

Oral Abstract

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Blockade of ephrinB2/EphB4 signaling within the osteoblast lineage reduces osteoblast differentiation and mineralization

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Ephrin and Eph family members are local mediators of cell function acting largely through contact-dependent processes in physiological development and maturity. Osteoclast-derived ephrinB2 has been shown to act upon the receptor tyrosine kinase, EphB4 in osteoblasts through a contact-dependent mechanism favouring bone formation. The aim of the present study was to investigate the consequences of interaction of the ligand, ephrinB2 with its receptor, EphB4 within the same cell lineage. A synthetic peptide antagonist of ephrinB2/EphB4 interaction and recombinant soluble extracellular domain of EphB4 (sEphB4) were used to block ephrinB2/EphB4 interaction. In murine mesenchymal cells (Kusa 4b10) grown under osteoblast differentiating conditions, the effect of both agents on osteoblast gene expression (real time RT-PCR) and mineralisation (Alizarin staining) were measured. Osteoblast genes expressed late in differentiation, DMP-1 and interferon induced transmembrane protein 5 were significantly down-regulated (2 fold) by both agents while mineralization was significantly inhibited in a dose-dependent manner. Immunohistochemistry of paraffin-embedded 3 week old male rat femur revealed differential ephrinB2 expression, with groups of osteoblasts on mature trabecular bone positive for ephrinB2, while immature (woven) bone osteoblasts were negative suggesting the action may be most relevant in mature bone. Osteoclasts also stained positively for ephrinB2, as previously reported.

Thus the functional consequence of blocking ephrinB2/EphB4 signaling in osteoblasts was to inhibit mineralization and expression of several osteoblast genes involved late in differentiation. This is consistent with ephrinB2/EphB4 signaling within the osteoblast lineage having a paracrine role in osteoblast differentiation, in addition to the proposed role of osteoclast-derived ephrinB2.