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• BASIC RESEARCH •

Therapeutic effects and molecular mechanisms of anti-fibrosis herbs and selenium on rats with hepatic fibrosis

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Abstract

AIM: To study the therapeutic effects of anti-fibrosis herbs and selenium on hepatic fibrosis induced by carbon tetrachloride (CCl_4) in rats and the underlining molecular mechanisms.

METHODS: Fifty-three Wistar rats were randomly divided into: normal control group, model control group, colchicine group, anti-fibrosis herbs group (AF group) and anti-fibrosis herbs plus selenium group (AS group). The last four groups were administered with CCl_4 at the beginning of experiment to induce hepatic fibrosis. Then colchicine, anti-fibrosis herbs and selenium were used to treat them. The normal control group and the model control group were given normal saline at the same time. At the end of the 6th week, rats in each group were sacrificed. Blood and tissue specimens were taken. Serum indicators (ALT, AST, HA, LN) were determined and histopathological changes were graded. Lymphocyte CD_4 and CD_8 were examined by flow cytometry. Expression of TGF-β_1 and NF-κB was detected by immunohistochemistry and expression of TGF-β_1 mRNA was detected by semi-quantified RT-PCR.

RESULTS: Histological grading showed much a smaller degree of hepatic fibrogenesis in AS group and AF group than that in colchicine group and model control group. The serum content of ALT, AST, HA and LN in AF group and AS group were significantly lower than that in colchicine group (ALT: 65.8±26.5, 67.3±18.4 and 96.2±20.9 in AF, AS and colchicine groups respectively; AST: 150.8±34.0, 154.6±27.3 and 215.8±24.6 respectively; HA: 228±83, 216±58 and 416±135 respectively; LN: 85.9±15.0, 80.6±18.6 and 106.3±14.2 respectively) ($P<0.05$). The level of CD_4 and CD_4/CD_8 ratio in AF group and AS group was significantly higher that those in cochicine group (CD_4: 50.8±3.8, 52.6±3.4 and 40.2±2.1 in AF, AS and colchicine groups respectively; CD_4/CD_8 ratio: 1.45, 1.46 and 1.26, respectively ($P<0.05$). The expression level of NF-κB and TGF-β_1 in the liver tissues of AF and AS treatment groups was markedly decreased compared with that in cochicine group, and TGF-β_1 mRNA was also markedly decreased (1.07±0.31 and 0.98±0.14 *vs* 2.34±0.43, $P<0.05$).

CONCLUSION: Anti-fibrosis herbs and selenium have beneficial effects on hepatic fibrosis in rats by enhancing immunity and inhibiting NF-κB and TGF-β_1 expressions.

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INTRODUCTION

Hepatic fibrosis is a common pathological process of chronic hepatic disease, leading to the development of irreversible cirrhosis[1-3]. The incidence of hepatitis is high in China[4-8]. If treated properly at fibrosis stage, cirrhosis could be prevented[9]. However, there are no effective antifibrosis drugs to date. Chinese herbs, which are well known for their long history of proven therapy of various diseases with low cost and few side effects, have particular potentials in the treatment of hepatic fibrosis[10-18]. In addition, some studies have indicated that selenium is closely related to the inhibition of hepatic fibrosis[19,20]. In the present study, we first established a rat model of chronic liver injury - hepatic fibrosis-cirrhosis and then tested the therapeutic effects of Chinese herbs and selenium on hepatic fibrosis. An array of indexes in proteins and mRNA levels were evaluated in order to understand the mechanism underlying the effects observed.

MATERIALS AND METHODS

Reagents

TGF-β_1 mRNA primers were purchased from Sangon Biological Technology Company, China. Anti-TGF-β_1 monoclonal antibody and anti-NF-κB polyclonal antibody were purchased from Santa Cruz Biological Technology Company, USA. Streptomycin avidin peroxidase immunohischemistry kit for immunohistochemistry and RNA isolation kit were purchased from Boster Biological Technology Ltd, China. Anti-CD_4 and CD_8 polyclonal antibodies were purchased from Caltag Biological Technology Company, USA. Serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the Laboratory Department of 4th affiliated hospital, Hebei Medical University, China. Serum hyaluronic acid (HA) and laminin (LN) concentrations were measured radioimmunologically using a commercial kit (Shanghai Navy Medical Institute, China).

