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Original

Endothelin-1 regulates rat bone sialoprotein gene transcription

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Abstract: Endothelin-1 (ET-1) was originally discovered as a vasoconstrictor protein excreted by vascular endothelial cells. Recently, tumor-produced ET-1 has been considered to stimulate osteoblasts to form new bone, and to be an important mediator of osteoblastic bone metastasis. ET-1 has high affinity for two different membrane receptors, ET_AR and ET_BR, which are expressed by many types of cells including osteoblasts. Bone sialoprotein (BSP) is a phosphorylated and sulfated glycoprotein associated with mineralized connective tissues. To investigate the effects of ET-1 on BSP transcription, we used rat osteoblast-like ROS17/2.8 cells. Levels of BSP and osteopontin mRNA were increased at 12 h after treatment with ET-1 (10 ng/ml), and ET-1 at the same concentration induced luciferase activity of a -116 to +60 BSP promoter construct at 6 h. Transcriptional activity of -84BSPLUC, which contains the cAMP response element (CRE), was increased by ET-1. Furthermore, at 6 h, ET-1 (10 ng/ml) increased the binding of nuclear protein to CRE, the FGF2 response element (FRE) and the homeodomain protein-binding site (HOX). Antibodies against CREB1, JunD and Fra2 disrupted the formation of CRE-protein complexes, while antibodies against Runx2 and Dlx5 reduced the formation of FRE- and HOX-protein complexes. These findings indicate that ET-1 increases BSP transcription via the CRE, FRE and HOX sites in the rat BSP gene promoter. (J Oral Sci 52, 221-229, 2010)

Keywords: bone sialoprotein; endothelin-1; bone formation; osteoblasts; transcription.

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Introduction

Endothelin (ET) was originally discovered as a vasoactive peptide produced by vascular endothelial cells, and was shown to cause constriction of capillary vessels (1-3). Subsequent gene cloning studies revealed the presence of three structurally and pharmacologically distinct isopeptides, ET-1, ET-2, and ET-3 in humans (4). They all consist of 21 amino acids and have two intramolecular disulfide bonds. The propeptide form of ET, known as big ET, consists of 38 amino acids, and is cleaved by an ET-converting enzyme to yield an active mature form (5). ET-1 is not organ-specific, and is expressed primarily by endothelial cells (1).

ETs exert their effects by binding to two distinct cell surface ET receptors, ET_AR and ET_BR. Both of them are transmembrane domain molecules that belong to class A of the G-protein-coupled heptahelical receptors with an external amino terminus, an internal carboxy terminus, and binding sites intrinsic to the heptahelical portions of the receptor (6). ET_BR binds to the three peptide isotypes with equal affinity. In contrast, ER_AR binds to ET-1 with higher affinity than the other isoforms (7). Therefore, the endogenous agonist ET-1 has high affinity for ET_AR and ET_BR. Upon activation by ET-1, ET_AR interacts with and activates a G-protein that triggers parallel activation of several signal-transducing pathways. These include

phospholipase C activity with a consequent increase of intracellular calcium levels, protein kinase C (PKC) (8), epidermal growth factor (EGF) receptor (9), phosphatidylinositol-3-kinase (PI3-K) (10), ras/raf/mitogen-activated protein kinase (MAPK) and p125 focal adhesion kinase (FAK) phosphorylation pathways (11). This cascade of events ultimately induces nuclear transcription of several proto-oncogenes, including *c-myc*, *c-fos*, and *c-jun*, which have the capability of influencing cell growth and proliferation (12). ET may also have significant effects on bone, affecting both bone-forming and bone-resorptive cells (13-16). ET-1 increases osteoblastic intracellular calcium concentrations, and reduces parathyroid hormone (PHT)-stimulated adenosine 3', 5'-cyclic monophosphate (cAMP) production by osteoblastic cells (17). ET-1 enhances the effect of other osteoblast-stimulatory factors, such as BMP-7, to induce bone formation (18-20). ET-1 stimulates phosphate transport, a process important for the initiation of bone matrix calcification, in osteoblast-like MC3T3-E1 cells, through ET_AR-mediated activation of PKC (21). Hypoplasia of the facial bones in ET-1 null mice suggests that matrix mineralization of the facial bones is disrupted in this strain (22). The ET-1 signature comprises several motifs, such as osteoblastic differentiation, invasion, and suppression of apoptosis. ET-1 activates calcineurin and causes nuclear translocation of NFATc1, implicating a pathway in the ET-1-mediated stimulation of osteoblasts. Suppression of apoptosis may be an important factor in the promotion of osteoblastic growth by ET-1 (23).

