The Role of Splice-Variants of Periostin in Bone Development and Disease

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A splice-variant of Periostin, henceforth referred to as Periostin-Like-Factor (PLF) was identified by using the 'READS' differential display of mRNAs isolated from mouse tissues during various stages of development. Comparison of the predicted amino acid sequence of Periostin in mouse and human tissues with Periostin-Like-Factor in mouse tissues, showed differences in two regions at the carboxy-terminus. These differences are consistent with the two proteins being isoforms resulting from an alternately spliced gene. The alterations in amino acid sequence in these regions are probably functionally significant since the rest of the amino acids are highly conserved across species. During normal mouse embryogenesis and in osteopetrotic bone the temporal and spatial patterns of expression of these isoforms were determined using in situ hybridization and immunohistochemical analyses. We found that they were expressed during embryogenesis in preosteoblast mesenchymal cells and in bone precursor cells in the peri- and endosteum in osteopetrotic bone. Levels of these isoforms were significantly up regulated in osteopetrotic bone compared to normal controls. The expression of these isoforms in preosteoblasts suggests a role in the early stages of osteoblast differentiation. In MC3T3-E1 osteoblast-like cells these proteins were highly expressed at the early stages of differentiation. Western blot analysis of proteins from bone isolated at various stages during development showed two isoforms. We evaluated the effect(s) of second-generation anti-sense PLF and Periostin oligonucleotides on several functional parameters associated with differentiation in MC3T3-E1 cells. Levels of alkaline phosphatase, osteopontin, Cbfa1 and osteocalcin and collagen type I were significantly reduced in anti-sense treated cells compared to controls. It has been shown that as an extracellular matrix product these proteins regulate osteoblast-extracellular matrix interactions. Since they are also located within cells we expect that they are involved in signal transduction via binding to integrin receptors. Collectively, data from our experiments suggest a role for PLF and Periostin in osteoblast differentiation and function.