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Regulation of Expression of Plasminogen Activator Inhibitor-1 in Cultured Rat Osteoblastic Cells by Osteogenic Protein-1 (BMP-7)

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Abstract Osteogenic Protein-1 (OP-1), a member of the bone morphogenetic protein (BMP) family that belongs to the TGF-β superfamily, induces bone formation in vivo and stimulates the synthesis of biochemical markers characteristic of osteoblast phenotypes in vitro. In the present study, effects of OP-1 on the expression of the plasminogen activator inhibitor-1 (PAI-1) in fetal rat calvaria (FRC) cells were examined. The PAI-1 protein levels in conditioned media of FRC cells treated with OP-1 or solvent control were determined by quantitative 2-dimensional polyacrylamide gel electrophoresis. The identity of PAI-1 was confirmed by mass spectroscopy. OP-1 increased the PAI-1 protein level by about 5-fold after 48 h. Northern blot analysis showed that the PAI-1 mRNA level was elevated by OP-1 by about 25% compared to the control. The observed increase in the PAI-1 mRNA and protein level was regulated post-transcriptionally as supported by the following observations: (a) OP-1 did not stimulate the cloned PAI-1 promoter-reporter gene activity in transient transfection studies, (b) inhibition of transcription by actinomycin D did not change the PAI-1 mRNA level in the OP-1-treated FRC cells, and (c) the stability of the PAI-1 mRNA in FRC cells treated with OP-1 was increased by about 28% compared to that in the control cells. Hence, the present study shows that primary cultures of rat osteoblastic cells synthesize and secrete PAI-1 protein and that OP-1 elevates the PAI-1 protein level. At least, one of the regulatory mechanism is by stabilizing the PAI-1 mRNA. J. Cell. Biochem. Suppl. 36:46–54, 2001.
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Bone remodeling is essential for skeletal growth and remodeling for the maintenance of normal bone structure. The remodeling process consists of a tight coupling of two processes: bone formation and bone resorption. Bone formation results from matrix synthesis and mineralization by the osteoblasts, whereas bone resorption results from matrix degradation and dissolution of the mineral phase by the osteoclasts.

During bone formation, osteoblastic cells not only synthesize their own extracellular matrix (ECM) proteins, but also secrete a variety of proteases, including the matrix metalloproteinases (MMPs; see review and references within [Partridge and Winschester, 1996]). The MMPs (e.g. collagenase, stromelysin, and gelatinase) are latent proteases and are secreted by osteoblasts as well as many other cell types. The MMPs participate in matrix remodeling and can be regulated via the plasminogen activator/plasmin pathway. Plasminogen activators (PAs) are specific serine proteases that cleave plasminogen to plasmin which is also a serine protease with broad substrate specificity. Tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) are the two types of PAs usually found in osteoblast [Allan et al., 1991] and osteoclast [Grills et al., 1990; Partridge and Winschester, 1996]. In addition to exhibiting protease activity, uPA has been shown to stimulate proliferation of fetal rat calvaria (FRC) cells and human osteoblastic cells [Rabbani et al., 1992]. Both PAs are inhibited by plasminogen activator inhibitor (PAI) type 1 (PAI-1), one of the two naturally occurring PAIs. PAI-1, a 50 kDa glycoprotein, is present in the conditioned media of rat osteoblastic-like cells and rat osteosarcoma cells [Allan et al., 1990]. Mature osteoblasts from fetal rat calvariae appeared to have a lower PA activity and a higher

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PAI activity than periosteal precursor cells [Pfeilschifter et al., 1990].

The expression of the PAIs is regulated by numerous growth factors and cytokines. For example, activin, glucocorticoids, interleukin-1, leukemia inhibitor factor, and TGF-β stimulate PAI-1 expression [Emeis and Kooistra, 1986; Lund et al., 1987; Allan et al., 1990, 1991; Fukumoto et al., 1992; Bruzdzinski et al., 1993; Partridge and Winschester, 1996; Dennler et al., 1998]. On the other hand, parathyroid hormone (PTH), PGE2, and 1,25-dihydroxyvitamin D3 decrease PAI-1 mRNA and protein levels [Fukumoto et al., 1992, 1994]. The combination of PTH and PA stimulates osteoblastic cell migration, increases TGF-β level and PA activity, but decreases PAI-1 level [Yee et al., 1993]. However, the effect of bone morphogenetic proteins (BMPs) on PAI-1 expression in osteoblastic cells is not known.

