Journal of Cellular Biochemistry Supplements 30/31:92–102 (1998)

Osteopontin Expression and Function: Role in Bone Remodeling

David T. Denhardt[1]* **and Masaki Noda**[2]

[1]Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ 08854
[2]Tokyo Medical Dental University, Medical Research Institute, Tokyo 101, Japan

Abstract The cytokine and cell attachment protein osteopontin (OPN) is not necessary for the development and survival of mice in a clean animal facility. The primary role of OPN appears to be that of facilitating recovery of the organism after injury or infection, which generally causes an increase in its expression. It also is essential for some forms of bone remodeling. OPN stimulates cellular signaling pathways via various receptors found on most cell types and can encourage cell migration. OPN modulates immune and inflammatory responses and possibly negatively regulates Ras signaling pathways. Its apparent ability to enhance cell survival by inhibiting apoptosis may explain why the metastatic proficiency of tumor cells increases with increased OPN expression. J. Cell. Biochem. Suppls. 30/31:92–102, 1998. © 1998 Wiley-Liss, Inc.

Key words: osteopontin; enhanced cell survival; inhibition of apoptosis; bone remodeling

Osteopontin (OPN) is a highly phosphorylated and glycosylated protein found in all body fluids and in the extracellular matrix of mineralized tissues. Background information and more thorough documentation of the assertions made in the report can be found in the reviews by Patarca et al. [1993], Giachelli et al. [1995], Butler et al. [1996], Oates et al. [1997], and Rittling and Denhardt [1999], which provide a more extensive discussion of what is known about OPN, particularly in the immune system, in the vascular system, in mineralized tissues, in cancer, and in the kidney, respectively. These investigators also cover material not covered here, for example, the structure of the protein and its influence on the precipitation of Ca^{2+} salts. OPN is widely expressed, particularly at sites of inflammation. Through its interactions with various cell surface receptors, it stimulates intracellular signaling pathways that modify cell behavior and alter gene expression. A principal function may be to support homeostatic processes that reduce cell death that results from potentially lethal insults.

Abbreviations used: ALP, alkaline phosphatase; βFGF, basic fibroblast growth factor; BSP, bone sialoprotein; CKII, casein kinase II; Con A, concanavalin A; CT, cytotrophoblast; dex, dexamethasone; EGF, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; ET, endothelin; FCS, fetal calf serum; iNOS, inducible nitric oxide synthase; IMH, immunohistochemistry; ISH, in situ hybridization; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NB, Northern blot; NR, nuclear runon; OC, osteocalcin; OPN, osteopontin; prog, progesterone; PKC, protein kinase C; PTH, parathyroid hormone; RA, retinoic acid; RT, *Rickettsia (Orientia) tsutsugamushi*); ST, syncytial trophopblasts; TGF-β, transforming growth factor-β; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; $VitD_3$, vitamin D_3.

Research supported by the National Institutes of Health: (AR44434; ES06897), and the Cancer Federation Inc.

*Correspondence to: David T. Denhardt, Nelson Biological Laboratories, 604 Allison Road, Piscataway, NJ 08854-8082. E-mail: denhardt@biology.rutgers.edu

Received 21 September 1998; Accepted 22 September 1998

REGULATION OF OPN EXPRESSION

OPN expression is enhanced by many agents acting on specific cell types through diverse signaling pathways, some of which involve protein kinase C (PKC). For example, PKC appears to be a downstream effector of OPN expression in $Src^{-/-}$ fibroblasts stimulated with epidermal growth factor (EGF) [Chackalaparampil et al., 1996]. Treatments known to affect OPN mRNA levels, which usually correlate with OPN secretion, are listed in Table I. In most, but not all, of the studies reported, control is exerted at the level of transcription, at least in large part. Although increases in OPN

expression are often the focus, Broess et al. [1995] have investigated the ability of $1,25(OH)_2$ vitamin D_3 to inhibit OPN expression in various populations of 17-day chicken embryo osteoblasts. These investigators make the important points that the developmental stage and species of the embryo and the treatment of the osteoblasts and their state of maturation determine how the cells will respond in any particular case.

Analysis of the mouse, human, chicken, and rat promoters has uncovered many potential sites for transcription factor interactions, and some transcription factors have been implicated in OPN transcription. These include nuclear receptors, including progesterone, glucocorticoids, and $1\alpha,25(OH)_2$ vitamin D_3; basic helix-loop-helix proteins; AP-1(fos/jun); and CBFA1/PEBP2αA. Increased OPN expression at sites of injury or infection likely results from the release of growth factors (e.g., platelet derived growth factor, PDGF) or cytokines (e.g., interleukin-1 [IL-1]) that activate various transcription factors, including Fos and Jun, that are capable of upregulating OPN transcription. Bonnelye et al. [1997] discovered that steroidogenic factor-responsive elements (SFREs) in the OPN promoter are targets for the estrogen-related orphan receptor ERR-1 in osteoblast cells. ERR-1 is clearly involved in controlling ossification. The POU transcription factor Oct-4, which is highly expressed in the mouse preimplantation embryo, binds to a sequence element, PORE, in the first intron in OPN and transactivates OPN transcription, which can be inhibited by the HMG protein Sox-2 in preimplantation embryo cell lines [Botquin et al., 1998].

Mice deficient in the *runt*-family transcription factor CBFA1/PEBP2αA fail to form bones, and unable to respire because of the absence of a mineralized rib cage, they die immediately after birth [Komori et al., 1997]. Osteoblasts and hypertrophic chondrocytes from the embryos of mutant mice expressed OPN (also osteocalcin, alkaline phosphatase) at much lower levels than cells from control embryos, suggesting that CBFA1/PEBP2αA is required for OPN expression in these cells. Overexpression of CBFA1/PEBP2αA augments OPN expression in the osteoblast-like cells MC3T3E1 [Tsuji et al., 1998]. Scleraxis, a basic helix-loop-helix transcription factor expressed in the sclerotome, enhances OPN expression when overexpressed in osteoblasts [Liu et al., 1997]. Since scleraxis is upregulated by transforming growth factor-β (TGF-β) it is likely to be one of effectors of TGF-β signaling [Liu et al., 1996].

OPN RECEPTORS: MEDIATORS OF ADHESION, MIGRATION, AND SIGNALING

OPN is a ligand for the integrins $\alpha_v\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$ [Liaw et al., 1995], and for $\alpha_4\beta_1$ [Bayless et al., 1998], $\alpha_8\beta_1$ [Denda et al., 1998], and $\alpha_9\beta_1$ [Smith et al., 1996]. CD44, the hyaluronic acid receptor, is also a receptor for OPN [Weber et al., 1996]. Interactions of immobilized OPN with $\alpha_v\beta_1$, $\alpha_v\beta_3$ or $\alpha_v\beta_5$ support the adhesion of smooth muscle cells, but only $\alpha_v\beta_3$ will promote a migratory response [Liaw et al., 1995]. Each of these three integrins also exhibits different distributions on the cell surface and sends different signals to the cell. Although the GRGDS sequence in OPN is a major integrin binding site, required for many cell types to adhere to OPN [Xuan et al., 1995], other sequences in OPN also interact with receptors. For example, Katagiri et al. [1996] have shown that B16-BL6 cells can bind to sequences on both the N-terminal and the C-terminal sides of the GRGDS sequence. The $\alpha_4\beta_1$ integrin, which is expressed on activated leukocytes, mediates adhesion via a sequence present in the thrombin-generated N-terminal fragment, which contains the GRGDS sequence. However, adhesion can be inhibited by a peptide containing an LDV sequence, but not an RGD sequence [Bayless et al., 1998]. Figure 1 illustrates the various integrins implicated in OPN binding.

