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• BASIC RESEARCH •

Transcriptional regulation of human α1(I) procollagen gene in dermal fibroblasts

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Abstract

AIM: To clarify the fractional activity of promoters from human α1(I) procollagen gene, the interaction between cis-elements and consensus DNA-binding proteins responsible for high promoter activity, and the potential application of promoter competitors as well as cytokines for antifibrogenesis.

METHODS: Sequence between 2 483 bp upstream of the start of transcription and 42 bp downstream of this site was investigated with serial 5'-deletion. The 5'-deleted promoters recombined with chloramphenicol acetyltransferase (CAT) as reporter gene were transiently transfected to human dermal fibroblasts. Electrophoretic mobility shift assay was performed to show the DNA-protein binding capacity of the promoter sequence. Cytokines including tumor necrosis factor α (TNFα) and interferons (INFs) were added to the culture medium of transiently transfected fibroblasts. Competitor DNA for the binding sites of Sp-1, Ap-1 and NF-1 was individually cotransfected transiently in order to block the promoter-driven CAT expression.

RESULTS: Sequences of -2 483 to +42 bp and -268 to +42 bp of human α1(I) procollagen gene had high activity as promoters. Binding sites for Ap-1 and Sp-1 were among the cis-regulatory elements recognizing consensus transcription factors responsible for basal promoter activity of sequence -268 to +42 bp. TNFα, IFNα, IFNβ showed inhibitory effects on sequence -2 483 to +42 bp as promoter with activities 43%, 62% and 60% of control respectively. Transfection of the promoter competitors could reverse the promoter activity of -268 to +42 bp 40-60%.

CONCLUSION: Sequences of -2 483 to +42 bp recombined with reporter gene provide an ideal construction for transcriptional study of α1(I) procollagen gene. The anti-collagen capacity of TNFα and IFNs is associated with their transcriptional regulation. Ap-1 and Sp-1 mediate the basal transcriptional activation of human α1(I) procollagen gene in dermal fibroblasts. Competitors for highly active promoters might be a novel potential candidate in fibrotic blockade.

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INTRODUCTION

Excessive accumulation of extracellular matrix (ECM) following chronic impairment of tissue gives rise to the development of fibrosis which might occur in skin and other organs, such as liver, kidney and lung[1]. Scarring or cirrhosis with the progression of fibrosis can cause functional failure of the organ due to the distortion of the structure. Type I collagen, composed of two chains of α1(I) and one chain of α2(I), is the most abundant component of ECM in most fibrotic tissues[1]. The expression of genes coding for the α1(I) and α2(I) chains of type I collagen is regulated coordinatively[2]. Researches on the expression of type I collagen in the past decades have been ascribed to fluctuations under various pathophysiological conditions at transcriptional and translational levels[3]. Most of the recent available evidence suggest that the principal mechanisms operate at the level of transcription, although control and changes in mRNA processing and stability may also play a role[4-7]. The mechanisms of transcriptional activation of collagen genes are poorly understood till now. Several putative regulatory elements that may determine the transcriptional efficiency of type I collagen gene have been identified in their corresponding promoters[8-11]. Fine mapping of the cis-acting elements as well as the identification of their consensus DNA-binding proteins involved in the modulation of collagen gene expression is crucial for understanding the pathological regulation of collagen accumulation. In our previous work, we analyzed the promoter activity from mouse α2(I) procollagen gene as well as its modulation by cytokines[12,13]. In this study, we investigated the fractional activity of promoter from human α1(I) procollagen gene and the interaction between cis-elements and consensus DNA-binding proteins responsible for high promoter activity. Sequence between 2 500 bp upstream of the start of transcription and 42 bp downstream of this site was studied with serial 5'-deletion. We report that regions from -2 483 to +42 bp, -268 to +42 bp of human α1(I) procollagen gene have higher promoter activities. Binding sites for Ap-1, Sp-1 may be among the cis-regulatory elements recognizing consensus transcription factors responsible for basal promoter activity of sequence -268 to +42 bp. The anti-fibrotic capacities of TNFα and IFNs are associated with their transcriptional regulation of type I collagen. Transfection of the promoter competitors can partially reverse the promoter activity, suggesting that promoter competitors for highly active promoters may be a novel antifibrotic tool.

