Transcriptional Activation of Type I Collagen Gene during Hepatic Fibrogenesis

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Abstract: Increased production of type I collagen is a common hallmark of fibrotic diseases in various organs including the liver. This increase is exerted mainly by transcriptional upregulation of the genes coding for the a1 and a2 chains of type I collagen. We have shown that the -378 to +58 upstream sequence of a2 (I) collagen gene (COL1A2) is essential for basal transcription in skin fibroblasts and hepatic stellate cells. In addition, the -313 to -183 region is responsible for stimulation of the gene expression by TGF-β. We therefore designated this region the TGF-β-responsive element, and revealed that a ubiquitous trans-activator Sp1 and unknown nuclear factor(s) bind to this region to mediate the stimulatory effect of TGF-β. Experiments using transgenic mice harboring the COL1A2 upstream sequence linked to a β-galactosidase gene have revealed that the same -313 COL1A2 promoter is activated in a cell type-specific manner during hepatic fibrogenesis in vivo.

Key words: collagen, transcription, hepatic fibrosis, hepatic stellate cells, TGF-β

INTRODUCTION

Collagens represent a family of proteins involved not only in the maintenance of organ architecture and tissue integrity but also in developmental program and tissue repair process. There is a dynamic balance between production and degradation of collagen. Alteration of this equilibrium results in either excessive collagen deposition or inadequate tissue integrity. Irrespective of the initial stimuli, fibrosis in different organs is caused commonly by a chronic and uncontrolled inflammatory/repair process leading to excessive deposition of collagen and other components of extracellular matrix. The liver is one of those organs undergoing progressive fibrosis as a result of chronic repeating inflammation.

Various cytokines and growth factors including TGF-β and TNF-α play important roles to orchestrate tissue remodeling in both normal and pathologic conditions. In general, they first bind to the specific receptors on cell surface, and their signals are transmitted to the cytoplasm and then to the nucleus. Eventually they modify the interaction between cis-acting DNA elements and trans-acting nuclear factors, which in turn stimulate or repress the gene transcription (Fig. 1). It is therefore important to identify the cis-acting element and trans-acting nuclear factors involved in collagen gene expression for the better understanding of fibrogenic process in the liver.

This review gives a brief summary of our work aiming at clarification of molecular pathogenesis underlying hepatic fibrogenesis. We first identified the cis-acting DNA elements and trans-acting nuclear factors essential for basal and TGF-β-stimulated transcription of a2(I) collagen gene (COL1A2) in skin fibroblasts. Then we used hepatic stellate cells, the major source of collagen in the liver, and revealed that the same transcriptional mechanisms are utilized for COL1A2 transcription in skin fibroblasts and hepatic stellate cells. Finally, experiments using transgenic mice harboring the COL1A2 upstream sequence indicated that the same promoter region is activated during hepatic fibrogenesis in vivo.

COL1A2 Transcription IN Skin Fibroblasts

Type I collagen, the major component of extracellular matrix in fibrotic tissues, is a heterotrimer composed of coordinately expressed two a1 chains and one a2 chain. Our studies using fetal skin fibroblasts have mapped the upstream sequence essential for COL1A2 transcription in skin fibroblasts and hepatic stellate cells. Furthermore, experiments using transgenic mice harboring the COL1A2 upstream sequence indicated that the same promoter region is activated during hepatic fibrogenesis in vivo.

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Fig. 1. Schematic representation of the intracellular signaling pathway through which a certain gene transcription is modulated by cytokines and growth factors. +1, transcription start site.

necessary for the high level of COL1A2 transcription. DNase I footprinting analysis identified at least two sites of DNA-protein interaction within this segment, Box A and Box B.

Gel mobility shift assays further divided Box A into two overlapping subregions, Box 5A and Box 3A (Fig. 2). Box 5A was found to be the binding site of an unknown repressor, whereas a ubiquitous transcription factor Sp1 binds to the 3A region and interacts with Box B-bound unknown factor(s). Both 3A and B regions are necessary to mediate the stimulatory effects of TGF-β on COL1A2 transcription. We therefore designated the 3A+B region the TGF-β responsive element (TbRE).

