대한피부연구학회지. 제 6 권, 제 1 호
Korean Journal of Investigative Dermatology
1999; Vol. 6, No. 1, pp. 9 - 16

Transcriptional Regulation of Type I Collagen Genes: Role of Transforming Growth Factor-β and Tumor Necrosis Factor-α

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In mammals, collagen accounts for about 30% of animal proteins and thus is the single most abundant protein[1]. In human dermis, the proportion is even higher, constituting about 70% of the dry weight and it functions as the main structural element[2]. The fluctuation and the resultant balance between production and degradation of collagen plays an essential role in maintaining the homeostasis of every tissue. For example, overproduction of type I collagen results in scleroderma or pulmonary fibrosis, and defect in its structure is manifested by osteogenesis imperfecta. Therefore, clarifying the mechanisms of how production and degradation of type I collagen is regulated is the basis for understanding and overcoming these disease processes which are still puzzling to mankind.

Among the 19 types of collagen discovered so far[3], type I collagen accounts for 80% of the total collagen in adult skin and the abundance in volume implies its importance in maintaining the integrity of the skin with diverse biologic functions such as the promotion of cell migration, differentiation, and tissue morphogenesis during development[4]. Type I collagen is a product of two genes, COL1A1 and COL1A2, which are located on different chromosomes but are coordinately regulated[5]. The regulatory mechanisms of the production of corresponding proteins, α1(I) and α2(I) procollagens, respectively, are not fully understood but the variations in collagen production in growth and differentiation[6-8], cytokine and growth factor modulation[9-11], and various fibrotic conditions[12-16] have been attributed to fluctuations in the steady-state collagen mRNA levels. The fluctuations can be attributed mainly to the regulation at the transcriptional level, although, to some degree, translational control and changes in mRNA processing and stability can be held responsible[17]. Generally, the tissue-specific transcription of gene expression in metazoans is regulated by a complex interaction between cis-regulatory DNA elements and sequence-specific trans-acting factors[17,18] and cytokines in various pathologic conditions are known to influence the production of diverse *trans*-acting factors. Type I collagen expression is stimulated by various agonists, including ascorbic acid, acetaldehyde, lipid peroxidation products, toxic oil, and transforming growth factor-β (TGF-β)[4] while it is inhibited by tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ)[19,20].

Of the diverse array of cytokines involved in development, pathologic processes, and tissue repair, transforming growth factor-β (TGF-β) has been noted to be the key factor with its potent ability to stimulate fibroblast proliferation and collagen production, as well as to inhibit collagenase synthesis[21-23]. TGF-β is produced and secreted mainly by platelets and inflammatory cells and is the most

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potent stimulant of type I collagen production. *In vitro* studies with scleroderma fibroblasts have shown that the stimulation of collagen production is dependent on TGF-β[24] and activated fibroblasts in idiopathic pulmonary fibrosis produce TGF-β along with abnormally elevated levels of collagen[25]. On the other hand, tumor necrosis factor-α, released by activated macrophages, is known to possess functions opposite to that of TGF-β[26], causing suppression of collagen production while stimulating synthesis of metalloproteinases[19,27-30]. Therefore, TGF-β and TNF-α are invaluable tools to study the factors and their mechanisms regulating the production of type I collagen at a transcriptional level. Early in vitro studies with cultured fibroblasts have shown that TGF-β stimulates the synthesis of type I collagen at the transcriptional level[22,31]. However, TNF-α -induced suppression of type I collagen production is a relatively late effect that requires protein synthesis[19] and the mechanisms underlying these antagonistic effects are not yet known. It is the aim of this review to summarize what is known about the regulatory factors that affect the homeostasis of type I collagen genes and to clarify the effects of TGF-β and TNF-α at the transcriptional level.