MATERIALS AND METHODS

Cell culture

Human dermal fibroblast culture was established by explanting tissue specimens obtained from the abdominal skin of a 3-year old male patient because of burn of his left arm and requiring skin transplantation. The cells were maintained under standard conditions in Dulbeco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 100 mL/L fetal calf serum (FCS, Gibco, USA). The cells in passages 3-8 were used for study.

Construction of plasmids

Plasmid pCAT3-enhancer (Promega, USA), a promoterless vector which contains SV40 enhancer and chloramphenicol

acetyltransferase (CAT) as reporter gene, was used as the recombinant plasmid backbone. The putative promoters in 6 constructions named pCOLH$_1$0.1, pCOLH$_1$0.27, COLH$_1$0.5, pCOLH$_1$0.9, pCOLH$_1$1.5 and pCOLH$_1$2.5 corresponded to sequences -105 to +42 bp, -268 to+42 bp, -496 to+42 bp, -829 to +42 bp, -1 448 to +42 bp, -2 483 to +42 bp, respectively in human α1(I) procollagen gene with the same 3' ends. They were obtained by PCR with p5.3K α1 containing 5' flanking region -5 300 to +42 bp of human α1(I) procollagen gene (gift from Dr. Sergio A. Jimenez) as template[14]. Sense primers for PCR were as follows: pCOLH$_1$0.1: 5' -ATGTCTACGCGTCTGATT GGCTGGGGCACGG-3' , pCOLH$_1$0.27: 5' -ATGTCTACGCGT TCTGAGGACCCAGCTGCAC-3' , pCOLH$_1$0.5: 5' -ATG TCTACGCGTGGAGAGGTCCTCAGC ATGC-3' , pCOLH$_1$ 0.9: 5' -ATGTCTACGCGTGGCTGCTC CATCACCCAAC-3' , pCOLH$_1$1.5: 5' -ATGTCTACGCGTCTCAGGACGAGGTA GATTG-3' , pCOLH$_1$2.5: 5' -ATGTCTACGCGTACATCTT CAGCCTGGGCAC-3' , Antisense primers for the 6 putative promoters were the same: 5' -ATAGTACTCGAGCGTGC CTCCTGCTCCGAC-3' . The sense and antisense primers contained *Ml*uI and *Xho*I adaptors (underlined part) which were 6 bases away from the 5' end of the primers. PCR was performed as routine with high-fidelity PCR kit (Roche) and PE-9600 PCR amplifier. The PCR products were digested with *Ml*uI and *Xho*I and then ligated to *M*luI-*Xho*I linearized pCAT3 vector with T4 DNA ligase (Promega). The ligation mixtures were transformed to competent *E.coli* (JM109), and the ampicillin resistant positive clones were further identified by small-scale restriction enzyme digestion and DNA sequencing (ABI 377). The correct clones were amplified and recombinant plasmids were extracted and purified with plasmid isolation kit (Qiagen). The purity and yield rate were determined in UV spectrophotometer (Du 600, Beckman).

Synthesis of oligonucleotide for binding sites of Ap-1, Sp-1 and NF-1

Consensus binding sites for Ap-1, Sp-1, and NF-1 were first synthesized as single stranded DNA (Sangon, Shanghai) and sequences were:Ap-1, 5' CGCTTGATGACTCAGCCGGAA 3' ; Sp-1,5' ATCGATCGGGGCGGGGCGCGC3' ; NF-1, 5' TTTTGGATTGAAGCCA ATATGATA3' . The synthesized sense and antisense single stranded DNAs were matched and mixed at a molar ratio of 1:1. After incubation of the mixtures at 95 ℃ for 10 min and then cooling slowly down to room temperature, the double stranded DNAs were stored at -20 ℃.

