PHOSPHOLIPASE Cγ2 IS REQUIRED FOR THE DEVELOPMENT OF CALCIUM-OSCILLATIONS IN OSTEOCLASTS

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Background. Osteoclasts are the unique bone-resorbing cells of hematopoietic origin, which are critically involved in diseases characterized by pathological bone loss, such as rheumatoid arthritis. Phospholipase Cγ2 (PLCγ2) is an important signaling molecule in hematopoetic lineages and it was shown to be required for inflammatory arthritis in mice. Here we aimed to test the role of PLCγ2 in *in vivo* bone metabolism, as well as in *in vitro* osteoclast cultures using PLCγ2-deficient (PLCγ2$^{-/-}$) mice.

Materials and methods. The trabecular architecture of the distal femoral metaphysis of wild-type (WT) and PLCγ2$^{-/-}$ mice was tested by micro-CT analysis. Bone marrow cells were isolated from long bones of WT and PLCγ2$^{-/-}$ mice, and then differentiated into osteoclasts *in vitro* in the presence of recombinant M-CSF and RANKL. For retroviral reconstitution of osteoclast precursors, Platinum-E cells were transfected with a bicistronic MSCV-based retroviral vector expressing PLCγ2 along with GFP from an internal ribosome entry site. Viral supernatants were collected and incubated with fetal liver cells obtained from WT and PLCγ2$^{-/-}$ embryos, and the cells were then differentiated into osteoclasts *in vitro*. Osteoclast development, function and gene expression was tested using *in vitro* osteoclast and macrophage cultures. For intracellular calcium measurements, the cells were loaded with 5 μM Fura-2-AM, 0.05% pluronic F127 and imaged with a fluorescent microscope.

Results. PLCγ2$^{-/-}$ mice had significantly higher trabecular bone mass under basal conditions than WT mice. PLCγ2 was required for *in vitro* development and resorptive function of osteoclasts, but not for the upregulation of osteoclast-specific genes. Bone marrow derived WT osteoclasts showed long lasting oscillations in the intracellular calcium concentrations, while the genetic deficiency of PLCγ2 completely blocked calcium-oscillations in the PLCγ2$^{-/-}$ osteoclast cultures. Retroviral reconstitution of PLCγ2 into PLCγ2$^{-/-}$ fetal liver derived osteoclast cultures - but not mock infection - restored the ability of the cells to show oscillations in the intracellular calcium levels.

Conclusion. Our results indicate that PLCγ2 participates in bone resorption under basal conditions, likely because of its role in the development of calcium-oscillations in osteoclasts.

Az absztrakt témája elméleti jellegű, és a poszter prezentációt választanám.