1. Title

2. Research Term
FY (2000) ~2002

3. Research Fields
Biomedical Science

4. Research Categories
Phase IB Research

5. Research Theme
Signal transduction of cells cultured in mechanical tension and microgravitational environment

6. Investigators
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8. Summary of Research
Physical environment including gravity are believed to play an important role in muscle and bone atrophy
and / or regeneration in space flight. We developed here new methods for studying the effects of magnetic
force and multidirectional gravity vector environment on cell differentiation.

I. Effect of magnetic force
We examined the effect of magnetic force on differentiation of cultured human osteoblasts. Magnetic
microparticles (MPs) were introduced into the cytoplasm of a human osteoblast cell line and the cells were
cultured in a magnetic field (MF) in group MP-MF. Three groups of controls were used: cells without MPs
were cultured out of MF (group C), cells without MPs were cultured in MF (group MF), and cells with MPs
were cultured out of MF (group MP). We did not notice the difference in cell numbers among the four
experimental groups throughout the culture. The cells in group MP-MF became larger and were elongated
along the axis of the magnetic poles. Appearance of alkaline phosphatase (AlPase) activity, formation of
bone nodules and calcium deposition were accelerated depending on the intensity of the magnetic field. It
takes longer culture in the other three groups to exhibit these phenomena. Core-binding factor A1 (Cbfa1:
transcription factor for osteoblast differentiation) and osteocalcin (a bone-matrix protein involved in
controlling osteogenesis) were expressed earlier or stronger in group MP-MF than the other groups.

Then we compared phosphorylation of MAPK between group MP-MF and group C. Phosphorylation of
p38 MAPK (p38) was increased in group MP-MF, while total p38 as well as total and phosphorylated forms of
MAPK/ERK 1/2 and SAPK/JNK were not changed between the two groups. When a p38 inhibitor, SB 203580, was added to the culture medium in group C, AlPase activity, formation of bone nodules and calcium deposits were completely inhibited. On the other hand, they were inhibited only partially by a MAPK/ERK 1/2 inhibitor, U-0126. Based on these results, it is concluded that 1) osteoblast differentiation is accelerated by a magnetic force, 2) this acceleration is mainly attributed to the activation of p38 phosphorylation, and 3) the stimulus induced by a magnetic field offers a new approach to osteoblast differentiation.

II. Effect of multidirectional gravity vector

A 3D-clinostat which is a three-dimensional (3D)-clinostat is a device for multi-direction G force generation. By controlled rotation of two axes, a 3D-clinostat cancels the cumulative gravity vector at the center of the device and produces an environment with an average of $10^{-3}$G over time. We cultured a human osteoblast cell line in a 3D-clinostat (group CL), examined differentiation of the cells including morphology, histological detection of calcification and MAPK cascades and compared to those in a normal 1G environment (group C). Cell numbers were not different between the two groups thought the culture. In the group C, AlPase activity was detected on day 7 of culture, bone nodules were formed on day 12, and calcium deposits were seen on day 20. In the group CL, the cells looked larger and bulged. AlPase activity was detected on day 10 of culture. However, neither bone nodules nor calcification was found up to day 21. The expression levels of Cbfa1, and osteocalcin increased in group C but decreased in group CL. Phosphorylation of p38\_MAPK (p38) was repressed in culture in group CL, while total p38 as well as total and phosphorylated forms of ERK1/2 and SAPK/JNK were not changed in group CL. When a p38 inhibitor, SB 203580, was added to the culture medium in group C, AlPase activity, formation of bone nodules and calcium deposits were strongly inhibited. On the other hand, they were inhibited only partially by a MAPKK inhibitor, U-0126. Based on these results, it is concluded that 1) osteoblast differentiation is inhibited in culture in a 3D-clinostat, and 2) this inhibition is mainly due to the suppression of p38 phosphorylation.

Then, we cultured human mesenchymal stem cells (hMSC) in Dulbecco’s MEM for 3 days in the 3D-clinostat (group CL) or in 1G environment (group C), and the cell pellet was obtained from each culture. The pellet of hMSC was 8-times larger in group CL than in group C in 2 weeks. The pellet of each group was further cultured in a chondrocyte differentiation medium for 2 weeks in 1G. Type II collagen production was increased in group C, while it was not expressed in group CL. Phosphorylation of MAPK/ERK was repressed in group CL, while total MAPK/ERK was not changed. Then we transplanted the pellets into the cartilage defect made in the intercondylar fossa of the mouse femur. After 2 weeks, necrosis of the transplanted cells from group C was often seen. When the transplants were from group CL, necrosis was scarcely seen, and the transplants formed hyaline cartilage. These results suggest that hMSC remain more undifferentiated in culture in a 3D-clinostat, which makes better differentiation after transplantation.

9. Publication List