In pathological situations, tumor-derived ET-1 may induce localized bone formation when certain types of breast and prostate cancer metastasize to bone (24,25). Human breast and prostate cancer cell lines abundantly secrete ET-1. The bone anabolic response to ET-1 is blocked by the selective ET_AR antagonist atrasentan (ABT-627), but not by inhibition of ET_BR. ET_AR blockade significantly reduces osteoblastic bone metastases and the tumor burden in bone (26). The molecular mechanisms by which ET_AR activation stimulates bone responses are unknown. Some data have indicated that ET-1 regulates message and protein secretion of dickkopf homolog 1 (DKK1), a secreted inhibitor of the Wnt signaling pathway, in osteoblasts and can mediate the anabolic responses of osteoblasts to ET-1 (27).

Bone sialoprotein (BSP) is a highly sulfated, phosphorylated and glycosylated protein that is characterized by its ability to bind to hydroxyapatite through polyglutamic acid sequences and to mediate cell attachment through an Arg-Gly-Asp (RGD) sequence. The expression of BSP is essentially restricted to mineralized connective tissues, and BSP mRNA is produced at high levels at the onset of bone, dentin and cementum formation (28-29). The temporo-spatial deposition of BSP to nucleate hydroxyapatite crystal formation indicates its potential role in the initial mineralization of bone (30-31). BSP is also expressed in osteotropic cancers such as breast, lung, thyroid and prostate cancers. Thus, regulation of BSP gene expression plays an important role in the differentiation of osteoblasts, bone matrix mineralization and tumor metastasis (32). The human, mouse and rat BSP genes have been cloned and partially characterized. The BSP gene promoter includes a highly conserved region that extends upstream from the transcription start site to nts -370 (33-35). This region includes a functional inverted TATA box (36) overlapping with the vitamin D response element (37), an inverted CCAAT box that is required for basal promoter transcription activity (38,39). In addition, a cAMP response element (CRE) (40-42), a fibroblast growth factor 2 (FGF2) response element (FRE) (43-45), a pituitary-specific transcription factor-1 (Pit-1) motif (46) and a homeodomain protein-binding site (HOX) (45,47) have all been characterized. Further upstream, a transforming growth factor-β (TGF-β) activation element (TAE) (48) and a glucocorticoid response element (GRE) overlapping an AP1 site (49-51) have also been identified.

ET-1 modulates the expression of osteopontin (OPN) and osteocalcin mRNA in rat osteoblastic osteosarcoma (ROS17/2.8) cells (52). In the present study, we used ROS17/2.8 cells to determine the molecular mechanism responsible for the effects of ET-1 on BSP transcription. Our findings indicated that ET-1 increases BSP transcription through the mediation of CRE, FRE and HOX sites in the rat BSP gene promoter.

Materials and Methods

Materials

Alpha-minimum essential medium (α-MEM), fetal calf serum (FCS), penicillin, streptomycin, trypsin-EDTA and lipofectamine were obtained from Invitrogen (Carlsbad, CA). The PGL3-basic luciferase plasmid, pSV-β-galactosidase control vector (β-Gal) and U0126 were purchased from Promega Co. (Madison, WI, USA). The protein kinase inhibitors H89 and H7 were from Seikagaku Corporation (Tokyo, Japan). Tyrosine kinase inhibitor, herbimycin A (HA) and guanidium thiocyanate were purchased from Wako Pure Chemical Industries (Tokyo, Japan), and Src tyrosine kinase inhibitor PP1 was from Biomol Research Lab. (Plymouth Meeting, PA). ET-1 was purchased from Merck Japan (Tokyo, Japan). Rat osteoblast-like ROS17/2.8 cells were a gift from Dr. Jaro Sodek (49).

