Phosphate Regulates Expression of SIBLINGs and MMPs in Cementoblasts

BL. Foster[1], EC. Swanson[1], D. Matsa-Dunn[1], S. Sato[1], RB. Rutherford[2], and MJ. Somerman[1,2]

University of Washington, School of Dentistry, Seattle, WA [1]Dept of Periodontics; [2]Dept of Oral Biology
*Corresponding Author: Brian L. Foster, blfoster@u.washington.edu

ABSTRACT

Introduction: Cementoblasts, the cells responsible for tooth root cementum formation, are especially sensitive to local phosphate and pyrophosphate during development, as evidenced by cementum phenotypes resulting from altered phosphate/pyrophosphate distribution. SIBLING family members BSP, OPN, and DMP-1 are regulated by phosphate in cementoblasts and have been shown to activate three specific matrix metalloproteinase (MMP) partners: MMP2, MMP3, and MMP9, respectively, *in vitro*. The aim of this study was to examine regulatory effects of phosphate on SIBLING and MMP expression in cementoblasts, *in vitro*.

Materials & Methods: Immortalized murine cementoblasts were treated with inorganic phosphate, *in vitro*, and effects on gene expression (by real time RT-PCR and mouse total genome microarray) were observed. Dose-response (0.1-10 mM phosphate) and time-course (1-48 hr) assays were performed. A sodium-phosphate uptake inhibitor, foscarnet, was used to better define phosphate-mediated effects on cells.

Results: Three SIBLING family members were regulated by phosphate: OPN (increased over 300% of control), DMP-1 (increased over 3,000% of control), and BSP (decreased). MMP3 was dramatically increased (4,000% of control), paralleling regulation of its partner OPN. Both MMP2 and MMP9 were slightly down-regulated. Time-course experiments indicated a response for SIBLING and MMP genes within 24 hr. Use of foscarnet demonstrated that phosphate uptake was required for observed changes in gene expression.

Discussion: These results indicate an effect of phosphate on cementoblast SIBLING and MMP expression *in vitro*. During cementum formation, phosphate may be an important regulator of cementoblast activity, including modulation of biomineralization, attachment, and matrix modification.

Key Words: cementoblasts, phosphate, SIBLING, matrix metalloproteinase, osteopontin

INTRODUCTION

The periodontium consists of the supportive tissues of the tooth, including the tooth root dentin and cementum, the periodontal ligament (PDL), and the surrounding alveolar bone. The cementum is the thin mineralized tissue covering the tooth root that plays a role in anchoring cementum to the alveolar bone via the periodontal ligament. Cementoblasts and cementum are sensitive to levels of phosphate (P_i) and pyrophosphate (PP_i), as evidenced by mutant and knock-out phenotypes with altered P_i/PP_i levels (Beertsen *et al.*, 1999; Nociti *et al.*, 2002; van den Bos *et al.*, 2005; Whyte, 1994). While this sensitivity may result in part from physicochemical interactions, there is also mounting evidence that P_i may serve as a signaling molecule (Adams and Shapiro, 2003; Beck, 2003). In cementoblasts specifically, we have shown intriguing Pi regulation of several genes involved in the processes of differentiation and mineralization, and metabolism of P_i and PP_i in the cell local environment (Foster *et al.*, In press; Rutherford *et al.*, Submitted).

The SIBLING (Small Integrin-Binding Ligand N-linked Glycoprotein) family includes noncollagenous extracellular matrix-associated proteins with several organizational features in common, as well as similar, functionally important post-translational modifications (Fisher and Fedarko, 2003; Qin *et al.*, 2004). SIBLINGs have been intimately associated with mineralized tissue formation, though continuing research has revealed additional functions and tissue localizations (Jain *et al.*, 2002; Ogbureke and Fisher, 2004).

Matrix metalloproteinases (MMPs) are a family of more than twenty endopeptidases with the capacity to degrade components of the extracellular matrix. MMPs have traditionally been regarded as being involved in activities including tissue remodeling, facilitation of cell migration, and cell response to microenvironment. Reports have additionally supported a role for MMPs in the generation of active epitopes of sequestered growth factors, adhesion molecules, cytokines, chemokines, and receptors (Sternlicht and Werb, 2001). Up-regulation of certain MMPs has also been linked to pathological conditions, including oral diseases (Sorsa *et al.*, 2004).

It has been demonstrated that three SIBLING family members partner specifically with three MMPs, and reversibly activate the proMMP forms of these enzymes without removal of the inhibitory propeptide. Bone sialoprotein (BSP) partners with MMP-2, osteopontin (OPN) with MMP-3, and dentin matrix protein-1 (DMP-1) with MMP-9 (Fedarko *et al.*, 2004; Ogbureke and Fisher, 2004). MMPs are typically inactivated by tissue inhibitors of MMPs (TIMPs), but SIBLING partners were shown to protect MMPs from this inactivation. Thus, co-expression of SIBLING and MMP partners provides a potential mechanism for localized and finely regulated MMP function, even with constitutive TIMP expression. While the SIBLING proteins can activate their MMP partners, it has been proposed that the MMPs may proteolyti-