In vivo bone formation can be induced by OP-1 [Sampath et al., 1992; Cook, 1999; Geesink et al., 1999], a member of the BMP family that belongs to the TGF-β superfamily [Wozney et al., 1988; Ozkaynak et al., 1990; Kingsley, 1994; Reddi, 1994; Hogan, 1996]. OP-1 also stimulates the synthesis of biochemical markers characteristic of osteoblast phenotypes [Asahina et al., 1993; Knutsen et al., 1993; Andrews et al., 1994; Maliakal et al., 1994; Yeh et al., 1996]. In view of the demonstrated role of OP-1 in bone growth and repair and the role of PAI in matrix remodeling, we hypothesized that OP-1 plays an important role in the regulation of the cell matrix formation system of osteoblastic cells. In the present study, we examined the effect of OP-1 on the expression of PAI-1 in primary cultures of FRC cells.

MATERIALS AND METHODS

Materials

FBS, HBSS, αMEM, penicillin/streptomycin, and trypsin-EDTA, were purchased from Life Technologies (Grand Island, NY). 5,6-dichloro-1-β-ribofuranosylbenzimidazole (DRB) and collagenase were obtained from Sigma Chemical Co. (St. Louis, MO). Recombinant human OP-1 was provided by Stryker Biotech (Hopkinton, MA) and was dissolved in 47.5% ethanol/0.01% trifluoroacetic acid. All other reagents were of molecular biology grade. All buffers were prepared with diethylpyrocarbonate-treated water. Restriction enzymes were purchased from New England Biolabs (Beverly, MA). RNeasy kit and Qiagen plasmid kit were from Qiagen (Santa Clarita, CA). DECAprimeII system and Strip-EZ DNA kit were from Ambion (Austin, TX). Oligonucleotide primers for PCR were synthesized by the Center for Advanced DNA Technologies, The University of Texas Health Science Center (San Antonio, TX). The Dual-light chemiluminescent reporter gene assay system for the combined detection of luciferase and β-galactosidase was from Tropix (Bedford, MA). Ampholytes were purchased from Amersham Pharmacia Biotech (Piscataway, NJ).

Fetal Rat Calvaria Cell Culture

Animals were handled and euthanized following the procedures approved by the Institutional Animal Care and Use Committee of The University of Texas Health Science Center. Primary osteoblastic cell cultures were prepared by subjecting the calvarium from the 19-day old fetus of timed pregnant Sprague-Dawley rats to sequential digestion with trypsin/collagenase as described previously [Yeh et al., 1996]. Digestions 3–5, containing cells that were previously shown to express the osteoblastic phenotype, were pooled and plated at 2×10^5/ml in complete αMEM containing 10% FBS, penicillin and streptomycin, and vitamin C (100 μg/ml). Cells were grown to confluence and frozen in liquid N_2. For experimentation, cells were cultured from the frozen stock in T-75 flasks for 4 days until confluent and subcultured (passage 3) at a cell density of 4×10^4/ml in T-75 flasks or 6-well plates. After an additional 4 days, cells became confluent and were treated with serum-free αMEM in the absence or presence of OP-1. Control cultures contained equal amount of solvent vehicle. The duration of treatment is indicated in each figure legend.

Analysis of Secreted Proteins in the Conditioned Media

Total proteins in the conditioned media of FRC cells treated with solvent vehicle or OP-1 (300 ng/ml) for 48 h were recovered by TCA precipitation. Equal amounts of proteins were dissolved in 9 M urea, 8% β-mercaptoethanol, 6% Triton X-100, and 9% ampholytes (50/50 vol/vol, pH 3.5–9.5 and pH 5.0–8.0) and subjected to 2-D electrophoresis basically as described [O'Farrell, 1975]. After electrophoresis, proteins were detected by Coomassie blue staining. The staining intensity of the putative

PAI-1 protein spot in the control sample was normalized to that of protein in the same gel whose intensity remained unchanged in both the control and the experimental sample. The normalized intensity in the control was compared with that in the OP-1-treated sample using the 2-D gel analysis software (Visage 100, BioImage, MI). The putative PAI-1 protein spot was excised and digested with trypsin following published methods [Shevchenko et al., 1996; Wilm et al., 1996]. Tryptic peptides were analyzed by Drs. M. Kinter and N. Sherman (University of Virginia) using capillary LC-MS on a Finnigan LCQ ion trap mass spectrometer with a Protana nanospray ion source interfaced to a self-packed C18 reversed phase capillary column. The data were analyzed by searching the NCBI non-redundant database using the Sequest algorithm.

