Ets-1 Stimulates Platelet-Derived Growth Factor A-Chain Gene Transcription and Vascular Smooth Muscle Cell Growth via Cooperative Interactions With Sp1

Fernando S. Santiago, Levon M. Khachigian

Abstract—The platelet-derived growth factor (PDGF) family of ligands (composed of A-, B-, C-, and D-chains), potent mitogens, and chemoattractants for cells of mesenchymal origin has been implicated in numerous vascular pathologies involving smooth muscle cell (SMC) hyperplasia. Understanding the molecular mechanisms mediating PDGF transcription would provide new insights into strategies to control PDGF-dependent pathophysiologic processes. We have demonstrated previously that PDGF-A expression is under the positive regulatory influence of Sp1, Sp3, and Egr-1 and is negatively controlled by GCF2, NF-1(X), and WT-1. In this article, we demonstrate that Ets-1 induces PDGF-A expression in primary rat aortic SMCs at the level of transcription and mRNA expression. Electrophoretic mobility shift, supershift, and mutational analyses revealed a functional role for the \( ^{555}\text{TTCC}^{555} \) motif in the PDGF-A promoter that binds endogenous Ets-1. Chromatin immunoprecipitation analysis showed the interaction of endogenous and exogenous Ets-1 or glutathione S-transferase-tagged Ets-1, bearing only the DNA-binding domain with the authentic PDGF-A promoter. Conversely, dominant-negative mutant of Ets-1 blocked the promoter interaction of endogenous Ets-1. Overexpression of Ets-1 but not the mutant form of Ets-1 activates the PDGF-A promoter cooperatively with Sp1. Sp1, which interacts with Ets-1, failed to induce PDGF-A promoter-dependent expression if the promoter contained a site-specific mutation in this novel Ets-binding site. Small interfering RNA to Ets-1 and Sp1 blocked PDGF-BB- and serum-inducible PDGF-A expression. SMC growth was stimulated by Ets-1 and Sp1 separately and further increased by both factors together. Ets-1-inducible mitogenesis is blocked by antibodies neutralizing PDGF-A and involves activation of the PDGF \( \alpha \)-receptor, which binds PDGF-A. These findings identify a functional \textit{cis}-acting element for Ets-1 in the PDGF-A promoter and demonstrate that Sp1 and Ets-1 cooperatively activate PDGF-A transcription in vascular SMCs. (Circ Res. 2004;95:000-000.)

Key Words: Ets-1 ■ smooth muscle cells ■ transcription ■ growth ■ Sp1 ■ autocrine

Platelet-derived growth factors (PDGFs), the family of which comprises A-, B-, C- and D-chains,\(^1\)\(^-\)\(^3\) are potent mitogens and chemoattractants implicated in various vascular pathologic settings.\(^4\) For example, PDGF-A is expressed by smooth muscle cells (SMCs) after balloon injury\(^5\)\(^,\)\(^6\) and in human atherosclerotic\(^7\) and restenotic lesions after percutaneous transluminal coronary angioplasty.\(^8\) Of the 2 high-affinity PDGF receptors (PDGFRs), PDGF-A binds to PDGFR-\( \alpha \)-but not receptor-\( \beta \).\(^9\) Somatic cell hybrid chromosome segregation analysis and in situ hybridization studies assigned PDGF-A to chromosome 7 (7p21–7p22).\(^10\)\(^,\)\(^11\) Previous studies by our group have revealed that PDGF-A gene expression in SMCs and endothelial cells is under the positive transcriptional control of the zinc finger transcription factors specificity protein 1 (Sp1), Sp3, and early growth response-1 (Egr-1).\(^12\)\(^-\)\(^15\) These transcription factors interact with overlapping nucleotide recognition elements located at bp \(-71/-55\) in the proximal region of PDGF-A promoter. Conversely, PDGF-A is repressed by the Wilms’ tumor suppressor gene product WT-1,\(^16\)\(^,\)\(^17\) GCF2,\(^18\) and NF-1(X).\(^19\)

