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Ets-1 Regulates TNF-α-Induced Matrix Metalloproteinase-9 and Tenascin Expression in Primary Bronchial Fibroblasts

Yutaka Nakamura,* Stéphane Esnault,† Takashi Maeda,‡ Elizabeth A. B. Kelly,* James S. Malter,† and Nizar N. Jarjour‡*†

Increased subepithelial deposition of extracellular matrix proteins is a key feature in bronchial asthma. Matrix metalloproteinase-9 (MMP-9) is a proteolytic enzyme that degrades the extracellular matrix. Tenascin is an extracellular matrix glycoprotein that is abundant in thickened asthmatic subbasement membrane. The expression of MMP-9 and tenasin reflects disease activity in asthma and airway remodeling. The molecular mechanisms regulating the expression of these proteins remain unknown. Both MMP-9 and tenasin promoters contain an Ets binding site, suggesting control by Ets-1. Thus, we hypothesized that Ets-1 expression is increased in asthma and that it contributed to enhanced MMP-9 and tenasin expression. To test this hypothesis, we determined the expression of Ets-1 in bronchial biopsies obtained from asthmatic subjects and determined the expression of Ets-1, MMP-9, and tenasin by bronchial fibroblasts activated ex vivo. We observed that nuclear extracts from TNF-α-activated fibroblasts showed increased Ets-binding activity. In addition, TNF-α-activated fibroblasts had increased expression of Ets-1 mRNA and protein, which preceded an increase in MMP-9 and tenasin mRNA. Furthermore, treatment of fibroblasts with Ets-1 antisense oligonucleotides down-regulated TNF-α-induced Ets-1, MMP-9, and, to a lesser extent, tenasin protein expression or activity. Taken together, these data demonstrate that TNF-α increases MMP-9 and tenasin expression in bronchial fibroblasts via the transcription factor Ets-1, and suggest a role for Ets-1 in airway remodeling in asthma. The Journal of Immunology, 2004, 172: 1945–1952.

Airway inflammation and remodeling are key histopathologic features in bronchial asthma (1, 2). Airway remodeling is thought to lead to irreversible airway obstruction, making treatment of asthmatic patients more difficult (3). Bronchial biopsies from asthmatic patients consistently show thickening of the reticular layer of the subepithelial basement membrane (4). The major components of the basement membranes are type IV collagen, laminin, nidogen, and proteoglycans (5). Several collagens, including types I, III, and V, have been described in the bronchial subepithelial basement membrane zone, which, along with tenasin, are elevated in asthmatic airways (4, 6). Tenasin is an extracellular matrix glycoprotein expressed during morphogenesis and tissue repair. In normal human bronchi, tenasin is typically absent, but accumulates within the epithelial subbasement membrane of asthmatics (6). Airway obstruction has been shown to be inversely correlated with amounts of subepithelial tenasin in patients with asthma (7).

Development, cell migration, wound healing, and tissue remodeling are physiologic processes in which the matrix metalloproteinases (MMPs) play crucial roles (8). The 92-kDa type IV collagenase/gelatinase (MMP-9) is capable of degrading type I, IV, V, VII, and XI collagens and laminin (9–12). MMP-9 is expressed in a wide variety of human malignancies, whereas lymphocytes, neutrophils, eosinophils, and fibroblasts use MMP-9 to degrade and migrate across basement membranes or through extracellular matrix. In addition, MMP-9 can contribute to inflammation and remodeling by releasing chemokines and growth factors that are bound to extracellular matrix proteins (8). We have previously shown that local airway allergen challenge in atopic subjects results in increased levels of MMP-9, suggesting that this enzyme may play a role in the pathogenesis of allergic asthma potentially through its effects on inflammatory cell migration, angiogenesis, and tissue remodeling (13). Finally, MMP-9 immunoreactivity is significantly increased in both the epithelium and submucosa of patients with asthma (7, 14). Several transcription factors have been implicated in MMP-9 and tenasin gene expression (15–20), including Ets-1. Ets was originally identified as the transforming oncogene in the E26 avian erythroblastosis virus and is involved in the invasion and metastasis of human malignant tumors (21, 22). Ets transcription factors bind via an 80-aa C-terminal domain to a GGA (A/T) consensus sequence called the Ets binding site (EBS) or PEA3 element (21, 22). EBSs are present in the promoters of many genes involved in cellular proliferation, differentiation, development, hemopoiesis, apoptosis, metastasis, tissue remodeling, and angiogenesis (21, 22).