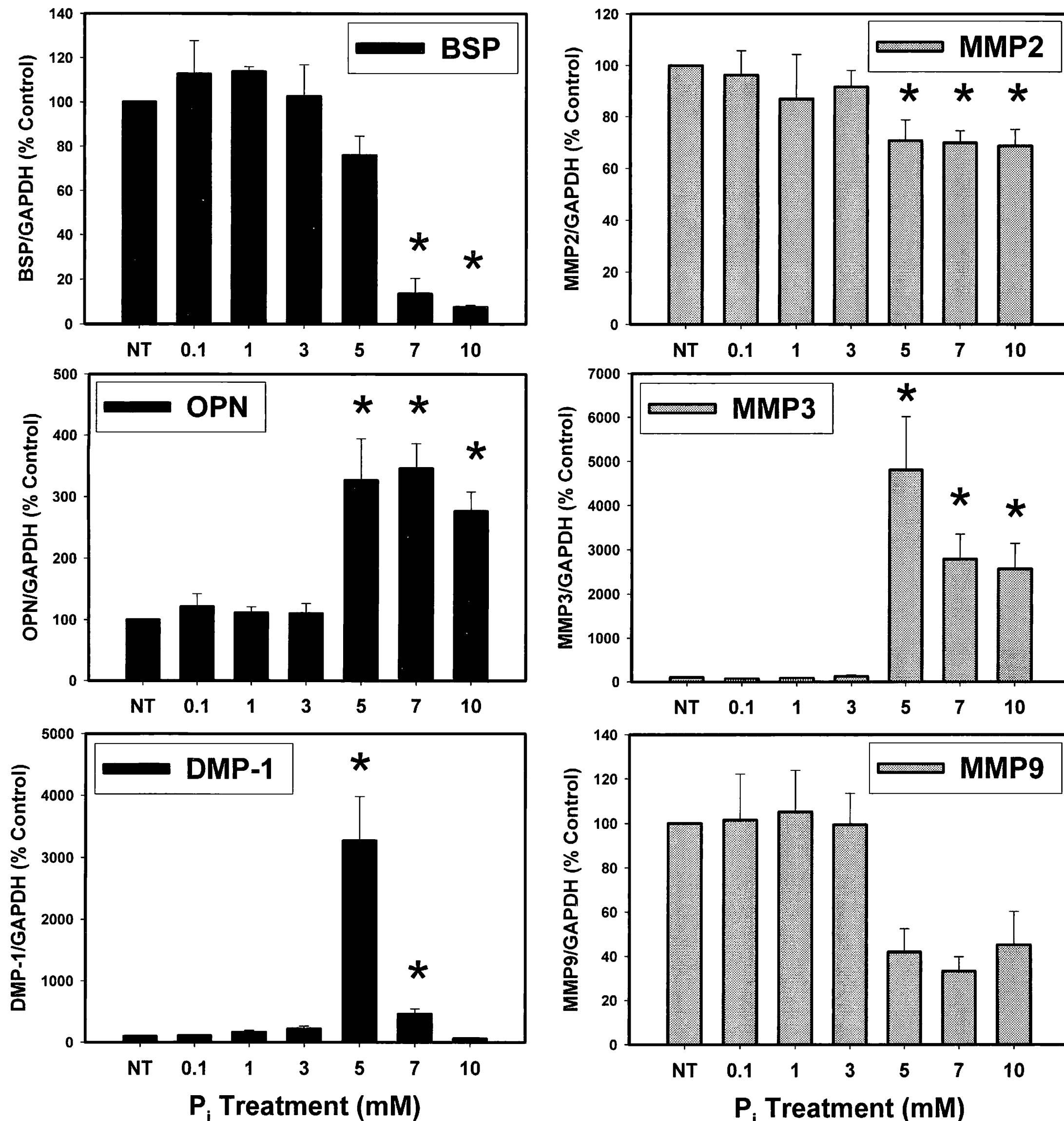

Fig 1. Phosphate regulates SIBLING and MMP genes in cementoblasts: Dose-response. Addition of 0.1-10 mM Pi to cementoblast cell cultures regulated SIBLING genes BSP, OPN, and DMP-1, and MMP genes MMP2, MMP3, and MMP9. Partners BSP and MMP2 were both decreased, OPN and MMP3 were increased, and in contrast, DMP-1 was increased though partner MMP9 was decreased, although the latter was not found to be statistically significant (see text for discussion). NT=no treatment control; bars represent SE; * is significance at $p < 0.05$.

cally process the SIBLINGs (Gao *et al.*, 2004).

The aim of this study was to examine the regulation of SIBLING proteins and MMPs by Pi in cementoblasts, *in vitro*. While we have shown previously that Pi mediates regulation of several SIBLING genes, here we additionally consider parallel effects on MMP expression. Both SIBLINGs and MMPs play critical roles during formation of mineralized tissues, and further study of the co-regulation of these factors may improve understanding of their contributions to oral mineralized tissue development, maintenance, and their potential use in regenerative therapies.