Ets-1 belongs to the \textit{ets} gene family, which has been implicated in a variety of biological pathways regulating cell growth, differentiation, and apoptosis. It is defined by a highly conserved DNA-binding domain comprising \( \approx85 \) amino acids recognizing the central core motif \( ^{7}\text{GGAA}^{17} \) of the PDGF-A promoter.\(^20\)\(^,\)\(^21\) Ets-1, like PDGF-A, is upregulated at exposure to agonists such as serum in vitro\(^22\)\(^-\)\(^25\) and is expressed in injured vasculature.\(^26\)\(^-\)\(^28\) It is also expressed by SMCs in human carotid atherosclerotic lesions.\(^29\) However, whether PDGF-A transcription is influenced by Ets-1 is presently unknown.

Here we have identified a functional \textit{cis}-acting element for Ets-1 in the PDGF A-chain promoter that mediates cooperative activation by Sp1. Gel shift, chromatin immunoprecipitation (ChIP), pull-down, and transient transfection analyses

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From the Centre for Vascular Research, The University of New South Wales, and the Department of Haematology, The Prince of Wales Hospital, Sydney, Australia.

Correspondence to Levon M. Khachigian, PhD, Centre for Vascular Research, Department of Pathology, The University of New South Wales, Sydney NSW 2052, Australia. E-mail L.Khachigian@unsw.edu.au

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Materials and Methods

Transfections and Luciferase Assays
Early-passage (used between passages 2 and 6) primary rat aortic SMCs were obtained from Cell Applications, Inc. and maintained in Waymouth’s medium (Life Technologies), pH 7.4, and supplemented with 1 mmol/L L-glutamine (Invitrogen), 10 U/mL penicillin, 10 μg/mL streptomycin, and 10% FBS at 37°C in a humidified atmosphere of 5% CO2. Transient transfections were performed with cells at 60% to 70% confluence with indicated constructs and 1 μg of the internal control plasmid pRL-TK using FuGENE6 transfection agent (Roche Molecular Biochemicals). Luciferase activity was measured 24 hours after transfection using the Dual Luciferase Assay System (Promega). Firefly luciferase activity was normalized with Renilla luciferase generated by pRL-TK to correct for transfection efficiency.

Plasmid Constructs
Cytomegalovirus (CMV)-Sp1 was obtained from Robert Tjian (Howard Hughes Medical Institute, University of California). p643A-luc, containing 643 bp of the human PDGF-A promoter upstream of Firefly luciferase, was generated in our laboratory.30 mEts-1-643A-luc plasmid was generated using the QuickChange Site-Directed Mutagenesis Kit (Stratagene). Ets-1 cDNA was excised from pKCR3-Ets-1 (a gift from Ian Cassidy, Department of Biochemistry and Molecular Biology, University of Queensland, Australia) and cloned into protamine complementary DNA 3 (pcDNA3). pKCR3-DN-Ets-1 was generated previously by us.29

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assays
Cells, transfected as indicated, were washed and scraped in 10 mL of cold PBS, pH 7.4, and transferred to precooled centrifuge tubes. Cells were pelleted by centrifugation at 1200 rpm for 10 minutes at 4°C and lysed by incubation in Solution A (10 mmol/L Heps, pH 8.0, 1.5 mmol/L MgCl2, 10 mmol/L KCl, 0.5% Nonidet P-40, 1 mmol/L dithiothreitol [DTT], 0.5 mmol/L phenylmethylsulfonyl fluoride [PMSF], 4 μg/mL aprotinin, and 10 μg/mL leupeptin). Samples were spun at 14 000 rpm for 40 seconds. The pellet was resuspended in Solution C (29 mmol/L Heps, pH 7.9, 1.5 mmol/L MgCl2, 420 mmol/L NaCl, 0.2 mmol/L EDTA, 1 mmol/L DTT, 0.5 mmol/L PMSF, 4 μg/mL aprotinin, and 10 μg/mL leupeptin) and incubated by gently shaking for 20 minutes at 4°C. Supernatants were transferred to precooled microfuge tubes containing an equal volume of Solution D (20 mmol/L Heps, pH 7.9, 100 mmol/L KCl, 0.2 mmol/L EDTA, 20% glycerol, 1 mmol/L DTT, 0.5 mmol/L PMSF, 4 μg/mL aprotinin, and 10 μg/mL leupeptin) and stored immediately at −80°C until use. Nuclear extracts were incubated with the indicated 32P-labeled double-stranded oligonucleotides (150 000 cpm) in a total volume of 20 μL containing 10 mmol/L Tris-HCl, pH 8.0, 50 mmol/L MgCl2, 1 mmol/L EDTA, 1 mmol/L DTT, 5% glycerol, 1 μg salmon sperm DNA, 5% sucrose, 1 μg of poly(dIdC), and 1 mmol/L PMSF. The mixture was incubated for 35 minutes at 22°C. In supershift experiments, nuclear extract was incubated with 2 μg of antibody before addition of the probe. Samples were loaded onto 6% nondenaturing polyacrylamide gel electrophoresis and visualized by autoradiography.

