· Original Article ·

The expression of TGF-β1-Smads pathway in mice liver fibrosis induced by *Schistosoma japonicum*

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【**Abstract**】 **Objective** To investigate the transcription level expression of TGF-β1, its two transmembrane receptors TGF-β receptor I (TβRI) and TGF-β receptor II (TβRⅡ) and Smad2, Smad3, Smad4 and Smad7 during the development of liver fibrosis in the BALB/c mice infected with *Schistosoma japoncum*. **Methods** Fifty BALB/c mice infected with cercariae of *Schistosoma japonicum* (20 ± 1) were used as the liver fibrosis model and 10 uninfected mice belonged to the normal control group. Liver specimens were got at the 8th, 12th, 16th and 24th week post infection respectively and the normal controls were sacrificed at the same period mentioned above. Some liver tissues were frozen in the fluid nitrogen immediately and then conserved in the -80 ℃ refrigerator for reverse transcription polymerase chain reaction (RT-PCR) to detect the mRNA level of TGF-β1, TβRI, TβRⅡ, Smad2, Smad3, Smad4 and Smad7. The final value was expressed by the ratio of the scan density of the amplified fragment with the internal control of β-actin, which is a relative value. Other liver pieces were fixed in 10% buffered formalin for histology assay to detect the size of egg granuloma measured by the product of maximum width and the maximum length and expressed in square micrometers and the final results were the average of the five readings randomly under the microscope and thus to determine the liver fibrosis degree, by the criteria: grade Ⅰ: 2^0=1 no significant collagen was observed in liver tissue; grade Ⅱ: 2^1=2 collagen distributed around and in the granuloma; grade Ⅲ: 2^2=4 more collagen appeared in the portal tracts while few collagen among liver lobules; grade Ⅳ: 2^3=8 fibrous tissues penetrated into liver lobules. **Results** Collagen fibers appeared around egg granulomas after 8 weeks of *Schistosoma japoncum* infection and increased gradually. At the 16th week after infection, fibrous tissue distributed evidently in liver lobules and the score of liver fibrosis was 4.27 ± 1.03 and fibrosis degree peaked at the 24th week when scores amounted to 6.90 ± 1.57, while the score of normal mice liver fibrosis was 1. The normal level of TGF-β1 mRNA in liver was 0.30 ± 0.18. It reached the peak 0.87 ± 0.76 at the 8th week, and then decreased, elevated again (1.34 ± 0.52) at the 24th week. TβRⅡ mRNA detection demonstrated that reduction (0.60 ± 0.30) at the 8th week, elevation to the normal level 0.92 ± 0.21 at the 12th week, reduction again (0.76 ± 0.16) at 16th week, and increase again (1.16 ± 0.73), at the 24th week compared with the normal level of mRNA of TβRⅡ (1.16 ± 0.25). After infection, Smad2 mRNA diminished to 0.41 ± 0.23 and 0.50 ± 0.16 at the 12th and 24th week post-infection, respectively, compared with the normal mRNA level of Smad2 (0.85 ± 0.10) while levels of Smad3 mRNA elevated (0.62 ± 0.09) at the 16th week and remained the higher level (0.61 ± 0.14) till the 24th week. No significant changes were observed in TβRⅠ mRNA, Smad4 mRNA and Smad7 mRNA during the mice liver fibrogensis compared with those in the normal control group. **Conclusion** In liver fibrogensis induced by *Schistosoma japonicum*, The down regulation of the following factors may play the induction roles: TβRⅡ mRNA, Smad3 mRNA and Smad2 mRNA in latter stage of post-infection, as well as the normal level of Smad7 mRNA may play a positive role in fibrogensis. On the contrary, the reduction of Smad2 mRNA in the early period of post-infection may inhibit liver fibrogensis.