MATERIALS AND METHODS

Cell culture. Immortalized murine cementoblasts (OCCM-30) were maintained in Dulbecco' s Modified Eagle Medium (DMEM) with 10% (v/v) fetal bovine serum (FBS) and Penicillin/Streptomycin/L-Glutamine (all reagents from Invitrogen, Carlsbad, CA). Isolation and characterization of OCCM-30 cementoblasts has been previously described (D'Errico *et al.*, 2000). OCCM-30 cells were plated (2.56 x 10^4 cells/cm^2) and upon confluence media were changed to DMEM with 5% FBS, and treatments were added. Pi levels for dose-response were 0.1, 1, 3, 5, 7, and 10 mM, and untreated control. For time-course studies, 5 mM Pi was used. Total RNA was isolated by Trizol® reagent (Invitrogen, Carlsbad, CA) at 48 hr for dose-response experiments and at times 1, 6, 24, and 48 hr for time-course. For studies with foscarnet (phosphonoformic acid, PFA), an inhibitor of Na/Pi co-transport into the cell, cells were incubated with 3 mM

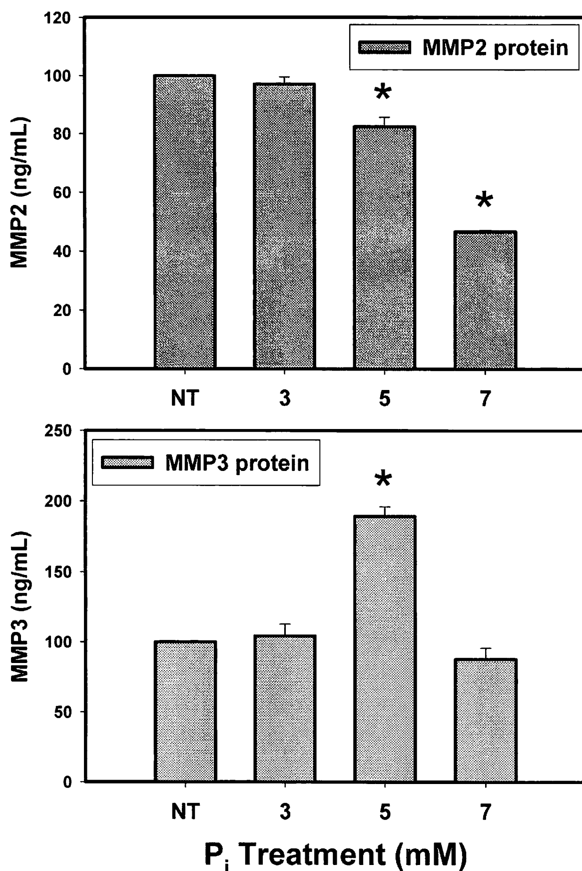

Fig 2. Phosphate regulates MMP protein levels in cementoblasts. Experiments were carried out as described under Dose-Response section, with cell media supernatant samples taken at 96 hr and analyzed by ELISA. MMP protein levels were regulated similarly to gene expression as described in Figure 1 (t=48 h), with a significant increase in MMP3 and decrease in MMP2 at 5 mM P_i. NT=no treatment control; bars represent SE; * is significance at $p < 0.05$.

PFA for 30 min prior to P_i addition, with PFA levels maintained in media until RNA isolation at 24 hr.

Real-time quantitative RT-PCR. Total RNA was DNAse treated (DNA-free™, Ambion Inc., Austin, TX), and two step RT-PCR was performed with a first-strand AMV cDNA synthesis kit and DNA Master SYBR Green I kit (Roche Diagnostics North America, Indianapolis, IN) according to manufacturer directions. A Roche Lightcycler system (Roche Diagnostics, Basel, Switzerland) was used for real time quantitative PCR and subsequent melting curve analysis of products to ensure product specificity. Primers were designed by Lightcycler probe design software and sequences checked by BLAST search. Primers (forward / reverse) designed for mouse sequences included: bone sialoprotein (BSP, 5' -GAGACGGCGATAGTTCC-3' / 5' -AGTGCCGCTAACTCAA-3'), OPN (5' -TTTACAGCCTGCACCC-3' / 5' -CTAGCAGTGACGGTCT-3'), dentin matrix protein-1 (DMP1, 5' -ATGATAACGCAATGGGT-3' / 5' -GTAATGCCTCAATGGCAC-3'), MMP2 (5' -AGATTGACGCTGTGTATG-3' / 5' -GCGATGAGCTTAGGGA-3'), MMP3 (5' - GGCTATACGAGGGCAC -3' / 5' - CAAATTCCAACTGCGAAG -3'), MMP9 (5' - CACCGAGCTATCCACT -3' / 5' -ACCTGAACCATAACGC ? 3') MMP23 (5' - GGTAACCCGAAGACGC-3' / 5' - CTCGCTGTCATCAAAGT-3'), and GAPDH (5' -ACCACAGTCCATGCCATCAC-3' / 5' -TCCACCACCCTGTTGCTGTA-3').

Relative quantification of PCR products was achieved using the LightCycler Relative Quantification Software version 1.0 (Roche Diagnostics, Basel, Switzerland) using GAPDH as a reference gene, with calibrator normalization and amplification efficiency correction.

ELISA. MMP proteins in cell culture supernatant samples were analyzed using Quantikine MMP2 and MMP3 mouse ELISA kits (R&D Systems, Minneapolis, MN). No commercially available mouse-specific MMP-9 ELISA kit was available, so MMP9 protein levels cannot be reported at this time.