Although several cytokines elicit MMP-9 and tenasin expression, TNF-α is particularly potent (23, 24). TNF-α markedly up-regulated MMP-9 production in human endothelial cells (25), fibroblasts (26), and leukemia cells (27) and was the most potent tenasin inducer in fibroblastic cells (28). These data lead us to hypothesize that Ets-1 contributes to airway remodeling by driving pulmonary volume in 1 s; PC_{20}, provocative dose that induces a 20% drop in lung function (FEV_{1}).

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MMP-9 and tenasin production. As Ets-1 expression in the asthmatic airway has not been established, we first examined Ets-1 expression within the bronchial mucosa of patients with asthma, then demonstrated the expression of Ets-1, MMP-9, and tenasin mRNA and protein by TNF-α-activated airway fibroblasts. Finally, we evaluated the effect of antisense oligonucleotides to the EBS on the expression of MMP-9 and tenasin to further define the potential role for Ets-1 in airway remodeling.

Materials and Methods

Subjects and selection and biopsy handling

Bronchial biopsy specimens were obtained from subjects with mild to moderate asthma and from normal subjects. Asthmatic subjects were selected using American Thoracic Society criteria. These subjects had physician-diagnosed asthma, airway responsiveness to methacholine (PC20 provocative dose that induces a 20% drop in lung function), <8 mg/ml, and/or reversibility to β-agonists (>12%; Table I). All subjects used only an inhaled β-agonist on demand. None had used inhaled or systemic corticosteroids or any other anti-inflammatory drugs, such as leukotriene modifiers, sodium cromoglycate, or nedocromil sodium, in the previous 3 mo. All asthmatic patients were atopic nonsmokers. Normal subjects were nonatopic nonsmokers with no history of asthma or systemic diseases and had normal spirometry (FEV1, forced expiratory volume in 1 s, >80%) and airway responsiveness (methacholine PC20 >16 mg/ml). Bronchial biopsy specimens were obtained by bronchoscopy. Bronchial tissues from seven asthmatic and seven normal controls were evaluated for the histologic study. Biopsy specimens from another three moderate asthmatics and three normal subjects were obtained for fibroblast isolation. The study was approved by the University of Wisconsin-Madison Center for Health Sciences human subjects committee. Informed consent was obtained from each subject before participation.

Immunohistochemistry

Paraffin-embedded biopsies were fixed using a formaldehyde-free glyoxal fixative (Prefer Fixative; Anatech, Battle Creek, MI). Immunolocalization of Ets-1 in bronchial tissue was performed using monoclonal anti-Ets-1 (dilution, 1/20; NeoMarkers, Fremont, CA); Ets-1 was detected using an Envision Plus System (DAKO, Carpinteria, CA) according to the manufacturer's instructions. Bronchial sections were counterstained with hematoxylin. Negative controls human vimentin (dilution, 1/50; NeoMarkers) and the fast red chromogen. Finally, the sections were counterstained with DAB, the specimens were stained using the alkaline phosphatase-anti-alkaline phosphatase technique to ensure fibroblast phenotype. Neither the passage number (three to five) nor the patient source (asthma vs normal) affected responsiveness to TNF-α.

RNA isolation and RT-PCR

RNA was extracted from cultured fibroblasts by RNeasy (Qiagen, Valencia, CA) according to the procedure provided by the vendor. The concentration of RNA was determined spectrophotometrically. RT-PCR was performed according to the manufacturer's instructions. Total RNA was reverse transcribed using Omniscript reverse transcriptase (Qiagen). PCR was performed with the AmpliTaq Gold (PE Applied Biosystems, Foster City, CA) using 1-μl reverse transcriptase samples in a 25-μl final reaction mixture. The following primer pairs were used for amplification: Ets-1: forward, 5′-AGCGGACTCCTCACATCA-3′; reverse, 5′-TCTGCAAGGTTGTCCTGTCTG-3′; MMP-9: forward, 5′-GGCCTCTATGACCCTGTAATTGT-3′; reverse, 5′-TACCAAGGCGGATCTGCTC-3′; tenasin: forward, 5′-CAGGCTCAACTTACCAAGTAC-3′; reverse, 5′-CTTCGCGTGGGCTCTGAAGG-3′; and β-actin: forward, 5′-TACCAACTGGGACGACATG-3′; reverse, 5′-GTACAGGATGACAGGCTT-3′. DNA amplification was obtained by annealing at 60°C for Ets-1, at 58°C for MMP-9 and tenasin, and at 65°C for β-actin for 30 s each. The PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide. The sizes of the PCR products for Ets-1, MMP-9, tenasin, and β-actin were 669, 468, and 200 bp, respectively. Ets-1 and β-actin sequences were amplified for 27 cycles. MMP-9 and tenasin sequences needed 35 amplification cycles to be detected. PCR without RT samples was performed as a negative control. Signals were quantified by densitometric scanning (α Imager 2200 version 5.5; α Innotech, San Leandro, CA) and normalized against β-actin.

