Transcription Factor Ets-1 Regulates Gelatinase A Gene Expression in Mesangial Cells

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Abstract. Ets transcription factors are involved in cell growth and angiogenesis. Ets-1 targets include members of the matrix metalloproteinase superfamily. In inflammatory glomerular diseases, the patterns and regulation of Ets expression have not been fully characterized. In the present study, nuclear binding activities to the consensus Ets-1/PEA3 motif were detected in mesangial cells (MC), and the Ets-1 protein was positively identified by Western blotting, reverse transcription PCR (RT-PCR), and DNA-binding studies. The 5′ flanking regions of the human and rat gelatinase A genes contain clusters of potential Ets-1 binding motifs, one of which is evolutionarily conserved. Using a series of 5′ deletion reporter constructs of the rat gelatinase A gene and an Ets-1 expression plasmid, a concentration-dependent threefold trans-activation of gene expression mapped to the conserved Ets-1 binding motif at −1004/−1053 bps, designated responsive element-2 (RE-2). The RE-2 was operative within the context of the homologous gelatinase A promoter but not with a heterologous simian virus 40 promoter. Specific Ets-1 binding to this sequence was demonstrated by DNA-binding studies. Transient expression of an Ets-1 expression plasmid increased gelatinase A protein expression. Our findings identify an additional matrix metalloproteinase family member, gelatinase A, as an Ets-1 responsive gene in MC that may play a role in the high level expression of this enzyme in inflammatory glomerular diseases.

Mesangial cell (MC) proliferation, increased matrix turnover, and accumulation of interstitial collagens are common findings in most forms of progressive glomerular diseases (1). These changes generally continue even after removal of the initial causative event, progressively resulting in destruction of the glomerular architecture with the final outcome of glomerular scarring (1). Recent findings have elucidated a pivotal role for the “activated MC” phenotype (2). In contrast to the quiescent MC found within normal glomeruli, activated MC predominantly secrete interstitial type I and III collagens. Concurrently, high-level expression of matrix metalloproteinase-2 (MMP-2, 72-kD type IV collagenase, gelatinase A) is found (3,4). Gelatinase A belongs to the matrix metalloproteinase gene superfamily, all of which are secreted as latent proenzymes with subsequent activation by proteolysis in the extracellular space. The enzymatic activity of the MMP is dependent on the presence of zinc and divalent cations (5), and these enzymes exhibit broad substrate specificity (6). In addition to the role of gelatinase A in glomerular extracellular matrix turnover, the enzyme has a direct influence on the MC phenotype. Turck et al. (7) have shown that targeted deletion of gelatinase A in MC results in an exit from the cell cycle and development of a phenotype resembling quiescent MC in vivo. Addition of exogenous gelatinase A, but not gelatinase B, promoted MC proliferation with reacquisition of the prosclerotic phenotype (7). Concordant gelatinase A expression and MC proliferation has been detected in vivo and is a common finding in mesangiproliferative diseases (8). Altogether, these findings lend support for the important role of gelatinase A in the progression of glomerular sclerosis.

To gain insight into the regulatory events orchestrating gelatinase A gene expression, extensive studies have been performed on the human and rat gelatinase A regulatory sequences. As a result, an evolutionarily conserved enhancer element, designated response element-1 (RE-1; −1282/−1322 bps), and a silencer element were isolated (9–11). The RE-1 confers approximately 80% of the transcriptional activity controlling constitutive gene expression. In addition to the proximal promoter and the RE-1, a third region at −1004/−1053 bps (rat) exhibits a high degree of sequence similarity with the human gelatinase A gene (at −1245/−1293 bps). Within this sequence, a consensus Ets-1 binding site was identified, whereas other transcription factor binding motifs were absent. Our results demonstrate a MC-specific increase of gelatinase A gene transcription through transcription factor Ets-1 binding to this element.

Materials and Methods

Cells and Culture Conditions

Rat MC were established and characterized as described previously (7,12) and were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM l-glutamine, 100 µg/ml streptomycin,
and 100 U/ml penicillin at 37°C in humidified 5% CO₂ in air. The immortalized human MC line was kindly provided by Bernhard Banas (Ludwig-Maximilians-University, Munich, Germany) and cultured as described previously (13). Human umbilical vascular endothelial cells (HUVEC) were isolated as described (14). The cells were cultured on gelatin-coated culture flasks in medium M199 withEarle’s salts supplemented with20% fetal calf serum, 25 μg/ml endothelial growth supplement (Collaborative Research, Bedford, MA), and 25 μg/ml heparin.