【**Key words**】 *Schistosoma japonicum*; Smad; Fibrosis; Liver; TGF-β1; Receptor

TGF-β1-Smads 信号传导通路在感染日本血吸虫小鼠肝纤维化中的表达

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【摘要】 目的 探讨在 BALB/c 小鼠感染日本血吸虫后形成肝纤维化的过程中，转化生长因子-β（transforming growth factorβ, TGF-β）1 及其两类受体：TGF-β 受体Ⅰ（TβRⅠ）、TGF-β 受体 Ⅱ（TβRⅡ）以及 Smad2、Smad3、Smad4 和 Smad7 在转录水平的表达。 **方法** 50 只 BALB/c 小鼠感染日本血吸虫尾蚴，（20 ± 1）条/只，用于构建肝纤维化模型，10 只未感染的 BALB/c 小鼠作为健康对照组，分别在感染后 8、12、16 和 24 周处死小鼠取肝组织，同时取对照组小鼠肝组织。所获肝组织一部分立即液氮冷冻后，保存于−80 ℃，通过 RT-PCR 方法测定 TGF-β1、TβRⅠ、TβRⅡ和 Smad2、Smad3、Smad4 以及 Smad7 的 mRNA 水平，结果为相对值，以待测 mRNA 密度扫描计数与内参照 β-actin mRNA 密度扫描计数的比值表示。另一部分肝组织置入常规 10%甲醛固定液中，用于伊红-苏木素（HE）染色和天狼猩红染色，其中 HE 染色用于测定血吸虫虫卵肉芽肿面积（每一个虫卵肉芽肿的最大长度与最大宽度的乘积，以 mm^2 表示），每份标本随机测量 5 个虫卵肉芽肿面积，求平均值，天狼猩红染色用于判断肝纤维化程度，并以下述方法计分：正常肝组织为 0 级，以 2^0=1 计分；胶原纤维包绕肉芽肿周围并插入其中为Ⅰ级，以 2^1=2 计分；汇管区有大量纤维化，小叶间仅有少量纤维为Ⅱ级，以 2^2=4 计分；纤维组织大量延伸至小叶间为Ⅲ级，以 2^3=8 计分。 **结果** 小鼠感染日本血吸虫 8 周后其肝脏中形成的虫卵肉芽肿周围出现胶原纤维，并随着感染时间的延长，胶原纤维逐渐增加。感染后 16 周，胶原纤维在肝小叶中分布明显，其肝纤维化程度计分为 4.27 ± 1.03 分；至感染 24 周时，胶原沉积量达到顶峰，为 6.90 ± 1.57 分；而正常小鼠肝组织的肝纤维化计分为 1 分。正常小鼠肝脏中 TGF-β1 mRNA 的表达水平为 0.30 ± 0.18，其表达量在感染后 8 周达到高峰（0.87 ± 0.76），而后下降，但在感染 24 周时，其表达量再次升高（1.34 ± 0.52）。TβRⅡmRNA 在感染 8 周时有所下降，为 0.60 ± 0.30，在感染 12 周时回升到正常水平，为 0.92 ± 0.21，在感染 16 周时，其表达量又下降为 0.76 ± 0.16，而在感染 24 周时升至 1.16 ± 0.73；而正常小鼠肝组织中 TβRⅡmRNA 的表达水平为 1.16 ± 0.25。感染后，Smad2 mRNA 在感染 12 周时和感染 24 周时均较正常对照（0.85 ± 0.10）有所下降，分别为 0.41 ± 0.23 和 0.50 ± 0.16。Smad3 mRNA 在感染 16 周时有所升高（0.62 ± 0.09），这种高水平表达持续到 24 周（0.61 ± 0.14）。在肝纤维化形成过程中，Smad4 mRNA 和 Smad7 mRNA 以及 TβRⅠmRNA 的表达水平与正常对照组比较无明显差异。 **结论** 在日本血吸虫性肝纤维化形成过程中，下述因子的下调可能诱导肝纤维化形成：TβRⅡmRNA、Smad3 mRNA 和处于感染后期的 Smad2 mRNA，而 Smad7 mRNA 的正常水平表达在肝纤维化形成中发挥促进作用。在感染早期，Smad2 mRNA 表达的下调可能抑制肝纤维化的形成。