DNA transfection

DNA transfection was performed with Dosper liposomal transfecting reagent (Roche, Germany) according to manufacturer' s instruction. Briefly, the day before transfection, dermal fibroblasts were seeded at 5×10^5/well in a 6-well plate (Nunc, USA) in 2 mL DMEM. The cells were incubated until 60-80% confluence. Then the medium was replaced with fresh culture medium without FCS shortly before adding transfection reagent. Two micrograms of construct plasmid together with 1 μg of pSV β-gal (Promega,USA) as internal standard were cotransfected. CAT expression plasmid pCAT6.2 (Invitrogen, USA) was also transfected as CAT expression positive control. One milliliter medium containing 20 mL/L FCS was added to each well 6 h later. The medium was then replaced with fresh normal culture medium 24 h later. Two days after transfection, the cells were ready for reporter gene (CAT) expression measurement. For the cells transfected with pCOLH$_1$0.27, 10 μg of DNA for binding sites of Ap-1, Sp-1 or NF-1 was transfected to the cells 24 h after pCOLH$_1$0.27 transfection. Mock DNA transfection was included. The transfection was terminated 24 h later and ready for reporter gene detection. To study the regulatory effect of cytokines, cells were starved for 4 h after being transfected with pCOLH$_1$2.5 for 24 h, then culture medium was replaced by fresh medium containing TNFα 10 μg/L (R&D, USA), IFNα 1×10^5 U/L (PBL, England), IFNν 1×10^5 U/L (Roche, USA) respectively. Another 24 h later, the transfection was terminated and ready for reporter gene detection.

Determination of CAT and b-galactosidase

Forty-eight hours after initial transfection, cells were washed with precooled phosphate buffered solution (PBS, 0.1 mol/L, pH7.4) and lysed with lysis buffer (Roche, USA). Aliquots of cell extracts were made for protein determination as described prevoiously[15]. CAT was measured by ELISA (Roche, USA) and β-galactosidase with enzyme activity analysis method (Promega, USA).

Electrophoretic mobility shift assay (EMSA)

EMSA was performed as described previously[16]. Briefly, a fragment spanning -268 to +42 bp of the human α1(I) procollagen gene was obtained by PCR with p5.3K α1 as template. The fragment obtained from PCR was purified, digested with *Eco*RII and 3' end-labeled with digoxigenin (Roche). Crude nuclear extracts from early passage confluent human fibroblasts were prepared according to the method described by Erdos *et al*[17]. The protein concentration in nuclear extract was determined by Bradford method[15]. DNA-protein binding reactions (20 μL) were performed in a buffer containing 1 μg of poly[d(A-T)], 1 μg of poly L-lysine, 15 μg of crude nuclear extract, 50 fmol of dig-labeled probes. Unlabeled probes and synthetic binding sites of Ap-1, Sp-1 and NF-1 were applied with 100-fold molar excess for competition tests. Following 20-min incubation at room temperature, DNA-protein complexes were resolved from free DNA probes by electrophoresis on 60 g/L non-denatured polyacrylamide gels. Transfer of DNA was finished by electroblotting. Signals were captured by chemiluminescent detection with alkaline phosphatase labeled anti-dig antibody and CSPD (Roche) as substrate.

RESULTS

Construction and transfection of 5'-deleted recombinant plasmids

Constructions of the 6 recombinant plasmids were verified by small-scale restriction enzyme digestion and DNA sequencing. Figure 1 shows the correct digestion of the constructs. The putative promoters in the constructions were 0.1 kb, 0.27 kb, 0.5 kb, 0.9 kb, 1.5 kb, and 2.5 kb, corresponding to -105 to +42 bp, -268 to +42 bp, -496 to +42 bp, -829 to +42 bp, -1 448 to +42 bp, -2 483 to +42 bp of human α1(I) procollagen gene. DNA sequencing indicated that the inserted sequences of the putative promoters were the same as that published in GeneBank (accession No X98705). The 6 constructs containing serial 5' -deleted promoters were transiently transfected to early passage confluent human fibroblasts. Forty-eight hours after transfection, the expression of reporter gene CAT was determined by ELISA with the detection of β-galactosidase activity and protein for normalization. The expression of normalized CAT in the cells transfected with pCOLH$_1$2.5 was set as 1, the relative expression level of normalized CAT in cells transfected with other constructs is shown in Table 1 (mean±SD of three independent experiments), indicating that the highest CAT expressions were driven by -2 483 to +42 bp and -268 to +42 bp as promoters while the lowest by -105 to +42 bp.