Statistical analysis. All experiments were performed in triplicate (N=3) with dose-response experiments (without and with foscarnet) analyzed by one-way ANOVA (Sigmastat 3.1) and pairwise comparisons by SNK test, and time-course experiments log-transformed (ratio of treated/untreated) and a t-test applied (MS Excel). Log transformation was used to control passage-to-passage variation in basal cell gene expression. A p-value less than 0.05 was used to indicate significance.

RESULTS

Dose-response (Figures 1 and 2). Increasing the extracellular phosphate (P_i) in OCCM-30 cementoblast cell cultures regulated SIBLING and MMP genes (Figure 1). At 5 mM P_i, both OPN and DMP-1 were significantly increased (300% and 3,000% percent of untreated control, respectively), while BSP was decreased to about 80% of control. While the decrease in BSP was significant at higher P_i doses, these conditions were stressful for the cells (as indicated by cell morphology) and the doses were not used in subsequent experiments. MMP-2, partner of BSP, was also down-regulated to approximately 80% of untreated control. Like its partner OPN, MMP-3 was up-regulated by 5 mM P_i, to nearly 5,000% of control. In contrast to MMP2 and MMP3, both of which were regulated similarly to their SIBLING partners, MMP9 was seemingly decreased at higher P_i doses, but variable constitutive expression of DMP-1 mRNA in OCCM-30 cementoblast cultures confounded any determination of significance through statistical analysis.

For ELISA experiments, the same experimental design was employed, but with doses of 3, 5, and 7 mM P_i, and cell culture supernatant and cell lysates were collected at 48 hr. MMP2 and 3 protein levels corresponded with the pattern of regulation established from real-time PCR in Figure 1. MMP2 protein decreased with increasing P_i doses whereas 5 mM Pi significantly increased MMP3 protein. Though 7 mM Pi increased MMP3 mRNA (though less than the 5 mM Pi dose), this dose did

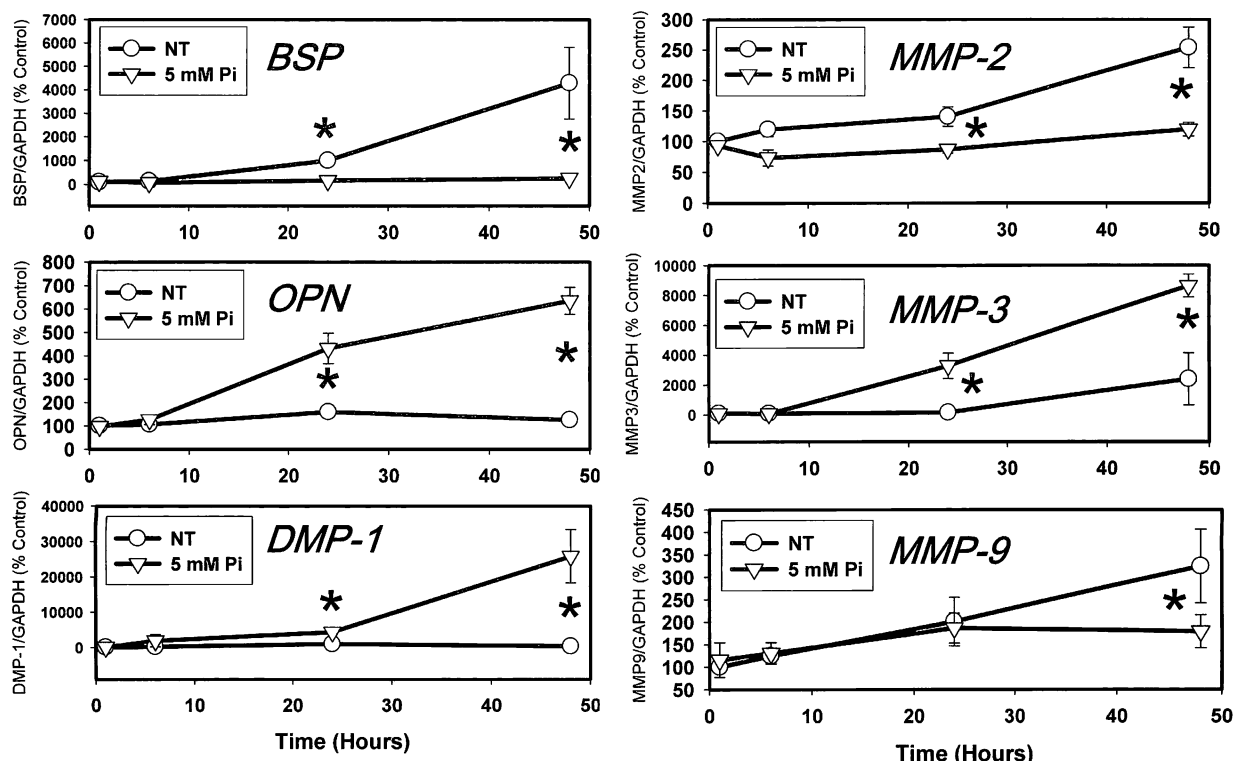

Fig 3. Phosphate regulates SIBLING and MMP genes in cementoblasts: Time-course. Addition of 5 mM Pi to cementoblast cell cultures with isolation of RNA from cementoblast cultures at 1, 6, 24, and 48 hr. Regulation of SIBLING and MMP genes was observed by t=24 hr. NT=no treatment control; bars represent SE; * is significance at $p < 0.05$.

not change MMP3 protein levels. No commercially available mouse specific MMP-9 ELISA kit was available, so MMP9 protein levels can not be reported at this time.

