Osteoactivin-Derived Peptides Induce Osteoblast Differentiation in MC3T3-E1 Cells

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We previously identified novel gene called osteoactivin (OA) in bone. OA was identified by differential display using total RNA from wild type compared to osteopetrotic (op) long bone and calvaria. In this study, we examined the role of OA in osteoblast differentiation in vitro using two anti-OA antibodies: anti-OA antibody 27 (Ab-27) and anti-OA 551 (Ab-551). These antibodies were raised against different regions of the molecule; Ab-27 was raised against the N-terminus and Ab-551 was raised against the C-terminus, a sequence that contains an RGD motif. We found that only Ab-551 significantly decreased osteoblast differentiation including, alkaline phosphatase activity, nodule formation and matrix mineralization. In order to test the role of the RGD motif of OA protein in osteoblast differentiation, we designed two peptides that mimic the sequence of the OA peptide used to generate Ab-551. The first peptide (OA-D) has the RGD domain and the second peptide (OA-E) has E (Glutamic acid) in the place of D (Aspartic acid). We examined the effect of these two peptides on osteoblast proliferation and differentiation in vitro. Although both peptides had no significant effect on osteoblast proliferation and/or viability, they significantly induced alkaline phosphatase activity, nodule formation and calcium deposition. Bioinformatic analysis of these peptides showed the presence of a serine residue that is potentially phosphorylated by casein kinase II (CK-II). Further analysis of other OA protein family members showed that there is conserved serine residue close to C-terminus, which matches the position of serine residue of the OA peptides. CK-II is known to phosphorylates many osteoblast-related proteins that regulate osteoblast development and differentiation such as osteopontin and vitronectin. Collectively, these data show that both OA-D and OA-E peptides significantly induced osteoblast differentiation in vitro and the effect of these peptides is RGD independent. Additional studies are warranted to determine if phosphorylation of the OA peptides by CK-II might be involved in their mechanism of action during osteoblast differentiation.