The centrally located thrombin cleavage site, 6 or so residues on the C-terminal side of the GRGDS sequence in OPN, is strictly conserved. In a study of the ability of OPN to promote haptotaxis (migration promoted by bound ligand) and chemotaxis (migration in response to soluble ligand) of two different cancer cell lines, Senger et al. [1996] found that OPN promoted haptotaxis, but not chemotaxis, and that only the N-terminal GRDGS-containing portion of OPN was active. Cleavage of OPN by thrombin enhanced the ability of the GRGDS sequence to bind $\alpha_v\beta_3$ and to promote haptotaxis. The $\alpha_9\beta_1$ binding site, which also involves the GRGDS sequence, is masked in intact OPN, but revealed upon cleavage by thrombin [Smith and Giachelli, 1998]. These results suggest a role

TABLE I: Regulation of Osteopontin Expression*

Agent/treatment	Cells/Tissues	Changes in OPN mRNA/protein	Comment	Reference
Concanavalin A *R. tsutsugamushi*	T-lymphocytes Peritoneal macrophages	mRNA increased (NB) by Con A or in RT-sensitive macrophages	Original cloning of early T-lymphocyte activation gene-1	Patarca et al. 1989 J Exp Med 170:161
TGF-β1,2 (0.4–4 ng/ml)	ROS 17/2.8 osteosarcoma MC3T3E1 cells	mRNA synthesis increased (48 h) (NR, NB), half-life unchanged	TGF-β opposed reduction caused by dexamethosone, no $VitD_3$ synergism	Noda et al. 1988 J Biol Chem 263:13916
TGF-β1 (1 ng/ml)	ROS 17/2.8, RCA11 cells Primary rat calvaria cultures	mRNA (NB), radiolabeled protein variably upregulated	No increase in OPN mRNA in ROS 17/2.8 cells at 24 h	Kubota et al. 1989 BBRC 162:1453
TGF-β1 (2 ng/ml)	Primary cultures of rat calvaria cells	2 ng/ml out to 20 days had little effect on mRNA levels (NB)	Inhibited OC, BMP-2 and nodule formation	Harris et al. 1994 J Bone Miner Res 9:855
TGF-β1 (10 ng/ml) EGF (50 ng/ml)	Rat kidney epithelial cell line NRK52E	mRNA (NB, NR) and protein (ELISA) both increased at 48 h	No increase induced by IGF-1, angiotensin II, PDGF-BB, bFGF	Malyankar et al. 1997 Kidney Int 51:1766
Endothelin (1-100 nM)	ROS 17/2.8 osteosarcoma cell (osteoblast-like)	ET-1, but not ET-3, caused 3X increase in mRNA (NB) at 24 h	OC increased also, max at 24 h (ET inhibits osteoclastic bone resorpt.)	Shioide and Noda 1993 J Cell Biochem 53:176
Endothelin (10-100 nM)	Rat mesangial cell line	1.3x increase in mRNA (NB) with 10nM and 100 nM	ET-1 may cause the increased OPN expression in the ischemic kidney	Nambi et al. 1997 BBRC 241:212
Angiotensin II (1 μM) bFGF, TGF-β	Confluent vascular smooth muscle cells	Increase in mRNA (NB) and protein (WB) 1-3 days	Increased OPN in rat neointimal smooth muscle after angioplasty	Giachelli et al. 1993 J Clin Invest 92:1686
Retinoic acid (1 –100 nM)	Day-18 chick embryo chondrocytes	RA caused progressive increase in mRNA (NB) out to 6 days	ALP expression and mineralization also increased	Iwamoto et al. 1993 Exp Cell Res 207:413
Retinoic acid (1 μM)	Preosteoblastic UMR201 Rat cell line	mRNA increased by 1 h (NB), 3x increase after 4 h (NR)	Nuclear processing of primary OPN transcripts enhanced by RA	Manji et al. 1998 J Gen Physiol 176:1
Dexamethasone (10 mM) BMP-2, VitD	Rat bone marrow stromal cell culture	mRNA strongly increased by Dex+BMP2+VitD (NB, ISH)	Correlates with osteoblastic differentiation and increased ALP	Rickard et al. 1994 Dev Biol 161:218
Dexamethasone (also LPS, IL1, INF-γ)	Cardiac ventricular myocytes Microvascular endothel. cells	Increase in mRNA (NB) and protein (WB), half-life unchanged	OPN may serve to inhibit induction of inducible nitric oxide synthase	Singh et al. 1995 J Biol Chem 270:28471
Dexamethasone (0.1 – 10 nM)	Freshly isolated human bone marrow stromal cells	Reduction in mRNA levels (NB) during 4-wk mineralization	OC and BSP also decreased; ALP increased	Cheng et al. 1996 J Cell Biochem 61:182
Progesterone (50 μg) 17β-estradiol (10 μg)	Mouse skin	Enhanced mRNA levels (NB) Also in pregnant or lactating mice	Agent applied to the skin of non-pregnant females for 6 or 24 h	Craig and Denhardt 1991 Gene 100:163
Progesterone	Cytotrophoblasts differentiating into syncytia	Increased mRNA (NB) result of endogenous or exogenous progest	RU486 blocked progest enhancement but upregulated OPN in its absence	Omigbodun et al. 1997 Endocrinology 138:4308
1α,25$(OH)_2$VitaminD_3 (25 pM to 2.5 nM)	ROS 17/2.8 osteosarcoma cells	Increased synthesis of phosphorylated protein	Increase inhibited by actinomycin	Prince and Butler 1987 Collagen Rel Res 7:305
1α,25$(OH)_2$VitaminD_3 (1-100 ng/ml)	Mouse epidermal JB6 C141.5a cells	Increased mRNA levels (NB) by 4 h, blocked by actinomycin D	Nonphosphorylated OPN produced; no change in OPN mRNA stability.	Chang et al. 1994 Endocrinology 135:863
1α,25$(OH)_2$VitaminD_3 Dexamethasone	Rat bone organ culture (10 mM VitD, 100 nM Dex)	mRNA expression elevated by both (in situ hybridization)	BSP expr. suppressed by $VitD_3$; OPN expressed by more cell types than BSP	Chen et al. 1996 Connect Tissue Res 34:41
1,25$(OH)_2$VitaminD_3 (1-10 nM)	ROS17/2.8 osteosarcoma cells Rat calvaria osteoblastic cells	Enhanced mRNA levels (NB)	Decrease in the transcriptional inhibitor HES1 regulates OPN expression	Matsue et al. 1997 Bone 20:329
TPA (10 ng/ml) (also PDGF, EGF)	JB6 Cl22 cells Swiss 3T3 cells	Increased mRNA (NB, NR); Induced by PDGF in 3T3 cells	Transient in subconfluent JB6 cells, stable induction in confluent JB6 cells	Smith and Denhardt 1987 J Cell Biochem 34:13