Time-course (Figure 3). A dose of 5 mM Pi was added to OCCM-30 cementoblast cell cultures, with RNA samples taken at times 1, 6, 24, and 48 hrs. All SIBLING genes were significantly up- or down-regulated by 24 hr. MMP2 and 3 were also regulated by 24 hr, though MMP9 was not significantly different from control until the 48 hr time. Over this time in culture, basal BSP expression (i.e., that of untreated controls) increased, while OPN and DMP-1 were at stable levels. These trends for gene expression in (untreated) cementoblasts over time in culture have been reported previously (D'Errico *et al.*, 2000) and are similar to those reported for some other mineralizing cell types of cells, such as osteoblasts (Beck *et al.*, 2000; Franceschi *et al.*, 1994). Expression of all three MMPs was observed to increase in untreated cementoblasts over 48 hr, with MMP3 increasing to about 1,000% of control (NT at time 1 hr), and MMPs 2 and 9 both increasing to about 300% of control. Thus, the three MMPs examined here increased with time in untreated OCCM-30 cementoblast cell cultures.

Blocking Pi import (Figure 4). In addition to doses of 1, 3, and 5 mM Pi, 3mM PFA (foscarnet, phosphonoformic acid) was included in OCCM-30 cementoblast cell cultures, with RNA samples taken at 24 hrs. Inclusion of PFA partially abrogated the regulation of SIBLING and MMP genes. While 5 mM Pi increased OPN mRNA to 250% of control, inclusion of PFA limited increase to 150%. Similarly, MMP3 was up-regulated to 2,000% by 5 mM Pi, but PFA limited MMP3 increase to only about 300% of control. GAPDH reference gene was not affected by PFA, and PFA regulation was similarly abrogated in other SIBLINGs/MMPs tested (data not shown). The blocking of Pi regulation of genes observed when 3 mM PFA was included may be incomplete due to less than 100% Na-Pi transporter blockage, and also due to potentially additional means of Pi entry that are not affected by PFA.

DISCUSSION

These studies demonstrate transcription-level regulation of SIBLING and MMP genes by Pi in cementoblasts, *in vitro*. SIBLING genes OPN and DMP-1 were increased, while BSP was decreased. MMP3 expression was increased, while MMP2 and 9 were decreased. Blocking Pi uptake partially abrogated regulation of SIBLING and MMP genes, indicating that Pi transportation into cells is likely necessary for gene regulation.

SIBLING family. SIBLING (Small Integrin-Binding Ligand N-linked Glycoprotein) extracellular matrix proteins play important roles in mineralized tissue formation in the tooth, as supported by studies of temporal and spatial expression of SIBLINGs during development (Baba *et al.*, 2004a; Baba *et al.*, 2004b; D'Errico *et al.*, 1997; Feng *et al.*, 2003; Hao *et al.*, 2004; MacNeil *et al.*, 1995), and reports of altered phenotypes resulting from human

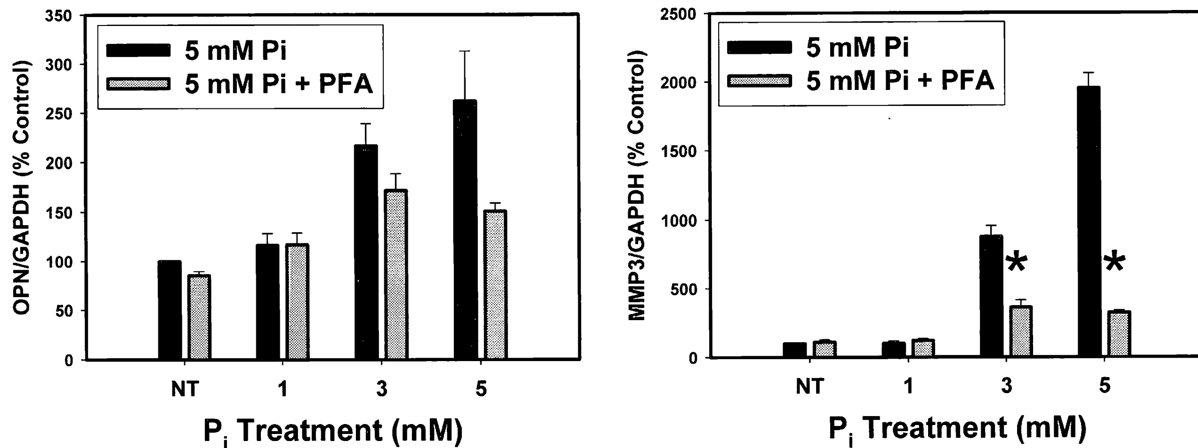

Fig 4. Blocking phosphate uptake partially abrogates regulation of SIBLING and MMP genes. A dose of 3 mM phonofosformic acid (PFA) was added to cementoblasts 30 minutes prior to and along with doses of P_i, and RNA was isolated after 24 hr. PFA blocks some P_i uptake into cells, also partially abrogating Pi-induced effects on SIBLING and MMP gene expression. This observation indicates that P_i enters the cell to regulate gene expression. NT=no treatment control; bars represent SE; * is significance at $p < 0.05$.