Western Blot Analysis
Total cell lysates were resolved by denaturing SDS-PAGE and then transferred into polyvinylidene difluoride membrane (Immobilon; Millipore). The membrane was blocked for 1 hour with 5% skim milk in PBS with 0.05% Tween 20. Ets-1 and Sp1 were detected with appropriate antibodies (1:1000; Santa Cruz Biotechnology) and by chemiluminescence. BSA–PBS–TWEEN 20 buffer was used for blocking and incubation purposes only when phosphotyrosine monoclonal antibodies (1:1000; BD Transduction Labs) were used.

Reverse Transcription–Polymerase Chain Reaction
Total RNA was extracted using TRIzol reagent (Invitrogen), and cDNA was generated using Superscript II reverse transcriptase (Invitrogen) with random primers according to the instructions of the manufacturer. Polymerase chain reaction (PCR) for PDGF-A was done in 20 μL reaction containing 1 mmol/L MgCl2, 50 μmol/L 2′-deoxynucleoside 5′-triphosphate (dNTP), 0.1 μmol/L primers, 1 μL cDNA, and 1 U of Platinum Taq Polymerase (Invitrogen) using an Applied Biosystem GeneAmp PCR system 2400 (Perkin Elmer). Amplification conditions are as follows: 94°C for 1 minute, 25 cycles of 94°C for 10 seconds, 57°C for 30 seconds, 72°C for 1 minute, and an extension at 72°C for 4 minutes.

demonstrate that Ets-1 physically and functionally interacts with the PDGF-A promoter. We show that Ets-1 and Sp1 stimulate primary rat aortic SMC growth. This is attributable, at least in part, to Ets-1–dependent autocrine PDGF-A activation of PDGFR-α.
Sequences of the primers were as follows: forward ratPDGFAf97 5′/CACCCCTGCTTTCCTCGAGGAAAGCC/3′, reverse ratPDGFAr710 5′/CCCGCCCCCTTTCCCAAGACTGAC/3′.

For GAPDH amplification, essentially the same conditions as those of PDGF-A amplification were used except 1.5 mmol/L MgCl₂, 21 cycles with annealing temperature of 58°C. The reaction was loaded onto 1.2% agarose gel, and amplicons were visualized by ethidium bromide staining.

**Immunohistochemistry**

Paraffin-embedded sections of human atherosclerotic lesions were immunostained with rabbit polyclonal antibodies to Ets-1 and Sp1 (Santa Cruz Biotechnology) or PDGF-A (Genzyme) as described previously.31

**ChIP Analysis**

SMCs seeded into 100-mm Petri dishes were transfected overnight with 20 μg of indicated plasmids. Cells were washed with PBS, pH
7.4, before ChIP using the appropriate antibody. PCR was performed in 1 mmol/L MgCl₂, 0.1 mmol/L dNTP, 0.1 μmol/L primers, and 1 U Platinum Taq Polymerase (Invitrogen). Cycling conditions were as follows: 94°C for 2 minutes; 40 cycles of 94°C for 30 seconds; 56°C for 10 seconds; and 72°C for 1 minute; and with another extension time of 4 minutes. Rat PDGF-A promoter was amplified using primers ratprom800-1000F and ratprom500-800R.