TPA, other PKC activators	JB6 Cl22, both confluent and subconfluent cultures	Increase in mRNA (NB) blocked by H7, RA, dexamethasone	Inhibitors of tumor promotion diminished OPN induction by TPA	Smith and Denhardt 1989 J Cell Physiol 139:189
TPA (50 ng/ml) EGF (10 ng/ml)	MC3T3E1 (mouse osteoblastic cell line)	Increase in mRNA (NB, NR) Reduced by cycloheximide	Maximal at 4-8 h, EGF and Ras-transformation also induced OPN	Nose et al. 1990 Cell Growth Diff 1:511
TPA, VitD, TGF-β, RA, ConA, PTH	ROS 17/2.8 osteosarcoma RCA 11 osteoplastic line RC/V primary calvaria cells	Cell-dependent increases or decreases in mRNA levels in response to different agents	Both 55 kDa and 44 kDa phosphorylated proteins examined Some post-transcriptional regulation	Kasugai et al. 1991 Bone Miner 13:235
TPA (1.5 nM)	HL-60 cells, induced to differentiate by TPA	PKC-dependent increase in mRNA (NB) 6-24 h	Increase also required MAP kinase No effect of RA, $VitD_3$ or butyrate	Atkins et al. 1997 Arch Biochem Biophys 343:157
TPA (0.5-1.5 nM) RA (0.01-1 μM)	HL-60 cells differentiating into monocytes/macrophages	Increased mRNA (NB) and protein (ELISA)	Increased integrin and CD44 receptors also; no effect of RA	Atkins et al. 1998 J Cellu Physiol 175:229
TNFα (10 ng/ml) Fetal calf serum (10%)	Rat mesangial cells	2-3x increase in mRNA (NB)	PDGF, IL-1β and TGF-β had little effect on OPN expression	Nagasaki et al. 1997 BBRC 233:81
Lipopolysaccharide (1μg/ml)	Various macrophage lines and mouse peritoneal macrophages	Immediate and prolonged increase in mRNA levels (NB)	Delayed increase with a "crude" lymphokine preparation	Miyazaki et al. 1990 J Biol Chem 265:14432
IL-1α (4-200 pM) also TNFα, LPS, VitD3	MC3T3-E1 cell line Primary mouse calvaria cells	Increase in mRNA (NB, NR) and protein expression	Similar increases induced by TNF-α, LPS, and $1\alpha,25(OH)_2$vitaminD_3	Jin et al. 1990 Mol Cell Endocrinol 74:221
IL-1β	Human knee articular chondrocytes	Enhanced mRNA levels (NB, RT-PCR)	OPN identified by differential display RT-PCR	Margerie et al. 1997 Osteoarthr Cartilage 5:129
IL-2 (500 U/ml human recombinant IL-2)	Resting mouse spleen NK cells	mRNA up-regulated (NB) starting by day 1, maintained for 14 days	OPN expression turned on during activation of natural killer cells	Pollack et al. 1994 J Leukoc Biol 55:398
Calcitonin (10 nM)	Rabbit osteoclasts	Inhibited mRNA expression (NB) and bone-resorbing activity	Possibly by activating Ca^{2+}/PKC- and PKA-dependent pathways	Kaji et al. 1994 Endocrinology 135:484
Parathyroid hormone (1-100 nM)	ROS 17/2.8 Primary rat calvaria	Protein (WB) and mRNA (NB, NR) reduced for 1-4 days	PTH effect mimicked by cholera toxin, 8-Br-cAMP, forskolin, isoproterenol	Noda and Rodan 1989 J Cell Biol 108:713
cAMP (as a second messenger)	Human cytotrophoblasts and syncytial trophoblasts	CTs, but not STs, synthesized mRNA, protein (NB, IHC)	cAMP appeared to reduce OPN mRNA as CTs differentiated into STs	Daiter et al. 1996 Endocrinology 137:1785
Fetal calf serum (10 %)	Rat mesangial cells in 2D or 3D type I collagen cultures	FCS increased mRNA in 2-dimensional cultures (NB)	OPN expression in 3-dimensional cultures independent of FCS	Prols et al. 1998 FEBS Lett 422:15
3-dimensional culture in type I collagen gels	Microvascular endothelial cells (also protein in tissues)	mRNA 10x higher in differentiating cells	Correlated with the reorganization of the cells into vascular tubes	Prols et al. 1998 Exp Cell Res 239:1
Al^{3+}, (also Co, Cr, Ni, Ti, V ions)	ROS17/2.8 cells	Only Al reduced mRNA levels at noncytotoxic ion concentrations	Also ALP and OC mRNAs reduced Other ions effective at cytotoxic levels	Sun et al. 1997 J BioMed Mater Res 34:29
PDGF (1 nM) angioplasty	Rat aortic smooth muscle cells, carotid arteries	mRNA up 2-7X, 3 h to 3 days, mediated by the PDGF-β receptor	PDGF-AB&BB, but not AA, effective; bFGF, EGF, IL-1β, TGF-β tested also	Wang et al. 1996 Arterioscl Thromb Vasc Biol 16:1365
Attachment of cells to fibronectin	Embryonic chicken calvaria osteoblasts	Increased mRNA (NB) result of receptor occupancy by FN	No increase when cells plated on plastic alone	Carvalho et al. 1998 J Cell Biochem 70:376
Glyoxylic acid (120 mg/kg/day, 14 d)	Rat kidney, agent induces stone formation in the kidney	Increased mRNA (ISH) in distal convoluted tubules	Model for renal stone formation Inhibited by estrogen or progesterone	Umekawa et al. 1995 Biochem Mol Bio 35:223
cGMP-dependent protein kinase (PKG)	Vascular smooth muscle cells	Decreased mRNA (NB) and protein (WB)	Thrombospondin also decreased. Mediator of NO and cGMP signaling	Dey et al. 1998 Circ Res 82:139
Trypsin treatment, v-Myc	Chicken chrondrocytes Virus-expressed v-myc	Increase in mRNA (NB) by trypsin or v-*myc* expression	Chicken OPN, which has two RGD sequences, cloned	Castagnola et al. 1991 J Biol Chem 266:9944

*Publications in which OPN expression has been shown the respond to a particular perturbation are listed. Omitted are publications cited in the text and others that report enhanced OPN expression in malignancies or in response

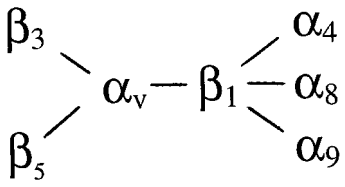

Fig. 1. Integrins that bind OPN. Faccio et al. [1998] reported that the $\alpha_v\beta_3$ integrin on the osteoclast-like cell line GCT23 could exist in an inactive or an active state and that the activated integrin was more effective at binding OPN and promoting migration.

for OPN in the clotting process because thrombin formed from prothrombin at the site of vascular injury initiates clot formation by cleaving fibrinogen. Cleavage of OPN during clot formation would expose the $\alpha_9\beta_1$ binding site, which could serve to attract and promote the adherence of cells expressing $\alpha_9\beta_1$ (also $\alpha_v\beta_3$). Possibly this underlies the observation by Liaw et al. [1998] that skin wounds healed aberrantly in mice lacking OPN. The removal of tissue debris was retarded, apparently the result of impaired macrophage function rather than reduced macrophage infiltration. Although the newly formed skin matrix appeared less well organized, and the collagen fibrils were smaller, the tensile properties of the healed wound appeared normal.