PAI-1 Promoter Constructs

The 1.65 kb PAI-1 promoter fragment (from nucleotides −1510 to +136. GenBank Accession no. J05206) was generated by PCR using rat liver genomic DNA as a template and the following primers: 5′ sense primer (GATCCCCGGGTCCCAGAC) and 3′ antisense primer (CTCGAGCTTTCGGAAGTG). The DNA was inserted into pGL2-Basic in front of the reporter gene Luciferase as previously described [Yeh et al., 1998]. The base sequence of the insert was confirmed by double-stranded DNA sequencing. Subsequently, five DNA constructs with different 5′-end deletions of the promoter were generated using appropriate, unique restriction endonucleases. The DNA constructs were used in transient transfection studies of sub-confluent FRC cells and the promoter activity determined as described below.

Transient Transfections

All plasmid DNAs were isolated using the Qiagen's plasmid Maxi kit and checked for purity on 1% agarose gels. Only the pure DNA preparations were used for transfection. FRC cells were grown in 6-well plates for 3 days or to near confluency and transiently transfected with promoter constructs by the DNA-calcium phosphate co-precipitation method [Chen and Okayama, 1987]. Twelve micrograms of plasmid DNA were used for each 6-well plate. After 3 h of transfection at 37°C, the media was replaced by 15% glycerol in HEPES-buffered saline. After 2 min at room temperature, the transfected cells were briefly rinsed with HBSS and allowed to recover in fresh complete αMEM plus 10% FBS for 20 h. Cells were treated with serum-free αMEM containing vehicle or 300 ng/ml OP-1 for 24 h (3 wells/treatment). Cells were lysed with 100 μl 1X lysis buffer (Tropix). A 50 μl aliquot was used to measure the reporter luciferase and the β-galactosidase activity. The degree of luminescence was detected by the OPTOCOMP I luminometer ILA911 (Tropix). The luciferase activity was normalized to the β-galactosidase activity in the lysate.

Studies With Actinomycin D, Cycloheximide, Hydroxyurea, and DRB

To examine effects of inhibition of RNA synthesis, de novo protein synthesis and cell replication, actinomycin D (0.5 μM), cycloheximide (3.6 μM) and, hydroxyurea (1 mM), respectively was added with OP-1 simultaneously to confluent FRC cells. After 6–24 h, total RNA was isolated using the Qiagen's RNeasy kit and treated with DNase. The level of PAI-1 mRNA was measured by Northern analysis, quantified by PhosphorImaging, and normalized to that of the 18S rRNA.

For mRNA stability studies, confluent FRC cells were treated with serum-free αMEM containing vehicle or 200 ng/ml OP-1 for 24 h. The RNA polymerase II inhibitor, 5,6-Dichloro-1-β-ribofuranosylbenzimidazole (DRB, 100 μM) was added to both vehicle- or OP-1-treated cells to arrest transcription. At different time intervals after DRB addition, total RNA was isolated as described above.

Northern Blot Analysis

Northern analyses were carried out as described previously [Yeh et al., 1996]. Briefly, total RNA blots were prehybridized at 42°C for 4 h and the PAI-1 mRNA level was measured using a 0.98 Kb cDNA fragment as a probe. The 0.98 Kb fragment contains the base sequence from nucleotides 213 to 1194 of the PAI-1 coding region (GenBank Accession no. M24067) and was excised from the parent plasmid with PvuII, fractionated on agarose gel, and purified using Geneclean III (BIO 101, Vista, CA). The parent plasmid contains a 3.05 Kb PAI-1 cDNA sequence cloned in the pBluescript SK vector. Probes were labeled with [^{32}P]dCTP using the DECAprimeII system or [^{32}P]dATP using the Strip-EZ DNA kit. After overnight incubation, the blots were washed and exposed to a

Phosphor screen (Molecular Dynamics, Sunnyvale, CA) for signal detection. The intensity of the radioactive mRNA band was scanned with the PhosphorImager and quantified with the ImageQuant software. Blots were stripped at 68°C with the Strip-EZ Probe Degradation Buffer and checked to ensure that the level of radioactivity was reduced to background before probing with a 18S rRNA oligonucleotide probe that was ^{32}P end-labeled to check for proper sample loading.