**Nuclear and Cytoplasmic Cell Extracts**

Cells were grown to 80% confluence in tissue culture flasks, washed twice with ice-cold phosphate-buffered saline (PBS) without calcium and magnesium, and scraped in 10 ml of cold PBS. Nuclear cell extracts were prepared as described previously (12). Nuclear protein concentrations were determined by the Bio-Rad (Hercules, CA) protein assay using bovine serum albumin (BSA) as standard. Nuclear extracts were stored at −80°C until performance of electrophoretic mobility shift analysis, Western blotting, or Southwestern blotting.

**Electrophoretic Mobility Shift Analyses**

Double-stranded probes were generated by heating complementary synthetic oligonucleotides for 10 min at 95°C with subsequent cooling to room temperature over 6 h. The PEA3/Ets-1 probe harboring the sequence 5’-GATCTCACGAGCAATTTGA-3’ contains an Ets-1 consensus binding site (15). The evolutionarily conserved sequence 5’-GATCTCGAGCAAGGA-3’ contains an Ets-1 consensus binding site (15). The evolutionarily conserved sequence 5’-GATCTCAGCTTCAGCTTCAGCTTC-1004 of the rat gelatinase A regulatory sequence was performed within the Ets-1 core-binding motif “AGGA.” The remainder probes used for binding assays consisted of 21-mers harboring the putative Ets-1 binding sites depicted in Figure 3A. All probes were radiolabeled by means of T4-polynucleotide kinase using [γ-32P]ATP and were purified on 14% polyacrylamide gels and eluted, and 6 × 10⁶ cpm of labeled probe was included per binding reaction. Binding reactions were performed at 22°C for 30 min in binding buffer (20 mM HEPES [pH 7.9], 20% glycerol, 0.1 M NaCl, 0.2 mM EDTA) containing 0.2 mM PMSF, 0.5 mM DTT, 300 μg/ml acetylated BSA, and 2 μg of poly(dI-dC) in a total volume of 25 μl upon addition of nuclear or cytoplasmic extracts (15 μg total protein/binding reaction if not otherwise indicated). Samples were electrophoresed on nondenaturing 4% polyacrylamide, 7.5% glycerol gels in a buffer containing 1 × Tris/borate/EDTA before autoradiography.

**Reverse Transcription PCR**

Isolation of mRNA was performed with the Oligotex mRNA kit (Qiagen, Chatsworth, CA) according to the manufacturer’s instructions. Reverse transcription was achieved by addition of AMV reverse transcriptase (Promega, Madison, WI) in a reaction buffer containing 4 μl (40 U/μl) of RNase inhibitor (Roche Molecular Biochemicals, Summerville, NJ), 0.5 μg of oligo d(T)18, 0.5 mM dNTPs, and incubation for 2 h at 42°C and for 5 min at 95°C. Subsequently, cDNAs were precipitated and PCR were performed by addition of 2 U Taq polymerase (Life Technologies-BRL, Grand Island, NY) in reaction buffer (200 mM TrisHCl [pH 8.3], 0.5 M KCl, 1.5 mM MgCl₂), 0.25 pmol dNTPs, 25 pmol primers (specific Ets-1 primers for full-length rat ets-1 transcripts were: 5’-ATGAAGGCAGCGGCCTGATCT-3’ and 5’-TTACTCTACAGCATCCGCTTCCA-3’). A high sequence homology of these primers with the human ets-1 gene exists, whereas other Ets-1 members do not show sequence similarities. After heating at 95°C for 2 min, a total of 40 cycles were performed with 30 s at 95°C, 30 s at 53°C, and 30 s at 72°C. The final reaction was kept at 72°C for 7 min, and the amplified fragments were subsequently electrophoresed on an 1.5% agarose/ethidium bromide gel.