mutations and knock-out animals carrying SIBLING gene mutations (Dong *et al.*, 2005; Ye *et al.*, 2004). SIBLINGs have been individually characterized as nucleators, promoters, regulators, and/or inhibitors of mineralization, though this is often dependent on post-translational modifications, especially phosphorylation (Gericke *et al.*, 2005; He *et al.*, 2003; Tartaix *et al.*, 2004), but also including glycosylation, proteolytic processing, and transglutaminase cross-linking (Kaartinen *et al.*, 2005; Qin *et al.*, 2003; Qin *et al.*, 2004). Although the acidic natures of most of the SIBLING proteins (except for MEPE, not discussed here) are partly responsible for interaction with and regulation of hydroxyapatite formation, other characteristics of the SIBLING proteins relate to additional roles, including cell attachment (via $\alpha_v\beta_3$ integrin binding).

Matrix metalloproteinases. MMPs are a family of more than 20 related endopeptidases enzymes that are capable of degrading the various components of the extracellular matrix (ECM). MMPs are critical for operations including tissue development, remodeling, wound healing, and also releasing sequestered bioactive molecules from the ECM (Mott and Werb, 2004; Sternlicht and Werb, 2001; Visse and Nagase, 2003). MMPs may also contribute to a multitude of pathologies including arthritis, cancer, and oral diseases (Baker *et al.*, 2002; Sorsa *et al.*, 2004; Sternlicht and Werb, 2001). MMPs are expressed during tooth formation, and there is evidence that disruption or absence of MMPs has serious negative consequences for tooth development (Bourd-Boittin *et al.*, 2004; Bourd-Boittin *et al.*, 2005; Fanchon *et al.*, 2004; Goldberg *et al.*, 2003). MMP expression in the region of the developing tooth root has not been thoroughly characterized, but as with enamel and dentin they likely play an important role here as well. More complete characterization of MMPs in the periodontium would advance understanding of their potential roles during development of this region.

SIBLING-MMP partners. MMPs are typically secreted in a latent, inactive form (proMMP) that requires proteolytic removal of the inhibitory propeptide to activate. MMP protein activity is further regulated by the expression of tissue inhibitors of MMPs (TIMPs), a family of four endogenous protein inactivators of MMP activity that are also carefully regulated in local cell environments (Sternlicht and Werb, 2001; Visse and Nagase, 2003). Some very exciting studies have supported a role for SIBLING proteins in activating latent propetide forms of MMPs, and additionally protecting activated MMPs from TIMP inhibition or even reactivating TIMP-inhibited MMPs (Fedarko *et al.*, 2004). The specific SIBLING-MMP binding relationships are BSP-MMP2, OPN-MMP3, and DMP-1-MMP9. Additionally, these SIBLING-MMP binding pairs have been identified in several tissues including salivary glands and kidney, and in multiple types of cancer (Chaplet *et al.*, 2005; Fisher *et al.*, 2004; Karadag *et al.*, 2004). While SIBLING-MMP complexes may aid in the metastatic and osteotropic processes of some cancers, a role in normal development of teeth (and bones) can also be conceived, and in fact seems likely. There is some evidence that osteoblasts may even take part directly in bone matrix dissolution via MMPs (Parikka *et al.*, 2005). SIBLING proteins are important constituents of the ECM, MMPs are critical in matrix modeling and maturation, and both are expressed during tooth formation. Concomitant expression of SIBLING and MMP partners may be a novel mechanism for the exquisite regulation of local MMP activity during tooth development. Here we show *in vitro* that MMP2, 3, and 9 are expressed constitutively in cementoblast cell cultures, and additionally that their transcription can be regulated by inorganic phosphate (P_i).

Inorganic phosphate as a signaling molecule. Accumulating evidence supports a role for P_i as a cell signaling molecule, in addition to its role as a constituent of hydroxyapatite in teeth and bones. P_i regulates OPN expression in MC3T3-E1 pre-osteoblasts (Beck *et al.*,

2000), and much work has been done to elucidate regulatory mechanisms of OPN and other genes in osteoblasts (Beck, 2003; Beck and Knecht, 2003; Beck *et al.*, 2003; Conrads *et al.*, 2004). Pi may also serve as an apoptogen in terminally differentiated chondrocytes and osteoblasts as a mechanism for cell removal (Adams *et al.*, 2001; Adams and Shapiro, 2003). Furthermore, we have shown P_i regulation of several genes in cementoblasts related to functions of cell differentiation, mineralization, P_i/PP_i transport and metabolism, including the SIBLINGs BSP, OPN, and DMP1 studied here (Foster *et al.*, Accepted; Foster *et al.*, In press). Initial steps toward defining the mechanisms involved in P_i regulation of cementoblasts have been made and are currently being explored in greater depth. Levels of local P_i and PP_i may vary during tooth development in relation to activities such as matrix synthesis, mineralization, remodeling, resorption, and matrix maturation, and cells in the local area may be regulated in multiple ways by these processes. The importance of P_i and PP_i in formation of hydroxyapatite is well-established, but P_i may also serve as signaling molecule regulating cell behavior, for example expression of SIBLING and MMP proteins that control properties of the developing matrix. By better understanding the role of Pi in periodontal development, there is potential to advance our abilities to provide predictable therapies for periodontal regeneration, and also understand and treat genetic defects that negatively affect periodontal tissues.