【关键词】 日本血吸虫；Smad；纤维化；肝脏；TGF-β1；受体

Transforming growth factor-β（TGF-β）is a member of a family of growth factors that regulates cellular proliferation, cellular differentiation, embryonic development, wound healing, and angiogenesis in a cell-specific manner. In mammalian, TGF-β family consists of three members, including TGF-β1,TGF-β2 and TGF-β3. Among others, TGF-β1 is an important cytokine in liver fibrogenesis, which has been confirmed by a lot of studies. In general, the TGF-β1 response is mediated by its receptors. Three types of TGF-β1 receptors are identified: TGF-β type Ⅰ receptor（TβRⅠ）, TGF-β type Ⅱ receptor（TβRⅡ）and TGF-β type Ⅲ receptor（TβRⅢ）. The former two are transmembrane serine/threomine kinases with a signaling role. Type Ⅲ receptor, a proteoglycan, which may participate in ligand binding and presentation, supports an essential and non-redundant role of TGF-β signaling, especially for TGF-β2 [1]. TβRⅡ is constitutively active kinases that leads to ligand-binding specific signaling. TβRⅡ docking leads to TβRⅠ recruitment, phosphorylation, and subsequent cellular signaling. TβRⅡ is an critical receptor for TGF-β signaling and many researchers investigate TGF-β1 role through suppress TβRⅡ expression, which also provide the strategy for the liver fibrosis therapy[2-11]. All those studies demonstrated that the response of TGF-β1 was inhibited by suppressing the binding of TGF-β1 with TβRⅡ while they did not reveal the expression of TβRⅡ in liver disease. That gives rise to a problem that different expression level of TβRⅡ during the course of the disease needs to be modulated in different ways.

Smads family, an important substrate of TGF-β receptor Ⅰ type, is the downstream intracellular effectors of the activated TGF-β/TGF-β receptor signaling. Smads have involved in the biological effects of TGF-β1, for example, Smad3 is correlated with increased （collagen type Ⅰ 2, COL1A2）and （plasmihogenactivator-1,PAI-1）gene transcription in activated hepatic stellate cells（HSC）[12]. Smad-containing complexes do not interact with the Timp-1 AP1 site, and over expression of Smads does not

substitute the induction of the gene by TGF-β1. Furthermore, tissue inhibitor of metalloproteinase 1 (TIMP-1) is still induced by TGF-β1 in Smad knockout cell lines, though to varying extents. In contrast, Smads do interact with the MMP-1 AP1 site and mediate the repression of induced matrix metalloproteinase 1 (MMP-1) gene expression by TGF-β [13]. Smads family includes Smad1-Smad8 while Smad2, Smad3, Smad4, and Smad7 are members of Smads mediating the response of TGF-β1. TGF-β1 signals through the heterometric complexes of type I and type II transmembrane Ser/Thr kinase receptors. The activated type I kinase by type II receptor at the Gly-Ser (GS) domain associates transiently with, and also phosphorylates receptor-regulated Smad2 and Smad3. Once phosphorylated, receptor-regulated Smads dissociate from the type I receptor, bind to Smad4 and then the complexes enter the nucleus and bind to target promoters to fulfill the effects of TGF-β1. Smad7 belongs to the inhibitory member of Smads and acts in opposition to signaling and inhibits the signal transduction. Therefore, in the base state, it can modulate the TGF-β1 response to make its effect in balance.

At present, Smads expression in HSC has been studied widely [14-16]while the results are not very consistent. The reasons may be related the souse of HSC: passage or immortalized HSC, primary activated HSC and HSC from the normal rat or the model one by bile duct ligation or by CCl_4 intoxication. Till now, the dynamic observation seems few on Smads expression in liver fibrosis *in vivo* although skin or pulmonary fibrosis *in vivo* has been studied[17-18]. The murine model of intestinal schistosomiasis shows similar pathological sequel of infection compared with human disease [19]. To clarify the role of TGF-β-Smads signal pathway in the progression of liver fibrosis *in vivo*, we reported this pathway expression in mice of liver fibrosis induced by *Schistosoma japoncum*.

1 Methods

1.1 Establish the mice liver fibrosis

Sixty BALB/c mice, aged 6 to 8 weeks, weighing 18-20 g, were obtained from Chinese Science Academy Animal Experiment Center, and were maintained with Rodent Diet (Animal Experiment Center of medical school of Zhejiang University) and water ad lib. Mice were cared for and used in accordance with the Declaration of Helsinki. After maintenance for a week, 50 mice were infected with 20 ± 1 cercariae of *Schistosoma japonicum* at the Medicine and Science Academy of Zhejiang Province and 10 uninfected mice were used as the normal control. In the process of liver fibrogenesis, 36 mice survived and 24 mice died.

1.2 Histological evaluation

Eight mice were killed for liver sample at the 8th, 12th, 16th, and 24th week post-infection, respectively, and 10 normal mice were sacrificed. Liver samples were placed in 10% formalin for histomorphometric studies. Consecutive serial sections of 5 μm were cut from formalin-fixed, paraffin-embeded tissue, and stained with picric acid-sirius red. Liver egg granuloma was measured in hematoxylin-eosin stain by the product of the maximum width and the maximum length and expressed in square micrometers. The value of granuloma size was the average of five readings in one liver tissue section. Sirius red stain was used to determine liver fibrosis degree by below criteria: 2^0=1 no significant collagen was observed in liver tissue; 2^1=2 collagen distribute around and in the granuloma; 2^2=4 more collagen appeared in the portal tracts while few collagen among liver lobules; 2^3=8 fibrous tissues penetrate into liver lobules.