**Southwestern Blot Analyses**

Nuclear extracts from MC (7.5 μg/lane) were electrophoresed on a 12.5% sodium dodecyl sulfate (SDS)–polyacrylamide gel and transferred onto nitrocellulose membranes by electroblotting. Membranes were blocked in 25 mM HEPES [pH 8.0], 10% glycerol, 50 mM NaCl, 1 mM EDTA, 2% BSA for 12 h at 4°C, washed for 5 min in TNE-50 buffer (10 mM Tris/HCl [pH 7.5], 50 mM NaCl, 1 mM EDTA, 1 mM DTT), and probed for 4 h at 22°C in TNE-50 containing radiolabeled double-stranded oligonucleotide (10⁵ cpm/ml). The membranes were washed three times in TNE-50 buffer at 4°C for 1 min each, before autoradiography.

**Western Blot Analyses**

Nuclear extracts (7.5 μg/lane) from MC were electrophoresed on 12.5% SDS-polyacrylamide gel and transferred onto nitrocellulose membranes. Membranes were blocked for 2 h at 22°C in TTBS (10 mM Tris [pH 8.0], 150 mM NaCl, 0.05% Tween-20) containing 2% BSA before three washes with TTBS and incubation with primary antibodies (at 1:2000 dilution) overnight at 4°C. As primary antibodies polyclonal rabbit anti–Ets-1 antibody N276 (Santa Cruz Biotechnology, Heidelberg, Germany) raised against a peptide mapping with the amino terminal domain of Ets-1 (AA 55–70) or polyclonal rabbit anti-ets-1 antibodies C20 (AA 422–441) and C275 (both from Santa Cruz Biotechnology) raised against peptides mapping within the carboxy terminal domain of Ets-1 were used. The C20 and C275 antibodies exhibit a broad crossreactivity with other members of the Ets transcription factor family, whereas the N-276 antibody is specific for Ets-1. As secondary antibody, donkey anti-rabbit HRP-linked F(ab)2 fragment immunoglobulin (1:5000; Amersham/Pharmacia Biotech, Piscataway, NJ) was used, and band detection was achieved using ECL detection reagent (Amersham/Pharmacia Biotech).

**Transient Transfection Studies**

Transient transfection of MC was performed with liposomal preparation TfX-50 (Promega) as described (16). Purified plasmid DNA was diluted in 1000 μl of RPMI 1640 medium, mixed with sterile TfX-50 preparation (4.5 μl/μg DNA), and incubated at room temperature for 15 min. MC were grown to 60 to 70% confluency in six-well culture plates and washed twice with PBS. To each well, 1 ml DNA/liposome mixture was added and incubated for 2.5 h at 37°C with subsequent addition of complete 10% FCS/RPMI medium. Cell lysis, β-galactosidase, and luciferase assays were performed after 48 h (10). Eukaryotic expression plasmids used include the vector pEVRF harboring the full-length ets-1 sequence (pEVRF-ets-1) and pSG5 (Stratagene, La Jolla, CA) containing full-length YB-1 (pSG5-YB-1) (12). A series of luciferase reporter constructs harboring rat gelatinase A regulatory sequences of up to 1686 bps relative to the translational start site subcloned into pGL2-basic (Promega) were evaluated (9). In cotransfection experiments, 1 μg of luciferase reporter plasmid was combined with increasing amount of pEVRF-ets-1 plasmid ranging from 0.1 μg to 4.0 μg DNA/well. The total DNA content was equalized by inclusion of pEVRF plasmid. As control for transfection efficiency, pSV40-βGal plasmid (1 μg/well; Promega) was included.
Zymography

For semiquantitative gelatin zymography cell culture medium was exchanged with serum-free medium containing 0.2% bovine albumin and conditioned for 48 h. Before conditioning, cells were transiently transfected with plasmids pEVRF (1 μg/well), pEVRF-ets-1 (1 μg/well), pSG5-YB-1 (1 μg/well), and pSG5-YB-1/pEVRF-ets-1 combined (0.5 μg each/well) in 6-well plates, as described above. Ten microliter aliquots of conditioned media were suspended in nonreducing sample buffer, electrophoresed on a 7.5% SDS-polyacrylamide gel containing 2 mg/ml gelatin, washed in renaturating buffer (50 mM Tris/HCl [pH 8.0], 2.5% Triton X-100), and incubated overnight at 37°C in 50 mM Tris/HCl [pH 8.0], 1 μM ZnSO₄, 5 mM CaCl₂. Gels were methanol-fixed, stained with Coomassie Blue R-250 before destaining. Relative band intensities were assessed using Phoretix computer densitometry software (Biostep, Jahnsdorf, Germany).