Transfection of promoter competitors

For the cells transfected with pCOLH$_1$0.27, 10 μg of consensus recognition DNA for Ap-1, Sp-1 or NF-1 was transfected to

cells 24 h after pCOLH$_1$0.27 transfection. Reporter gene (CAT) was determined another 24 h later. Relative CAT expression values in different transfection groups were calculated relative to that of mock DNA transfection. The result shown in Figure 2 indicted that transfection of Ap-1 or Sp-1 DNA inhibited CAT expression approximately by 25% and 20% respectively compared to mock DNA transfection (P<0.05). Transfection of NF-1 DNA did not show definite effect on reporter gene expression.

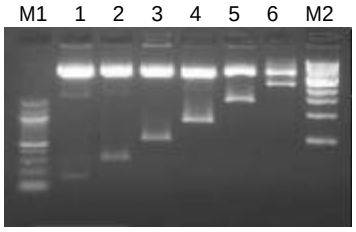

Figure 1 Electrophoresis of six constructs digested with *Ml*uI and *Xho*I. M1: 100 bp DNA ladder marker, M2: 1 kb DNA ladder marker, Lane1: pCOLH$_1$0.1, lane2: pCOLH$_1$0.27, lane3: pCOLH$_1$0.5, Lane4: pCOLH$_1$0.9, Lane5: pCOLH$_1$1.5, Lane6: pCOLH$_1$2.5. Six recombinant plasmids containing serial 5'-deleted flanking sequences of human α1(I) procollagen gene as putative promoter were digested with *Ml*uI and *Xho*I at 37 ℃ for 1 h. The digested DNAs were fractionated on 15 g/L agarose gel showing vector DNA (4.3 kb) and insertion promoters with different sizes.

Table 1 Summary of CAT expression driven by various lengths of the 5'flanking sequence from human α1(I) procollagen gene

Name of transfected constructions	Putative promoters' length (bp)	Relative activities of reporter gene (CAT, mean±SD)[1]
pCOLH$_1$0.1	-105 to 42	0.10±0.02
pCOLH$_1$0.27	-268 to 42	0.97±0.04
pCOLH$_1$0.5	-496 to 42	0.20±0.05
pCOLH$_1$0.9	-829 to 42	0.36±0.09
pCOLH$_1$1.5	-1 448 to 42	0.73±0.11
pCOLH$_1$2.5	-2 483 to 42	1.0

[1]The expression of normalized CAT in the cells transfected with pCOLH$_1$2.5 was set as 1, the relative expression level of normalized CAT in the cells transfected with other constructs is shown in Table 1 (mean±SD of three independent experiments).

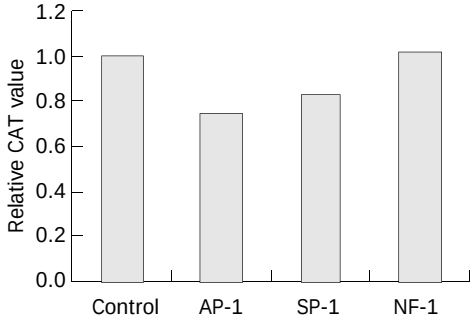

Figure 2 Effects of consensus DNA on CAT expression in pCOLH$_1$0.27 transfected cells. Control: mock DNA transfection, Ap-1: transfection of Ap-1 consensus DNA (10 μg), Sp-1: transfection of Sp-1 consensus DNA (10 μg), NF-1: transfection of NF-1 consensus DNA (10 μg). For the cells transfected with pCOLH$_1$0.27, transfection of consensus recognition DNA for Ap-1, Sp-1 or NF-1 was performed 24 h after initial transfection. Reporter gene CAT was determined with ELISA after another 24 h. Relative CAT values in different transfection groups were calculated relative to that in mock DNA transfection. The result represented three independent experiments. [a]P<0.05 compared to control.