Binding of OPN to any of the above receptors impacts on cellular signaling pathways. Changes in intracellular Ca^{2+} levels, in protein tyrosine phosphorylation, in oxidant levels, and in phosphoinositide metabolism have been reported [e.g., Denhardt et al., 1995; Chellaiah and Hruska, 1996]. When integrin receptors cluster in an adhesion plaque, interactions among associated proteins, including paxillin, vinculin, focal adhesion kinase, *src, cas,* actin, and α-actinin result in changes in protein phosphorylation and cytoskeletal structure. Elgavish et al. [1998] discovered a subpopulation of primary prostate epithelial cells that, under growth-restricting conditions, could proliferate on a substrate coated with OPN, but not on substrates coated with collagen or fibronectin. This suggests that certain cells can be stimulated to proliferate by OPN in the extracellular matrix, possibly explaining the tendency of prostate tumors to metastasize to bone. Rat aortic endothelial cells undergo apoptosis when plated on plastic in the prolonged absence of serum. However, if the cells are plated on plastic coated with OPN the cells survive, apparently as the result of a Ras- and Src-dependent activation of NFκB mediated by the $\alpha_v\beta_3$ integrin [Scatena et al., 1998]. OPN, previously reported to inhibit the induction of inducible nitric oxide synthase by inflammatory mediators in cell culture [Hwang et al., 1994], has also been shown to inhibit induction of iNOS by LPS in tissue from rat thoracic aortae [Scott et al., 1998].

Soluble OPN partially inhibits the apoptotic response of human umbilical vein endothelial cells deprived of necessary growth factor stimulation (C.A. Lopez, J. Zhang, and D.T. Denhardt, in preparation). The results presented in Figure 2 show that OPN affects the apparent distribution of Bcl-$X_{S/L}$ in the cell. In contrast to the control cells in Figure 2A, the growth factor-deprived cells shown in Figure 2B are undergoing apoptosis (data not shown) and exhibit a change in the localization of Bcl-$X_{S/L}$ from a predominantly perinuclear location to a more diffuse cytoplasmic distribution. Independent preparations of OPN from two different sources inhibited apoptosis and the dispersal of Bcl-$X_{S/L}$ (Fig. 2C,D). Bcl-X_L is a member of the anti-apoptotic Bcl-2 family that appear to function by inhibiting activation of procaspases and maintaining the integrity of organelle membranes. By contrast, Bcl-X_s is pro-apoptotic. Although the details of the signaling pathway remain unclear, OPN evidently facilitates the survival of stressed cells by suppressing apoptosis.

OPN IN MINERALIZED TISSUES: ROLE IN BONE REMODELING

OPN, one of the more abundant noncollagenous proteins in bone, is localized to cell–matrix and matrix–matrix interfaces in mineralized tissues, notably the lamina limitans and cement lines, where it is deposited as the result of osteoclast action [Dodds et al., 1995]. OPN may protect the exposed bone surface or prime it for subsequent cell–matrix interactions. McKee and Nanci [1996] have proposed that OPN acts as an opsonin, facilitating macrophage adhesion and phagocytosis of particulate mineralized tissue debris. OPN can be cross-linked by transglutaminase; it can bind to various extracellular molecules, including type I collagen, fibronectin, and osteocalcin. This might be expected to add physical strength to extracellular matrices. OPN promotes the attachment of bone cells to bone matrix, although there is disagreement regarding its colocalization with the $\alpha_v\beta_3$ integrin in the osteoclast sealing zone [Butler et al., 1996].