TGF-β treatment of skin fibroblasts increased the binding of both Sp1 and Box B-bound unknown factors when using the 3A+B fragment as a gel-shift probe. However, when using the 3A oligonucleotide or Sp1 consensus sequence as a probe, there was no increase in Sp1 binding following TGF-β treatment. It is therefore suggested that TGF-β stimulates COL1A2 transcription not by increasing the amount of Sp1 but by modifying the interaction between Sp1 and the B-bound factors, which in turn increases the binding affinity of Sp1 to the 3A region (Fig. 2).

Fig. 2. Schematic representation of cis-acting DNA elements and trans-acting nuclear factors modulating COL1A2 transcription in skin fibroblasts and its regulation by TGF-β and TNF-α. R, an unknown repressor bound to the 5A region.

It has been also revealed that counter-repression of COL1A2 transcription by TNF-α is mediated through the same TbRE as well as by increasing the amount of a repressor protein bound to the immediately upstream 5A region (Fig. 2). Thus, the convergence of the TGF-β and TNF-α signals on the same COL1A2 upstream sequence represents an example of combinatorial gene regulation achieved through composite responsive elements.

There has been a controversy as to what binds to the COL1A2 upstream sequence to mediate the stimulatory effect of TGF-β. First, NF1 binding to the upstream sequence of the mouse α2(I) collagen gene corresponding to Box A was implicated in TGF-β-elicited stimulation of gene transcription. Our study using the human gene indicated that Sp1 but not NF1 binds to the region and mediates TGF-β action. More recently, it was proposed that an API binding site present at the 3′ boundary of Box B is important for TGF-β stimulation of COL1A2 transcription. Although they implicated a switch from c-Jun to Jun B bound to the API site in mediating TGF-β action, it was subsequently refuted by our study using an API inhibitor and antibodies against c-Jun and Jun B.

COL1A2 Transcription in Hepatic Stellate Cells

Hepatic stellate cells, also known as fat-storing cells and Ito cells, are sinusoidal cells localized within the space of Disse, and are considered to be the main producers of type I collagen in fibrotic liver. During development of hepatic fibrosis, they undergo an activation process which is characterized by a decrease in vitamin A-containing fat droplets, increased expression of α-smooth muscle actin and enhanced production of type I collagen. These activated stellate cells are named myofibroblasts. Freshly isolated stellate cells have a lot of fat droplets containing vitamin A and produce little, if any, type I collagen. When cultured, they gradually loose the fat droplets and produce type I collagen while acquiring the phenotype resembling myofibroblasts. Such a phenotypic change of cultured stellate cells is thus considered to be a good model for studying the pathogenesis underlying the process of hepatic fibrosis. However, the molecular events responsible for pathologic activation of collagen gene expression in stellate cells during hepatic fibrogenesis have not been fully understood.

Based on the results with skin fibroblasts, we next examined COL1A2 transcription in cultured stellate cells. We used two distinct stellate cell clones, CFSC-2G and CFSC-5H, derived from a single cirrhotic liver induced by carbon tetrachloride injection. They have different phenotypes: CFSC-2G resembles the freshly isolated stellate cells and thus expresses little TGF-β and collagen mRNAs, whereas CFSC-5H mimics the activated myofibroblasts and therefore contains larger amounts of both TGF-β and collagen mRNAs.

Cell transfection experiments indicated that the upstream sequence between nucleotide -378 and -183...
is essential for COL1A2 transcription in both stellate cell clones as well as in skin fibroblasts. It was also shown that the 5A region acts as a negatively cis-acting element, as deletion of the region resulted in an increase in transcriptional activity. In contrast, substitution of the Sp1-bound 3A region with an unrelated polylinker sequence reduced COL1A2 expression in two different cell types of mesenchymal origin. Contribution of Sp1 has been also implicated by others in activation of the coordinately expressed α1(I) collagen promoter in cultured stellate cells.

When incubated with TGF-β, there was an increase in COL1A2 transcription in CFSC-2G, the lower expressor of TGF-β and collagen (Fig. 3). In contrast, the other clone, CFSC-5H, containing larger amounts of both TGF-β and collagen mRNAs was not stimulated any more by TGF-β treatment (Fig. 3). Nuclear proteins prepared from CFSC-2G and CFSC-5H clones bind to the TbRE more strongly than those from skin fibroblasts.