Preparation of cell lysates and probe for EMSA

Nuclear extracts were prepared from fibroblasts as described previously (29). Bronchial fibroblasts were treated with 0, 10, 50, and 100 ng/ml TNF-α for 1 h, then suspended in 400 μl of a hypotonic buffer containing 10 mM HEPES, 15 mM MgCl2, 10 mM KCl, and 0.5 mM DTT. After incubation on ice for 10 min, the mix was vortexed in a microcentrifuge tube. The cytoplasmic fraction was discarded after centrifugation at 4°C for 10 s at 14,000 rpm. The pellet was resuspended in 30 μl of hypotonic buffer containing 25% glycerol, 20 mM HEPES, 15 mM MgCl2, 420 mM NaCl, 0.2 mM EDTA, 0.5 mM DTT, and 0.5 mM PMSF and incubated on ice for 30 min. The mix was vortexed and homogenized by passing the lysate through a needle fitted to a syringe (1-cc insulin syringe U-100 28 G1/2; BD Biosciences, Rutherford, NJ). After centrifugation for 2 min at 14,000 rpm, the soluble nuclear extract was collected. The fraction was stored at −80°C until use. A protein assay (Bio-Rad, Hercules, CA) was performed to determine the protein concentration. Complementary oligonucleotides (5′-TGAACGGAGGAGGAGAACG-3′ and 5′-ACTACGCTTCCCTCCTGC-3′) containing Ets binding sites in the human MMP-9 promoter region with four-base overhangs were combined, boiled in 0.5 M NaCl, and cooled to room temperature. Mutated Ets-binding sites (5′-TGAAGCGAGGAGGAGAACG-3′ and 5′-ACTACGCTTCCCTCCTGC-3′) (underline shows mutated sequences) were used as a negative control. Duplexes were end-labeled with [γ-32P]ATP with terminal transferase (Promega, Madison, WI) using the manufacturer’s recommendations. Denatured micrograms of nuclear extract and [32P]-labeled (3000 Ci/mmol) double-strand oligomers (50 fmol) were incubated in binding buffer (20% glycerol, 20 mM HEPES, 100 mM KCl, 20 mM Tris-HCl, 1 mM EDTA, and 1 mM DTT) with or without unlabeled competitors for 30 min at room temperature. For the supershift experiments, 3 μg of the Ets-1 or NF-κB Ab (Santa Cruz Biotechnology, Santa Cruz, CA) was added to the incubation (1/1000; NeoMarkers) to allow visualization of the probe. After a 30 min pre-run of the gel, samples were loaded onto 7.5% native polyacrylamide gels in 0.25× Tris-borate-EDTA buffer and run at 120 V for 3 h at 4°C. Gels were dried and visualized by autoradiography.

Oligonucleotide transfection

Antisense, sense, or mismatch phosphorothioate oligonucleotides homologous with the Ets-1 gene in the vicinity of the initiation codon were commercially synthesized (Invitrogen, Carlsbad, CA). The sequences were: sense, 5′-ACCATGAGGGGCGGCGGCTACTCAATCTGTTTG-3′ (−3 to 22); antisense, 5′-TGAATGACGCGCGCCCTGACCTGTTTAC-3′ (22 to −3); and

| Table I. Clinical characteristics of subjects used for biopsy* |
|-----------------|----------------|
| Asthma (n = 7)  | Normal (n = 7) |
| Gender (male/female ratio) | 5:2 / 4:3 |
| Age (range, years) | 19–39 / 19–43 |
| FVC% (predicted) | 77 ± 1 / 98 ± 4 |
| PC20 (mg/ml) | 1.6 ± 0.4 / >20.0 |
| Reversibility (%) | 18 ± 3 / 6 ± 1 |

