Role of Cadherins in Bone Biology

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Human osteoblasts express a repertoire of cadherins and that perturbation of cadherin-mediated cell-cell interaction reduces bone morphogenetic protein 2 (BMP-2) stimulation of alkaline phosphatase activity. We tested whether inhibition of cadherin function interferes with osteoblast function *in vitro* and *in vivo*. To inhibit the cadherin function *in vitro*, we expressed a truncated N-cadherin mutant (NCadΔC) with dominant negative action in MC3T3-E1 osteoblastic cells. In stably transfected clones, calcium-dependent cell-cell adhesion was decreased by 50%. Analysis of matrix protein expression during a 4-week culture period revealed that bone sialoprotein, osteocalcin, and type I collagen were substantially inhibited with time in culture, whereas osteopontin transiently increased. Basal alkaline phosphatase activity declined in cells expressing NCadΔC, relative to control cells, after 3 weeks in culture, and their cell proliferation rate was reduced moderately (17%). Finally, ^{45}Ca uptake, an index of matrix mineralization, was decreased by 35% in NCadΔC-expressing cells compared with control cultures after 4 weeks in medium containing ascorbic acid and β-glycerophosphate. Similarly, BMP-2 stimulation of alkaline phosphatase activity and bone sialoprotein and osteopontin expression also were curtailed in NCadΔC cells. Therefore, expression of dominant negative cadherin results in decreased cell-cell adhesion associated with altered bone matrix protein expression and decreased matrix mineralization. We next studied the function of osteoblast cadherins *in vivo* by transgenic expression of a truncated N-cadherin with dominant-negative action, driven by an osteoblast specific promoter (OG2-NcadΔC). Duringthe first 3 months of life, bone mineral density was reduced whereas percent body fat was increased in transgenic animals compared to wild type littermates, with associated decreased bone formation rate and osteoblast number, but normal osteoclast number. Osteoblast differentiation was delayed in calvaria cells isolated from transgenic mice. Likewise, the number of osteoblast precursors in bone marrow stromal cells from OG2-NcadΔC mice was decreased compared to wild type cultures, whereas the number of adipogenic precursors was increased. In vitro, a transcriptionally active β-catenin mutant reversed the delay in osteoblast differentiation and the exuberant adipogenesis. Thus, in vivo disruption of cadherin function hinders osteoblast differentiation and favors, indirectly, bone marrow progenitor cell commitment to the alternative adipogenic lineage via interference with β-catenin signaling. This results in decreased bone formation, delayed acquisition of peak bone mass

and increased body fat.

Next we tested whether disruption of N-cadherin function in stromal cells by dominant negative N-cadherin affects their ability to support osteoclastogenesis by altering heterotypic interaction with osteoclast precursors. ST2 cells were transduced with retrovirus dominant negative N-cadherin (NCad C) and cocultured with bone marrow macrophages (BMMs) to investigate the ability to support osteoclastogenesis. As a downstream target of NCad ΔC, β-catenin/TCF transcriptional activity was analyzed using TOPflash reporter construct. Real-time RT-PCR analysis and RANKL-luciferase reporter assay were performed to study the effects of NCad ΔC on the OPG/RANKL system. Immunoblotting analysis showed that primary bone marrow stromal cells and ST2 cells as well as bone marrow macrophages (BMM) expressed N-cadherin. Retroviral expression of NCad ΔC in ST2 cells did not significantly inhibit cell adhesion but markedly impaired the formation of tartrate-resistant acid phosphatase-positive osteoclasts (>40%) when cocultured with BMMs. However, the inhibition of osteoclastogenesis was not reproduced by neutralizing antibody against N-cadherin. Expression of NCad ΔC, however, strongly suppressed β-catenin/TCF transcriptional activity in ST2 cells, which was rescued by constitutively-active β-catenin adenovirus (Aḍ Δ N46 β-catenin) or constitutively-active TCF mutant (pCS2-VP16 ΔβXTCF-3). As a potential downstream target of Wnt signaling, we have found that the expression of RANKL was reduced in ST2 cells expressing NCad ΔC. Moreover, Wnt-3A, Aḍ ΔN46 β-catenin, or VP16 Δβ XTCF-3 increased the expression of RANKL and enhanced the transcriptional activity of mouse RANKL promoter in ST2 cells. Our data suggest that expression of dominant negative N-cadherin in ST2 cells suppressed osteoclastogenesis by interfering with β-catenin regulation of RANKL independent of cell-cell adhesion. Together with the previous data, which found that N-cadherin mediated cell-cell interaction is critical in osteoblast maturation and function, these results indicate that cadherin mediated signaling is involved in both arms of bone remodeling, bone formation and resorption.