The Effects of Modulating Osteoactivin Function on Osteoblast Differentiation

Presentation Time: Tuesday, 10:45 a.m. - 11:00 a.m.

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In a study examining differential gene expression in bone from normal and *osteopetrotic* (*op*) rats, we isolated a novel cDNA, termed osteoactivin (rOA), that was over-expressed in *op* when compared to normal bone. Subsequent *in situ* hybridization and immunohistochemical localization demonstrated that rOA mRNA and protein are expressed by osteoblasts. In primary osteoblast cultures, rOA mRNA levels exhibited a temporal pattern of expression being expressed at highest levels during the later stages of matrix maturation and mineralization. Furthermore, the protein is synthesized by osteoblasts and secreted into the medium. In this study we attempted to block rOA expression and function using an rOA anti-sense oligonucleotide or an anti-rOA antibody. Using an rOA anti-sense oligonucleotide, we were able to block rOA expression in primary osteoblast cultures resulting in decreased alkaline phosphatase activity, osteocalcin production, nodule formation and matrix mineralization. Using the Chariot protein transfection reagent as a vehicle to deliver the anti-rOA antibody inside the cells, we demonstrated that anti-rOA antibody treatment also showed a dose-dependent inhibition of alkaline phosphatase activity, osteocalcin production, nodule formation and mineralization. Conversely, a CMV-rOA construct was generated and used to examine the effect of rOA over-expression on osteoblast development and function in MC3T3-E1 osteoblast-like cells. rOA over-expression in cells transiently transfected with CMV-rOA increased nodule formation, alkaline phosphatase activity, osteocalcin production and matrix mineralization compared to cultures following transfection with an empty vector or treated with transfection reagent alone. These data suggest that rOA plays a major role in the regulation of osteoblast differentiation and function.