Pro-inflammatory cytokine-mediated transcription of type VII collagen gene (COL7A1) in epidermal keratinocytes and dermal fibroblasts

OAtsushi Kon, Noniko Ito, Yuka Kudo, Fengchao Chen, Keiichi Takagaki
Department of Biochemistry, Hirosaki University School of Medicine

Recent studies have demonstrated that diminishment of anchoring fibrils (AF), which stabilize the attachment of the basement membrane zone to the dermis, are involved in the pathophysiology such as wound healing and skin aging. Expression of COL7A1, encoding type VII collagen that is a major component of AF, is also decreased in wounded and aged skin. In this study, we have investigated COL7A1 transcription by pro-inflammatory cytokines, TNF-α and IL-1β, which relate to wound healing and skin aging. Nuclear run-on assay, luciferase assay and gel shift mobility assay revealed that both TNF-α and IL-1β tissue-specifically downregulated COL7A1 transcription in epidermal keratinocytes, whereas COL7A1 transcription was upregulated by each pro-inflammatory cytokine in dermal fibroblasts. The responsive elements of both TNF-α and IL-1β in epidermal keratinocytes were located between nucleotide -144 and +92 of COL7A1 promoter. On the other hand, TNF-α and IL-1β upregulated COL7A1 transcription in dermal fibroblasts through the induction of transcriptional factors, NF-κB and AP-1, which bind to nucleotide -252/-230, and -422/-396, respectively. Specifically, AP-1 family members, Jun (JunB, JunD) and Fos (FosB, Fra1, Fra2), are crucial for IL-1β-mediated upregulation of COL7A1 transcription. Taken together, these data suggest that the decrease of COL7A1 transcription in epidermal keratinocytes, main cells expressing COL7A1 in the skin, and subsequent diminishment of AF formation result in pathophysiology of wound healing and skin aging characterized by scar and wrinkle formation.

Treatment strategy for organ fibrosis by cell type-specific intervention of TGF-β/Smad signaling

OYutaka Inagaki1, Miwa Kushida1, Jobhu Itoh1, Reieichi Higashiyama2, Yun Yu Hong2, Tetsu Watanabe3, Isao Okazaki4, Kiyoshi Higashi5, Kazuo Ikeda6
Tokai University School of Medicine1, Sumitomo Chemical Co., Ltd2, Osaka City University Graduate School of Medicine3

Background & Aims: Irrespective of the initial stimuli, excessive deposition of extracellular matrix (ECM) is a common hallmark of fibrotic diseases in various organs. Expression of type I collagen, the major ECM component in the fibrotic tissue, is strictly controlled by a number of growth factors and cytokines, of which TGF-β is the most potent factor in stimulating gene transcription. Recently, a family of proteins, termed Smad, has been identified as intracellular mediators of the signal transduction pathways of the TGF-β superfamily members. Thus, TGF-β and Smad proteins could be the targets for preventing and treating organ fibrosis. However, intervention of TGF-β/Smad signaling affects physiological signal transduction as well, and may cause serious adverse effects upon clinical application. Here we have attempted to suppress hepatic fibrosis by expressing a TGF-β/Smad antagonist selectively in collagen-producing cells only in the fibrotic liver.

Materials & Methods: Recombinant adenoviral vectors were constructed on the basis of Cre/loxP system. One of them expresses Cre recombinase under the control of the -17.0 to -15.5 kb tissue-specific enhancer of the a 2(I) collagen gene (COL1A2). A potent CAG expression unit was used to drive Cre in a positive control vector. Other vectors encode either green fluorescence protein (GFP) or a TGF-β/Smad signal repressor, YB-1. They are expressed after the 10xP-f1anked stuffer sequence was excised by Cre recombinase. Adenoviruses were injected intravenously to the mice untreated or treated with carbon tetrachloride (CCL4). GFP expression was analyzed under a confocal laser-scanning microscopy. Inhibitory effects of YB-1 overexpression on the progression of hepatic fibrosis were estimated by luciferase assays and histological examination using collagen promoter/luciferase transgenic reporter mice.

Results: When using the CAG expression unit as a control, GFP was strongly expressed in a large number of hepatocytes in both normal and CCI4-treated liver. In contrast, GFP expression under the control of the tissue-specific COL1A2 enhancer was detected in activated hepatic stellate cells in CCl4-induced fibrotic liver, but not in untreated normal liver. There was no GFP fluorescence observed in any other organs including skin, lung, kidney and spleen, when using the COL1A2 enhancer. Adenovirus-mediated YB-1 expression under the control of the COL1A2 enhancer significantly decreased COL1A2 promoter activity following CCl4 injection and subsequently suppressed the progression of hepatic fibrosis.

Conclusion: By using the tissue-specific COL1A2 enhancer, we have succeeded to interfere with the TGF-β/Smad signal in a cell type-specific manner and suppress the progression of experimental hepatic fibrosis. These results may also provide novel insights for organ fibrosis to achieve cell type-specific gene expression only in the fibrotic tissue with little damage to other organs.

TGF-β/Smadシグナル伝達からみた線維線維症の治療戦略

O稲垣 一豊1、柳田美和1、伊東丈夫2、東山礼一3、洪 雲玉2、渡辺 哲3、岡崎 賢2、東 清史1、池田一雄1
東海大学医学部1、住友化学工業環境科学研究所3、大阪市立大学大学院4

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