EMSA

The fragment spanning -268 to +42 bp in human α1(I) procollagen gene was digested into three smaller ones (42 bp, 113 bp, 155 bp) with *Eco*RII (Figure 3). EMSA with these three labeled oligonucleotides mixture as probes showed that DNA-protein complexes were generated and detected in form of retardation bands. Competition with molar excesses of the same unlabeled probe prevented the formation of DNA-protein complexes, suggesting the specificity of the binding between DNA and protein. Excess consensus DNA for Sp-1, Ap-1 or NF-1 partially inhibited the occurrence of retardation differently, indicating the potential binding sites for Sp-1, Ap-1 and NF-1 in -268 to +42 bp of human α1(I) procollagen gene (Figure 4).

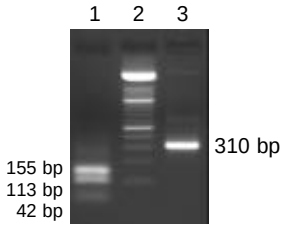

Figure 3 Electrophoresis (20 g/L agarose gel) of the fragment -268 to +42 bp digested with *Eco*RII. Lane1: *EcoRII*-digested fragment, Lane2: 100 bp DNA ladder, Lane3: 310 bp length of fragment spanning from -268 to +42bp. The 310 bp fragment spanning -268 to +42 bp of the human α1(I) procollagen gene was obtained by PCR with p5.3Kα1 as template. The fragment was digested with *Eco*RII. Electrophoresis (20 g/L agarose gel) of the digested mixture showed 3 bands with different sizes (42 bp, 113 bp, 155 bp) which were labeled and used as probes in EMSA.

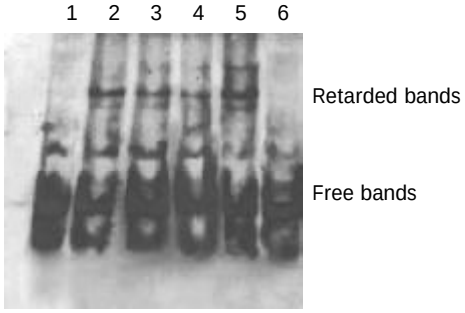

Figure 4 Result of EMSA with *EcoRII*-digested -268 to +42 bp as probe. Lane1: Labeled DNA, Lane2: Labeled DNA+ nuclear protein +NF-1 consensus DNA, Lane3: Labeled DNA + nuclear protein + Sp-1 consensus DNA, Lane4: Labeled DNA + nuclear protein + Ap-1 consensus DNA, Lane 5: Labeled DNA + nuclear protein, Lane 6: Labeled DNA + excess unlabeled DNA + nuclear protein. The existence of several retarded bands in EMSA indicated that there were several nuclear protein binding sites in sequence –268 to +42 bp (lane 5). No retardation occurred when excess unlabeled DNA probe was added to the DNA-protein reaction, confirming the specificity of the retardation (lane 6). Consensus DNAs for Sp1, Ap-1 and NF-1 were among the possible regulatory elements since the molar excess of the consensus unlabeled probe (Sp1, Ap-1, NF-1) inhibited partially the formation of retardation bands differently (lanes 2,3 and 4).

Effect of cytokines on reporter gene activity

TNFα, IFNα and IFNγ inhibited the reporter gene activity by 40-60% in the cells transfected with pCOLH$_1$2.5 (P<0.05) compared to the control. The strongest inhibitory effect appeared in TNFα group (Table 2).