Statistical Analysis

Multiple means were compared with one-way ANOVA, followed by Student's *t*-test for paired comparisons with the control. The statistical programs in the PSI-Plot (Poly Software International, Salt Lake City, UT) for personal computers were used for the analyses.

RESULTS

In the present study, 2-D polyacrylamide gel electrophoresis analysis was used to examine the effect of OP-1 on the expression level of secreted proteins in the conditioned media of FRC cells. A typical electrophoretic profile of secreted proteins stained by Coomassie blue is shown in Figure 1. OP-1 stimulated the synthesis/secretion of numerous proteins, but the present study focused on a protein with an approximate molecular weight of 40 kDa and a pI of 6.4. OP-1 stimulated the level of this protein 4 to 5 fold. Sequence determination of several tryptic peptides derived from this protein followed by gene and protein data banks searches of the sequences revealed that the protein is PAI-1 with a calculated molecular weight of 45 kDa and a pI of 6.2.

To determine whether the increase in PAI-1 protein was the result of an increase in PAI-1 mRNA, the steady-state PAI-1 mRNA level in control and OP-1-treated FRC cells was measured as a function of time of OP-1 treatment. Figure 2 shows that a significant level of PAI-1 mRNA was detectable in control cultures during the time period examined. The basal level was not altered during the culture period. However, the mRNA level was significantly elevated after 24 h of OP-1 treatment and reached a maximum (about 25% above the control) after 48 h. The mRNA level dropped significantly but it remained elevated above the control (by 11%) after 72 h of OP-1 treatment. The mRNA level remained elevated until about 144 h (6 days) after OP-1 treatment, and then declined gradually

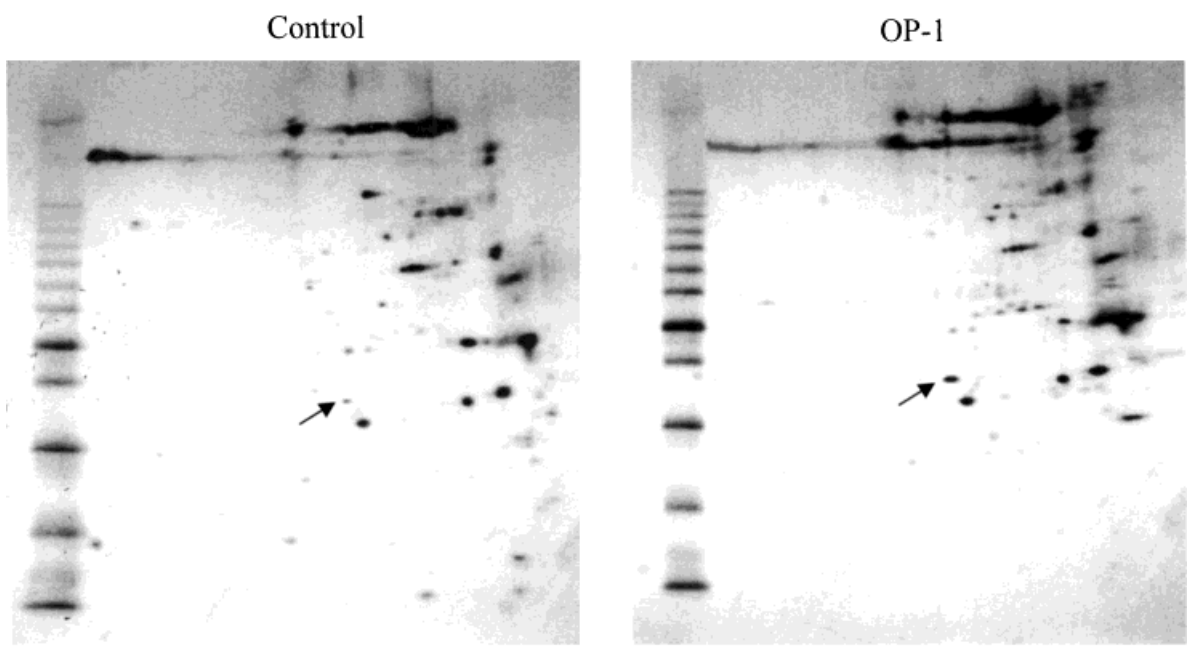

Fig. 1. A representative electrophoretic pattern of proteins in conditioned media of FRC cells treated with solvent or OP-1 for 48 h. Proteins were analyzed by electrophoresis on 2-dimensional polyacrylamide gels and detected by Coomassie blue staining. The PAI-1 protein spot is indicated by an arrow.