We interpreted these findings as suggesting that collagen production in CFSC-5H has been already activated by the autocrine stimulation of TGF-β, whereas CFSC-2G is only partially activated, but can be easily recruited to produce more collagen once stimulated by exogenous TGF-β. We propose that stellate cell activation is probably a multi-step process that involves autocrine and paracrine stimulation of collagen gene expression by TGF-β (Fig. 4).

Activation of COL1A2 Promoter during Hepatic Fibrogenesis in vivo

The results of cell transfection and DNA binding assays using cultured stellate cells may not be fully applicable to hepatic fibrogenesis in vivo. Because of the obvious limitations of these in vitro experiments, we sought further confirmation in transgenic mice harboring COL1A2 upstream sequences containing the TbRE linked to either a luciferase or β-galactosidase reporter gene.

Transgenic mouse lines were generated using the mouse or human α2(I) collagen promoter-reporter gene constructs shown in Fig. 5. One of the two constructs (pGB17) contains the −17 kb to +54 sequence of the mouse α2(I) collagen promoter linked to a firefly luciferase gene. Strong enhancer activity has been found between −13.5 kb and −17 kb of the mouse promoter sequence. The other construct (pSY313) contains the −313 to +58 upstream sequence of the human proα2(I) collagen promoter linked to a bacterial β-galactosidase gene (LacZ). Acute and chronic liver injury was introduced by injecting once a week 0.1 mL/kg body weight of carbon tetrachloride intraperitoneally.

A single or repeated intraperitoneal CCl₄ adminis-
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Fig. 6. A histogram showing quantification of the \( \alpha 2(1) \) collagen and \( \beta \)-galactosidase mRNA levels in hepatocytes (HEP) and non-parenchymal cells (NP) freshly obtained from untreated or CCl\(_4\)-treated (CCl\(_4\)) mouse liver.

The values are standardized for the GAPD mRNA levels, and are expressed relative to those in non-parenchymal cells from untreated animal. The asterisk means that the value is significantly higher than that in cells from untreated animals.

tration increased the \(-17\) kb COL1A2 promoter activity more than 10-fold\(^{10}\). The activity went down in parallel with recovery from acute liver injury following a single CCl\(_4\) injection. In contrast, high level of luciferase enzyme activity was constantly observed in chronically CCl\(_4\)-treated mouse liver\(^{10}\). It is therefore suggested that continuous activation of both \( \alpha 1(1) \) and \( \alpha 2(1) \) collagen promoters plays a critical role in the development of hepatic fibrosis.

We next examined activation of the \(-313\) COL1A2 promoter, which had been implicated in in vitro COL1A2 stimulation, in liver tissues following CCl\(_4\) administration. X-Gal staining of liver sections indicated that the promoter seemed to be activated mainly in the necrotic areas and around fibrous septa. However, the LacZ expression driven by the \(-313\) COL1A2 promoter was rather weak, and it was difficult to clearly indicate cellular localization. Thus, the identity of the LacZ-expressing cells was confirmed by isolating parenchymal and non-parenchymal cell fractions and by culturing stellate cells. The results indicated that the \(-313\) COL1A2 promoter was utilized only in non-parenchymal cells, and that the activity was significantly higher in the cells from CCl\(_4\)-treated mice than in those from untreated animals (Fig. 6). Interestingly, TGF-\( \beta \) treatment increased the promoter activity and the amount of endogenous COL1A2 mRNA in stellate cells from untreated, but not CCl\(_4\)-treated mice\(^{10}\). From these findings, we conclude that the \(-313\) COL1A2 promoter is activated in a cell type-specific manner to stimulate gene expression during hepatic fibrogenesis in vivo.

CONCLUSION

Altogether, the data indicated that the upstream sequence of COL1A2 promoter containing the TGF-\( \beta \)-responsive element is responsible for stellate cell activation in vitro and is activated in a cell type-specific manner during hepatic fibrogenesis in vivo. Experiments in progress are characterization of the cell type-specific molecular mechanisms controlling collagen gene transcription as well as identification of the nature of Box B-bound factors and their interaction with Sp1. They will help our better understanding of the molecular pathogenesis underlying hepatic fibrogenesis and may eventually contribute to the development of therapeutic means that prevent hepatic fibrosis by suppressing pathologic collagen gene activation in stellate cells.

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