ACKNOWLEDGMENTS

The authors would like to thank the University Of Washington Department Of Periodontics for their support, and Drs. Francisco H. Nociti, Jr., Fernanda Boabaid, Eiji Nemoto, Hanson Fong, and Sahba Fatherazi for their assistance. Thanks also go to Dr. Brian Leroux for his advice on statistical analysis. This study was supported by NIH/NIDCR grant DE15109 and funding from Nestlé Purina PetCare Research.

REFERENCES CITED

Adams CS, Mansfield K, Perlot RL, Shapiro IM (2001). Matrix regulation of skeletal cell apoptosis. Role of calcium and phosphate ions. *J Biol Chem* 276(23):20316-22.

Adams CS, Shapiro IM (2003). Mechanisms by which extracellular matrix components induce osteoblast apoptosis. *Connect Tissue Res* 44 Suppl 1(230-9.

Baba O, Qin C, Brunn JC, Jones JE, Wygant JN, McIntyre BW, Butler WT (2004a). Detection of dentin sialoprotein in rat periodontium. *Eur J Oral Sci* 112(2):163-70.

Baba O, Qin C, Brunn JC, Wygant JN, McIntyre BW, Butler WT (2004b). Colocalization of dentin matrix protein 1 and dentin sialoprotein at late stages of rat molar development. *Matrix Biol* 23(6):371-9.

Baker AH, Edwards DR, Murphy G (2002). Metalloproteinase inhibitors: biological actions and therapeutic opportunities. *J Cell Sci* 115(Pt 19):3719-27.

Beck GR, Jr., Zerler B, Moran E (2000). Phosphate is a specific signal for induction of osteopontin gene expression. *Proc Natl Acad Sci U S A* 97(15):8352-7.

Beck GR, Jr. (2003). Inorganic phosphate as a signaling molecule in osteoblast differentiation. *J Cell Biochem* 90(2):234-43.

Beck GR, Jr., Knecht N (2003). Osteopontin regulation by inorganic phosphate is ERK1/2-, protein kinase C-, and proteasome-dependent. *J Biol Chem* 278(43):41921-9.

Beck GR, Jr., Moran E, Knecht N (2003). Inorganic phosphate regulates multiple genes during osteoblast differentiation, including Nrf2. *Exp Cell Res* 288(2):288-300.

Beertsen W, VandenBos T, Everts V (1999). Root development in mice lacking functional tissue non-specific alkaline phosphatase gene: inhibition of acellular cementum formation [In Process Citation]. *J Dent Res* 78(6):1221-9.

Bourd-Boittin K, Septier D, Hall R, Goldberg M, Menashi S (2004). Immunolocalization of enamelysin (matrix metalloproteinase-20) in the forming rat incisor. *J Histochem Cytochem* 52(4):437-45.

Bourd-Boittin K, Fridman R, Fanchon S, Septier D, Goldberg M, Menashi S (2005). Matrix metalloproteinase inhibition impairs the processing, formation and mineralization of dental tissues during mouse molar development. *Exp Cell Res* 304(2):493-505.

Chaplet M, Waltregny D, Detry C, Fisher LW, Castronovo V, Bellahcene A (2005). Expression of dentin sialophosphoprotein in human prostate cancer and its correlation with tumor aggressiveness. *Int J Cancer*.

Conrads KA, Yu LR, Lucas DA, Zhou M, Chan KC, Simpson KA, Schaefer CF, Issaq HJ, Veenstra TD, Beck GR, Jr., Conrads TP (2004). Quantitative proteomic analysis of inorganic phosphate-induced murine MC3T3-E1 osteoblast cells. *Electrophoresis* 25(9):1342-52.

D'Errico JA, MacNeil RL, Takata T, Berry J, Strayhorn C, Somerman MJ (1997). Expression of bone associated markers by tooth root lining cells, in situ and in vitro. *Bone* 20(2):117-26.

D'Errico JA, Berry JE, Ouyang H, Strayhorn CL, Windle JJ, Somerman MJ (2000). Employing a transgenic animal model to obtain cementoblasts in vitro. *J Periodontol* 71(1):63-72.

Dong J, Gu T, Jeffords L, MacDougall M (2005). Dentin phosphoprotein compound mutation in dentin sialophosphoprotein causes dentinogenesis imperfecta type III. *Am J Med Genet A* 132(3):305-9.

Fanchon S, Bourd K, Septier D, Everts V, Beertsen W, Menashi S, Goldberg M (2004). Involvement of matrix metalloproteinases in the onset of dentin mineralization. *Eur J Oral Sci* 112(2):171-6.

Fedarko NS, Jain A, Karadag A, Fisher LW (2004). Three small integrin binding ligand N-linked glycoproteins (SIBLINGs) bind and activate specific matrix metalloproteinases. *Faseb J* 18(6):734-6.