Experimental Anti–Thy-1.1 Nephritis Model and Immunoperoxidase Staining

Anti–Thy-1.1 mesangial proliferative glomerulonephritis was induced in male Wistar rats (Charles River, Sulzfeld, Germany) weighing 140 to 160 g at the start of the experiment by injection of 1 mg/kg monoclonal anti–Thy-1.1 antibody (clone OX-7; European Collection of Animal Cell Cultures, Salisbury, UK). Animals were killed, and kidney specimens for immunohistology were obtained before disease induction and on day 7 after disease induction (n = 4). Four-micrometer sections of methyl Carnoy fixed biopsy tissue were processed by an indirect immunoperoxidase technique as described previously (17). Primary antibodies included polyclonal rabbit anti-human Ets-1 antibody (N-276, Santa Cruz Biotechnology) and a murine monoclonal antibody (clone 1A4) directed against α-smooth muscle actin. Negative controls consisted of substitution of the primary antibody with equivalent concentrations of normal rabbit IgG. Double immunostaining for the identification of Ets-1–expressing MC was performed as reported (18) by first staining the sections for Ets-1 with an anti–Ets-1 antibody (N-276, Santa Cruz Biotechnology) using an indirect immunoperoxidase procedure. Sections were then incubated with the IgG₁ monoclonal antibody 1A4 against α-smooth muscle actin.

Materials

Cell culture materials, radioisotopes, and electrophoresis materials were purchased from Life Technologies-BRL, Amersham-Pharmacia International, and Bio-Rad, respectively.

Results

MC Expression of Ets Transcription Factor Family Members

Although previous studies have shown Ets family member expression by endothelial cells (EC), epithelial cells, and fibroblasts, studies on MC have not been performed. Using complementary methodological approaches, Ets-1 expression was analyzed in human and rat MC. Two polyclonal antibodies raised against different Ets protein epitopes were used for Western blot analyses of rat and human MC nuclear and cytosolic extracts. Anti–Ets-1 antibody C275 recognizes a conserved epitope in the protein C-terminus, and anti–Ets-1 antibody N276 was raised against an epitope in the N-terminus and is specific for Ets-1. As shown in Figure 1A, four distinct bands of molecular sizes 15, 33, 54, 65, and 97 kDa were detected under reducing conditions with antibody C275, which was used for Western blot analysis of rat and human MC nuclear and cytosolic extracts. A similar band pattern was observed with antibody N276. Southwestern blotting was performed using a radiolabeled oligonucleotide encompassing the consensus Ets-1/PEA3–binding motif (C), yielding a similar band pattern as the one obtained with crossreactive antibody C275 with molecular sizes of 15, 33, 54, and 65 kDa, whereas the 97 kDa band was absent. In the cytoplasmic fraction, similar binding activities were present, but with lower amounts of the 54 and 65 kDa proteins.
The Gelatinase A Regulatory Sequence Contains Several Putative Ets-1 Binding Sites

Although Ets-1 regulates the expression of several MMP family members, an influence on gelatinase A expression is yet not reported. As a first step, a computerized search for potential Ets-1 binding sites within the human and rat gelatinase A regulatory sequences was performed using the MatInspector program version 2.2 (Genomatix, Munich, Germany) (20). The application of such sequence analysis algorithms does not permit an exact prediction of Ets-1 binding and trans-regulation, but it may hint at potential regions of importance. Within the published rat regulatory sequence up to 1686 bp, a total of 16 putative Ets-1 binding sites containing the GGA core sequence were detected, the locations of which are given in Figure 3A (+ and − denote sense and antisense strands). Sequences with matrix similarities higher than 0.85 were selected. The expected number of sites due to random event (re) is 1.53. In a similar search performed with the human gelatinase A regulatory sequence, a total of 12 potential Ets-1 binding sites were detected (not shown). Among these, a region...
Control reactions performed with the empty reporter construct pGL2basic demonstrated no effect of pEVRF–ets-1.
It can be concluded from these results that in the context of the previously described rat gelatinase A regulatory sequence Ets-1 trans-activates gene transcription.

