Osteoactivin: A Novel Factor That Regulates Osteoblast Development and Function

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Osteoblast development is a complex process involving the expression of specific growth factors and regulatory proteins that control cell proliferation, differentiation and maturation. We have previously identified a novel gene, termed osteoactivin (OA), which is more highly expressed in the bones of *osteopetrotic* mutant rats compared to their normal littermates. OA is a glycoprotein of 572 amino acids with a predicted molecular weight of 63.8 kDa and has 13 potential sites for *N*-linked glycosylation. In this study, we further examined OA expression in bone and its role in the differentiation of osteoblasts in primary cultures. In a comparison of long bones and calvaria with other tissues, Northern blot and RT-PCR analyses showed that OA is most highly expressed in bone compared with any of the other non-osseous tissues examined. *In situ* hybridization and immunohistochemical analyses of OA in normal bone revealed that it is primarily expressed in osteoblasts. In primary rat osteoblast cultures, OA showed a temporal pattern of expression, being expressed at highest levels during the later stages of matrix maturation and mineralization. To further study the properties of the OA protein, primary osteoblasts were cultured for 1 week, fixed, and incubated with a polyclonal antibody raised against amino acids 551-568 at the C-terminal end of the protein. OA co-localized with the endoplasmic reticulum and Golgi apparatus, suggesting that OA is a secreted protein. These results were confirmed by the detection of osteoactivin in the conditioned medium of primary osteoblast cultures by Western blot analysis. To test the effects of OA on osteoblast differentiation and function, we used an anti-OA antibody to block the OA that is constitutively produced in culture. Treatment with neutralizing OA-antibody inhibited nodule formation and mineralization in a dose-dependent manner. Collectively our results demonstrate that OA is a novel protein that is produced by osteoblasts and plays an important role in regulating osteoblast development and function.