* Data are presented as the mean ± SE unless otherwise noted, p < 0.05.
FIGURE 1. Bronchial biopsies contain Ets-1-positive cells. Representative photomicrographs show bronchial mucosal biopsy specimens stained with anti-Ets-1 Ab. A, Normal healthy control (×200). B, Asthmatic subject (×200). C, Higher magnification of biopsy from an asthmatic patient (×600). D, The number of Ets-1-positive cells in bronchial biopsies from normal (n = 7) and asthmatic subjects (n = 7). E, Colocalization of Ets-1 immunoreactivity (brown) and anti-vimentin Abs (red; ×1000). Double-positive cells with fibroblast morphology are indicated by arrows. F, The number of Ets-1 and vimentin double-positive fibroblasts in bronchial biopsies from normal (n = 7) and asthmatic subjects (n = 7). * p < 0.05.

Recombinant human pro-MMP-9 (92 kDa; Oncogene, San Diego, CA) and active MMP-9 (83 kDa; Oncogene) were used as positive controls. Photographs of the gels were scanned by an imaging densitometer system (α In Image 2200 version 5.5).

Immunoblotting

Fibroblasts treated with or without 100 ng/ml TNF-α for 24 h were collected and lysed in TNE buffer (10 mM Tris-HCl (pH 7.8), 1% Nonidet P-40, 1 mM EDTA, and 150 mM NaCl) as described previously (32). The lysate was mixed with sample buffer and boiled for 3 min. Twenty micrograms of protein was resolved by 10% SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad). The membrane was incubated with rabbit polyclonal anti-human Ets-1 Ab (Oncogene), anti-human β-actin Ab (Sigma-Aldrich, St. Louis, MO), which were detected using a colorimetric HRP detection method (Bio-Rad) according to the manufacturer’s instructions. Photographs of the gels were scanned with an imaging densitometer system. Signals were analyzed by densitometric scanning and were normalized against β-actin.

Statistical analysis

All data are expressed as the mean ± SEM. The numbers of cells expressing Ets-1 in biopsies from asthmatics and normal subjects were compared

FIGURE 2. Time course of Ets-1, MMP-9, and tenascin mRNA induction by TNF-α. Fibroblasts were treated for the times shown with 100 ng/ml TNF-α. Total RNA was isolated and subjected to RT-PCR analysis. β-Actin was used as a cDNA control. A, Representative RT-PCR. The values in B are the mean ± SEM of Ets-1 (▪), MMP-9 (●), and tenascin (□) expression from three different donors. * p < 0.05 vs 0 h.

FIGURE 3. TNF-α induces Ets-1 protein. Bronchial fibroblasts were pretreated with or without TNF-α (100 ng/ml) for 24 h, and cellular extracts were immunoblotted for Ets-1 and β-actin.
using unpaired Student’s t test. In time-course experiments, values from fibroblasts without stimulation by TNF-α were standardized to 1.0. In transfection experiments, results from fibroblasts stimulated with 100 ng/ml TNF-α for 24 h without transfection of oligonucleotides were standardized to 1.0. To assess the differences among six groups (0, 1, 3, 6, 12, and 24 h) or three groups (no oligonucleotides, sense oligonucleotides, and antisense oligonucleotides), the Tukey multiple comparison procedure was used to evaluate the statistical significance of a set of multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

**Results**

**Detection of Ets-1 expression in bronchial mucosa**

Ets-1 protein expression was investigated by immunohistochemistry (Fig. 1, A–C and E). Compared with biopsy specimens from normal subjects (Fig. 1A), the number of cells expressing Ets-1 protein was significantly increased in biopsies from asthma patients (Fig. 1, B–D); mean ± SEM, 19.0 ± 2.4 vs 4.7 ± 2.9/100 μm² ($p < 0.05$). To determine whether fibroblasts expressed Ets-1, double immunohistochemistry was employed. As shown in Fig. 1E, double-positive cells with the morphology of fibroblasts and exhibiting both brown color in the nucleus (Ets-1, DAB) and red color in the cytoplasm (vimentin, Fast Red) were detected. The number of fibroblasts expressing Ets-1 protein was significantly increased in biopsy specimens from asthma patients (Fig. 1F, $p < 0.05$; 4.0 ± 0.5/100 μm²) compared with those from normal subjects (0.9 ± 0.3/100 μm²). Thus, airway fibroblasts in asthmatics had increased expression of Ets-1 compared with that in normal subjects.