### Mapping of the Ets-1 Binding Site in the Gelatinase A Promoter to an Evolutionarily Conserved Region

Two approaches were chosen to map the Ets-1 binding site(s) responsible for trans-regulation of the gelatinase A gene. The flanking sequences of the Ets “GGA” core determine binding specificities and affinities of the different Ets-1 family members. To identify the specific Ets-1 binding sites involved in gelatinase A gene regulation, a series of gelatinase A deletion constructs (1 μg/well) were cotransfected with the Ets-1 expression plasmid pEVRF–ets-1 or empty control plasmid (1 μg/well) into rat MC. The lengths of the luciferase deletion constructs are depicted in Figure 5, together with the location of the RE-1 (9). The conserved sequence harboring the potential Ets-1 binding site is denoted response element-2 (RE-2; compare Figure 3B). Relative luciferase values were calculated for differences between control and Ets-1–transfected cells, with control values set at 100%. Results were confirmed in three experiments performed in triplicate with SD less than 10%. All of the Ets-1–dependent trans-activation was lost when sequences located between −1004/−1053 bps (rat) relative to the translational start site were omitted. Adjacent to the RE-2 motif, another potential Ets-1 binding motif is located that shows similarities with the interferon-γ responsive element (IRE) (21). Notably, the IRE lies outside of this sequence element and did not confer trans-activation. In addition, the RE-2 was tested for potential cis-regulation within the context of the heterologous SV40 promoter. Here, no enhanced trans-activation by co-expressed Ets-1 was detected. These results indicate that Ets-1 trans-activates gelatinase A transcription in MC via the evolutionarily conserved sequence located between −1004/−1053 bps of the rat gelatinase A gene, and this activity is only operative within the context of the intrinsic promoter. A similar stimulatory response was detected in NRK fibroblasts (22).

To confirm that endogenous Ets-1 binds to this sequence motif, a series of electrophoretic mobility shift analyses were performed. First, a double-stranded oligonucleotide harboring the RE-2 sequence was prepared and used for assessment of nuclear protein binding. As shown in Figure 6A, a distinct nucleoprotein complex forms with MC nuclear extract and the double-stranded RE-2 as probe (Figure 6A, lane 2). Upon inclusion of homologous DNA in 100-fold and 500-fold molar excess, complex formation was competed, whereas equal amounts of unrelated heterologous DNA did not affect nucleo-complex formation (compare lanes 3 and 5 in Figure 6A). When a mutated oligonucleotide with base changes in the “GGA” core sequence (GG to TT transition) was used as probe, no nuclear protein binding was detected (lane 6 in Figure 6A).

Supershift studies were performed using the specific anti–Ets-1 antibody N276 and an unrelated antibody raised against...
nuclear factor-1. Inclusion of antibody N276 did not change the mobility of free probe (Figure 6A, lane 7), whereas a dramatic change of binding was observed with addition of N276 to the nuclear extract binding reaction. The major complex "1" was no longer detected, and a weaker supershifted band, indicated by "2", was present. At the same time, a major high mobility band appeared (indicated by 3), the identity of which is unclear. One may speculate that the anti-Ets antibody stabilizes Ets/DNA-complex formation in the context of the RE-2 probe; however, the detected supershift (indicated by *) using antibody N276 confirmed the identity of Ets-1 in the low mobility complex. A similar set of experiments is shown in Figure 6B, where anti-Ets antibodies C275 and N276 were tested for binding to the DNA probe alone was not detected. When binding reactions of nuclear proteins were performed with the antibodies, low mobility bands were detected (indicated by <3) and a similar high mobility complex formed (indicated by <4). These supershifts are in concordance with endogenous Ets-1 binding to the Ets consensus motif present in the RE-2.

Additional DNA binding studies were performed with potential Ets-1 binding sites from the computerized search (compare Figure 3A). In these studies, no complex formation could be detected (Figure 6C), indicating the specificity of Ets-1 binding to the RE-2 sequence.

**Influence of Ets-1 on Gelatinase A Protein Expression**

The potential effect of Ets-1 on gelatinase A protein expression was analyzed in MC by directly measuring gelatinolytic activities after transfection with either the empty expression plasmid pEVRF (CON, 1 µg/well) or pEVRF–ets-1 (1 µg/well). In the same series of experiments, a known trans-activator of gelatinase A expression, YB-1, was co-expressed and served as positive control (23). Transfection efficacy determined by β-galactosidase staining was approximately 30 to 40% (data not shown). Cell culture medium was exchanged 24 h after transfection and substituted by serum-free albumin-containing medium. Supernatants were collected after 48 h and assayed for gelatinolytic activities by means of zymography. As can be seen in Figure 7A, overexpression of Ets-1 enhanced gelatinase A synthesis about twofold. YB-1 had a similar stimulatory effect on gelatinase A expression. When both plasmids were combined (0.5 µg/well) and introduced into MC, gelatinase A expression was not synergistically stimulated. Densitometric results of zymography are depicted in Figure 7B.
Ets-1 Expression in Healthy Rat Kidney and in Mesangioproliferative Glomerular Disease