1.3 RNA isolation and RT-PCR

Liver samples were immediately frozen in liquid nitrogen and stored at -80 ℃ for RNA extraction and mRNA analysis. Total RNA was isolated from about 100 mg liver tissue by extraction in TRIzol (Life Technologies, Inc.). 2 μl of RNA were mixed with 1 μl random primer and 7 μl deionize water treated with diethyl pyrocarbonate (DEPC) for the incubation at 70 ℃ for 5 min, and then was put on ice immediately. Add the following components

to above mixture: 5 × first strand buffer 4 μl, 100 mmol/L dithiothreitol 2 μl, moloneymurine leukemia virus (M-MLV, Superscript II kit. Life Technologies, Inc.) 0.5 μl, 2.5 nmol/L dNTP 2 μl, deionize water treated with DEPC 1.5 μl. The whole mixture was incubated at 42 ℃ for 1 h and then at 70 ℃ for 10 min. The following mouse gene specific primers were used for RT-PCR amplification (Table 1).

Table 1 Primer sequence

Name	Amplified fragment length(bp)	Upstream primer	Downstream primer
β-actin[21]	940	5′-GTGACGAGGCCCAGAGCAAGAG-3′	5′-AGGGGCCGGACTCATCGTA-3′
TGF-β1[22]	279	5′-GGT TTTCTCATAGATGGCGT-3′	5′-ACCTGCAAGACCATCGACAT-3′
TGFβRI[23]	240	5′-TGCGGTTATGGCAGATATAGACC-3′	5′-TAGCTGAAATTGACCTAATTCCTCG-3′
TGFβRII[24]	505	5′-CAGGGACCTCAAGAGCTCTAAC-3′	5′-GTCCATATGCTCCAGCTCACTG-3′
Smad2[25]	205	5′-GGAAAGGGTTGCCACATGTT-3′	5′-AGAATCTCCGTGTGCCGAGG-3′
Smad3[26]	706	5′-TGACTACAGCCATTCCATTC-3′	5′-TCACTGTCTGTCTCCTGTAC-3′
Smad4[25]	648	5′-ACGGCCATCTTCAGCACCAC-3′	5′-AGAATGCACAATCGCCGGAG-3′
Smad7[27]	489	5′-GCATTCCTCGGAAGTCAAGAGG-3′	5′-TGCGGTTGTAAACCCACACG-3′

The whole PCR processes: (1) Pre-denature 95 ℃ for 3 min. (2) Cycles were as follows: 94 ℃ for 30 s; 57 ℃ (TGF-β1, TβR Ⅰ, TβR Ⅱ), 65 ℃ (Smad2, 3, 4), 55 ℃ (Smad7) for 30 s, 72 ℃ 30 s (Smad2, 3, 4 for 35 cycles and Smad7 for 30 cycles); 72 ℃ for 7 min. The products were electrophoresed by 1.5% agarose gel and the results were analyzed with IS-1000 Digital Imaging System (Alpha) while β-actin was considered as the inter control. Through the above processes, the specific fragments of TGF-β1, TβR I, TβR Ⅱ and Smad2, Smad3, Smd4 and Smad7 were got.

1.4 Statistical analysis

The data was expressed as $\bar{x}\pm s$ and analyzed by SPSS 11.0.

2 Results

2.1 The histological features of the mice liver in fibrogenesis

Liver egg granuloma was measured in hematoxylin-eosin stain by the product of the maximum width and the maximum length and expressed in square micrometers. Liver fibrosis degree by followed criteria: 2^0=1, no significant collagen was observed in liver tissue; 2^1=2, collagen distribute around and in the granuloma; 2^2=4, more collagen appeared in the portal tracts while few collagen among liver lobules; 2^3=8, fibrous tissues penetrate into liver lobules. In the normal mouse liver, only little collagen surrounded the wall of the liver sinusoid (Fig. 1). At the 8th week post-infection, egg granuloma peaked and the collagen in liver appeared and wiped around the granuloma (Fig. 1). At the 12th week, some collagen stretched into the interior of granuloma. At the 16th week, amount of collagen was present in the portal area (Fig. 1). With the progression of disease, liver fibrosis of mice aggregated and fibrous tissue stretched into the lobe (Fig. 1). The areas of egg granuloma were reduction since 8th week post-infection while the liver fibrosis aggregated little by little with the time post-infection. The results of areas of egg granuloma and scores of liver fibrosis were displayed in Table 2.