Preparation of Chinese herbs

The anti-fibrosis herbs included Salvia miltiorrhiza, Sparganium stoloniferum, Angelica sinensis, Amyda sinensis, Curcuma aromatica, Carex phacota. These were purchased from Shijiazhuang Lerentang Pharmacy, China. The herbs were boiled with water and extracted by alcohol: put 95% alcohol in liquid of anti-fibrosis herbs, mixed the alcohol and herbs, filtrate the protein and amylum, then heat the liquid at 90-95 ℃ to evaporate the remained alcohol.

Establishment of animal model

Wistar rats, half males-half females, weighing 180-200 g were obtained from Experimental Animal Center of Hebei Medical

University, China. The rats were housed 5 heads per cage and subjected to 12 h-d/12 h-night cycle with free access to basic food and water. All animals were treated humanely according to the national guideline for the care of animals in the country.

Hepatic fibrosis was induced in rats by carbon tetrachloride (CCl_4). Wister rats were randomly assigned to normal control group (10), model control group (13), Colchicine group (10), anti-fibrosis herbs group (AF group, 10) and anti-fibrosis herbs plus selenium group (AS group, 10). On the first day of experiment, the rats in model group, colchicine group, AF group and AS group were given hypodermic injection of bean oil solution containing 400g/L CCl_4 (0.5 mL/100 g body mass), followed by injection of the same solution (0.3 mL/100 g body mass) every 4 d. The rats in normal control group received hypodermic injection of bean oil at the same dose and frequency. Fourteen times after CCl_4 administration, 3 rats in model control group were sacrificed to evaluate the liver histological change, which indicated the development of chronic hepatitis. Then colchicine group was given colchicine orally at a dose of 0.01 mg/100 g body weight daily, AF group was given anti-fibrosis herbs (2.11 g/mL) orally at a dose of 0.5 mL/100 g body mass daily, AS group was given orally anti-fibrosis herbs containing sodium selenite ($Na_2SeO_3.5H_2O$) at 3 μg/mL daily. Normal control group was given saline orally at a dose of 0.5 mL/100 g body mass daily. All the administrations lasted for 6 weeks.

Collection of specimens

At the end of the 6th week of the administration, rats in each group were sacrificed by amobarbital sodium anesthesia. Midline laparotomy was performed. Livers and thymus were excised and blood was collected through cardiopuncture.

Histological grading

Liver tissues were fixed in formalin and embedded in paraffin. Hematoxylin and eosin (HE) staining and Masson staining were performed according to the standard procedure. Histological grade of chronic hepatic fibrosis was determined by a semi-quantitative method based on the criteria described below: grade 0: normal liver, grade 1: few collagen fibrils extended from the central vein and portal tract, grade 2: collagen fibrils extension was apparent but had not yet encompassed the whole lobule, grade 3: collagen fibrils extended into and encompassed the whole lobule, grade 4: diffuse extension of collagen fibrils and pseudo-lobule formed.

Two pathologists who had no knowledge of their sources and each other's assessment examined the stained slide independently.