Table 2 Effect of cytokines on reporter gene activity in cells transfected with pCOLH$_1$2.5

Cytokines	Relative value of reporter gene activity[1]	*P* Value (compared to control)
Control	1.00±0.15	
TNFα 10 μg/L	0.43±0.17	<0.05
IFNα 1×10^5 U/L	0.62±0.15	<0.05
IFNν 1×10^5 U/L	0.60±0.16	<0.05

[1]The reporter gene activity of the cells transfected with pCOLH$_1$2.5 was set as 1, the relative expression level of normalized CAT in transfected cells treated with cytokines was expressed as mean±SD of three independent experiments.

DISCUSSION

The mechanisms involved in the regulation of collagen production under fibrotic conditions are not yet completely understood. The synthesis of collagen might be modulated transcriptionally and post-transcriptionally, similar to the regulation of most of the other proteins in eukaryotic cells. Evidence suggests that the stability of newly synthesized mRNA as well as some enzymes devoted to collagen synthesis and degradation may play important roles in excessive accumulation of collagen in tissues[18]. Recently, studies focused on transcriptional regulation revealed that there existed several important cis-acting elements in the upstream region of human or rodent type I procollagen genes[8-11,19]. Activation of type I collagen gene was regarded to be related to the MAP kinase cascade pathway[6,20]. Newly identified tissue specific transcription factors for transcription of collagen genes have been increasing[21-26]. Some responsive elements of cytokines, including TGFβ1, TNFα and IFNγ have been reported to be located in procollagen genes[7,27-29]. In previous work, we analyzed the promoter activity from mouse α2(I) procollagen gene as well as its modulation by cytokines and retinoic acid[12,13,30]. Sequence spanning from -348 bp to +54 bp in mouseα2(I)procollagen was found to be of the highest promoter activity and partial cell specificity. The activity was influenced by TGFβ, TNFα and IFNs. In order to elucidate the transcriptional regulation of type I collagen in humans, especially the cis-acting elements and consensus transcription factors involved, six recombinant plasmids containing serial 5' -deleted flanking sequences of α1(I) procollagen gene as putative promoter were constructed here. Transient transfection of these constructions into human dermal fibroblasts showed that the sequences spanning from -2 483 bp to +42 bp and from -268 to +42 bp could drive the reporter gene with higher activity, while -105 to +42 bp had lower activity, indicating that there might be some positive and negative elements in the 2.5 kb flanking region. Our result was in agreement partially with that of Jimenez *et al*. though the fine mapping was different because of different constructs and host cells used[14]. Further deduction from the ranked driving activity of the putative promoter suggests that positive elements may be localized at -2 483 to 1 448 bp, -1 448 to 829 bp, -829 to -498 bp, -268 to -105 bp and negative ones at -498 to 268 bp, -105 to +42 bp. Computer- based prediction (DNAssist 1.0) of this 2.5 kb flanking sequence revealed that there might be 5 binding sites for *Sp-1* (-123 bp, -1 615 bp, -1 628 bp, -2 170 bp, -2 176 bp), 1 for *NFkB* (-1 571 bp), 2 for *c-myc* (-1 118 bp, -2 406 bp), 2 for *Ap-1* (-103 bp, -1 985 bp), 1 for *NF-1* (-101 bp). Obviously, one site for *NF-1* (-101 bp), *Ap-1* (-103 bp) and *Sp-1* (-123 bp) might be located in -268+42 bp.

To further characterize the existence of Ap-1, Sp-1, and NF-1 as potential transcription factors transactivating α1(I) procollagen gene, we studied the DNA-binding capacity of sequence -268+42 bp, which showed a higher promoter activity in transfection experiment with modified EMSA. Restriction enzyme *Eco*R II cut the target sequence into 3 smaller fragments with different sizes, *i.e.* -268 to -227 bp (42 bp), -226 to114 bp (113 bp), and -113 to +42 bp (155 bp). The digested fragments were end-labeled with digoxigenin and used as probes in DNA-protein binding reaction (see details in MATERIALS AND METHODS). DNA-protein complexes were shown in form of DNA bands with low mobility. The existence of several retarded bands in EMSA indicates there are several nuclear protein binding sites in sequence -268 to +42 bp. No retardation could be found if excess unlabeled DNA probe was added to the DNA-protein reaction, confirming the specificity of the retardation. Consensus DNAs for Sp1, Ap-1 and NF-1 were among the possible regulatory elements since the molar excess of the consensus unlabeled probe (*Sp1, Ap-1, NF-1*) partially inhibited the formation of retardation bands differently (Figure 4).