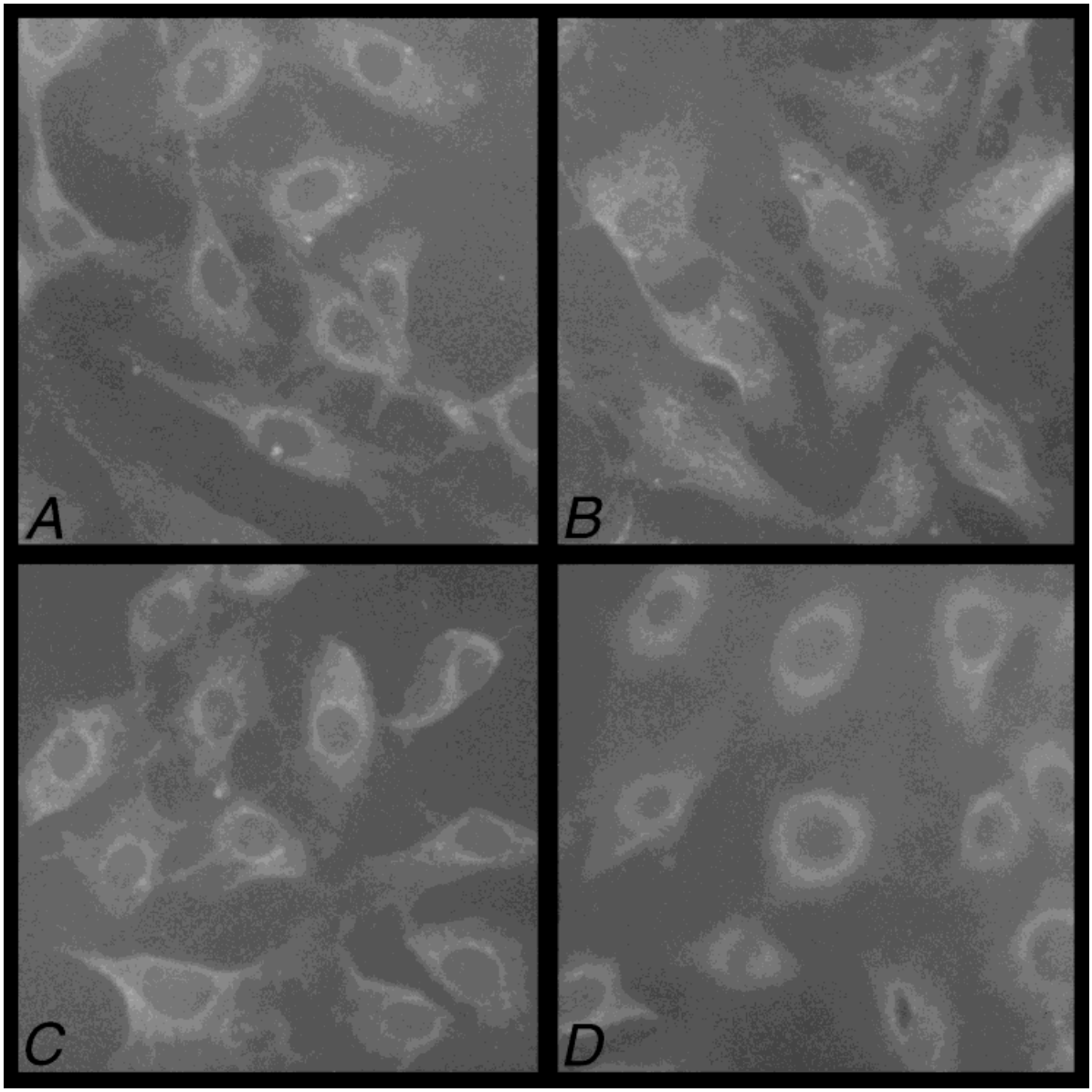

Fig. 2. OPN inhibits the redistribution of Bcl-$X_{S/L}$ induced in human umbilical vein endothelial cells by growth factor deprivation. Cells (2×10^4) were plated on gelatin-coated glass coverslips in M199 medium supplemented with 10% fetal calf serum (FCS) (HyClone, Logan, UT), 4 ng/ml acidic fibroblast growth factor (Sigma Chemical Co., St. Louis, MO), 7.5 µg/ml endothelial cell growth factor supplement (Sigma) and 5 U/ml heparin. Two days later, the medium was removed and replaced with M199 plus 1% bovine serum albumin (BSA) and other factors as indicated below; 12 h later, the cells were fixed in methanol at 0°C for 6 min at −20°C, washed 3× with phosphate-buffered saline (PBS), and incubated with 0.3% Triton X100 + 5% BSA for 1 h at 25°C. The coverslips were then incubated for 1 h with 1 µg/ml anti-Bcl-$X_{S/L}$ (Santa Cruz Biotechnology, Santa Cruz, CA), washed 3× with PBS, and then incubated further with FITC-conjugated Affinipure goat anti-rabbit IgG(H + L) (Jackson ImmunoResearch Laboratories, West Grove, PA). Fluorescence in the cells was visualized using a Nikon Diaphot 300 microscope. **A:** Cells in M199/BSA with fibroblast growth factor-α (FGF-α) ECGS (endothelial cell growth supplement), and FCS. **B:** Cells in M199/BSA alone. **C:** Cells in M199/BSA without supplements but with 5 pM human OPN. The human OPN was purified from conditioned medium as described by Hwang et al. [1994]. **D:** Cells in M199/BSA without supplements but with 5 pM bovine OPN purified from milk, a gift from Dr. E. Sörensen (Aarhus, Denmark). **Color plate on page 318.**

OPN is not required for the development of bones and teeth because these mineralized tissues are essentially normal in mice lacking OPN, even though there is an apparent oversupply of osteoclast precursors in the spleen and marrow compared with control mice [Rittling et al., 1998]. It has been shown that ovariectomized OPN-deficient mice do not exhibit the decrease in bone density that occurs in wild-type mice (H. Yoshitake, S.R. Rittling, D.T. Den-

hardt, and M. Noda, submitted). It appears that bone lacking OPN is less readily remodeled. Chackalaparampil et al. [1996] observed that $Src^{-/-}$ mice, which develop osteopetrosis due to impaired osteoclast action, expressed significantly less OPN than controls. In contrast, OPN was expressed at higher levels in rats bearing any one of three different mutations (*ia*, op, *tl*) that cause osteopetrosis, which results from a defect in bone resorption [Shalhoub et al., 1994]. The observed increased accumulation of OPN in the bones of these animals, which is possibly the consequence of higher levels of circulating $1\alpha,25(OH)_2$vitaminD_3, may represent an effort to compensate for the defect in bone resorption.

OPN can be phosphorylated intracellularly by the Golgi apparatus casein kinase [Lasa et al., 1997] and extracellularly by cell surface ectoenzymes with a specificity resembling that of casein kinase II (CKII) [Zhu et al., 1997]. In reviewing the different types of serine-phosphorylated and tyrosine-sulfated forms of OPN produced by pre-osteoblasts and osteoblasts, Sodek et al. [1995] report that bisphosphonates markedly suppress the expression of OPN by rat calvarial cells, possibly accounting for the ability of bisphosphonates to inhibit bone resorption. Some experiments suggest that OPN species with different post-translational modifications may have different functions in bone modeling or remodeling. The strong conservation of the phosphorylated serine residues and their sequence contexts suggests that they present a configuration of charges that is necessary for a crucial action, possibly that of binding bone mineral matrix in a very specific conformation that facilitates unique interactions of OPN with bone cells. Since dephosphorylated OPN does not support osteoclast attachment, it is possible that the tartrate-resistant acid phosphatase (TRAP), which is also secreted by osteoclasts, regulates osteoclast motility and detachment by its action on OPN [Dodds et al., 1995]. Phosphorylation of recombinant OPN by CKII affects its ability to support cell adhesion in a cell- or receptor-specific manner [Katayama et al., 1998]. Phosphorylation increased the capacity of OPN to stimulate osteoclast attachment about twofold. Whereas osteoclast attachment depended on a β_3 integrin and the GRGDS sequence, osteoblast attachment was independent of the β_3 integrin, though it still required the GRGDS sequence. Unlike osteoclast attachment, osteoblast attachment was not enhanced by CKII phosphorylation of OPN.

Mechanical stresses on cells influence OPN expression. Intermittent hydrostatic compression of various osteoblast cell types in culture augmented OPN (and alkaline phosphatase) expression [Kubota et al., 1993; Klein-Nulend et al., 1997]. Harter et al. [1995] found that osteoblast-like cells exposed to chronic intermittent mechanical strain contained elevated levels of OPN and type I collagen mRNA. In the intact mouse OPN mRNA expression was increased, and myeloperoxidase expression decreased, after mechanical loading (repeated rapid bending for 3 min) of the rat tibia [Miles et al., 1998]. These mechanical forces are thought to act at the sites of cell attachment, perhaps involving OPN, and to generate a shear stress at adhesion plaques. Mechanical stresses transmitted by the integrins to the cytoskeleton modify cell shape and gene expression. Increased $[Ca^{2+}]_i$ resulting from the stimulation of mechanosensitive channels likely also modifies gene expression. These studies are consistent with a function for OPN in bone remodeling, but they do not indicate whether the function is primarily that of a static attachment factor or a dynamic signaling molecule.

OPN IN EXTRACELLULAR FLUIDS: ROLES IN IMMUNITY, INFECTION, INFLAMMATION, AND CANCER

Activation of T lymphocytes results in a substantial increase in OPN transcription, hence the alternative name early T-lymphocyte activation gene 1, or Eta-1 [Patarca et al., 1993]. Eta-1/OPN is able to enhance resistance to infection by flaviviruses (cause of yellow fever and viral encephalitis) and *Rickettsia tsutsugamushi* (now called *Orientia tsutsugamushi*). Weber and Cantor [1996] have presented arguments that Eta-1/OPN is T-lymphocyte suppressor factor, which acts to inhibit the helper activity of $CD5^+$ T cells and thereby suppress primary antibody responses. They also summarize evidence that Eta-1/OPN stimulates immunoglobulin production by B lymphocytes, possibly causing some forms of autoimmune disease when overproduced. Rotavirus infection of the intestinal epithelia stimulates OPN expression, a response that seems to hinder the course of the disease since infected OPN-deficient mice exhibit a more severe diarrhea than is seen in the control mice (E.E.

Rollo, S.J. Hempson, S.R. Rittling, D.T. Denhardt, E.R. Mackow, and R.D. Shaw, in preparation).