Flow cytometry of CD_4 and CD_8 positive cells

Sample fluorescence staining was performed using indirect immufluoredence labeling method. Sample cells were washed in 10 mL Na-azide-PBS and centrifuged. Primary mAb to human CD_4 and CD_8 was added to each tube. The tube was vortexed and incubated at 37 ℃ for 30 min, 10 mL azide-PBS was added for inactivation and the cells were centrifuged. The supernatant was sucked away. The second antibody of FITC-IgG was added to each tube. The tube was vortexed and incubated in the dark at 37 ℃ for 30 min. 10 mL azide-PBS was added for inactivation and the cells were centrifuged. The samples were stored at 4 ℃ in the dark for FACS analysis. The primary antibody and secondary antibody were replaced by 30g/L BSA in azide-PBS as negative controls, the primary antibody was replaced by 30g/L BSA in azide-PBS as the second antibody control. The stained samples were analyzed in a FACS 420 flow cytometer (FACS 420 Fluorescence Activited Cell Sorting, Becton. Dickinson, Sunnyvale. California, USA.) The light source was a 2W argon ion laser using a wave-length of 488 nm. The working power was 300 mW. Single parameter was measured respectively. Usually, 10 000 cells for each sample were analyzed. The analytic data were processed with a HP-Consort 30 computer. The coefficient of variation of the instrument was adjusted within 5% using PI staining chicken red blood cells.

Immunohistochemistry

Liver samples were formalin-fixed, paraffin-embedded and sectioned serially at 5 μm thickness. Immunohistochemistry was performed as described in streptomycin avidin peroxidase immunohischemistry kit (Boster). The sections were treated with 30mL/L H_2O_2 methanol at room temperature for 10 min and then washed with PBS for 5 min. After antigen retrieval, nonspecific binding sites were blocked by normal non-immune goat serum. The sections were incubated with primary antibody overnight at 4 ℃, secondary antibody at 37 ℃ for 30 min, and avidin peroxidase at 37 ℃ for 20 min, followed by DAB visualization. After several washings, the sections were counterstained with hematoxylin. Negative control slides were treated with PBS.

Semi-quantitative PCR

Total RNA was extracted using an RNA isolation kit, and quantity and quality of the RNA extracted were measured on a spectrophotometer. Purified RNA 2 μg and primer Oligo (dT) were used for reverse transcription (Promega). 5 μL reverse transcription template was used for amplification through PCR. The primers were: TGF-β_1, 113 bp: forward: 5'-AGGGCTACCATGCCACTTC-3', reverse: 5'-GCGGCACGCAGCACGGTGAT-3', GAPDH, 299 bp: forward: 5'-GTGAAGGTCGGAGTCAACG-3', reverse: 5'-GGTGAAGACGCCAGTGGACTC-3'. Amplification conditions included initial denaturation for 5 minutes at 94 ℃, 30 cycles of amplification with denaturation at 94 ℃ for 45 seconds, annealing at 61 ℃ for 45 s, and extension at 72 ℃ for 1 min. PCR products were analyzed by agarose gel electrophoresis (15 g/L) and visualized by ethidium bromide staining and ultraviolet illumination. Expression of each TGF-β_1 was scanned by a computer. The obtained values were related to housekeeping gene GAPDH, and the resulting relative ratios were analyzed statistically.

Statistical analysis

Data were analyzed with SPSS software. Quantitative data were presented as mean±SD and compared using one way ANOVA procedure. Frequency data were compared using Ridit procedure.

RESULTS

Anti-fibrosis herbs and anti-fibrosis herbs plus selenium treatment suppressed fibril deposition in and ameliorated liver function of hepatic fibrosis

Specimens from normal control group showed normal structures. Specimens from model control group showed apparent formation of fibrotic septa, encompassing regenerated hepatocytes into pseudo-lobules. Regenerated hepatocytes underwent severe lipoid degeneration. Specimens from AF and AS groups showed only mild fibrogenesis without pseudo-lobule formation. Statistical analysis presented significant differences between either AF group or AS group and model control group in histological gradings, indicating that fibrogenesis in both AS and AF groups was much less severe than that in colchicine group and model control group (Table 1).

Serum content of ALT, AST, HA and LN in AF group and AS group was slightly higher than that of normal control

group, but significantly lower than that in model control group (P<0.05). Serum content of ALT, AST, HA and LN in AF group and AS group was also lower than that in colchicine group (P<0.05). These data confirmed the histological findings that anti-fibrosis herbs and anti-fibrosis herbs plus selenium could inhibit hepatic fibrogenesis and ameliorate liver function (Table 2).