In a previous study performed by Naito et al. (24), MC Ets-1 expression has not significantly been detected by immunohistochemistry in healthy kidney as well as in a rat model of accelerated nephrotoxic serum-induced anti-GBM nephritis. Contrary to these findings, immunohistochemistry using the monospecific anti-Ets-1 antibody N-276 (Santa Cruz Biotechnology), which does not crossreact with other Ets family members, demonstrated a heterogeneous distribution of Ets-1–positive cells in healthy rat kidneys (CON) with staining of glomerular, tubular, and vascular cells (Figure 8). Exclusively nuclear staining was detected. Controls that included incubation with a nonspecific primary antibody showed no staining (not shown). On day 7 after induction of the anti-Thy-1.1 glomerulonephritis, profound changes of Ets-1 protein expression were detected. In these experiments, a counterimmuno-staining for α-smooth muscle actin was performed to identify MC. Ets-1 staining was intense in MC, whereas podocyte and EC staining was rather unchanged (Figure 8), suggesting that MC Ets-1 expression is upregulated in this model.

Discussion

Members of the large Ets transcription factor family are expressed by many tissues and are highly regulated at different stages during development. Ets-1 expression is not developmentally restricted (25) and is involved in as different processes as angiogenesis, inflammation, and tumorigenesis. Original work indicated a direct influence of Ets-1 expression on T cell commitment (26,27); however, recent work also shows highly regulated expression in nonhematopoietic tissues. Ets-1 expression has been observed in human embryos and granulation tissue and during tumor vascularization (28). Furthermore, Ets-1 is widely expressed during cardiac morphogenesis, a process accompanied by cell transdifferentiation and migration. Ets-1 may, in this process, trans-activate a number of genes involved in degradation of extracellular matrices and cell migration (29). In EC lines, angiogenic properties are correlated with elevated levels of Ets-1 and matrix metalloproteinases 1, 3 and 9, as well as integrin beta 3. Invasiveness was enhanced in high Ets-1–expressing cells and reduced in low Ets-1–expressing cells (30,31).
Figure 6. Binding of endogenous MC Ets protein(s) to the response element-2 (RE-2). To confirm that endogenous Ets-1 binds to this sequence motif, a series of electrophoretic mobility shift analyses were performed. First, a double-stranded oligonucleotide harboring the RE-2 sequence was prepared and used for assessment of nuclear protein binding. A distinct nucleoprotein complex forms with MC nuclear extract and the double-stranded RE-2 as probe (lane 2 in panel A). With inclusion of homologous (s) DNA in 100-fold and 500-fold molar excess complex formation was competed, whereas unrelated heterologous (ns) DNA did not affect nucleocomplex formation. When a mutated oligonucleotide with base changes in the “GGAA” core sequence was used as probe, no binding could be detected (lane 6). Supershift studies were performed using the specific anti–Ets-1 antibody N276 and an unrelated antibody raised against nuclear factor-1. Inclusion of antibody N276 did not change the mobility of free probe (lane 7), whereas a dramatic change of binding was observed with addition of N276 to the nuclear extract binding reaction, that is the major complex “1>” was no longer detected and a weaker supershifted band, indicated by “2>,” was present. At the same time, a major high mobility band appeared (indicated with “3>).” With anti-Ets antibodies C275 and N276, binding studies were performed in the absence (lanes 2 and 3, panel B) and presence of MC nuclear extract (lanes 4 and 5). Addition of antibodies to the nuclear protein binding reactions led to low mobility bands (indicated by “<3”) and major high mobility complex (indicated by “<4”). Additional DNA binding studies were performed with potential Ets-1 binding sites from the computerized search. In these studies, no complex formation could be detected (panel C), supporting the importance of the adjacent sequence for binding of Ets transcription.
stratate the constitutive expression of Ets-1 by cultured MC. Cross-reactive Western blotting using a polyclonal anti-Ets-1 antibody suggests that additional, as yet uncharacterized Ets-related proteins are also expressed by MC, the masses of which are approximately 15, 33, and 65 kD. Among the known Ets family members, approximate sizes of 65 kD have been reported for Elf-1 and PEA3. Another member of the Ets protein family, PU.1, with a calculated molecular weight of 30.7 kD has recently been identified in MC as negative regulator of gelatinase A transcription that acts via an upstream silencer element (11). Ets transcription factors with molecular sizes of 15 kD have not been described, suggesting that this band constitutes a proteolytic cleavage product. We are currently attempting to characterize the other Ets proteins using an expression cloning strategy.