OPN expression is enhanced as a consequence of tissue injury. It is found at high levels in granulomas associated with *Mycobacterium tuberculosis*, silicosis, reactions to foreign material, or certain immunologic disorders [Nau et al., 1997; Carlson et al., 1997]. OPN produced at sites of injury or infection is a potent chemoattractant for macrophages, which themselves often express high levels of OPN. Giachelli et al. [1998] showed that macrophage accumulation induced by intradermal injection of N-formyl-met-leu-phe (FMLP) was substantially inhibited by anti-osteopontin antibody. OPN expression is upregulated during the maturation of monocytes into macrophages [Krause et al., 1996], a process that presumably occurs as circulating monocytes extravasate and migrate through the tissue (up a cytokine/chemokine/OPN? gradient) to the site of injury.

Feuerstein and colleagues studied OPN expression in infarcts caused by occlusion of the middle cerebral artery of spontaneously hypertensive rats [Ellison et al., 1998]. The resulting focal ischemia was associated with a subset of ED-1 positive (possibly microglia-derived) macrophages that accumulated in the ischemic zone, and by 5 days after occlusion a maximal increase in OPN mRNA of about fiftyfold was reached. They suggested that OPN be considered a stress-inducible protein necessary during the healing process to form the peri-infarct scar and a new glial-limiting membrane. OPN expression is strongly elevated in the kidney by ischemic injury [Padanilam et al., 1996] or hydronephrosis [Diamond et al., 1995]. Ischemic injury induced in the kidney of the OPN-deficient mouse by clamping the renal artery appears to be more severe in comparison with the wildtype animal, possibly because there is also an increase in iNOS activity (E. Noiri, K. Dickman, F. Miller, G. Romanov, V.I. Romanov, R. Shaw, S.R. Rittling, D.T. Denhardt, and M.S. Goligorsky, submitted).

OPN may cause the restenosis observed after angioplasty. Restenosis results from local inflammation, thrombosis, and invasion and proliferation of smooth muscle cells within the intima of coronary arteries. OPN generated at the site of the angioplasty-induced injury to the endothelium interacts with $\alpha_v\beta_3$ integrins on coronary artery smooth muscle cells, causing the cells to migrate along the putative OPN gradient to the site of injury and to proliferate there in a PDGF-dependent manner. Evidence for this includes the ability of antisense oligonucleotides and antibodies targeting either OPN or $\alpha_v\beta_3$ to inhibit the migratory, invasive and proliferative abilities of coronary artery smooth muscle cells [Panda et al., 1997]. Weintraub et al. [1996] found that the ability of vascular smooth muscle cells to adhere and to invade collagen gels correlated with the amount of OPN they produced. This implies an autocrine/paracrine signaling pathway. Other examples of OPN expression associated with inflammation include renal tubulointerstitial disease, atherosclerosis, and myocardial necrosis [Giachelli et al., 1995]. Clearly, OPN can stimulate macrophage accumulation. Although this is likely important in combatting infections, in other circumstances the release of inflammatory mediators by the activated macrophages may actually increase the amount of tissue damage.

OPN, known earlier as transformation associated phosphoprotein, enhances the metastatic potential of transformed cells [Feng et al., 1995; Oates et al., 1996]. It is often expressed at high levels by malignant cells, correlating with the levels of Ras activation. A sequence element in the OPN promoter that contributes to increased OPN expression in Ras-transformed mouse 3T3 fibroblasts was described by Guo et al. [1995]. OPN is abundant in many human carcinomas, particularly at the invasive edge of the tumor. In different tumor types it may be produced by the tumor cells themselves and/or by macrophages associated with the tumor [Brown et al., 1994; Tuck et al., 1998]. Expression of OPN and CD44v9 by tumor cells strongly correlates with the extent of lymphatic vessel invasion and distant metastases in gastric cancer [Ue et al., 1998]. Weber et al. [1997] have suggested that the interaction of a modified OPN species with CD44, dysregulation of which has long been associated with the malignant phenotype, fosters the metastatic process by stimulating migration of the cancer cell. Chambers and her colleagues used a newly developed quantitative enzyme-linked immunosorbent assay (ELISA) for OPN to establish that elevated OPN levels in the plasma of patients with metastatic disease are associated with reduced patient survival [Singhal et al., 1997].

Shanmugam et al. [1997] showed that Rat-1 cells transformed with a temperature-sensitive

RSV mutant secreted at 41°C a highly sialylated 69-kDa form of OPN that was able to bind to the cells. At 34°C, the 69-kDa form was converted by a cell surface sialidase to a 62-kDa form that could not bind to cells. It was suggested that the inability of the 62-kDa species to bind to the cell might augment the transformed phenotype. In addition to inhibiting apoptosis (Fig. 2), OPN may promote the survival of transformed cells by reducing NO production by cytotoxic macrophages [Rollo et al., 1997] or by facilitating tumor cell adherence and invasion [Weintraub et al., 1996]. OPN, whose expression is increased by Ras activation, may negatively regulate Ras signaling pathways. This hypothesis is supported by the observation that 3T3 cells able to synthesize OPN appear able to support higher levels of oncogenic Ras expression than cells unable to synthesize OPN (Wu, D.T. Denhardt, and S.R. Rittling, submitted). We suggest that a high level of Ras signaling, as from a Ras oncogene, causes the death of the cell (by apoptosis?) in the absence of OPN.

REFERENCES

Bayless KJ, Meininger GA, Scholtz JM, Davis GE (1998): Osteopontin is a ligand for the $\alpha_4\beta_1$ integrin. J Cell Sci 111:1165–1174.

Bonnelye E, Vanacker JM, Dittmar T, Begue A, Desbiens X, Denhardt DT, Aubin JE, Laudet V, and Fournier B (1997): The ERR-1 orphan receptor is a transcriptional activator expressed during bone development. Mol Endocrinol 11: 905–916.

Botquin V, Hess H, Fuhrmann G, Anastassiadis C, Gross MK, Vreind G, Schöler HR (1998): New Pou dimer configuration mediates antagonistic control of an osteopontin preimplantation enhancer by Oct-4 and Sox-2. Genes Dev 12:2073–2090.

Broess M, Riva A, Gerstenfeld LC (1995): Inhibitory effects of $1,25(OH)_2$ Vitamin D_3 on collagen type I, osteopontin, and osteocalcin gene expression in chicken osteoblasts. J Cell Biochem 57:440–451.

Brown LF, Papadopoulos-Sergiou A, Berse B, Manseau EJ, Tognazzi K, Perruzzi CA, Dvorak HF, Senger DR (1994): Osteopontin expression and distribution in human carcinomas. Am J Pathol 145:610–623.

Butler WT, Ridall AL, McKee MD (1996): Osteopontin. In Bilezikian JP, Raisz LG, Rodan GA (eds): "Principles of Bone Biology." San Diego: Academic Press, pp 167–181.

Carlson I, Tognazzi K, Manseau EJ, Dvorak HF, Brown LF (1997): Osteopontin is strongly expressed by histiocytes in granulomas of diverse etiology. Lab Invest 77:103–108.

Chackalaparampil I, Peri A, Nemir M, McKee MD, Lin PH, Mukherjee BB, Mukherjee AB (1996): Cells in vivo and in vitro from osteopetrotic mice homozygous for c-src disruption show suppression of synthesis of osteopontin, a multifunctional extracellular matrix protein. Oncogene 12: 1457–1467.