Small Interfering RNA
SMCs were seeded in Petri dishes and growth arrested with serum-free medium 6 hours before infection with 0.2 μmol/L small interfering RNA (siRNA; Qiagen) targeting endogeneous rat Ets-1 and Sp1. PDGF-A was inducibly expressed by the addition of FBS (Invitrogen) to the final concentration of 10% for 1 hour. Total RNA was extracted using the Trizol method; cDNA synthesis and RT-PCR were done as mentioned previously in this section. The sequence of siRNA Sp1 targeting nucleotides 418 to 438 (accession No. D12768) was 
mRNA targeting endogeneous rat Ets-1 and Sp1. G-Sepharose 4 Fast Flow Beads (Amersham) for 2 hours at 4°C. Cytoplasmic or nuclear extracts were precleared with Protein immunoprecipitation

Immunoprecipitation

Total Cell Count
SMCs were seeded into 96-well titer plates and rendered growth arrested with serum-free medium 6 hours before infection with 0.2 μmol/L small interfering RNA (siRNA; Qiagen) targeting endogeneous rat Ets-1 and Sp1. PDGF-A was inducibly expressed by the addition of FBS (Invitrogen) to the final concentration of 10% for 1 hour. Total RNA was extracted using the Trizol method; cDNA synthesis and RT-PCR were done as mentioned previously in this section. The sequence of siRNA Sp1 targeting nucleotides 418 to 438 (accession No. D12768) was 
mRNA targeting endogeneous rat Ets-1 and Sp1. G-Sepharose 4 Fast Flow Beads (Amersham) for 2 hours at 4°C. Cytoplasmic or nuclear extracts were precleared with Protein immunopercipitation

Results and Discussion

Ets-1 Activates PDGF-A Transcription and mRNA Expression in Primary Rat Aortic SMCs
To determine the influence of Ets-1 on the expression of PDGF-A, primary rat vascular SMCs were transfected with the CMV-based expression vector pcDNA3-Ets-1, together with construct p643A-luc, a Firefly luciferase reporter construct driven by 643 bp of the PDGF-A promoter. Ets-1 increased luciferase activity from p643A-luc within 24 hours (Figure 1A). Ets-1 also increased PDGF-A mRNA (Figure 1B, top) expression without affecting GAPDH levels (Figure 1B, bottom). Basal Ets-1 protein expression was below the limit of detection by Western blot analysis (Figure 1C). pcDNA3-Ets-1, as opposed to its backbone counterpart (pcDNA3), produced immunoreactive Ets-1 (Figure 1C). In contrast, Sp1 levels did not change during Ets-1 overexpression (Figure 1C). Immuno-}

Endogenous Ets-1 Interacts With the 5'-TTCC-3' Motif in the PDGF-A Promoter
Inspection of the PDGF-A promoter revealed a putative reverse Ets binding motif (TTCC) located at bp −555/−552 relative to the transcriptional start site. To determine whether this element could support an interaction with Ets-1, we performed an electrophoretic mobility shift assay (EMSA) in which we incubated nuclear extracts of SMCs with [32P]-Oligo A -568/−540, a [32P]-labeled double-stranded oligonucleotide spanning bp −568/−540 in the PDGF-A promoter. This produced several discreet nucleoprotein complexes (Figure 2A, lane 2). However, only 1 of these complexes failed to form when the putative Ets binding motif was mutated from 5'-TTCC-3' to 5'-TACA-3' in probe [32P]-Oligo ma-568/−540 (Figure 2A, lane 3) or [32P]-Oligo ma-560/−540 (Figure 2A, lane 4). Preincubation of the extracts with Ets-1 antibodies reduced formation of this specific complex (Figure 2A).