In order to confirm the potential binding sites and their transcriptional regulatory effects on -268 to +42 bp flanking sequence, a set of competitors in forms of double stranded DNA consensus to Ap-1, Sp-1 and NF-1 were transfected to cells 24 h after pCOLH$_1$ 0.27 transfection. The competitive inhibitory effects were found in *Ap-1* (25%) and *Sp-1* (20%), indicating the positive effects of the sequences on *Ap-1* and *Sp-1* in -268 to +42 bp. The transfected sequence for *Ap-1* or *Sp-1* thus decreased -268 to +42 bp activity as promoter due to competition for binding of nuclear protein. The results were similar to those reported by Sugiura and Inagaki *et al*. who showed that some cytokines or calcium channel blockers could modulate the expression of collagen via Ap-1 and Sp-1[9,31]. The anticipated inhibitory effect of *NF-1* competitor has not been found due to unknown reasons. No effect of *NF-1* on basal transcription or the weak effect of *NF-1* beyond detection limit might be the explanation.

The Sp1 is a ubiquitously expressed zinc-finger transcription factor recognizing GC rich sequence that is widely distributed in the promoters of various genes and is thought to be a target of intracellular signaling[32]. It is regarded that Sp1 plays an important role in both basal and inducible regulation of type I collagen expression, and may be implicated in the increased production of collagen during the development of pathological fibrosis[9]. Ap-1 consists of either Jun homodimers or Fos/Jun heterodimeric complexes which bind the palindromic TRE sequence TGA(C/G)TGA. Ap-1 is subjected to regulation by both phosphorylation and chemical oxidation of specific cysteine residues mapping within the DNA binding domains[33]. In this study transfection of Ap-1 and Sp-1 oligonucleotides to pCOLH$_1$ 0.27 transfected cells inhibited the promoter activity of -268 to +42 bp, suggesting that Ap-1 and Sp-1 sites are important for the basal promoter activity of the α1(I) procollagen gene besides mediating the response of cytokines and chemicals[31-34]. Increased promoter activity of procollagen α2(I) induced by TGFβ1 or acetaldehyde is mediated through NF-1. The existence of NF-1 in -268 to +42 bp of human α1(I) gene has been shown by our competitive EMSA. Transfection of consensus DNA for NF-1 failed to inhibit the promoter activity of -268 bp to +42 bp in our experiment. The possible reason might be due to no or weak effect of NF-1 and thus its weak competition for the basal promoter activity.

The antifibrotic capacity of TNFα and IFNs has been reconfirmed in our study and their trancriptional regulation on collagen promoter definitely play a role in their anti-collagen production effect.

In conclusion, we find that sequences spanning from -2 483 bp to +42 bp and -268 to +42 bp in 5' -flanking region from α1(I) procollagen gene are highly active as promoters. The inhibitory cytokines including TNFα and IFNs downregulate collagen production via at least partially transcriptional regulation. The promoter activity of -268 bp to +42 bp shows

that binding sites for Sp-1, Ap-1 and NF-1 are existing candidate cis-element for transcriptional regulation in sequence -268 to +42 bp. Binding sites for Sp-1, Ap-1 are positive for basal transcription since transfections of their competitor oligo DNAs decrease the promoter activity of sequence -268 to +42 bp. Thus, transfection of competitor DNAs is applied for the first time to confirm that the sites for Sp-1 and Ap-1 are important for basal highly promoter activity. Competitors for the high active binding sites for transcription factors may be novel and promising tools for fibrotic blockade.

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