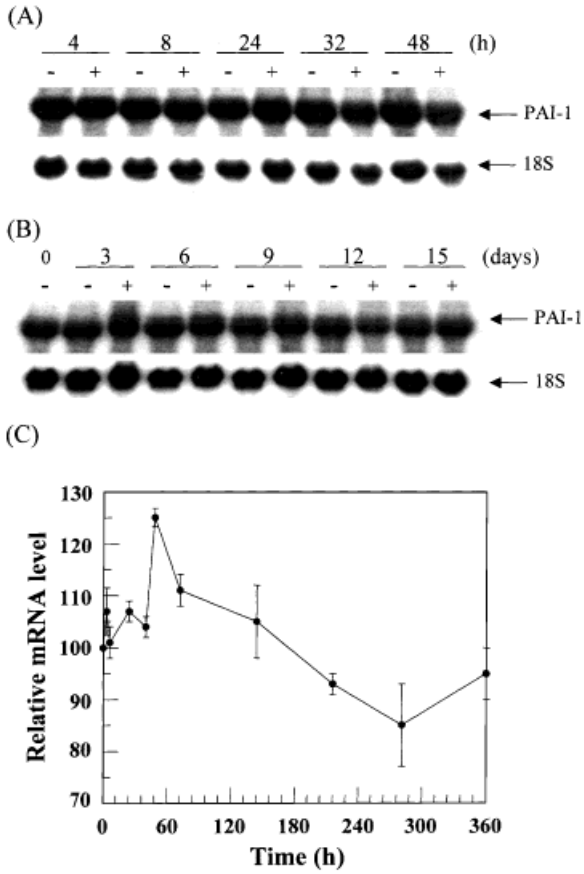

Fig. 2. Time course of OP-1 action on the steady-state PAI-1 mRNA level in FRC cells. Total RNA was isolated from confluent FRC cells treated with vehicle (–) or 300 ng/ml of OP-1 (+) for **(A)** a short-term (4, 8, 24, 32, and 48 h), and **(B)** long-term (3, 6, 9, 12, and 15 days). The level of PAI-1 mRNA was analyzed by Northern analysis using a PAI-1 cDNA probe. Representative blots showing levels of PAI-1 mRNA and 18S rRNA. **(C)** Quantitative data showing the relative PAI-1 mRNA level (corrected with the 18S rRNA level). Values are mean±SE for four independent determinations.

to about 10–15% below the control level subsequently.

To determine whether the increase in the PAI-1 mRNA level was due to an increase in the transcription of the PAI-1 gene, we tested the effect of OP-1 on the PAI-1 promoter activity. Accordingly, a 1646-bp DNA fragment that contains the putative PAI-1 promoter sequence and several 5′ deletion constructs (Fig. 3) were generated and tested. Figure 4A shows the reporter gene activity of the various promoter constructs in transfected FRC cells treated with solvent vehicle or OP-1. The promoter activity for the pGL2-Basic vector in transfected FRC cells treated with solvent was very low and OP-1 did not affect the control promoter activity. Sequential deletion of the 5′-end sequence did not reduce the basal promoter activity to any significant extent. However, deletion of the 299 nucleotides from the 5′-end (the $+23/+136$ construct) resulted in a promoter with about 15–20% of the promoter activity of the $-1510/+136$ construct but was still about 50-fold more active than the parent pGL2-Basic (Fig. 4A). OP-1 did not stimulate the cloned PAI-1 promoter activity (Fig. 4A and B). The observation implies that the increased in PAI-1 mRNA in OP-1-treated FRC cells is not due to a stimulation of transcription. Sequential removal of its 5′-end sequence did not produce any significant differences in promoter activity suggesting that the DNA region under examination does not contain a transcriptional inhibitory sequence.

Previously, TGF-β has been reported to stimulate PAI-1 mRNA synthesis in rat osteoblastic cells [Lund et al., 1987; Allan et al., 1991]. Moreover, the sequence consisting of $-794/-532$ of the PAI-1 promoter is sufficient for a 5-fold stimulation by TGF-β [Keeton et al., 1991; Hua et al., 1998] and that a 12-base pair sequence ($-732/-721$) is capable of conferring TGF-β responsiveness [Song et al., 1998]. We used TGF-β treatment as a control and tested our clones for the TGF-β stimulatory effect. Figure 4C shows that TGF-β indeed stimulated the promoter activity of all clones by about 2-fold, except the clone containing only $+23$ to $+136$ sequence. The data suggest that the PAI-1 promoter constructs used in the present study contained sufficient response element(s) and that the lack of response to OP-1 was not likely due to an abnormality in our DNA clones.