COL1A1

Regulation of the human α1 (I) procollagen gene is known to be modulated by the elements located in the promoter and 5′ flanking region, and the introns. Both positive and negative elements are known to reside in the 5′ flanking region and the introns, and the interactions between the regulatory elements may determine the tissue-specific activity of the COL1A1 promoter[4]. However, layout of the sequence motifs in the promoter and intronic segments of the COL1A1 is poorly defined and the importance of the individual cis-acting sequences in transcriptional regulation is somewhat controversial. The presence of negative regulatory elements has been shown by Boast et al[32], who removed upstream sequences from -2500 to -331 from a COL1A1 promoter /chloramphenicol acetyltransferase (CAT) construct containing the sequences that span from nucleotides -2500 to +123 (pWS-2.5/CAT) which resulted in the increase of CAT activity. Jimenez et al[17] have used 5.3 kilobase pairs (kb) of the human COL1A1 promoter/CAT reporter gene, and their deletion constructs, to transiently transfect NIH/3T3 (collagen-producing) cells and HeLa (collagen-nonproducing) cells. It was concluded that there are both positive and negative *cis*-acting regulatory elements in the corresponding promoter and that these regions function differently in collagen-producing and nonproducing cells. Study with the deletion constructs also showed that TGF-β1 responsive sequences were located between -174 and -84 bp from the transcription initiation site. The TGF-β1 responsive element was narrowed down by electrophoretic mobility shift assays (EMSA) to a region encompassing -164 and -142 bp of the promoter which contained an Sp1-like binding site that interacts with a nuclear factor markedly stimulated by TGF-β1.

Although the existence of an enhancer-like positive regulatory element of 782 bp (+820 to +1602) in the first intron of the human COL1A1 was first suggested by Rossouw et al[33], subsequent investigations[32,34,35] failed to confirm the observation. Moreover, it was found in recent studies that the role of the intronic sequences in the regulation of COL1A1 transcription is more complicated than was initially perceived, and the exact role of the intronic sequences in the expression of the human COL1A1 promoter has been made even more obscure. Olsen et al[36] showed that almost complete deletion of the first intron from a mini gene construct did not inhibit COL1A1 transcription in stably transfected NIH/3T3 cells. In contrast, Stack et al[37] introduced intron-sufficient or intron-less collagen promoter-reporter CAT constructs into transgenic mice and comparative expression of the reporter gene showed that the first intron contributed to the tissue-specific expression of the COL1A1 promoter. However, deletion of a 360 bp fragment (+494 to +854) from a COL1A1/CAT construct severely diminished its transcriptional activity[38,39], while deleting or mutating the putative AP-1 binding element within the sequence either decreased of increased the transcriptional expression of a COL1A1 promoter/CAT construct[40]. It was shown from this experiment that the AP-1 binding site in the first intron of the COL1A1 can act as a positive or negative element depending on the target cell. Another study done with human liver Ito cells has clearly shown that the AP-1 binding site, which spans from +598 to +604 relative to the transcription starting site, is the

main element responsible for TGF-β responsiveness of the COL1A1[41]. As AP-1 expression is influenced by TGF-β and since AP-1 is an essential factor in the transcriptional regulation of COL1A2 in response to TGF-β stimulation[42], it is conceivable that this AP-1 binding sequence might be one of the major elements which govern the response of the COL1A1 to TGF-β.

COL1A2

It has been demonstrated that the 3.5kb upstream sequence of the human α2(I) procollagen gene, linked to the CAT reporter gene, contains all elements necessary for high, tissue-specific transcription[32]. Further deletion of the 3.5kb sequence and reporter gene assay of their CAT constructs showed that direct high and tissue-specific transcription of the human COL1A2 promoter is mediated by sequences that lie between nucleotides -376 and -108 and it also suggested a presence of negative *trans*-acting factors between nucleotides -772 and -376. Similar deletion assay[43] denoted a GC-rich region located between nucleotides -303 and -271 as the constitutive responsive element in human fibroblasts. The region contains three binding sites for the transcriptional factor Sp1 and their mutation resulted in a 90% reduction in basal transcriptional activity of the promoter. This Sp1-binding site is located in a larger segment of the COL1A2 promoter which has also been shown to confer both TGF-β[44]and TNF-α[45] responsiveness. It was shown in these studies that TGF-β stimulates transcription of the COL1A2 promoter by increasing the affinity of an Sp1-containing protein complex for its corresponding DNA-binding site but they failed to precisely characterize the TGF-β or TNF-α response element (s) within the COL1A2 promoter. It was concluded that a 105 bp region of the promoter within -330 bp of the transcription start site could confer responsiveness to both TGF-β[44] and TNF-α[45]. This rather large promoter segment was shown to contain two smaller regions referred to as Box 3A, including nucleotides -313 to -286, and Box B, from nucleotides -271 to -255, relative to the transcription initiation site (Fig. 1). The upstream element, box A, was shown to bind Sp1 and to confer high basal activity to the promoter[43,44]. Box B harbors both a putative AP-1-binding element (CGAGTCA) and a non-canonical NF-κB-binding site (AGAGTTTCCC). Although no specific transcription factor could be identified by Inagaki et al.[45], they postulated that unknown factor (s) interacting with both Box B and the upstream Sp1-binding sites present in Box A to -313 were necessary for promoter response to either TGF-β or TNF-α. Also, using antibody interference assays, the authors were unable to demonstrate the involvement of either NF-κB (p50/p65) or AP-1 in the transcriptional response to TNF-α.