Cell culture

ROS 17/2.8 cells were used in this study as osteoblast-like cells that synthesize BSP, and cultured at 37°C in 5% CO_2, 95% air in α-MEM containing 10% FCS. The cells were first grown to confluence in 60-mm tissue culture dishes in α-MEM medium containing 10% FCS, then cultured in α-MEM without serum for 12 h and incubated with ET-1 (0, 0.1, 1, 10 and 100 ng/ml) for 12 h or incubated with ET-1 (10 ng/ml) for 3-24 h. Total RNA was extracted with guanidinium thiocyanate as described previously (49). RNA was isolated from triplicate cultures and analyzed for the expression of BSP, OPN and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA by Northern hybridization, as described below.

Northern hybridization

Twenty-five-microgram aliquots of total RNA were fractionated on a 1.2% agarose gel and transferred to a Hybond N+ membrane. Hybridizations were performed at 42°C with ^{32}P-labeled rat BSP, OPN and GAPDH cDNA probes. Following hybridization, the membranes were washed four times for 5 min each at 21°C in 300 mM sodium chloride, 30 mM trisodium citrate, pH 7.0, containing 0.1% SDS. This was followed by two 20-min washes at 55°C in 15 mM sodium chloride, 1.5 mM trisodium citrate, pH 7.0, 0.1% SDS. The hybridized bands, representing the rat BSP mRNA (two polyadenylated forms: 1.6 and 2.0 kilobases) and OPN mRNA were scanned in a Bio-imaging analyzer (Fuji BAS 2500, Tokyo, Japan).

Transient transfection assays

Exponentially growing ROS 17/2.8 cells were used for transient transfection assays. Twenty-four hours after plating, cells at 50-70% confluence were transfected using Lipofectamine reagent. The transfection mixture included 1 μg of a luciferase (LUC) construct (pLUC1, -18~+60; pLUC2, -43~+60; pLUC3, -116~+60; pLUC4, -425~+60; pLUC5, -801~+60) and 2 μg of β-Gal vector as an internal control. Two days after transfection, the cells were deprived of FCS for 12 h, and ET-1 (10 ng/ml) was added for 6 h prior to harvesting. The luciferase assay was performed in accordance with the supplier's protocol (picaGene, Toyo Inki, Tokyo, Japan) using a luminescence reader (Acuu FLEX Lumi 400; Aloka, Tokyo, Japan) to measure luciferase activity.

Gel mobility shift assays

Confluent ROS 17/2.8 cells in T-75 flasks incubated for 3 to 12 h with ET-1 (10 ng/ml) in α-MEM without FCS were used to prepare nuclear extracts, with the addition of extra proteinase inhibitors (the extraction buffer was 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM dithiothreitol, 25% (v/v) glycerol, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/ml pepstatin A, 1 μg/ml aprotinin, pH 7.9). For gel shift analysis, double-stranded oligonucleotides encompassing the inverted CCAAT (nt -61 to -37, 5'-CCGTGACCGTG**ATTGG**CTGCTGAGA), CRE (nt -84 to -59, 5'-CCCACAGCC**TGACGTCG**CACCGGCCG), FRE (nt -98 to -79, 5'-TTTTCT**GGTGAGAA**CCCACA), and HOX (nt -204 to -179, 5'-TCCTCAGCCT**TCAATTAAAT**CCCACA) sequences in the rat BSP promoter were prepared by Bio-Synthesis, Inc. (Lewisville, TX). These double-stranded oligonucleotides were end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Nuclear protein extracts (3 μg) were incubated for 20 min at room temperature with 0.1 pM radiolabeled double-stranded oligonucleotide in buffer containing 50 mM KCl, 0.5 mM EDTA, 10 mM Tris-HCl, pH 7.9, 1 mM dithiothreitol, 0.04% Nonidet P-40, 5% glycerol, and 1 μg of poly (dI-dC). Following incubation, the protein-DNA complexes were resolved by electrophoresis on 5% non-denaturing acrylamide gels (38:2 acrylamide/bis acrylamide) run at 200 V at room temperature. After electrophoresis, the gels were dried, and autoradiograms were prepared and analyzed using an image analyzer.

Statistical analysis

Quadruplicate samples were analyzed for each experiment, and experiments were replicated to ensure the consistency of the responses to the drugs. Significance of differences between treatments and controls was determined using unpaired Student's *t*-test.

