AAO Foundation Award Final Report

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Award Type	Orthodontic Faculty Development Fellowship
Project Title	Expression of Bone Regulatory Genes (OPG and RANKL) in an *in vitro* osteoblast differentiation system.
Project Year	2002
Institution	University of California San Francisco Department of Growth and Developments Division of Orthodontics Veterans Affairs Medical Center Endocrine Research Unit
Summary/Abstract	Brief Summary : RANKL and OPG gene expressions were measured ± PTH at different stages of osteoblast development. Mouse stromal cells were cultured in osteoblast differentiating conditions, and RANKL, OPG, COL1, ALP, OC, PTHRec genes were measured using qRT-PCR. OPG:RANKL ratios indicate that PTH may induce a possible switch in the regulatory mechanism of osteoclastogenesis where OPG is inhibited early and RANKL is increased at late stages of osteoblast differentiation. Summary/Abstract: Receptor Activator of Nuclear Factor κB-Ligand (RANKL) is essential for osteoclastogenesis, and its decoy receptor osteoprotegerin (OPG) negatively regulates this process. Both genes are expressed in cells of the osteoblast lineage, but the precise relationship between the state of osteoblast differentiation and RANKL and OPG expression is not clearly defined. The goal of this project was to quantify changes in RANKL and OPG gene expression in response to parathyroid hormone (PTH) at different stages of osteoblast differentiation. In this study, mouse primary bone marrow stromal cells (BMSC) were cultured for up to 28 days. At specific time points of cell culture, cells were stimulated with bovine PTH peptide (bPTH[1-34]) for 2 hours. Levels of RANKL, OPG, alpha-1

	(Type I) collagen (COL1), alkaline phosphatase (ALP), osteocalcin (OC), and PTH receptor (PTHRec) mRNA were assayed using quantitative real-time RT-PCR (qRT-PCR). In control cells, there was a gradual increase of RANKL gene expression with murine osteoblastic stromal cell maturation to a three-fold level at Day 28. In contrast, OPG mRNA levels were maximal at day 14 of cell culture and decreased through the latter stages of osteoblast differentiation. Exposing the cells to 100 ng/ml of bPTH[1-34] induced minimal increases in RANKL mRNA levels from day 7 to day 14, but elevated expression significantly at days 21 (two-fold) and 28 (three-fold). PTH inhibited OPG gene expression maximally at Day 14, but continued to have inhibitory effects on cultured cells at Days 21 and 28. Alterations of RANKL and OPG mRNA levels by PTH in day 14 osteoblasts were sufficient to sustain a 5.6-fold increase in the number of $TRAP^+$ (tartrate-resistant acid phosphatase) cells when cocultured with osteoclast precursor cells. Cells in culture after 28 days showed a 1.9-fold increase in $TRAP^+$ cells following PTH treatment. We conclude that: 1) PTH significantly upregulates RANKL mRNA in primary bone marrow stromal osteoblasts with maximal sensitivity occurring late in osteoblast differentiation; 2) PTH inhibits OPG gene expression at all stages of osteoblast differentiation, and 3) changes in RANKL and OPG mRNA levels following exposure to PTH are associated with increased osteoclastogenesis as demonstrated by increased numbers of $TRAP^+$ cells in cocultures. The results further suggest that the osteoclastogenic activity of PTH occurs primarily by suppression of OPG gene expression in early osteoblasts and elevation of RANKL gene expression in mature osteoblasts.