Inhibition of transcription by actinomycin D did not change the PAI-1 mRNA level in both the control and the OP-1-treated FRC cells (Table I). Cycloheximide treatment did not affect the PAI-1 mRNA level. The concentration of actinomycin and cycloheximide used was previously established to effectively block RNA and protein synthesis, respectively in FRC cells [Centrella et al., 1991]. To determine whether the increase in PAI-1 expression was related to the mitogenic activity of OP-1, FRC cells were treated with OP-1 in the presence or absence of hydroxyurea to block DNA synthesis. The concentration of hydroxyurea used was also previously established to effectively block DNA synthesis in FRC cells [Centrella et al., 1991]. Inhibition of DNA

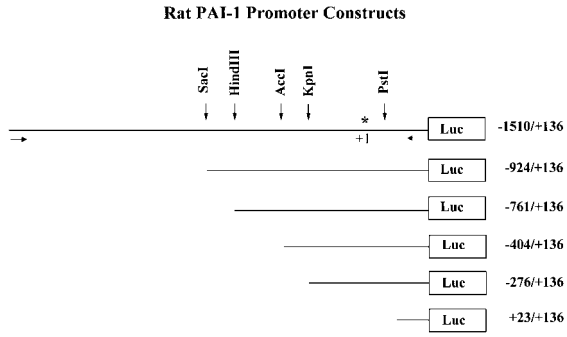

Fig. 3. DNA constructs containing the different rat PAI-1 promoter regions. The transcription start site is +1. The DNA fragment from −1510 to +136 was generated first by PCR and cloned into the pGL2-Basic vector containing the luciferase reporter gene (Luc). Positions of the primers are indicated by horizontal arrows. The 5′ end deletion fragments were generated by digestion of the parent DNA with unique restriction enzymes. These sites are shown (perpendicular arrows).

synthesis by hydroxyurea did not have any significant effect on PAI-1 mRNA expression.

The above findings suggest that the observed increase in PAI-1 mRNA and protein level was independent on new transcription of the PAI-1 gene, but the steady-state PAI-1 mRNA level was elevated. To determine whether OP-1 changed the stability of the PAI-1 mRNA, confluent FRC cells were treated with DRB, which inhibits RNA polymerase II activity. The half-life of PAI-1 mRNA in the transcriptionally arrested control FRC cells was long and was increased by 28% in OP-1-treated FRC cells (Fig. 5). The half life ($t_{\frac{1}{2}}$) of PAI-1 mRNA was estimated to be 74 h in the control and was increased to about 95 h in the OP-1-treated cells. The observation suggests that at least one of the mechanism for the increase in the synthesis/secretion of PAI-1 protein in OP-1 treated FRC cells is the result of an increase in the stability of the PAI-1 mRNA.

DISCUSSION

The rate of ECM deposition is regulated by the synthesis and degradation of extracellular proteins. One of the most important protease systems in regulating ECM degradation is the PA/PAI system. In the present study, we confirmed previous reports that PAI-1 mRNA is synthesized in FRC cells. In addition, in view of the critical role of OP-1 in bone formation, we further showed that OP-1 increased the steady-state PAI-1 mRNA level independent on new