Results

Effects of ET-1 on expression of mRNA for BSP and OPN

To study the regulation of BSP transcription by ET-1, we performed Northern hybridization analysis of total RNA extracted from osteoblastic ROS 17/2.8 cells. First, the dose-response relationship of BSP induction by ET-1 was established by treating the ROS 17/2.8 cells with different concentrations of ET-1 for 12 h. ET-1 increased the levels of BSP mRNA at 1 and 10 ng/ml and had a maximal effect at 10 ng/ml (Fig. 1A). When 10 ng/ml ET-1 was used to determine the time courses of expression of BSP and OPN mRNA, 10 ng/ml ET-1 upregulated BSP and OPN mRNA levels at 12 h, whereas no effect on GAPDH was observed (Fig. 1B).

ET-1 stimulates rat BSP gene transcription

To determine how ET-1 regulates BSP transcription,

transient transfection analyses were performed using chimeric constructs encompassing different regions of the rat BSP gene promoter ligated to a luciferase reporter gene (pLUC1~5) transfected into ROS 17/2.8 cells. After treatment with ET-1 (10 ng/ml) for 6 h, luciferase activities of BSP promoter constructs (pLUC3; nts -116~+60, pLUC4; nts -425~+60, pLUC5; nts -801~+60) were increased (Fig. 2). Included within the DNA sequence, which is unique to these regions, is an inverted CCAAT box (nts -50 to -46), and CRE (nts -75 to -68), Runx2 (nts -84 to -79), FRE (nts -92 to -85) and Pit-1 (nts -111 to -105) motifs (Fig. 3). To determine more precisely the target sites between nts -116 and -43 in the rat BSP gene promoter through which the effect of ET-1 was being mediated, we prepared a series of 5'-deletion constructs. The transcriptional activities of -84, -108 and -116BSPLUC were stimulated by ET-1 (10 ng/ml) at 6 h in ROS17/2.8 cells (Fig. 4). To determine the signaling pathways mediating the effects of ET-1, we used several protein kinase inhibitors of ET-1-regulated BSP transcription. Protein kinase A (PKA) inhibitor H89, tyrosine kinase inhibitor HA, Src tyrosine kinase inhibitor PP1 and mitogen-activated protein kinase (MAPK) kinase (MAPKK) inhibitor U0126 abolished the effects of ET-1 on -116BSPLUC transcriptional activity (data not shown). To confirm the functional elements, we performed luciferase analyses using single and double mutation constructs. After introducing 2 bp mutations in the CRE or/and FRE response elements within -116 BSPLUC, the basal activities of M-CRE, M-FRE and M-CRE/FRE were lower than the basal transcriptional activity of -116BSPLUC. The transcriptional stimulations by ET-1 were partially abrogated in the M-CRE and M-FRE constructs, whereas double mutation in CRE and FRE (M-CRE/FRE) abolished the stimulatory effects of ET-1 almost completely (Fig. 5).

