Transcriptional regulation of human \( \alpha 1(1) \) procollagen gene in dermal fibroblasts

Chun-Fang Gao, Hao Wang, Ai-Hua Wang, Wei-Dong Wan, Yan-Aan Wu, Xian-Tao Kong

**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\(^\text{[2]}\). 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 \( \alpha 1(I) \) and one chain of \( \alpha 2(I) \), is the most abundant component of ECM in most fibrotic tissues\(^\text{[1]}\). The expression of genes coding for the \( \alpha 1(I) \) and \( \alpha 2(I) \) chains of type I collagen is regulated coordinatively\(^\text{[1,2]}\). 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\(^\text{[1]}\). 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\(^\text{[3-5]}\). 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\(^\text{[6-11]}\). 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 \( \alpha 2(I) \) procollagen gene as well as its modulation by cytokines\(^\text{[1,2]}\). In this study, we investigated the fractional activity of promoter from human \( \alpha 1(I) \) procollagen gene and the interaction between cis-elements and consensus DNA-binding proteins responsible for high promoter activity.

**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 \( \alpha \) (TNF\( \alpha \)) 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 \( \alpha 1(1) \) 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. TNF\( \alpha \), IFN\( \alpha \), IFN\( \beta \) 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 \( \alpha 1(1) \) procollagen gene. The anti-collagen capacity of TNF\( \alpha \) and INFs is associated with their transcriptional regulation. Ap-1 and Sp-1 mediate the basal transcriptional activation of human \( \alpha 1(1) \) procollagen gene in dermal fibroblasts. Competitors for highly active promoters might be a novel potential candidate in fibrotic blockade.

**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

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acetyltransferase (CAT) as reporter gene, was used as the recombinant plasmid backbone. The putative promoters in 6 constructions named pCOLH0.1, pCOLH0.27, pCOLH0.5, pCOLH0.9, pCOLH1.5 and pCOLH2.5 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[16]. Sense primers for PCR were as follows: pCOLH0.1: 5’- ATGTCTACGGTCGTAGTTGCTCGGGCCAGG-3’, pCOLH0.27: 5’- ATGTCTACGGTCGTTGACGGCAGG-3’, pCOLH0.5: 5’- ATGTCTACGGTCTCTCGACTCCATCACCACAC-3’, pCOLH0.9: 5’- ATGTCTACGGTCGTAGCTCTCGAC-3’, pCOLH1.5: 5’- ATGTCTACGGTCTCTCGAC-3’, pCOLH2.5: 5’- ATGTCTACGGTCTCTCGAC-3’. 

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

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 synthesized as single stranded DNA (Sangon, Shanghai) and cloned into plasmids. Two micrograms of construct plasmid together with 60-80% confluence. Then the medium was replaced with fresh 75 cm² tissue culture flask (Nunc, USA) in 2 mL DMEM. The cells were incubated until 0.1, 0.5, 1.0, 2.5 kb, and 5.0 kb, corresponded 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, -4 96 to +42 bp, respectively in human α1(I) procollagen gene. The transfection was terminated and ready for reporter gene detection.

To study the regulatory effect of cytokines, cells were starved for 4 h after being transfected with pCOLH2.5 for 24 h, then culture medium was replaced by fresh medium containing TGFβ 10 μg/L (R&D, USA), IFNα 1x10^5 U/L (PBL, England), IFNγ 1x10^5 U/L (Roche, USA) respectively. Another 24 h later, the transfection was terminated and ready for reporter gene detection.

**Determination of CAT and β-galactosidase**

Forty-eight hours after initial transfection, cells were washed with precooled phosphate buffered solution (PBS, 0.1 mol/L, pH 7.4) and lysed with lysis buffer (Roche, USA). Aliquots of cell extracts were made for protein determination as described previously[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 EcoRI 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[18]. 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 pCOLH2.5 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 was -105 to +42 bp.

**Transfection of promoter competitors**

For the cells transfected with pCOLH0.27, 10 μg of consensus recognition DNA for Ap-1, Sp-1 or NF-1 was transfected to
cells 24 h after pCOLH.027 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 indicated 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.

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

| Name of transfected constructions | Putative promoters' length (bp) | Relative activities of reporter gene (CAT, mean\( \pm SD \)) |
|----------------------------------|---------------------------------|----------------------------------------------------------|
| pCOLH.0.1                        | -105 to 42                      | 0.10±0.02                                               |
| pCOLH.0.27                       | -268 to 42                      | 0.97±0.04                                               |
| pCOLH.0.5                        | -496 to 42                      | 0.20±0.05                                               |
| pCOLH.0.9                        | -829 to 42                      | 0.36±0.09                                               |
| pCOLH.1.5                        | -1448 to 42                     | 0.73±0.11                                               |
| pCOLH.2.5                        | -2483 to 42                     | 1.0                                                     |

\( ^1 \)The expression of normalized CAT in the cells transfected with pCOLH.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\( \pm SD \) of three independent experiments).

**Figure 1** Electrophoresis of six constructs digested with MluI and XhoI. M1: 100 bp DNA ladder marker, M2: 1 kb DNA ladder marker, Lane1: pCOLH.0.1, lane2: pCOLH.0.27, lane3: pCOLH.0.5, lane4: pCOLH.0.9, Lane5: pCOLH.1.5, Lane6: pCOLH.2.5. Six recombinant plasmids containing serial 5' - deleted flanking sequences of human \( \alpha 1(I) \) procollagen gene as putative promoter were digested with MluI and XhoI at 37°C 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.

**Figure 2** Effects of consensus DNA on CAT expression in pCOLH.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.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. \( ^2 P<0.05 \) compared to control.

