

ANXA6-REGULATED BONE MINERALIZATION BY SAOS-2 OSTEOBLASTS: LIGHT, FLUORESCENT AND ELECTRON MICROSCOPE ANALYSIS

Agnieszka Strzelecka-Kiliszek¹, Malgorzata Statkiewicz¹, Rafal Bartosiewicz¹, Rene Buchet², Slawomir Pikula¹

¹Nencki Institute of Experimental Biology PAS, PL-02093 Warsaw, Poland

²Universite Lyon 1, ICBMS CNRS UMR 5246, F-69622 Villeurbanne, France

E-mail: a.strzelecka-kiliszek@nencki.gov.pl

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ABSTRACT: Bone is highly specialized tissue composed of cells (osteoclasts, osteoblasts and osteocytes) and the extracellular matrix containing mostly type-I collagen (an organic phase providing tensile strength) and hydroxyapatite (HA, an inorganic phase providing mechanical resistance). Little is known about the mechanisms regulating bone mineralization. It has been reported that osteogenesis correlates with changes in acto-myosin cell cortex. Annexins, a family of Ca²⁺- and phospholipid-binding proteins, were also suggested to be associated with mineralization, being involved in the Ca²⁺ homeostasis and in the Ca²⁺ influx into matrix vesicles (MVs) [1]. We selected human osteosarcoma Saos-2 cells undergoing the osteoblastic differentiation program, producing the extracellular matrix and spontaneously releasing MVs as a model to determine the role of AnxA6 and acto-myosin cortex in bone mineralization. In this respect, the mineralization process was stimulated with ascorbic acid and β-glycerophosphate and monitored using different microscopy techniques. Calcium nodule detection by Alizarin Red-S (AR-S) staining and light microscopy analysis showed that mineral formation depends on cellular morphology. After stimulation, cells started to be round in shape and release MVs with a mineral inside. Spectrophotometric determination of AR-S concentration confirmed that stimulated cells produced a 5-fold higher amount of mineral within 12 days than control cells. We also demonstrated that mineralization index decreased in the presence of cytochalasin D (inhibitor of actin polymerization), whereas it increased upon blebbistatin (inhibitor of non-muscle myosin II) treatment. Immunofluorescence microscopy analysis showed an enrichment of AnxA6 in MVs which was correlated with acto-myosin cortex rearrangements under the plasma membrane releasing MVs. Transmission electron microscopy analysis showed that mineral deposition occurs inside two types of cellular vesicles: MVs with characteristic trilamellar membranes and apoptotic bodies (ABs) surrounded by a single membrane. Moreover immunogold staining of mineralizing cells indicated that only MVs contain AnxA6 in their lumen and that non-muscle myosin IIA ring is formed to cut them off from the plasma membrane. Electron probe X-ray microanalysis of the mineral phase composition demonstrated higher spectral peaks for Ca and P in mineralizing cells. The Ca/P ratio decreased after stimulation with slightly higher value for MV than the theoretical value in stoichiometrically pure HA (1.67) contrary to ABs containing other types of minerals. Electron probe combined elemental maps of mineral phase first time presented that some ionic substitutions known to occur in pathological mineralization [2] are observed also in the minerals formed by the cell cultures.

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