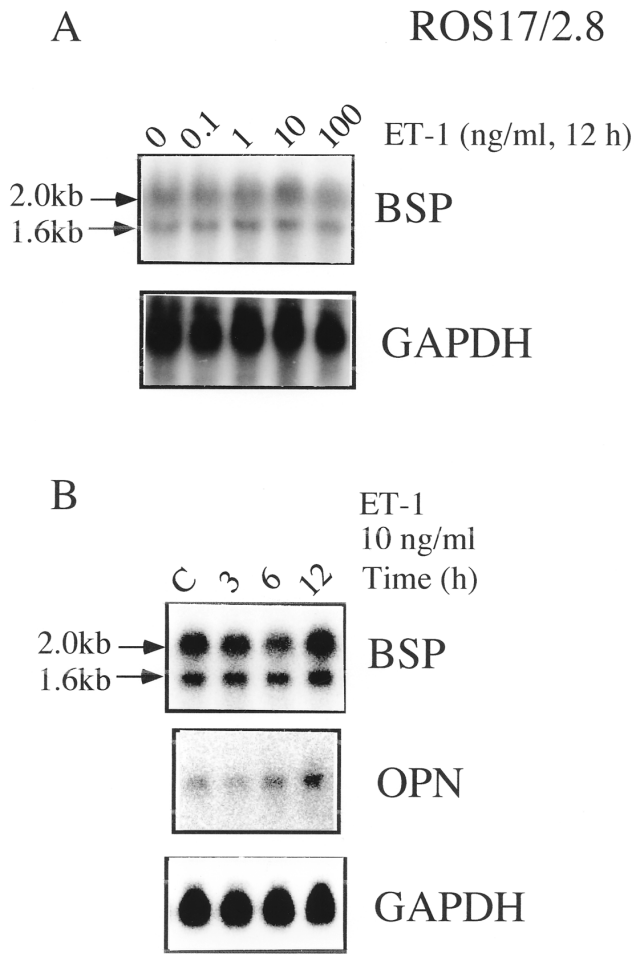

Fig. 1 Effects of ET-1 on levels of BSP and OPN mRNA in ROS17/2.8 cells. A: Dose-response effects of ET-1 (12 h) on levels of BSP mRNA in ROS17/2.8 cells. B: Time course effects of ET-1 (10 ng/ml) on expression of BSP and OPN. Total RNA was extracted with guanidinium thiocyanate, and Northern hybridizations were performed with ^{32}P-labeled rat BSP, OPN and GAPDH cDNA probes. The experiments were performed in triplicate for each data point. Results of a representative hybridization analysis for control and ET-1-treated cells are shown.

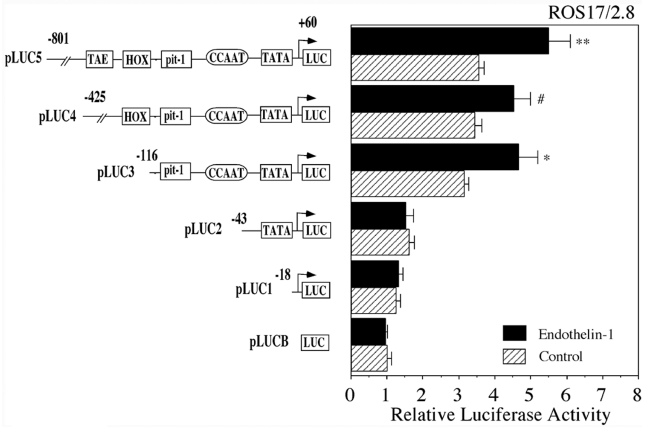

Fig. 2 ET-1 regulates BSP promoter activities in ROS 17/2.8 cells. Transient transfections of ROS 17/2.8 cells in the presence or absence of 10 ng/ml ET-1 for 6 h were used to determine transcriptional activities of chimeric constructs that included various regions of the rat BSP promoter ligated to a luciferase reporter gene. The results of transcriptional activity obtained from four separate transfections with constructs, pLUC basic (pLUCB) and pLUC1 to pLUC5, have been combined, and the values are expressed with standard errors. # ($P < 0.2$), * ($P < 0.1$); ** ($P < 0.05$).

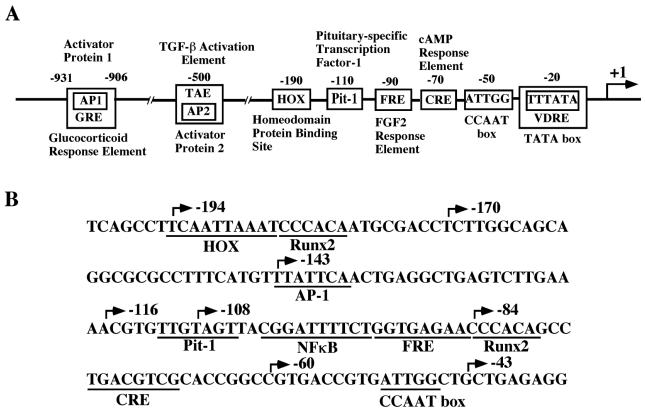

Fig. 3 Regulatory elements in the proximal promoter of the rat BSP gene. A: The positions of the inverted TATA and CCAAT boxes, the vitamin D response element (VDRE) that overlaps the inverted TATA box, CRE (a cAMP response element), FRE (a FGF2 response element), Pit-1 (which mediates the stimulatory effects of parathyroid hormone, PTH), the homeodomain protein-binding site (HOX), and TAE (a TGF-β activation element), which overlaps with AP2, and GRE (a glucocorticoid response element) overlapping with AP1, are shown. B: The nucleotide sequence of the rat BSP gene proximal promoter, encompassing an inverted CCAAT box, and the CRE, Runx2, FRE, NFκB, AP1, Runx2 and HOX elements, is shown from -201 to -35.