**EMSA**

The fragment spanning -268 to +42 bp in human \( \alpha 1(I) \) procollagen gene was digested into three smaller ones (42 bp, 113 bp, 155 bp) with EcoRII (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 \( \alpha 1(I) \) procollagen gene (Figure 4).

**Figure 3** Electrophoresis (20 g/ L agarose gel) of the fragment -268 to +42 bp digested with EcoRII. Lane1: EcoRII-digested fragment, Lane2: 100 bp DNA ladder, Lane3: 310 bp length of fragment spanning from -268 to +42 bp. The 310 bp fragment spanning -268 to +42 bp of the human \( \alpha 1(I) \) procollagen gene was obtained by PCR with p5.3k\( \alpha 1 \) as template. The fragment was digested with EcoRII. 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, Lane5: Labeled DNA + nuclear protein, Lane6: 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**

TNF\( \alpha \), IFN\( \alpha \) and IFN\( \nu \) inhibited the reporter gene activity by 40-60% in the cells transfected with pCOLH.2.5 (\( P<0.05 \)) compared to the control. The strongest inhibitory effect appeared in TNF\( \alpha \) group (Table 2).
Table 2  Effect of cytokines on reporter gene activity in cells transfected with pCOLH(2,5)

| Cytokines | Relative value of reporter gene activity | P Value |
|-----------|-----------------------------------------|---------|
| Control   | 1.00±0.15                               |         |
| TNFα      | 0.43±0.17                               | <0.05   |
| IFNα      | 0.62±0.15                               | <0.05   |
| IFNβ      | 0.60±0.16                               | <0.05   |

1The reporter gene activity of the cells transfected with pCOLH(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[6,11,19]. 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 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 TGFβ1, TNFα and IFNβ. 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 (DNAAssist1.0) of this 2.5 kb flanking sequence revealed that there might be 5 binding sites for Sp-1 (-123 bp, -615 bp, -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, -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 EcoRI 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.027 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 transcribed 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 Sugiuura 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[30,31]. 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[33]. Ap-1 consists of either Jun homodimers or Fos/Jun heterodimeric complexes which bind the palindromic TRE sequence TGA/C/G/TIGA. 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, 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 transcriptional 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, transcription 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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REFERENCES

1 Friedman SL. Seminars in medicine of the Beth Israel Hospital, Boston. The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. N Engl J Med 1993; 328: 1828-1835

2 Slack J, Lindahl GE, Bornstein P. Regulation of expression of the type I collagen genes. A M J Med 1993; 45: 140-151

3 Rockey DC, Chung JJ. Interferon gamma inhibits lipoycote activation and extracellular matrix mRNA expression during experimental liver injury: implications for treatment of hepatic fibrosis. J Invest Med 1994; 42: 660-670

4 Inagaki Y, Nenoto T, Kushida M, Sheng Y, Higashi K, Ikeda K, Kawada N, Shirasaki F, Takehara K, Sugiyama K, Fuji M, Yamauchi H, Nakao A, de Crombrugghe B, Watanabe T, Okazaki I. Interferon alfa down-regulates collagen gene transcription and suppresses experimental hepatic fibrosis in mice. Hepatology 2003; 38: 890-899

5 Buttrn C, Skupin A, Rieber EP. Transcriptional activation of the type I collagen genes COL1A1 and COL1A2 in fibroblasts by interleukin-4: analysis of the functional collagen promoter sequences. J Cell Physiol 2004; 198: 248-258

6 Papakrivopoulou J, Lindahl GE, Bishop JE, Laurent GJ. Differential roles of extracellular signal-regulated kinase 1/2 and p38 (MAPK) in mechanical load-induced procollagen alpha(1)(I) gene transcription. J Cell Physiol 2004; 199: 509-520

7 Kubota K, Okazaki J, Ooi L, Kent C, Liu B. TGF-beta stimulates collagen I in vascular smooth muscle cells via a short element in the proximal collagen promoter. J Surg Res 2003; 109: 43-50

8 Ratszu V, Lalazar A, Wong L, Dang Q, Collins C, Shaulian E, Jensen S, Friedman SL. 2000; 536: 190-1210, a Kupffer-like transcription factor up-regulated in vivo during early hepatic fibrosis. Proc Nat Acad Sci U S A 1998; 95: 9500-9505

9 Chen SJ, Artlett CM, Jimenez SA, Vargha. Modulation of human alpha(I) procollagen gene activity by interaction with Sp1 and Sp3 transcription factors in vitro. Gene 1998; 215: 101-110

10 Rippe RA, Schrum LW, Stefanovic B, Solis-Herruzo JA, Brenner DA. NF-kappaB inhibits expression of the alpha2(I) collagen gene. DNA Cell Biol 1999; 18: 751-761

11 Bergeron C, Page N, Joubert P, Barbeau B, Hamid Q, Chakir J. Regulation of procollagen I (alpha1) by interleukin-4 in human bronchial fibroblasts: a possible role in airway remodelling in asthma. Clin Exp Allergy 2003; 33: 1389-1397

12 Gao CF, Wang H, Huang C, Kong XT. Study of activity of promoter from mouse α2(I) procollagen gene. Clin Med 1993; 119: 316-320

13 Gao CF, Wang H, Wu Y, Kong XT. The effect of cytokines on promoter activity in mouse α2(I) procollagen gene. J Med Cell Biol 1999; 14: 12-16

14 Jimenez SA, Vargha J, Olsen A, Li L, Diz A, Herhal J, Koch J. Functional analysis of human alpha 1(I) procollagen gene promoter. Differential activity in collagen-producing and -nonproducing cells and response to transforming growth factor beta 1. J Biol Chem 1994; 269: 12684-12693

15 Bradford M. A rapid and sensitive method for the quantitation of microgram amounts of protein utilizing the principle of protein-