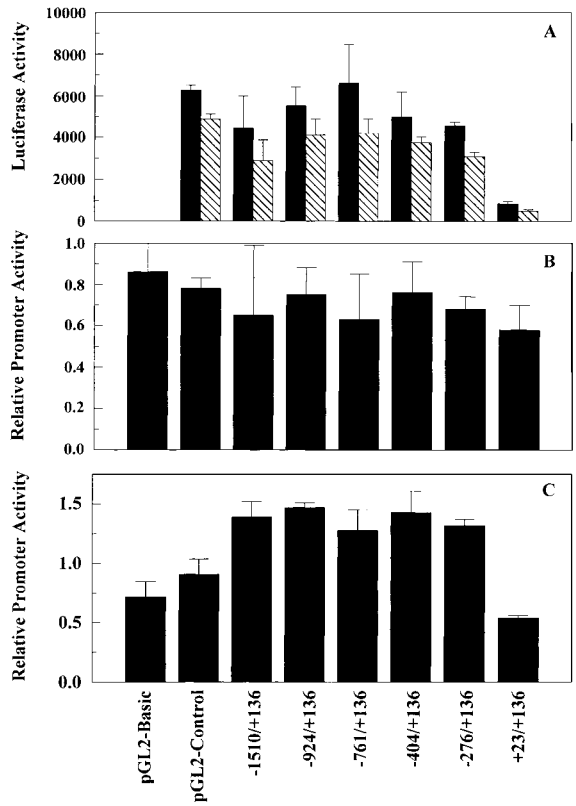

Fig. 4. Effects of OP-1 and TGF-β on the PAI-1 promoter activity in transiently transfected FRC cells. **(A)** Cultures were transfected with the DNA constructs containing the different deletions shown in Figure 3 and were treated with either solvent (solid bars) or OP-1 (hatched bars, 300 ng/ml) for 24 h. The luciferase activity was normalized to the β-galactosidase activity. **(B)** Ratios of the normalized luciferase activity in OP-1-treated culture/control culture as a function of the different promoter constructs. **(C)** Ratios of the normalized luciferase activity in TGF-β-treated culture/control culture as a function of the different promoter constructs. Cultures were transfected with the different DNA constructs and treated with solvent or TGF-β (5 ng/ml) for 24 h. Values represent mean ± SE of four independent determinations from two different FRC cell preparations.

TABLE I. Effects of Inhibitors on PAI-1 mRNA Expression in Control and OP-1-Treated FRC Cells*

Treatment	PAI-1 mRNA level in OP-1-treated cells/control cells
PBS	96±7
Act D	115±9
CHX	107±6
HU	94±8

*Confluent cells were treated with 200 ng/ml OP-1 in serum-free αMEM in the presence of PBS, actinomycin D (Act D, 0.5 μM) for 6 h, cycloheximide (CHX, 3.6 μM) for 24 h, or hydroxyurea (HU, 1 mM) for 24 h. Total RNA was isolated and the PAI-I mDNA level was probed by Northern analysis using a PAI-1 cDNA and quantified by PhosphorImaging. Values represent the mean ± SE of three determinations.

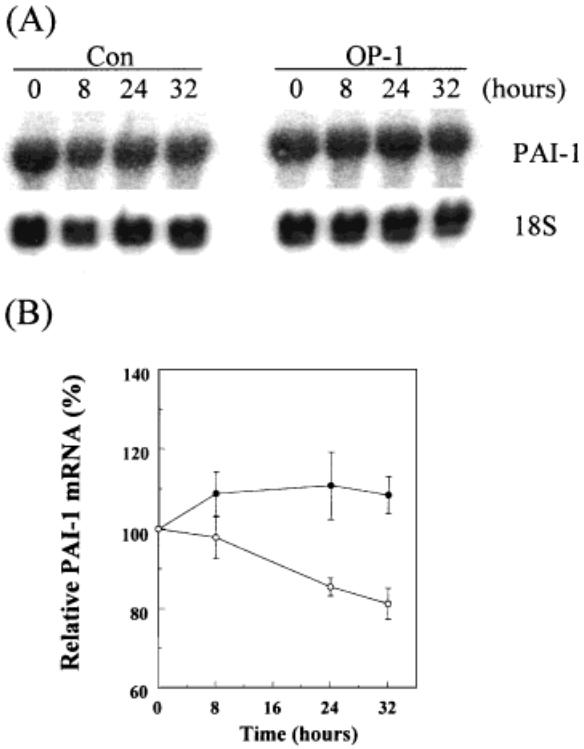

Fig. 5. Effects of OP-1 on PAI-I mRNA decay in FRC cells treated with DRB. Confluent FRC cells were treated with 200 ng/ml OP-1 or vehicle in serum-free αMEM for 24 h. DRB (100 μM) was added. At 8, 24, and 32 h after the addition of DRB, RNA was isolated and subjected to Northern analysis using a radiolabeled PAI-1 cDNA probe. **(A)** A representative blot showing the level of PAI-1 mRNA and 18S rRNA in control and OP-1-treated FRC cells is shown. **(B)** A quantitative plot of the relative PAI-1 mRNA (time 0 as 100), after correction with 18S rRNA as a function of DRB exposure time. (o) control cells. (•) OP-1-treated cells. Values are mean ± SE for three independent determinations.