**Induction of Ets-1, MMP-9, and tenasin mRNA by TNF-α**

As bronchial fibroblasts expressed the transcription factor Ets-1 in vivo, cultured fibroblasts from asthma or normal subjects were further analyzed in time-course experiments after treatment with TNF-α. Of note, there were no significant differences between cultured fibroblasts from normal or asthmatic subjects; therefore, only data from asthma patients are shown. Cultured cells were stained with anti-vimentin, which showed that 100% of these cells were fibroblasts (not shown). TNF-α increased Ets-1, MMP-9, and tenasin mRNA levels in a dose-dependent fashion (not shown). In time-course experiments performed with 100 ng/ml TNF-α, significant induction of MMP-9 and tenasin mRNA was observed after 6 h and continued to increased for at least 24 h (Fig. 2, A and B). In contrast, increased Ets-1 mRNA expression was detected within 1 h of stimulation, peaked at 3 h, and remained elevated for at least 24 h (Fig. 2, A and B). As a consequence, Fig. 3 demonstrates that the expression of Ets-1 protein was elevated after 24 h of TNF-α treatment.

**TNF-α increases Ets-1 DNA binding activity**

Gel mobility shift assays were performed to examine Ets binding activity in fibroblasts treated with TNF-α for 1 h (Fig. 4). Incubation of nuclear extracts from fibroblasts with double-stranded oligonucleotides containing the EBS site in the human MMP-9 (Fig. 4, A and B) and tenasin (Fig. 4, C and D) promoter regions showed mobility-shifted complexes. TNF-α treatment increased Ets binding activity to the MMP-9 (Fig. 4A) and tenasin (data not shown) promoter region oligonucleotides in a dose-dependent fashion. Furthermore, addition of anti-human Ets-1 Ab decreased the protein-oligonucleotide complex seen after TNF-α treatment. Irrelevant IgG or anti-NF-κB Ab had no effect (Fig. 4B). An excess of unlabeled specific competitor abolished binding to labeled Ets, whereas 10- and 50-fold molar excesses of mutant competitor oligonucleotide had no effect on Ets-1 binding activity (Fig. 4B). The same results were obtained with the tenasin promoter oligonucleotide (Fig. 4, C and D). Therefore, Ets-1 is activated in nuclear extracts from TNF-α-treated fibroblasts and is capable of interacting with fragments of the MMP-9 and tenasin promoters. These data suggested that Ets-1 may contribute to MMP-9 and tenasin up-regulation in fibroblasts exposed to TNF-α.

**Transfection of airway fibroblasts with FITC-conjugated phosphorothioate Ets-1 antisense oligonucleotides**

To further investigate the role of Ets-1 in the expression of MMP-9 and tenasin, fibroblasts were transfected with Ets-1 sense, mismatched, or antisense phosphorothioate oligonucleotides. To determine transfection efficiencies, phosphorothioate oligonucleotides were end-labeled with FITC and transfected into fibroblasts.
Homogenous staining of the nuclei with pronounced accumulation in the nucleoli was noted after 6 h (Fig. 5) in ~50% of the fibroblasts.

Ets-1 antisense oligonucleotides reduce DNA binding activity

To determine whether antisense oligonucleotides effectively blocked the DNA binding activity of Ets-1, we performed gel mobility shift assays. In nuclear extracts from nontransfected, TNF-α-treated fibroblasts, EBS binding activity was induced. As shown in Fig. 6, A and C, DNA binding activity to the MMP-9 promoter region oligonucleotides was reduced by 78% when fibroblasts were transfected with antisense oligonucleotides. Transfection of fibroblasts with sense or mismatched oligonucleotides did not affect the binding activity (Fig. 6A). Similar results were observed when extracts from transfected fibroblasts were incubated with tenascin promoter region oligonucleotides (Fig. 6, B and D). Therefore, DNA binding activity was substantially reduced by antisense oligonucleotides.