2.2 Transcription level of TGF-β1, TβR Ⅰ and TβR Ⅱ in liver fibrogenesis

The level of TGF-β1 mRNA peaked at the 24th week after infection. The level of TβR Ⅱ mRNA reduced at the 8th and the 16th week, respectively while its level amounted to normal level at the 24th week (Table 3). TβR Ⅰ remained the normal level during the development of liver fibrosis. The level of TGF-β1 mRNA was positively correlated with liver fibrosis degree, TβR Ⅰ mRNA level, and TβR Ⅱ mRNA level (r=0.661, 0.385, 0.340, all P<0.05). The expression of TβR Ⅰ mRNA was positively correlated with TβR Ⅱ mRNA level (r=0.829, P<0.05).

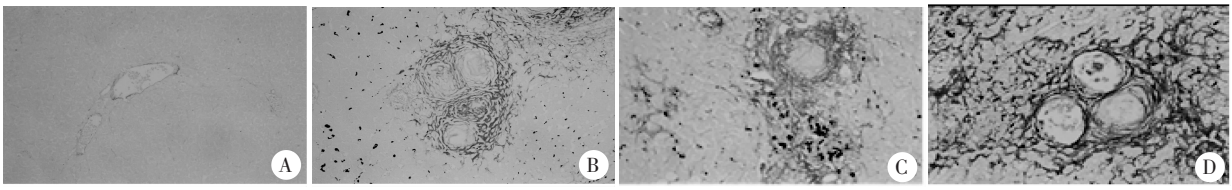

Fig. 1 Collagen expression in the liver tissue of mice (picric acid-sirius red straining, ×200)

A: normal BALB/c mice liver, B: BALB/c mice liver at the 8th week post-infection, C: BALB/c mice liver at the 16th week post-infection, D: BALB/c mice liver at the 24th week post-infection

Table 2 Results of ares of egg granuloma and scores of liver fibrosis degree post-infection ($\bar{x}\pm s$)

Group	No.	Ares of egg granuloma (mm^2)	Scores of liver fibrosis degree
Control	10	0	1
8 week post-infection	8	5.33±1.03[a]	2.03±0.52[a]
12 week post-infection	8	4.95±0.96[a]	3.22±0.63[a]
16 week post-infection	8	3.91±1.75[a]	4.27±1.03[a]
24 week post-infection	10	2.94±1.69[a]	6.90±1.57[a]

a: vs the control group, $P<0.05$

Table 3 Expression of TGF-β1, TβR Ⅰ, TβR Ⅱ mRNA at different stages after *Schistosoma* infection ($\bar{x}\pm s$)

Group	No.	Scores of liver fibrosis degree	TGF-β1, TβRI, TβRII mRNA/β-actin mRNA		
			TGF-β1	TβRI	TβRII
Control	10	1.0±0.0	0.30±0.18[a]	0.90±0.51	1.16±0.25
8 week post-infection	8	2.5±1.0	0.87±0.76	0.96±0.91	0.60±0.32[b]
12 week post-infection	8	3.5±1.0	0.59±0.11[a]	0.63±0.15	0.92±0.21
16 week post-infection	8	4.0±0.0	0.60±0.30	0.48±0.20	0.76±0.16[b]
24 week post-infection	8	6.2±2.1	1.34±0.52	0.68±0.70	1.16±0.73

a: $P<0.05$ vs the 24th week group, b: $P<0.05$ vs the control group

Table 4 Smad2, Smad3, Smad4 and Smad7 mRNA level in mice liver at different stages after *Schistosoma* infection ($\bar{x}\pm s$)