Ets-1 Binds to the Authentic PDGF-A Promoter
To provide confirmatory evidence for the physical interaction of Ets-1 with the endogenous PDGF-A promoter, we performed ChIP analysis using cells that had been transfected with pcDNA3-Ets-1 or pcDNA3, in combination with Ets-1 antibodies. Figure 2B demonstrates that Ets-1 binds to the endogenous PDGF-A promoter in SMCs transfected with

Figure 3. Integration of the 5'-TTCC-3' motif in the PDGF-A promoter and an intact Ets-1 transactivation domain are critical for Ets-1 activation of PDGF-A transcription. A, Cells were cotransfected with 10 μg of wild-type p643A-luc or mutant p643A-luc(5'-TACA-3') and 3 μg of pcDNA3-Ets-1 or pcDNA3, and 1 μg of pRL-TK. Firefly luciferase activity was determined in cell lysates after 24 hours. The y-axis represents the ratio of the Firefly luciferase activity over the Renilla to normalized for transfection efficiency. Error bars denote the SEM from 4 observations. B, Cells were cotransfected with 10 μg of p643A-luc and 3 μg of pcKCR3-Ets-1 or pcKCR3-DN-Ets-1 and 1 μg of pRL-TK. The y-axis represents the ratio of the Firefly luciferase activity over the Renilla to normalized for transfection efficiency 24 hours after transfection. Error bars denote the SEM from 4 observations.
pcDNA3. The intensity of the PDGF-A amplicon increased in cells transfected with pcDNA3-Ets-1. That the amplicon, the identity of which was confirmed by sequencing, could no longer be observed in the absence of the Ets-1 antibodies using extracts of cells transfected with either pcDNA3-Ets-1 or the empty expression vector demonstrates that Ets-1 interacts with the authentic PDGF-A promoter.

To provide additional evidence that Ets-1 interacts with the endogenous PDGF-A gene, we performed ChIP analysis with cells transfected with pKCR3-DN-Ets-1 or its backbone control, pKCR3. pKCR3-DN-Ets-1 generates a form of Ets-1 protein bearing only the DNA-binding domain and lacking the transactivation domain, thus functioning as a dominant-negative inhibitor of endogenous Ets-1. This mutant also lacks the C-terminal epitope of the Ets-1 antipeptide antibody and therefore should evade pull-down by the Ets-1 antibody. DN-Ets-1 decreased the interaction of endogenous Ets-1 with the PDGF-A promoter (Figure 2C, top). The nucleotide 500 to 700 region of the rat PDGF-A promoter (accession No. L06238) does not contain Ets consensus elements (\(\text{T}^9\)/GGA\(\text{T}\)) and therefore should not be pulled down with Ets-1 antibodies and amplified. This region is detected in the input but is not associated with an Ets-1 immunoprecipitate (Figure 2C, bottom).

To ensure that these observations were not an artifact of the Ets-1 antibody, we performed ChIP analysis using epitope-tagged Ets-1 in combination with antibodies to the tag. We transfected SMCs with pGST-Ets-1, which produces the DNA-binding domain of Ets-1 fused to GST. GST antibodies pulled down the authentic PDGF-A promoter in pGST-Ets-1 transfectants (Figure 2D) but not in GST-negative pcDNA3 transfectants (Figure 2D). These findings provide further evidence, independently of Ets-1 antibodies, that Ets-1 physically binds the endogenous PDGF-A promoter.
Mutation of the 555 TTCC 552 Motif Lowers Basal and Ets-1–Inducible PDGF-A Promoter Activity

To determine the functional importance of the 555 TTCC 552 element in PDGF-A promoter, we cotransfected pcDNA3-Ets-1 with p643A-luc (555 TACA 552), in which 555 TTCC 552 had been mutated to 555 TACA 552. This mutation significantly reduced basal activity of the PDGF-A promoter (Figure 3A), consistent with observations that Ets-1 is required for basal expression of certain other target genes. Ets-1, as expected, activated the wild-type PDGF-A promoter (Figure 3A). However, the mutant PDGF-A promoter failed to respond to Ets-1 (Figure 3A). Thus, introduction of a mutation in the PDGF-A promoter that ablates Ets-1 nucleoprotein complex formation compromised Ets-1 induction of the promoter. To complement these findings, we cotransfected SMCs with p643A-luc together with pKCR3-DN-Ets-1 or its backbone control, pKCR3. Unlike activation of PDGF-A promoter–dependent expression by wild-type Ets-1, this mutant form of Ets-1 failed to stimulate the promoter and even reduced basal promoter activity (Figure 3B).