Discussion

Several new findings have emanated from this study. First, we have demonstrated that expression of the transcription factor Ets-1 is significantly increased in the airways of asthmatic patients compared with normal control subjects. Some of the Ets-1-positive cells were phenotypically identified as fibroblasts. Second, we have shown that TNF-α induced Ets-1, MMP-9, and tenascin in fibroblasts derived from bronchial mucosal biopsies. Finally, we have shown that Ets-1 antisense oligonucleotides against Ets-1 dramatically prevented TNF-α-induced enhancement of MMP-9 and partially inhibited tenascin expression.
Bronchial asthma is a chronic inflammatory disease of the airways associated with variable remodeling. The histological hallmarks of airway remodeling are proliferation of myofibroblasts, angiogenesis, and increased connective tissue deposition (1–4). At the molecular level, the expression of many genes involved in these processes is regulated by Ets-1, including TCRαβ (33, 34), vascular endothelial growth factor receptor (Flt-1, Flk-1) (35, 36), IL-4 (37), IL-5 (38), GM-CSF (39), E-cadherin (40), MMP-9 (19), vascular endothelial growth factor receptor (Flt-1, Flk-1) (35, 36), and tenascin (20). Our hypothesis was that in vivo expression of Ets-1 was increased within the bronchial mucosa of individuals with asthma. Using double-staining immunohistochemistry, we showed that Ets-1 was significantly increased in fibroblasts in the airways of asthmatic patients compared with normal control subjects. Ets-1 was also identified in inflammatory cells, consistent with their role in producing IL-4, IL-5, MMP-9, and tenascin.

Given our in vivo data, we employed in vitro cultures of fibroblasts obtained from asthmatic or normal subjects to evaluate the effect of TNF-α on the expression of Ets-1, MMP-9, and tenasin.

MMP-9 promoter region, but whether they are important in regulating MMP-9 expression in airway fibroblasts is not known. Stimulation of MMP-9 gene expression by TNF-α is partly mediated through the NF-κB and Sp1 motifs located at −600 and −558 nt upstream of the transcriptional start site (15). Gum et al. (19) have shown that the mutation of previously undescribed EBS and AP-1 motifs located at −540 and −533, respectively, severely impairs the ability of Ras to induce the MMP-9 gene in an ovarian cancer cell line. Thus, the essential cis elements and the trans-acting factors regulating MMP-9 production differ depending on the cell type and the stimulus. The regulation of tenasin by Ets has been investigated in human fibroblasts. Four functional EBSs were identified in the human tenasin promoter. Shirasaki and co-workers (20) have demonstrated that Flt-1 (a member of the Ets family of transcription factors) strongly activated the tenasin promoter, whereas Ets-1 had a more modest effect. Although these EBS sites were important for the trans-activation of this promoter by exogenous Flt-1 and Ets-1, endogenous Flt-1 and Ets-1 protein could not be detected bound to the EBS by Ab supershift experiments. They speculated that Ets factors may play a role in tenasin induction by specific stimuli, such as TNF-α.

In our present study EMSAs were performed to determine whether Ets-1 interacted with the upstream EBS in the MMP-9 and tenasin promoters. Representative gel shifts (shown in Fig. 4, B–D) demonstrated that constitutive nuclear binding activities specific for the Ets consensus sites in the MMP-9 and tenasin promoters were present in bronchial fibroblasts. Supershift analysis suggested that some of the DNA binding activity in bronchial fibroblasts was related to Ets-1. As discussed above, the ability of Ets-1 to regulate MMP-9 and tenasin expression in vivo may be tissue or stimulus specific. Our study showed that in bronchial fibroblasts TNF-α sequentially induced Ets-1 followed by MMP-9 and tenasin expression. Treatment of fibroblasts with TNF-α also increased both endogenous Ets-1 and EBS binding activity, suggesting a direct role for Ets-1 in MMP-9 and tenasin expression.
We transfected bronchial fibroblasts with antisense oligonucleotides to confirm the role of Ets-1 in regulating MMP-9 and tenasin expression. Ets-1 antisense oligonucleotides reduced TNF-α-induced increases in MMP-9. These data show that Ets-1 is essential to MMP-9 expression after TNF-α stimulation of fibroblasts in vitro and suggests that TNF-α-induced MMP-9 may be Ets-1-dependent in vivo as well. Tenasin expression was decreased by 36%, suggesting that although Ets-1 may contribute to the up-regulation of tenasin, other transcription factors, such as Fli-1 (20) or AP-1 (47, 48), may also be involved.

Taken together, our data suggest the potential therapeutic utility of Ets-1 antisense oligonucleotides as a novel molecular approach for the treatment of patients with airway fibrosis. This approach has the potential advantage of simultaneously blocking the production of two cardinal molecules involved in airway remodeling and may be more effective than anti-TNF-α therapy.

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