To further characterize the TGF-β and TNF-α response elements within the human COL1A2 promoter, we have developed a repertoire of 5′ deletion constructs and generated site-directed mutagenesis of specific sequences characterized as essential for growth factor response[42]. This approach allowed us to exclude Sp1-binding sites, which are included in Box 3A, as being necessary for TGF-β response and to characterize a short fragment of the COL1A2 promoter, spanning from nucleotides -265 to -241, as an AP-1-binding site (Fig. 2). Using site-directed mutagenesis, we have demonstrated that this region plays a regulatory role in the basal activity of the promoter and is fundamental for TGF-β response of the COL1A2 (Fig. 3). Furthermore, our data suggested that this AP-1 site may be sufficient to mediate TNF-α inhibitory effect on TGF-β driven up-regulation of COL1A2 gene expression. This is in contrast

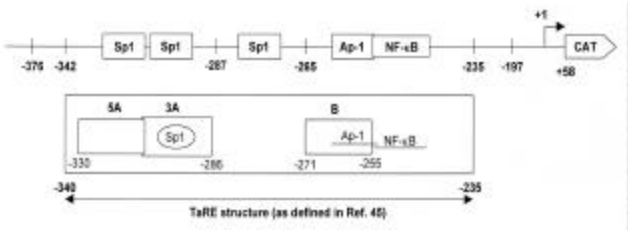

Fig. 1. Schematic representation of the promoter of the COL1A2. Shown above in boxes are the Sp1, AP-1, and NF-κB-binding sites in the order in which they are located. Below is the structure of the TaRE as defined by previous investigators. Box A was shown to bind Sp1 and to confer high basal activity to the promoter and box B harbors both a putative AP-1-binding element (CGAGTCA) and a non-canonical NF-κB-binding site (AGAGTTCC). It was postulated that unknown factor(s) interacting with both Box B and the upstream Sp1-binding sites present in Box A were necessary for promoter response to either TGF-β or TNF-α.

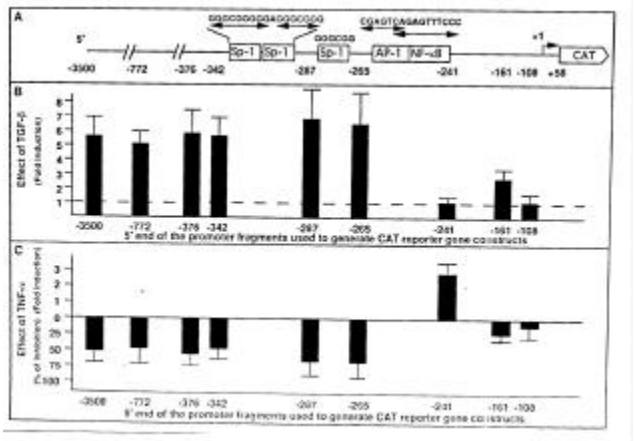

Fig. 2. Effects of TGF-β and TNF-α on the activity of 5′ deletions of the COL1A2 promoter in transient cell transfection experiments. TGF-β responsiveness of the COL1A2 is maintained even though all three of the Sp1- binding sites are removed but it showed dramatic decrease after removing the AP-1-binding site. This deletion study clearly proves that the Sp1-binding sites located between nucleotides -330 and -265, although important for basal expression of the promoter, are not required for TGF-β response (B). On the other hand, TNF-α is shown to suppress the transcriptional activity of the COL1A2 but looses its inhibitory effect as soon as the NF-κB-binding site is removed(C).