Group	No.	Scores of liver fibrosis degree	Smads/β-actin mRNA			
			Smad2	Smad3	Smad2	Smad3
Control	10	1.0±0.0	0.85±0.14	0.32±0.06	0.91±0.06	0.73±0.14
8 week post-infection	8	2.5±1.0	0.62±0.49	048±0.10	0.68±0.33	0.65±0.15
12 week post-infection	8	3.5±1.0	0.41±0.23[a]	0.40±0.11	1.12±0.44	1.45±1.10
16 week post-infection	8	4.0±0.0	0.69±0.24	0.62±0.09[a]	1.14±0.31	1.05±0.83
24 week post-infection	8	6.2±2.1	0.50±0.16[a]	0.61±0.14[a]	0.92±0.12	0.97±0.41

a: $P<0.05$ vs controls

2.3 Expression of Smad2, Smad3, Smad4 and Smad7

During the development of liver fibrosis, Smad2 mRNA and Smad3 mRNA changed significantly while the mRNA level of Smad7 and Smad4 remained constant. The level of Smad2 mRNA reduced at the 12th week post-infection and elevated at the 16th week. The mRNA level of Smad3 elevated at the 16th week and it remained its level till to the 24th week post-infection (Table 4).

3 Discussion

The pathogenesis of liver fibrosis remains incompletely understood. So the mice liver fibrosis model may provide us the mechanism of human liver fibrogensis. In previous works, the role of TGF-β1 in the mechanism of fibrosis has been confirmed. After the substrate of TGF-β receptor type Ⅰ, Smad family, was identified[28-29], researchers are eager to know how TGF-β1 plays its role to initiate fibrosis through Smad family. The expression of TGF-β1 receptors and Smads in the development of liver fibrosis *in vivo* have not been studied widely.

Our study revealed that TβR Ⅱ expression changed in the course of liver fibrosis while TβR Ⅰ remained constant. A lot of studies on TβR Ⅱ expression were in the field of hepatocellular

carcinoma [30-33] while only a few were in the field of liver fibrosis. We found that TGF-β1 was significantly correlated with its two transmembrane receptors-type Ⅰ and type Ⅱ, indicating that the interaction of these two receptors was essential to the TGF-β1 response.

During the fibrogenesis, the expression level of TβR Ⅱ mRNA reduced at the 8th week and the 16th week after infection, respectively. Our previous work[34] demonstrated that TGF-β1 was a kind of antiinflammation factor and inhibited the granuloma. Mola et al[35] also found that the size of granuloma was reduced by TGF-β1. The result revealed the reduction of TβR Ⅱ mRNA at the early stage after infection may inhibit the antiinflammation response of TGF-β1 and induce the progression of inflammation, resulting in liver fibrosis. At the 16th week after infection, fibrosis established in liver and HSC had activated into myofibroblasts. During the course of activation of HSC, TGF-β1 played an important role. But once activation of HSC, platelet derived growth factor-BB (PDGF-BB) seemed more important during the proliferation of myofibroblasts. Our previous works demonstrated that the sensitivity of PDGF-BB was better than that of TGF-β1 in the diagnosis of liver fibrosis [36]. Other results *in vivo* also revealed that TβR Ⅱ level reduced after activation of HSC, it may indicate that the sensitivity of myofibroblasts to TGF-β1 deceased[37-39]. The reduction of TβR Ⅱ could suppress some biological responses such as antiproliferation. That resulted in the proliferation of hepatic cells and enhanced fibrosis for we had noticed that expression of TGF-β1 and type Ⅰ and type Ⅱ receptor in hepatic cells. Date et al [40] also found that downregulation of TGF-β receptor occurred in hepatocytes after chemical insult and TGF-β1 could not transduce its antiproliferative signal. Recovery of TGF-β receptor expression caused the signal to transduce to the nucleus at 72 h.

The level of TGF-β1 peaked at the 24th week after infection. The possible reason was that the reduction of level of TβR Ⅱ at the 16th week leaded to the local concentration of TGF-β1 elevated with less to combine with the receptor.

TβR Ⅰ, a bridge between TGF-β1 and Smad, is phosphorylated by TβR Ⅱ. Our experiment revealed that TβR Ⅰ remained constant, which was consistent with the report of Wicker et al[39]. We thought the role of TβR Ⅰ was not influenced by its mRNA level, probably, it could play its transduction action as long as it was activated.