Feng JQ, Huang H, Lu Y, Ye L, Xie Y, Tsutsui TW, Kunieda T, Castranio T, Scott G, Bonewald LB, Mishina Y (2003). The Dentin matrix protein 1 (Dmp1) is specifically expressed in mineralized, but not soft, tissues during development. *J Dent Res*

Regulation of Hematopoietic Stem Cell and Its Interaction with Stem Cell Niche

F. Arai

Department of Cell Differentiation, The Sakaguchi Laboratory of Developmental Biology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan, farai@sc.itc.keio.ac.jp

ABSTRACT

Hematopoietic stem cells (HSCs) are responsible for Blood cell production throughout the lifetime of individuals. Interaction of HSCs with their particular microenvironments, known as stem cell niches, is critical for maintaining the stem cell properties, including self-renewal capacity and the ability of differentiation into single or multiple lineages. In the niche, the niche cells produce signaling molecules, extracellular matrix, and cell adhesion molecules, and regulate stem cell fates. Recently, long-term bone marrow (BM) repopulating (LTR) HSCs exist frequently in BM trabecular bone surface, and it was clarified that an osteoblast (OB) is a critical component for sustainment of HSCs. HSCs balance quiescence and cell division in the osteoblastic niche and also maintain the potential for long-term hematopoiesis. Especially, the quiescent state in the cell cycle is thought to be indispensable for the maintenance of hematopoietic stem cells (HSCs). We demonstrate that c-Kit^{+}Sca-1^{+}Lineage^{-} (KSL) HSCs expressing the receptor tyrosine kinase Tie2 are quiescent and anti-apoptotic, transplantable and comprise a side-population (SP) of HSCs, which contact closely to Angiopoietin-1 (Ang-1), a ligand for Tie2, expressing osteoblasts in the BM niche. Tie2 and Ang-1 are part of a key signaling interaction between HSC and osteoblasts. Tie2 and Ang-1 are expressed in a complementary pattern, and interaction of Tie2 and Ang-1 induced integrin dependent cell adhesion of HSCs to osteoblasts and extracellular matrix. This signaling pathway regulates functional criteria of HSC in the BM niche, including quiescence, anti-cell death and tight adhesion. These observations led us to a novel model in which Ang-1 produced by osteoblasts activates Tie2 on the HSCs and promote tight adhesion of HSCs to the niche, resulting in quiescence and enhanced survival of HSCs.

Key words: Quiescence, Tie2, Angiopoietin-1, N-cadherin, ATM

Tissue stem cells are characterized by their abilities to self-renew and to produce numerous differentiated daughter cells. These two special properties enable stem cells to play a central role in maintaining tissues. The activity of tissue stem cells is crucial for supply the mature cells in normal tissue turnover. Defective functional activity or low turnover of stem cells leads exhaustion of progenitor or mature cells in tissue and is causing in disease. Unregulated and over proliferation of stem cells is the leading cause of cancer. It now clear that the stem cell niche regulates the stem cell specific property including self-renewal, multi-potentiality, and relative quiescence.

The concept of the stem cell niche was first proposed for the human hematopoietic system in the 1970s (Schofield, 1978). A similar concept has also been proposed for the epidermis, intestinal epithelium, nervous system and gonads (Fuchs et al., 2004; Li and Xie, 2005). We hypothesized that cell cycle regulation by the niche is critical for the fate of HSCs. In the niche, signaling molecules, extracellular matrix, and cell adhesion molecules produced by niche cell regulate quiescence, self-renewal, and cell fate decision of the stem cell. Up to the present date, many adult tissue stem cells and their niches, including hematopoietic system, skin epidermis, gastrointestinal epithelium, brain, and lung were identified. There observation led us the understanding of the common property of the tissue stem cells. First, stem cells are relatively quiescent or slow cycling cells in the tissue. Second, stem cells adhere to the supporting cells (niche cells) in the stem cell niche. We found that the Tie2/Ang-1 signaling pathway between HSCs and OBs contributes to quiescence of HSCs in their stem cell niche, resulting in the maintenance of self-renewal ability and protection from stresses (Arai et al., 2004)

Identification of stem cell niche and niche cells in adult BM

A stem cell niche includes three compartments: localized niche cells (supporting cells), the extracellular matrix (ECM), and soluble factors derived from niche cells (Lin, 2002) (Figure 1). A unique feature of HSCs is that they migrate toward the stem cell niche during their ontogeny. During embryogenesis, development of the hematopoietic system occurs at various anatomical sites, including para-aortic splanchnopleural mesoderm (P-Sp)/ aorta-gonad-mesonephros (AGM) region, yolk sac, fetal liver, spleen, and bone marrow. Intraembryonic hematopoietic development may be associated with the major arterial region. Histological studies have demonstrated the presence of clusters of HSCs in close association with, and often adhering to, endothelial cells on the ventral surface (floor) of the aorta (North et al., 2002). It suggests that endothelial cells play as a niche for developing HSCs. In addition, recently two groups reported that the placental labyrinth region is a source of definitive hematopoiesis and plays as a niche for HSCs during midgestation (Gakas et al., 2005; Ottersbach et al., 2005).

OBs, which derived from mesenchymal stem cell, have long been known to play a central role in skeletal