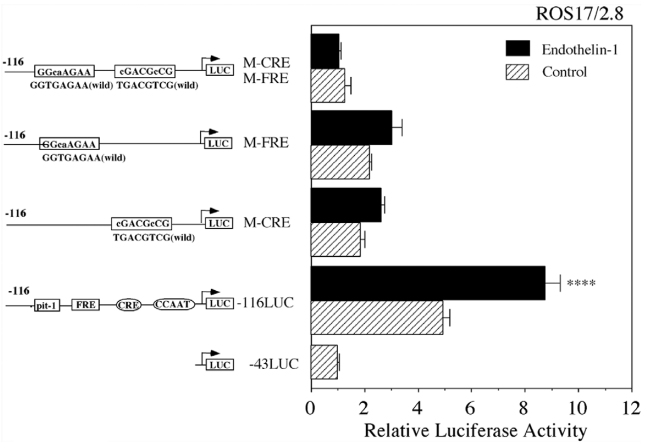

Fig. 5 Site mutation analyses of luciferase activities. Dinucleotide substitutions were made within the context of the -116BSP promoter fragment. M-CRE (cGACGcCG), M-FRE (GGcaAGAA) and M-CRE/FRE constructs were analyzed for relative promoter activity after transfection into ROS17/2.8 cells, and examined for regulation after treatment with ET-1 (10 ng/ml) for 6 h. The results of transcriptional activity obtained from four separate transfections with the constructs were combined, and the values are expressed with standard errors. **** ($P < 0.01$).

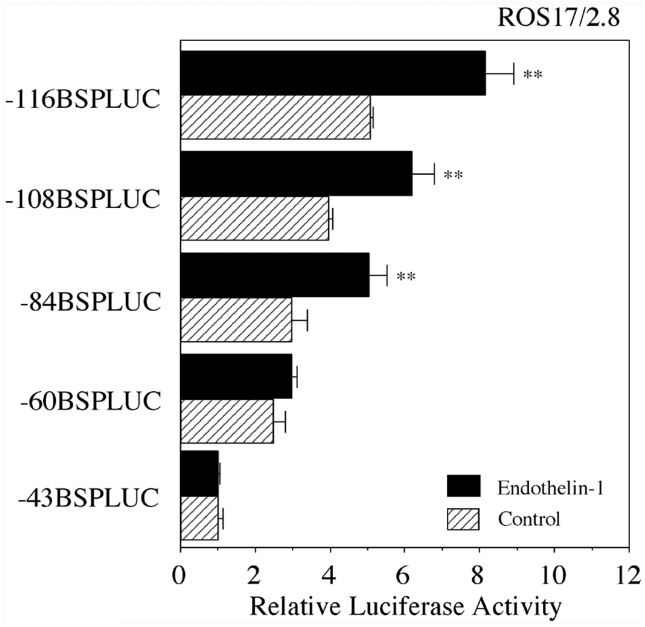

Fig. 4 Fine 5'-deletion mapping of the nts -116 to -43 elements in the BSP promoter. A series of rat BSP promoter 5'-deletion constructs were analyzed for relative promoter activity after transfection into ROS17/2.8 cells, which were subsequently treated with ET-1 (10 ng/ml) for 6 h. The results of transcriptional activity obtained from four separate transfections with constructs -43 BSPLUC (-43 to +60), -60 BSPLUC (-60 to +60), -84 BSPLUC (-84 to +60), -108 BSPLUC (-108 to +60), and -116 BSPLUC (-116 to +60) have been combined, and the values are expressed with standard errors. ** ($P < 0.05$).