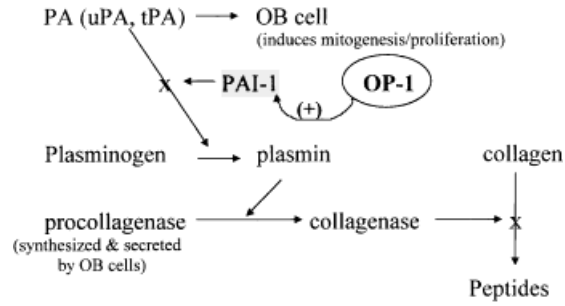

Fig. 6. A proposed model for the effects of OP-1 on PAI-1 expression in FRC cells and subsequent possible consequences.

transcription, protein synthesis, and cell replication. The PAI-1 protein level was elevated by about 5-fold via stabilization of the PAI-1 mRNA in FRC cells.

This is the first demonstration that a member of the BMP family affects PAI-1 gene expression in osteoblastic cells. The present observation thus adds to the list of hormones and cytokines that regulate PAI-1, the major physiologic regulator of plasminogen activation [Emeis and Kooistra, 1986; Lund et al., 1987; Allan et al., 1990; Allan et al., 1991; Fukumoto et al., 1992; Bruzdzinski et al., 1993; Partridge and Winschester, 1996; Dennler et al., 1998; Kretzschmar and Massague, 1998]. Of particular interest are the studies on the effect of TGF-β on PAI activity, since OP-1 is a member of the TGF-β superfamily. In osteoblast like cells (UMR 106-01), TGF-β stimulated PAI-1 mRNA expression 2 h after treatment and reached a maximum after 4–8 h [Allan et al., 1991]. The increase was not affected by cycloheximide treatment but was completely abolished by actinomycin D treatment. The PAI-1 protein level increased by about 5-fold. Our observations extended these findings by showing that TGF-β stimulated the activity of the PAI-1 promoter-luciferase reporter gene in FRC cells by 2-fold. In non-osteoblastic cells, such as WI-38 human lung fibroblasts, TGF-β showed even higher stimulation (50-fold) of PAI-1 mRNA expression after 8 h of treatment and the PAI-1 protein in the conditioned media was elevated by about 15-fold as determined by ELISA [Lund et al., 1987; Pfeilschifter et al., 1990; Allan et al., 1991]. In HepG2 and Hep3B human hepatoma cells as well as Mv1Lu mink lung epithelial cells, TGF-β induced PAI-1 both at the transcriptional level and post-transcriptional level by increasing the half-life of the PAI-1 mRNA by about 3-fold [Keeton et al., 1991; Westerhausen et al., 1991; Dennler et al., 1998]. The latter finding is in accord with our data in FRC cells. Also, in agreement with our findings in FRC cells, these authors reported that OP-1 did not stimulate the PAI-1 promoter in HepG2 hepatoma cells and Mv1Lu mink lung epithelial cells [Dennler et al., 1998]. Taken together, these findings suggest that the PAI-1 gene expression is subject to different modes of regulation at different levels, to different extents, and may be cell type specific.

Previously, other investigators suggested that the PA/plasmin pathway might play a role in coupling bone formation and resorption [Allan and Martin, 1995; Yang et al., 1997]. In view of the current observations and the previous proposal, we would like to propose a

model (Fig. 6). In this model, we speculate that the increase in PAI-1 protein would result in a net increase in the PA level secreted by the osteoblastic cells. The elevated PA level would in turn lead to an induction of mitogenesis and proliferation of the osteoblastic cells resulting in an increase in bone formation, a demonstrated role for OP-1. Our supposition is supported by previous findings which show that PAs, specifically uPA stimulates mitogenic activity and proliferation of human and rat osteoblastic cells [Rabbani et al., 1992]. On the other hand, an increase in PAI-1 protein would block the conversion of plasminogen to plasmin, which in turn would reduce the activation of procollagenase to active collagenase, leading to a cessation or decrease in collagen degradation. During bone formation, as induced by OP-1, ECM formation predominates and matrix remodeling including collagen degradation is minimal. In addition, it is tempting to speculate that the decrease in plasmin level, a serine protease with broad substrate specificity, might also decrease the turn-over rate of PAI-1, contributing to the elevation in PAI-1 level.

In conclusion, the present study demonstrates that OP-1 increases PAI-1 expression in rat osteoblastic cells. The observations suggest that OP-1 acts as an autocrine regulator of the ECM, and plays a role in the maintenance of the bone matrix.

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