Our study adds the gelatinase A gene as another Ets-1 target. The human and rat 5′ regulatory sequences of the gelatinase A genes display little similarities with other members of the MMP gene family (9) and lack a TATA-box within the proximal promoter and regulatory elements for transcription factor NF-κB. Extensive fine mapping of the human and rat gelatinase A gene for regulatory elements in different cell-types has been performed and demonstrate a complex pattern of transcriptional regulation through the action of several, cell-type specific cis-elements acting in concert with the homologous promoter (9,11,32,33). The enhancer element RE-1 at −1282 bp relative to the translational start site is responsible for about 80% of the trans-activity required for constitutive gelatinase A expression by cultured MC (9), with specific binding of transcription factors AP2, YB-1, and p53 (9,10,12,23,32). Conserved Ets-1/PEA3 elements that bind several members of Ets transcription factors have been found in all inducible MMP promoters. With the exception of MMP-12 these Ets-1/PEA3 elements are located adjacent to at least one AP-1 complex binding site. Ets-1 involvement in gelatinase A gene transcription was suggested by two observations: (1) several putative Ets-1 binding sites are present in the gelatinase A regulatory sequence; and (2) these sequences are conserved in the human and rat genes. In the present studies, MC nuclear Ets-1 was readily able to bind to a Ets-1 consensus oligonucleotide as well as to the RE-2 binding site. Binding specificity to the purine rich Ets-1/PEA3 element A/CGGAA/T is determined by flanking sequences (34,35). Ets proteins do not usually dimerize and bind to DNA alone but preferentially form complexes with other transcription factors, e.g., AP-1, for which they function as coactivators (34,36). AP-1 binding sites are not present in the vicinity of the RE-2; however, the results obtained with DNA binding studies suggest the presence of interacting proteins and direct interactions with homologous promoter elements were required for Ets-1 trans-activation in MC.

Given our knowledge concerning the role of gelatinase A in the transition of MC into the “activated” phenotype, trans-activation of gelatinase A gene transcription by Ets-1 may be of importance in the inflammatory response. Ets-1 is a potential key player in the molecular program responsible for the pro sclerotic MC phenotype by virtue of its target genes that include, among others, genes involved in cell-cycle progression (30,37) and extracellular matrix synthesis, e.g., extracellular matrix proteins osteopontin and tenascin (38). In addition, enzymes involved in extracellular matrix degradation and remodeling, collagenase-1 (MMP-1) (39), stromelysin (MMP-3) (30), and 92-kD type IV collagenase (MMP-9) (40) are also regulated by Ets-1. Given the observation of increased Ets-1 expression in a rat model of mesangioproliferative glomerulonephritis, in which increased gelatinase A expression has been demonstrated with MC activation (4), transcription factor Ets-1 has the potential of being critically involved in the progression of inflammatory glomerular disease.
Acknowledgments

We are grateful to the following people for kindly providing plasmid constructs: Barbara Graves (University of Utah, Salt Lake City, UT) for Ets-1 plasmid, Jenny P. Ting (University of North Carolina, Chapel Hill, NC) for YB-1 plasmid. We also thank Monika Cordes for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 542 project C4 to PRM and project C7 to JF) and National Institutes of Health grant DK39776 to DHL.

References


Figure 8. Immunostaining for Ets-1 in control Sprague Dawley rats and with mesangioproliferative glomerulonephritis. The distribution of Ets-1–positive cells in healthy rat kidneys (CON) was heterogeneous with exclusively nuclear staining of glomerular, tubular, and vascular cells. Profound changes of its expression were detected in the anti-Thy-1.1 glomerulonephritis (d7): Ets-1 staining was intense in MC, whereas podocyte and EC staining was unchanged. A counter-stain for α-smooth muscle actin identifies MC origin on day 7 after disease induction.


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