Gel mobility shift assays

To identify nuclear proteins that bind to the CCAAT, CRE, FRE and HOX elements and mediate the effects of ET-1 on transcription, double-stranded oligonucleotides were end-labeled and incubated with equal amounts (3 μg) of nuclear proteins extracted from confluent ROS 17/2.8 cells that were either not treated (control) or treated with ET-1 (10 ng/ml) for 3, 6 and 12 h. When we used the inverted CCAAT elements as probes, the DNA-protein complexes were not changed by ET-1 (10 ng/ml) stimulation (Fig. 6, lanes 1-4). When the CRE, FRE and HOX elements were used as probes, ET-1 (10 ng/ml) significantly increased the binding of nuclear proteins to CRE, FRE and HOX at 6 h (Fig. 6, lanes 7, 11 and 15). To further characterize the proteins in the complexes formed with CRE, FRE and HOX, we used antibodies against several transcription factors. Antibodies against CREB1, JunD and Fra2 disrupted the formation of the CRE-protein complexes (Fig. 7, lanes 4-6). Antibodies against

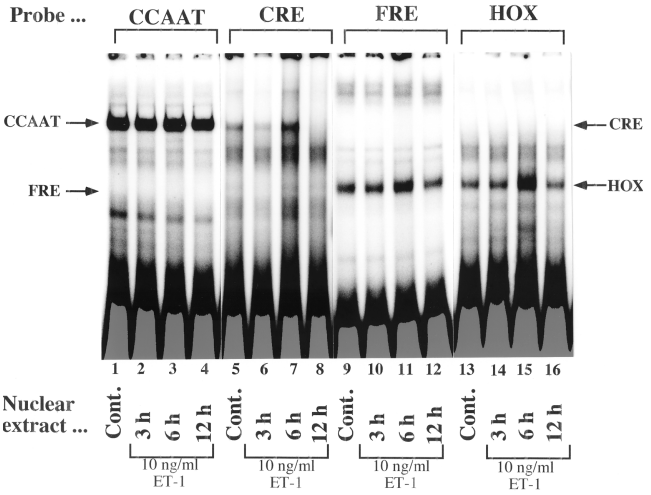

Fig. 6 ET-1 increases the binding of nuclear proteins to the CRE, FRE and HOX elements. Radiolabeled double-stranded inverted CCAAT (-61 CCGTGACCGTG **ATTGG**CTGCTGAGA -79), CRE (-84 CCCA CAAGCC**TGACGTCG**CACCGGCCG -59), FRE (-98 TTTTCT**GGTGAGAA**CCCACA -79) and HOX (-204 TCCTCAGCCT**TCAATTAAAT**CCCACA -179) oligonucleotides were incubated with nuclear protein extracts (3 µg) obtained from ROS17/2.8 cells stimulated without (lanes 1, 5, 9 and 13) or with ET-1 (10 ng/ml) for 3 h (lanes 2, 6, 10 and 14), 6 h (lanes 3, 7, 11 and 15), and 12 h (lanes 4, 8, 12 and 16). DNA-protein complexes were separated on 5% polyacrylamide gel in low-ionic-strength Tris-borate buffer, dried under vacuum, and exposed to an imaging plate for quantitation using an image analyzer.

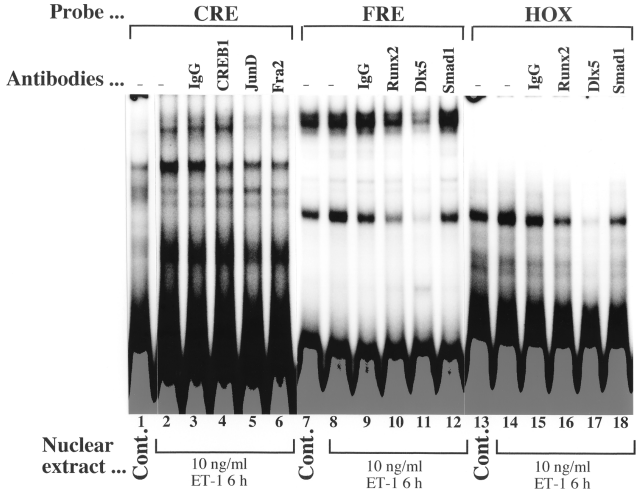

Fig. 7 Specific binding of nuclear proteins to the CRE, FRE and HOX elements. Radiolabeled double-stranded CRE, FRE and HOX were incubated with nuclear protein extracts (3 µg) obtained from ROS17/2.8 cells stimulated without (lanes 1, 7 and 13) or with ET-1 (10 ng/ml) (lanes 2-6, 8-12 and 14-18) for 6 h. Supershift experiments were performed with 0.4 µg of antibodies against IgG (lanes 3, 9 and 15), CREB1 (lane 4), JunD (lane 5), Fra2 (lane 6), Runx2 (lanes 10 and 16), Dlx5 (lanes 11 and 17) and Smad1 (lanes 12 and 18), added separately to each gel shift reaction.