Ets-1 Activates PDGF-A Transcription Cooperatively With Sp1

We demonstrated previously that Ets-1 and Sp1 physically interact and cooperatively activate the FasL promoter in the spontaneously transformed WKY12-22 (pup rat-derived) SMC line. To our knowledge, Ets-1/Sp1 interaction and cooperativity has not been demonstrated in SMCs. We had also established the existence of a functional cis-acting element for Sp1 in the proximal region (located at −71/−55) of the PDGF-A promoter. We hypothesized that Ets-1 and Sp1 together may activate the PDGF-A promoter in a cooperative manner, a mechanism hitherto not demonstrated in the context of any PDGF gene. Immunoprecipitation of nuclear extracts with polyclonal Sp1 antibodies followed by Western blot analysis with Ets-1 antibodies revealed that endogenous Ets-1 and Sp1 physically interact (Figure 4A). Conversely, precipitation with polyclonal Ets-1 antibodies followed by Western analysis with Sp1 antibodies further confirmed the interaction (Figure 4B). Immunohistochemical analyses using these antibodies indicate that Ets-1 and Sp1 are expressed together with PDGF-A in human atherosclerotic lesions (Figure 4C).

Overexpression of Sp1 alone increased PDGF-A promoter–dependent expression by almost 3-fold (Figure 5). However, Sp1 failed to activate the promoter when the Ets binding site had been mutated (Figure 5). The promoter was induced 6-fold when Ets-1 and Sp1 were coexpressed with the wild-type PDGF-A promoter–reporter construct (Figure 6A). Ets-1 activation of PDGF-A transcription was blocked by construct pEBG-DN-Sp1 (Figure 6B), which generates a mutant form of Sp1 containing only the DNA-binding domain. This dominant-negative form of Sp1 reduced cooperative activation of PDGF-A promoter-dependent expression with Ets-1 (Figure 6B, compare bars 6 and 7). That Ets-1–inducible PDGF-A transcription is blocked by siRNA targeting Sp1 and Ets-1 (Figure 6C) but not by an Irr siRNA molecule of identical size and concentration (Figure 6C) strengthens the evidence for cooperative Ets-1/Sp1 transactivation of the PDGF-A promoter.

We used siRNA to Ets-1 to provide further evidence for the positive regulatory role of endogenous Ets-1 in the regulation of PDGF-A gene expression. Ets-1 siRNA inhibited serum-inducible PDGF-A mRNA levels within 6 hours of transfection, siRNA targeting Sp1, which we demonstrated previously, plays a critical role in PDGF-A transcription—abrogated serum-inducible PDGF-A expression (data not shown). To demonstrate that a defined agonist can induce PDGF-A transcription via an Ets-1/Sp1–dependent mechanism, we used PDGF-BB, which stimulates Ets-1 expression in SMCs. PDGF-BB–inducible PDGF-A mRNA expression was inhibited by Ets-1 siRNA and Sp1 siRNA (Figure 6D) but not the Irr siRNA (Figure 6D).

Ets-1 and Sp1 Stimulate Primary Rat Aortic SMC Proliferation

Mitogenesis is blocked by neutralizing PDGF-A antibodies. Flow cytometric analysis and Coulter quantitation demonstrate that Sp1 and Ets-1 each increase both S-phase entry (Figure 7A) and total cell counts (Figure 7B) compared with their backbone control. Proliferation stimulated by Ets-1 together with Sp1 was blocked when Ets-1 was cotransfected with mutant Sp1 or when Sp1 was cotransfected with mutant Ets-1 (Figure 7B). Ets-1–inducible SMC proliferation is inhibited by Sp1 siRNA and Ets-1 siRNA (Figure 7C) but not by the Irr siRNA (Figure 7C). Conversely, Sp1–inducible SMC proliferation is blocked by Ets-1 siRNA and Sp1 siRNA (Figure 7C). Ets-1–inducible SMC mitogenesis is blocked using neutralizing PDGF-A antibodies but not species-matched and isotype-matched IgG (Figure 7D).