Previous researc hes showed that specific functions of Smad2 and Smad3 in TGF-β1 signaling although they have higher homology [41]. TGF-β1–mediated induction of matrix metalloproteinase-2 (MMP-2) was selectively depended on Smad2[41]. MMP-2 could degrade the normal base membrane (BM) in the sinusoid. That resulted in activating HSC to secrete type I collagen, depositing in the BM and impairing liver function. We found that the mRNA expression of Smad2 reduced at the 12th week post-infection. That indicated that the reduction of Smad2 mRNA inhibited the expression of MMP-2 and reduced type I collagen deposition. That demonstrated that down regulation of Smad2 suppressed liver fibrogenesis in the early stage. In contrast, Dooley et al[42] found that Smad2 remained unchanged during the activation process of HSC. The reason for the discrepancy may be that Smad2 mRNA was detected through the whole liver tissue, which differed from the detection of it from only one kind of cell in vitro. Smad2 mRNA reduced while TGF-β1 mRNA increased at the 24th week post-infection. The reduction of Smad2 mRNA may lead to the reduction of MMP-2, which resulted in the reduction of decomposition of type Ⅳ collagen and the increase of deposition and then aggregated the liver fibrosis. That indicated the reduction of Smad2 mRNA induced the live fibrosis at the later stage. From above, Smad2 played two sides effects in liver fibrogensis. Smad3 mRNA elevated at the 16th week post-infection and live fibrosis was also established at the same period. The activation of HSC depended mostly on autocrine of TGF-β1 while TGF-β1 autoinduction relied on the expression of Smad3[18]. So we thought Smad3 may induce liver

fibrosis, which was consistent with the result by knock-out Smad3[12] and other results *in vivo*[43]. *In vitro*, the similar results could be observed [44-45]. This result indicated that Smad3 mRNA probably became the target for antifibrosis treatment. Smad3 mRNA increased while TβR Ⅱ mRNA reduced at the 16th week post-infection. We thought that the effect induced by TβR Ⅱ mRNA reduction was the inducer for Smad3 to enhance the fibrogensis.

We found that Smad4 might play no role in the progression of liver fibrosis at transcription level, which was different to the importance of Smad4 in some cancer s[46-47]. During our study, mRNA level of Smad4 remained constant in the development of liver fibrosis in mice infected with *Schistosoma japoncum*. The similar results were also found in the HSC transformation and cirrhotic hepatocytes[16,48]. In contrast to that, Kitamura et al[49] observed that expression of Smad4 in the nucleus of the HSC of the cirrhotic liver was stronger than that in the non-cirrhotic liver. They also found that HSC line showed a stronger expression of Smad4 by TGF-β1 stimulation than that without TGF-β1 stimulation. Smad4 was identified as tumor suppressor at first, so it may play an important role in mediating the proliferation of TGF-β1 response while it seemed to have the minor role in fibrogenesis. The moderate proliferation was the feature of cirrhotic liver or cell line, which may give rise to the different results.

Smad7, as the negative regulator, plays an important role to balance the function of TGF-β1. On the contrary, Smad7 mRNA is controlled by TGF-β1. We observed that Smad7 mRNA remained unchanged during the development of liver fibrosis and did not increase with the elevation of TGF-β1. It may be that Smad7 lost the sensitivity to TGF-β1 and resulted in the liver fibrosis, which was consisted with the result of Tahashi et al [15] observed in chronic liver injuries. Smad7 mRNA remained the low level and the signals of liver fibrosis continued and resulted in liver fibrosis. This revealed that fibrogenesis would constantly progress without inhibition of Smad7. However, Song et al[50] demonstrated that Smad7 mRNA decreased in CCl_4-induced rat liver fibrosis model, which might be the net result of the competition with Smad3 up regulation. Kitamura et al [49] found that Smad7 mRNA increased in cirrhotic liver, which might inhibit the over expression of Smad4 mRNA to be moderate proliferation in liver for it increased correspondingly with Smad4 mRNA. Although Smad7 expression was not consistent in the present studies, the function, the regulation and the therapeutic role of Smad7 have been explored widely[51-55]. Whether Smad7 was inhibited or induced in the disease would be the key point to take the corresponding measures to terminate the adverse effects of TGF-β1.

In conclusion, in liver fibrogensis induced by *S. japonicum*, we found that the down regulation of the below factors may play the induction roles: TβR Ⅱ mRNA, Smad2 mRNA in latter stage post-infection, Smad3 mRNA while the normal level of Smad7 mRNA may play the positive role in fibro-gensis. On the contrary, the reduction of Smad2 mRNA may inhibit liver fibrogensis in the early period post-infection.

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勘 误

《国际医学寄生虫病杂志》2013 年第 40 卷第 2 期 P86、P87 页表 2、表 4 中,“有、无血吸虫病史者”对应栏目下的“DM+IFG”,修正为“IFG”。