AnxA6-regulated bone mineralization by Saos-2 osteoblasts is Src and rock kinase dependent

Agnieszka Strzelecka-Kiliszek, Slawomir Pikula

Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland.
E-mail: a.strzelecka-kiliszek@nencki.gov.pl

In this study we used human osteosarcoma Saos-2 cells undergoing the osteoblastic differentiation program, producing the extracellular matrix and spontaneously releasing matrix vesicles (MVs) as a model to determine the role of AnxA6, the acto-myosin cortex as well as of Src and ROCK phosphorylation in bone mineralization. The mineralization process was stimulated with ascorbic acid/β-glycerophosphate and modulated using specific inhibitors. Calcium nodule detection by Alizarin Red-S staining showed that mineral formation is accompanied by changes in cell morphology. After stimulation, cells become round and release MVs with a mineral nodules inside. Stimulated cells produced a 5-fold higher amount of mineral phase within 12 days than control cells. SDS-PAGE analysis of whole cell lysates indicated that stimulation of the cells for mineralization induces also changes in their protein profile. We observed that mineralization index decreased in the presence of cytochalasin D (inhibitor of actin polymerization), PP2 (inhibitor of Src kinase activity) and Y-27632 (inhibitor of ROCK kinase activity) whereas it increased in the presence of blebbistatin (inhibitor of non-muscle myosin II). Immunofluorescence analysis showed that the enrichment in AnxA6 in MVs correlated with acto-myosin cortex rearrangements and release of MVs. In addition, localization of AnxA6 and non-muscle myosin IIA was affected in Saos-2 cells treated with Y-27632 and blebbistatin suggesting the role of ROCK phosphorylation and myosin IIA in mineral formation.

This study is supported by a grant N301 025 32/1120 from the Polish Ministry of Science and Higher Education.