Chellaiah M, Hruska K (1996): Osteopontin stimulates gelsolin-associated phosphoinositide levels and phosphatidylinositol triphosphate-hydroxyl kinase. Mol Biol Cell 7:743–753.

Denda S, Reichardt LF, Müller U (1998): Identification of osteopontin as a novel ligand for the integrin $\alpha_8\beta_1$ and potential roles for this integrin-ligand interaction in kidney morphogenesis. Mol Biol Cell 9:1425–1435.

Denhardt DT, Lopez CA, Rollo EE, Hwang S-m, An X, Walther SE (1995): Osteopontin-induced modifications of cellular functions. Ann NY Acad Sci 760:127–142.

Diamond JR, Kees-Folts D, Ricardo SD, Pruznak A, Eufemio M (1995): Early and persistent up-regulated expression of renal cortical osteopontin in experimental hydronephrosis. Am J Pathol 146:1455–1466.

Dodds RA, Connor JR, James IE, Rykaczewski EL, Appelbaum E, Dul E, Gowen M (1995): Human osteoclasts, not osteoblasts, deposit osteopontin onto resorption surfaces: An in vitro and ex vivo study of remodeling bone. J Bone Miner Res 10:1666–1680.

Elgavish A, Prince C, Chang P-L, Lloyd K, Lindsey R, Reed R (1998): Osteopontin stimulates a subpopulation of quiescent human prostate epithelial cells with high proliferative potential to divide in vitro. Prostate 35:83–94.

Ellison JA, Velier JJ, Spera P, Jonak ZL, Wang X, Barone FC, Feuerstein GZ (1998): Osteopontin and its integrin receptor $\alpha_v\beta_3$ are upregulated during formation of the glial scar after focal stroke. Stroke 29:1698–1707.

Faccio R, Grano M, Colucci S, Zallone AZ, Quaranta V, Pelletier AJ (1998): Activation of $\alpha_v\beta_3$ integrin on human osteoclast-like cells stimulates adhesion and migration in response to osteopontin. Biochem Biophys Res Commun 249:522–525.

Feng B, Rollo EE, Denhardt DT (1995): Osteopontin (OPN) may facilitate metastasis by protecting cells from macrophage NO-mediated cytotoxicity: Evidence from cell lines down-regulated for OPN expression by a targeted ribozyme. Clin Exp Metast 13:453–462.

Giachelli CM, Schwartz SM, Liaw L (1995): Molecular and cellular biology of osteopontin: Potential role in cardiovascular disease. Trends Cardiovasc Med 5:88–95.

Giachelli CM, Lombardi D, Johnson RJ, Murry CE, Almeida M (1998): Evidence for a role of osteopontin in macrophage infiltration in response to pathological stimuli in vivo. Am J Pathol 152:353–358.

Guo X, Zhang YP, Mitchell DA, Denhardt DT, Chambers AF (1995): Identification of a *ras*-activated enhancer in the mouse osteopontin promoter and its interaction with a putative ETS-related transcription factor whose activity correlates with the metastatic potential of the cell. Mol Cell Biol 15:476–487.

Harter LV, Hruska KA, Duncan RL (1995): Human osteoblast-like cells respond to mechanical strain with increased bone matrix protein production independent of hormonal regulation. Endocrinology 136:528–535.

Hwang S-m, Lopez CA, Heck DE, Gardner CR, Laskin DL, Laskin JD, Denhardt DT (1994): Osteopontin inhibits induction of nitric oxide synthase gene expression by inflammatory mediators in mouse kidney epithelial cells. J Biol Chem 269:711–715.

Katagiri YU, Murakami M, Mori K, Iizuka J, Hara T, Tanaka K, Jia W-Y, Chambers AF, Uede T (1996): Non-RGD-domains of osteopontin promote cell adhesion without involving αv integrins. J Cell Biochem 62:123–131.

Katayama Y, House CM, Udagawa N, Kazama JJ, McFarland RJ, Martin TJ, Findlay DM (1998): Casein kinase 2 phosphorylation of recombinant rat osteopontin enhances adhesion of osteoclasts but not osteoblasts. J Cell Physiol 176:179–187.

Klein-Nulend J, Roelofsen J, Semeins CM, Bronckers ALJJ, Burger EH (1997): Mechanical stimulation of osteopontin mRNA expression and synthesis in bone cell cultures. J Cell Physiol 170:174–181.

Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T (1997): Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 89:755–764.

Krause SW, Rehli M, Kreutz M, Schwarzfischer L, Paulauskis JD, Andreesen R (1996): Differential screening identifies genetic markers of monocyte to macrophage maturation. J Leukoc Biol 60:540–545.

Kubota T, Yamauchi M, Onozaki J, Sato S, Suzuki Y, Sodek J (1993): Influence of an intermittent compressive force on matrix protein expression by ROS17/2.8 cells, with selective stimulation of osteopontin. Arch Oral Biol 38: 23–30.

Lasa M, Chang P-L, Prince CW, Pinna LA (1997): Phosphorylation of osteopontin by golgi apparatus casein kinase. Biochem Biophys Res Commun 240:602–605.

Liaw L, Skinner MP, Raines EW, Ross R, Cheresh DA, Schwartz SM, Giachelli CM (1995): The adhesive and migratory effects of osteopontin are mediated via distinct cell surface integrins. J Clin Invest 95:713–724.

Liaw L, Birk DE, Ballas CB, Whitsitt JS, Davidson JM, Hogan BLM (1998): Altered wound healing in mice lacking a functional osteopontin gene. J Clin Invest 101:1468–1478.

Liu Y, Nifuji A, Olson E, Noda M (1996): Sclerotome-related helix loop helix type transcription factor (scleraxis) mRNA is expressed in osteoblasts and its level is enhanced by type-beta transforming growth factor. J Endocrin 151: 491–499.

Liu Y, Watanabe H, Nifuji A, Yamada Y, Olson EN, Noda M (1997): Overexpression of a single helix-loop-helix type transcription factor, scleraxis, enhances aggrecan gene expression in osteoblastic osteosarcoma ROS17/2.8 cells. J Biol Chem 272:29880–29885.

McKee MD, Nanci A (1996): Secretion of osteopontin by macrophages and its accumulation at tissue surfaces during wound healing in mineralized tissues: A potential requirement for macrophage adhesion and phagocytosis. Anat Rec 245:394–409.

Miles RR, Turner CH, Santerre, Tu Y, McClelland P, Argot J, DeHoff BS, Mundy CW, Rosteck PR, Bidwell J, Sluka JP, Hock J, Onyia JE (1998): Analysis of differential gene expression in rat tibia after an osteogenic stimulus in vivo: Mechanical loading regulates osteopontin and myeloperoxidase. J Cell Biochem 68:355–365.

Nau GJ, Guilfoile P, Chupp GL, Berman JS, Kim SJ, Kornfeld H, Young RA (1997): A chemoattractant cytokine associated with granulomas in tuberculosis and silicosis. Proc Natl Acad Sci USA 94:6414–6419.

Oates AJ, Barraclough R, Rudland PS (1996): The identification of osteopontin as a metastasis-related gene product in a rodent mammary tumour model. Oncogene 13:97–104.