Runx2 and Dlx5 disrupted FRE- and HOX-binding proteins (Fig. 7, lanes 10, 11, 15 and 17), while antibody against Smad1 did not reduce the formation of FRE- and HOX-protein complexes (Fig. 7, lanes 12 and 18).

Discussion

In this study, we have shown that ET-1 enhanced BSP gene transcription by targeting CRE, FRE and HOX elements in the proximal promoter of the rat BSP gene. BSP is a valuable marker of osteogenic differentiation and bone formation, and through the RGD motif can facilitate attachment of normal bone or cancer cells to mineralized tissue surfaces. BSP is also highly expressed in osteotropic cancer cells and plays a role in the attachment the tumor metastasis to bone (28,29,32). BSP and OPN, as members of the family of small integrin-binding proteins (SIBLINGs), are the major constituents of non-collagenous proteins in bone matrix, and expression of their gene products has been shown to be under the control of calcitropic hormones and cytokines (28,53). ET-1 is a key mediator of osteoblastic bone metastasis, which is characteristic of breast and prostate cancers (24,25). In the present study, ET-1 increased the levels of BSP and OPN mRNA and transcriptional activity of the BSP gene (Fig. 1 and 2), thus supporting the idea that ET-1 is at least one of the regulators of osteoblastic function in the local bone environment.

From transient transfection assays, we initially located the ET-1-responsive region in the proximal promoter (pLUC3, -116 to -43) of the rat BSP gene, which encompassed an inverted CCAAT box, and CRE, Runx2, FRE and Pit-1 motifs (Fig. 3), and demonstrated no further increase in transcription of the pLUC4 construct, including a HOX site (Fig. 2), suggesting that the regulation of BSP transcription by ET-1 through HOX might be weaker than that of pLUC3, which contains the CRE and FRE elements. Using fine 5'-deletion constructs between nts -116 to -43 in the rat BSP promoter, -84BSPLUC was identified as a target for ET-1, which contains the CRE element. Luciferase activities of -108BSPLUC and -116BSPLUC were further enhanced by ET-1, suggesting that the ET-1 response region exists not only in -84BSPLUC but also in the -108BSPLUC and -116BSPLUC constructs (Fig. 4). The

transcriptional activities were totally abrogated by mutation in both the CRE and FRE sites of the -116 BSPLUC construct (Fig. 5), thus further confirming that both CRE and FRE are the functional elements for BSP transcriptional regulation of ET-1.

The involvement of the CRE, FRE and HOX sites was further supported by gel shift analyses (Fig. 6). Transcription factors that bind specifically to CRE, FRE and HOX were demonstrated by supershift assays. The results showed that antibodies against CREB1, JunD and Fra2 reacted with CRE-binding proteins, and that antibodies against Runx2 and Dlx5 interacted with FRE- and HOX-binding proteins (Fig. 7).

Recently, it has been demonstrated that tumor-produced ET-1 stimulates new bone formation *in vitro* and osteoblastic metastasis *in vivo*, and that these effects are mediated via ET_AR, blockade of which may be effective for prevention and treatment of osteoblastic bone metastasis caused by breast and prostate cancer (26). However, has also been reported that rat osteoblast-like osteosarcoma ROS17/2 cells express abundant ET_BR mRNA (54). Thus it is conceivable that ET-1 binds to ET_AR and/or ET_BR and activates multiple signaling pathways to alter the proliferation and/or differentiation of cultured osteoblasts-like cells to mediate the regulation of BSP expression. Further studies to clarify the receptors and signaling pathways affected by ET-1 need to be performed using multiple cultured osteoblast-like cells.

The present study has shown that ET-1 enhances transcription of the BSP gene through the PKA, Src tyrosine kinase and MAPKK-signaling pathways, and also identified the CRE, FRE and HOX sites in the rat BSP gene promoter as the target of ET-1-mediated regulation of BSP gene transcription.

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