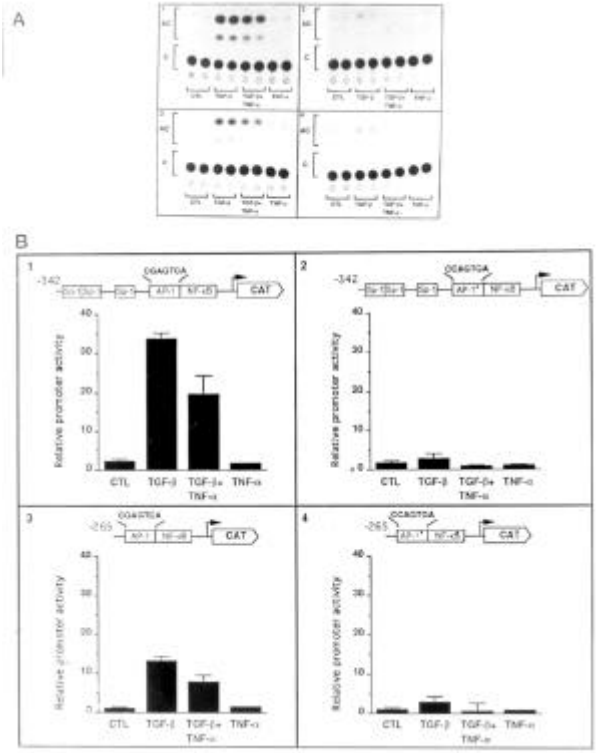

Fig. 3. Effect of a mutation in the AP-1-binding site on COL1A2 promoter activity and its regulation by growth factors. Site-directed mutagenesis was employed to modify the AP-1 site from its original sequence 5′-CGAGTCA-3′ to 5′-CCAGTGA-3′ in two constructs, the -342COL1A2/CAT and the -265COL1A2/CAT. Mutation of the AP-1 sites in both constructs led to a dramatic drop, ~50%, of basal activity as compared with their unmutated counterparts and resulted in almost complete of loss of TGF-β responsiveness (A and B, panels 1 and 3 versus panels 2 and 4, respectively).

with the results from studies done on rat and mouse α2 (I) collagen genes in which nuclear factor 1 (NF1) binding is responsible for the transcriptional activation of the promoter by TGF-β[46].

Previous studies suggested TNF-α-mediated suppression of COL1A2 transcription has been shown to be mediated by response elements residing in a region between nucleotides -378 and -235 relative to the transcriptional start site, as has been described above[45] (Fig. 1). The authors identified two protected regions (Box B, Box 3A) within this 134 bp element which, when placed in the context of a heterologous promoter, exhibited responsiveness to TNF-α but were unable to specifically localize the discrete sequences in question. However, we have shown that the region downstream of nucleotide -271, which contains an essential TGF-β upregulatory response element, was sufficient to allow TNF-α inhibitory response on the COL1A2 promoter[42] (Fig. 4) The un-canonical NF-κB binding site residing in the region has been proven to be the TaRE binding site (Fig. 5) and it has also been found that the regions of COL1A2 promoter upstream of nucleotide -271 are not required for TNF-α-mediated transcriptional repression[47]. It was concluded in this study that the solitary NF-κB- binding site rapidly binds NF-κB heterodimer in response to TNF-α challenge, which then results in a pronounced down-egulation of COL1A2 transcription in dermal fibroblasts.