Oates AJ, Barraclough R, Rudland PS (1997): The role of osteopontin in tumorigenesis and metastasis. Invas Metast 17:1–15.

Padanilam BJ, Martin DR, Hammerman MR (1996): Insulin-like growth factor I-enhanced renal expression of osteopontin after acute ischemic injury in rats. Endocrinology 137:2133–2140.

Panda D, Kundu GC, Lee BI, Peri A, Fohl D, Chackalaparampil I, Mukherjee BB, Li XD, Mukherjee DC, Seides S, Rosenberg J, Stark K, Mukherjee AB (1997): Potential roles of osteopontin and $\alpha_v\beta_3$ in the development of coronary artery restenosis after angioplasty. Proc Natl Acad Sci USA 94:9308–9313.

Patarca R, Saavedra RA, Cantor H (1993): Molecular and cellular basis of genetic resistance to bacterial infection: The role of the early T-lymphocyte activation-1/osteopontin gene. Crit Rev Immunol 13:225–246.

Rittling SR, Denhardt DT (1999): Osteopontin (OPN) function in pathology: Lessons from OPN-deficient mice. Exp Nephrol (in press).

Rittling SR, Matsumoto HN, McKee MD, Nanci A, An X-R, Novick KE, Kowalski AJ, Noda M, Denhardt DT (1998): Mice lacking osteopontin show normal development and bone structure but display altered osteoclast formation in vitro. J Bone Miner Res 13:1101–1111.

Rollo EE, Laskin DL, Denhardt DT (1996): Osteopontin inhibits nitric oxide production and cytotoxicity by activated RAW264.7 macrophages. J Leukocyte Biol 60:397–404.

Scatena M, Almeida M, Chaisson ML, Fausto N, Nicosia RF, Giachelli GM (1998): NF-κB mediates $\alpha_v\beta_3$ integrin-induced endothelial cell survival. J Cell Biol 141:1083–1093.

Scott JA, Weir L, Wilson SM, Xuan JW, Chambers AF, McCormack DG (1998): Osteopontin inhibits inducible nitric oxide synthase activity in rat vascular tissue. Am J Physiol (in press).

Senger DR, Perruzzi CA (1996): Cell migration promoted by a potent GRGDS-containing thrombin-cleavage fragment of osteopontin. Biochim Biophys Acta 1314:13–24.

Shalhoub V, Bettencourt B, Jackson ME, McKay CA, Glimcher MJ, Marks SC, Stein GS, Lian JB (1994): Abnormalities of phosphoprotein gene expression in three osteopetrotic rat mutations: Elevated mRNA transcripts, protein synthesis, and accumulation in bone of mutant animals. J Cell Physiol 158:110–120.

Shanmugam V, Chackalaparampil I, Kundu GC, Mukherjee AB, Mukherjee BB (1997): Altered sialylation of osteopontin prevents its receptor-mediated binding on the surface of oncogenically transformed tsB77 cells. Biochemistry 36:5729–5738.

Singhal H, Bautista DS, Tonkin KS, O'Malley FP, Tuck AB, Chambers AF, Harris JF (1997): Elevated plasma osteopontin in metastatic breast cancer associated with increased tumor burden and decreased survival. Clin Cancer Res 3:605–611.

Smith LL, Cheung H-K, Ling LE, Chen J, Sheppard D, Pytela R, Giachelli GM (1996): Osteopontin N-terminal domain contains a cryptic adhesive sequence recognized by $\alpha_9\beta_1$ integrin. J Biol Chem 271:28485–28491.

Smith LL, Giachelli CM (1998): Structural requirements for $\alpha_9\beta_1$-mediated adhesion and migration to thrombin-cleaved osteopontin. Exp Cell Res 242:351–360.

Sodek J, Chen J, Nagata T, Kasugai S, Todescan R, Li IWS, Kim RH (1995): Regulation of osteopontin expression in osteoblasts. Ann NY Acad Sci 760:223–241.

Tsuji K, Ito Y, Noda M (1998): Expression of the PEBP2α/AML3/CBFA1 gene is regulated by BMP4/7 heterodimer and its overexpression suppresses type I collagen and osteocalcin gene expression in osteoblastic and nonosteoblastic mesenchymal cells. Bone 22:87–92.

Tuck AB, O'Malley FP, Singhal H, Harris JF, Tonkin KS, Kerkvliet N, Saad Z, Doig GS, Chambers AF (1998): Osteopontin expression in a group of lymph node negative breast cancer patients. Int J Cancer 79:502–508.

Ue T, Yokozaki H, Kitadai Y, Yamamoto S, Yasui W, Ishikawa T, Tahara E (1998): Co-expression of osteopontin and CD44v9 in gastric cancer. Int J Cancer 79:127–132.

Weber GF, Cantor H (1996): The immunology of Eta-1/osteopontin. Cytokine Growth Factor Rev 7:241–248.

Weber GF, Ashkar S, Glimcher MJ, Cantor H (1996): Receptor–ligand interaction between CD44 and osteopontin (Eta-1). Science 71:509–512.

Weber GF, Ashkar S, Cantor H (1997): Interaction between CD44 and osteopontin as a potential basis for metastasis formation. Proc Assoc Am Physicians 109:1–9.

Weintraub AS, Giachelli CM, Krauss RS, Almeida M, Taubman MB (1996): Autocrine secretion of osteopontin by vascular smooth muscle cells regulates their adhesion to collagen gels. Am J Pathol 149:259–272.

Xuan J-W, Hota C, Shigeyama Y, D'Errico JA, Somerman MJ, Chambers AF (1995) Site-directed mutatgenesis of the RGD sequence in osteopontin destroys cell adhesion and migration functions. J Cell Biochem 57:680–690.

Zhu X, Luo C, Ferrier JM, Sodek J (1997): Evidence of ectokinase-mediated phosphorylation of osteopontin and bone sialoprotein by osteoblasts during bone formation in vitro. Biochem J 323:637–643.

NOTE ADDED IN PROOF

McKee and Nanci (1996) have commented on the ability of osteoblasts to deposit OPN in remodeling bone. Zohar et al. (1997) have observed what appears to be OPN in a perimembranous location (as opposed to the perinuclear localization of OPN destined for secretion) specifically in migrating calvarial cells. Crawford et al. (1998) have reported evidence that OPN derived from tumor cells may serve a different function from that performed by OPN derived from host cells. Rittling and Feng (1998) have noted that antisera against OPN may cross-react with other cellular proteins.

Crawford HC, Matrisian LM, Liaw L. (1998): Distinct roles of osteopontin in host defense activity and tumor survival during squamous cell carcinoma progression in vivo. Cancer Res 58:5206–5215.

McKee MD, Nanci A (1996): Osteopontin deposition in remodeling bone: An osteoblast-mediated event. J Bone Miner Res 11:873–874.

Rittling SR, Feng F (1998): Detection of mouse osteopontin by western blotting. Biochem Biophys Res Common 250: 287–292.

Zohar R, Lee W, Arora P, Cheifetz S, McCulloch C, Sodek J (1997): Single cell analysis of intracellular osteopontin in osteogenic cultures of fetal rat calvarial cells. J Cell Physiol 170:88–100.