" (l). lp, lower pharyngeal bone; c, cleithrum. GFP signals identify osteoclasts (OC). Credit: Tokyo Institute of Technology

Space travel in a reduced gravity environment can have lasting effects on the body. For example, studies clearly show that astronauts undergo a significant drop in bone mineral density during space missions, but the precise molecular mechanisms responsible for such changes in bone structure are unclear.

Now, Akira Kudo at Tokyo Tech, together with scientists in Japan in support of other countries, have performed remote, real-time, live imaging for fluorescent signals derived from osteoblasts and osteoclasts of medaka fish after only one day of exposure to microgravity aboard the International Space Station (ISS). They found increases in both osteoblast- and osteoclast-specific, promoter-driven GFP and DsRed signals one day after launch, which continued for up to eight days.

In their experiments, the team used four different double medaka transgenic lines focusing on up-regulation of fluorescent signals of osteoblasts and osteoclasts to clarify the effect of gravity on the interaction of osteoblast-osteoclast. They also studied changes in the gene expression in the transgenic fish by so-called transcriptome analysis.

These findings suggest that exposure to microgravity induced an immediate "dynamic alteration of gene expressions in osteoblasts and osteoclasts." Namely, these experiments based on real-time imaging of medaka from Earth and transcriptome analysis could be the prelude to the establishment of a new scientific research field of "gravitational biology."

To utilize transgenic medaka larvae in the space experiment, scientists and engineers carried fish eggs from Japan to Baikonur. They reared adult medaka fish in Moscow and collected eggs as a backup for delay of rocket launch (upper left). To perform in vivo imaging at international space station, they put hatching larvae into special gel in the fish chambers by using sterilized equipment (lower left). They carefully chose the first fish chambers judged by posture of fish (upper right) and put the chambers into blue boxes that were mounted in the Soyuz rocket (lower right; Mr. Tanigawa, the one on the far right and Dr. Chatani, the 2nd from the right). Credit: Tokyo Institute of Technology

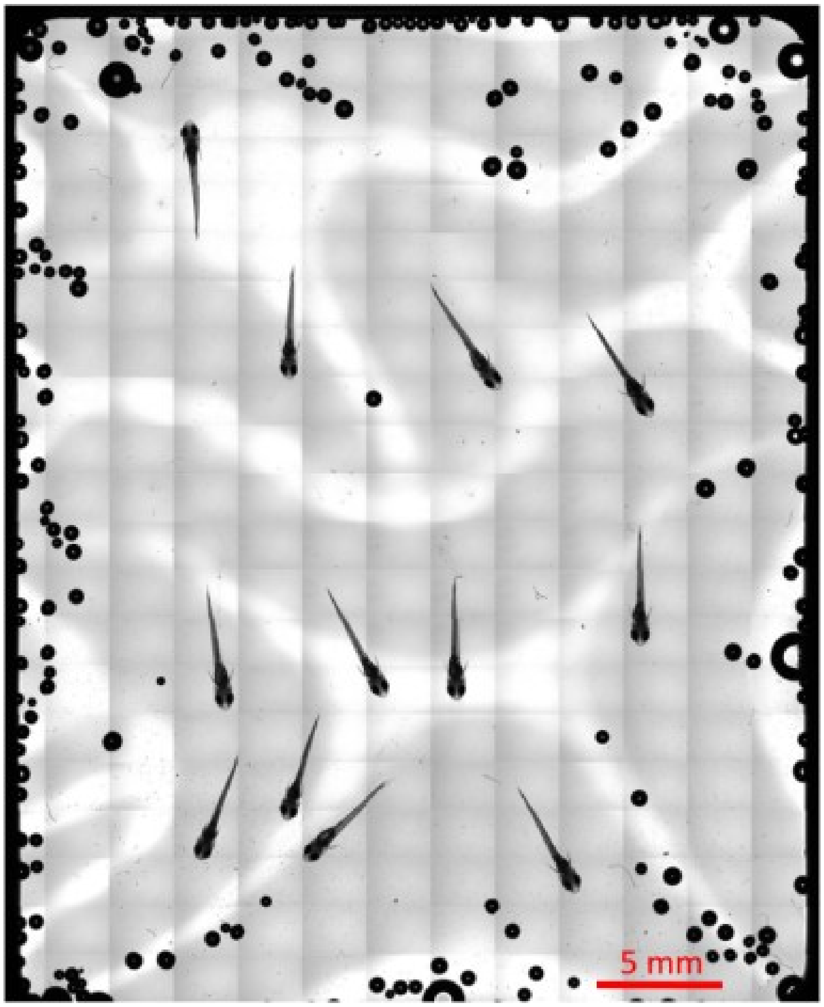
Methodology

The live imaging of fluorescence microscopy signals from the fish aboard the ISS were monitored remotely from Tsukuba Space Center in Japan.

Live imaging of osteoblasts showed an increase in the intensity of osterix- and osteocalcin-DsRed in pharyngeal bones one day after launch. This increased effect continued for eight days for osterix- and five days for osteocalcin.

In the case of osteoclasts, the fluorescent signals observed from TRAP-GFP and MMP9-DsRed increased significantly on the fourth and sixth days after launch.

The fluorescent analysis was complimented by using transcriptome analysis to measure gene expression in the transgenic fish. The researchers state that, "HiSeq from pharyngeal bones of juvenile fish at day two after launch showed up-regulation of two osteoblast- and three osteoclast- related genes".



Totally 273 images captured by 5x objective lens were integrated by Tiling method, showing an overall picture of the medaka chamber. This figure shows suitable localization of medaka fish for observation by 20x objective lens. Credit: Tokyo Institute of Technology

Also, transcription of the "nucleus" was found to be significantly enhanced based on whole-body gene ontology analysis of RNA-Seq, with the researchers observing transcription-regulators to be more up-regulated at day two compared with during day six.

Finally, Kudo and the team identified five genes: (c-fos and jun-b, pai-1 and ddit4, and tsc22d3) that were all up-regulated in the whole-body on days two and six, and in the pharyngeal bone on day two.

Life in so-called 'microgravity' environments—where the force of gravity is considerably less than on Earth—can cause significant problems for the human body. Astronauts who spend a number of months in space have been shown to suffer from reduced bone mineral density, leading to skeletal problems. Surprisingly, the loss of calcium starts at least 10 days after launch in astronauts in Skylab Flights.

In the next space experiment, Kudo and colleagues will clarify the role of glucocorticoid receptor (GR) on cells in microgravity.

More information: Masahiro Chatani et al, Acute transcriptional up-regulation specific to osteoblasts/osteoclasts in medaka fish immediately after exposure to microgravity, *Scientific Reports* (2016). DOI: 10.1038/srep39545

Provided by Tokyo Institute of Technology