Although IFN-γ inhibits type I collagen gene expression

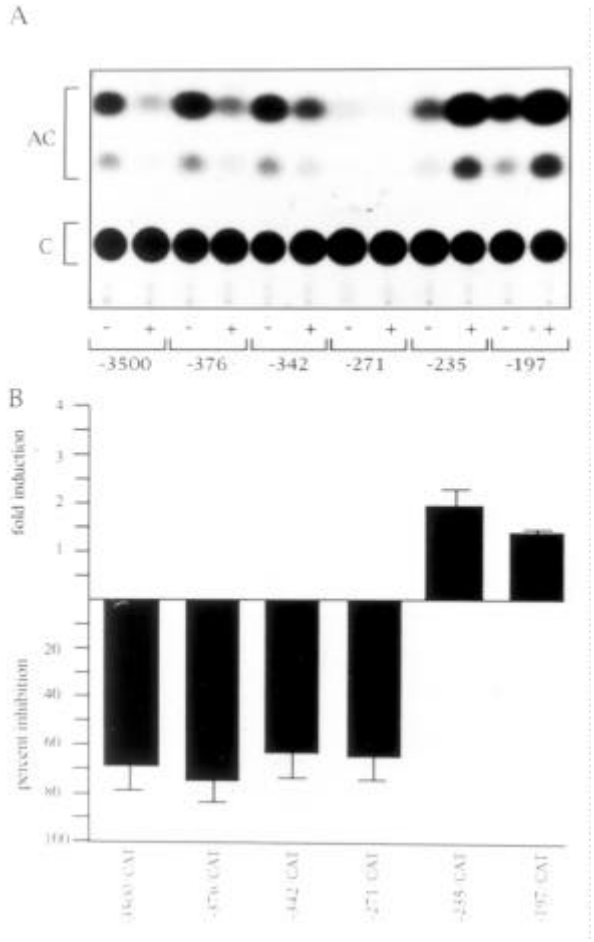

Fig. 4. Effects of TNF-α on the activity of 5′ deletions of the COL1A2 promoter in transient cell transfection experiments. Upstream deletion constructs between -3500 and -271 all had similar inhibitory response to TNF-α challenge (~50% to 75% inhibition) but the loss of elements between -271 and -235 entirely abolished the inhibitory response and even a slight reversal of the effect of TNF-α was noted.

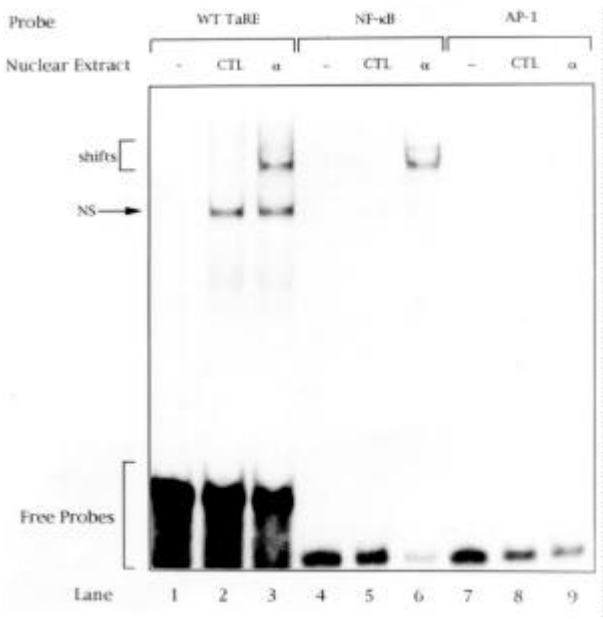

Fig. 5. Binding of nuclear proteins to the TaRE mimics NF-κB, not AP-1. TNF-α dramatically enhanced the binding of nuclear proteins to both TaRE and NF-κB probes. The relative migration rate of the shifted TaRE complexes (Lane 3) was similar to that of NF-κB complexes (Lane 6). No differential complex formation was observed with the consensus AP-1 probe. This lack of enhancement of AP-1 binding is likely to be due to the fact that AP-1 activation by TNF-α, and subsequent DNA binding activity, are transient phenomena that occur within a few hours after stimulation of the cells and can no longer be observed at the 24 h time point studied.

through both transcriptional and post-transcriptional mechanisms[20,48], the precise mechanism by which it induces such an effect has not been known. Recently, Higashi et al[49] showed that the IFN-γ-response element (IgRE) of the COL1A2 is located between nucleotides -161 and -125 relative to the transcription start site. The IgRE is clearly distinct from the previously described NF-κB binding site, which acts as the TaRE, and this could explain the additive inhibitory effect of these two cytokines[20].

In summary, recent studies have provided new insights into the activation or inhibition of type I collagen genes by various cytokines, but further clarification of the precise elements which are responsible for the regulation is desired. A better knowledge of the process will enhance our understanding of the connective tissue metabolism in physiologic as well as pathologic conditions and thus will lead us to the conquest of the debilatating and, at times, life-threatening diseases which result in sclerosis of the human body.

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