The autocrine mitogenic effect of PDGF-A on Ets-1 overexpression was further supported by immunoprecipitation experiments in which PDGFR-α was pulled down from total cell lysates followed by Western blot analysis with phosphotyrosine antibodies. Although PDGFR-α was expressed in SMCs transfected with the empty vector, it was inactive (Figure 7E). In contrast, tyrosine-phosphorylated PDGFR-α was readily

![Figure 5](http://circres.ahajournals.org/)

Figure 5. Sp1 activates the wild-type PDGF-A promoter but not the promoter bearing a mutation in the Ets-1 binding motif. The cells were cotransfected with 10 μg of either wild-type p643A-luc or mutant p643A-luc (555 TACA 552) and 1 μg of pRL-TK. The y-axis represents the ratio of the Firefly luciferase activity over the Renilla to normalized for transfection efficiency 24 hours after transfection. Error bars denote the SEM from 4 observations.
detectable in cells expressing Ets-1 without a change in total levels of PDGFR-α protein (Figure 7E).

Using a variety of approaches, including EMSA, ChIP, immunoprecipitation, and transient transfection analysis, this study provides the first demonstration that Ets-1 binds and activates the PDGF-A promoter. Confirmatory evidence was provided by dominant-negative siRNA and epitope-tagged approaches. Ets-1 binds to a distinct motif (TTCC) in the PDGF-A promoter located at bp -555/-552, a core Ets element in reverse. The integrity of this site is critical for Ets-1 binding and induction of PDGF-A expression. It is also required for cooperative Ets-1 and Sp1 activation of the promoter. Ets-1 activation of the PDGF-A promoter also requires an intact Sp1 binding site in the core region. These in vitro studies, which provide key insights into the role of Ets-1/Sp1 cooperativity in PDGF-A transcription, would be complemented by whole animal analyses in transgenic mice bearing PDGF-A promoter mutations in the Ets and/or Sp1 elements.

Gene expression is controlled in part at the level of transcription by the complex interplay of positive and negative regulatory factors. We and others have demonstrated that multiple nuclear factors interact with and regulate the PDGF-A promoter. These include Egr-1 and Sp3,12–15 WT-1,16,17 GCF2,18,19 BTEB236 NF-1(X),18,19 Sp1,12 and, as reported here, Ets-1. Whether Ets-1 cooperates with these other factors, as we show here it does with Sp1, to control PDGF-A transcription remains to be seen. The 2 CArG elements (putative SRF-binding motifs) in the vicinity of the TTCC-531 element (536CCCTTATG-531 and -536CAAAG-531) suggest the possibility of SRF/Ets cooperativity.

We have demonstrated here that Ets-1 and Sp1 stimulate the proliferation of early passage primary rat aortic SMCs. We have shown previously that Ets-1 inhibits apoptosis in these cells.77 The influence of Sp1 overexpression on SMC phenotype may depend on the amount of plasmid used and previous growth-arrest because previous studies by our group have revealed that
Sp1 inhibits SMC growth on transfection with 2- to 3-fold more of the Sp1 expression vector without previous growth-arrest. The present findings indicating cooperative Ets-1/Sp1 upregulation of PDGF-A expression in SMCs extend the scope of functional Ets-1/Sp1 interactions into cell growth. That Sp1, Ets-1, and PDGF-A are coexpressed in human atherosclerotic lesions and because Ets-1 but not Sp1 is inducibly expressed in SMCs after arterial injury suggests a role for Ets-1/Sp1 cooperativity in the process of neointimal thickening.

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References


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