Table 1 Histological grading of hepatic fibrosis

Group	Grade o	Grade I	Grade II	Grade III	Grade IV
Normal	10	0	0	0	0
Model	0	0	0	0	8[c]
Colchicine	0	1	3	4	1[c]
AF	4	3	1	1	0[a]
AS	3	3	2	1	0[ac]

[a]P<0.05, *vs* colchicine group; [c]P<0.05, *vs* normal control group.

Table 2 Serum content of ALT, AST, HA and LN

Group	ALT (u/L)	AST(u/L)	HA(μg/L)	LN (μg/L)
Normal	52.5±9.2[a]	137.8±18.7[a]	178±58[a]	59.8±21.8[a]
Model	165.6±32.7[ace]	257.4±22.6[c]	550±68[c]	130.0±30.5[c]
Colchicine	96.2±20.9[c]	215.8±24.6[c]	416±135[c]	106.3±14.2[c]
AF	65.8±26.5[a]	150.8±34.0[a]	228±83[ac]	85.9±15.0[c]
AS	67.3±18.4[a]	154.6±27.3[a]	216±58[ac]	80.6±18.6[c]

[a]P<0.05, *vs* colchicine group; [c]P<0.05, *vs* normal control group; [e]P>0.05, *vs* control group.

Anti-fibrosis herbs and anti-fibrosis herbs plus selenium treatment enhanced immunity of rats with hepatic fibrosis

The percentage of CD_4 and CD_8 and the ratio of CD_4 to CD_8 were significantly lower in model control group than that in normal control group. Both AF group and AS group showed a lower percentage of CD_4 and a lower ratio of CD_4 to CD_8 than normal control group. However, these values were significantly higher than those in colchicine group, suggesting that anti-fibrosis herbs and anti-fibrosis herbs plus selenium could enhance the immunity of rats with hepatic fibrosis (Table 3).

Table 3 Content of CD_4 and CD_8 in thymus

Group	Rats	CD_4(%)	CD_8(%)	CD_4/CD_8
Normal	10	54.1±1.4[a]	34.1±1.2	1.58
Model	8	40.2±2.1[c]	31.7±1.3	1.26[c]
Colchicine	9	42.1±2.0[c]	32.1±0.9	1.31[c]
AF	9	50.8±3.8[a]	34.8±2.1	1.45
AS	9	52.6±3.4[a]	35.9±2.2	1.46

[a]P<0.05, *vs* Colchicine group; [c]P<0.05, *vs* normal control group.

Anti-fibrosis herbs and anti-fibrosis herbs plus selenium treatment reduced TGF-b_1 expression

Positive staining of TGF-β_1 was found at central vein and Disse's areas but not at hepatocytes on sections of normal controls, whereas on sections of model control group, the positive staining was seen at interstitial cells, inflammatory cells, impaired hepatocytes as well as normal hepatocytes. Fibrotic septa were only slightly stained.

Compared with model control group, the staining index of TGF-β_1 in AF and AS groups was markedly decreased (P<0.05 in both groups). TGF-β_1 mRNA was detected in normal rat liver, but the expression level was increased significantly in model control group. Compared with colchicine group, TGF-β_1 level in AF and AS groups was markedly decreased (P<0.05, Table 4). Thus, the data at both transcript and protein levels suggested that anti-fibrosis herbs and anti-fibrosis herbs plus selenium could reduce TGF-β_1 expression in hepatic fibrosis.

Table 4 Level of TGF-β_1 mRNA in relation to GAPDH

Group	Rats	Ratio
Normal	10	0.57±0.11
Model	8	2.34±0.43[c]
Colchicine	9	1.88±0.21[c]
AF	9	1.07±0.31[a]
AS	9	0.98±0.21[a]

[a]P<0.05, *vs* Colchicine group; [c]P<0.05, *vs* normal control group.

Anti-fibrosis herbs and anti-fibrosis herbs plus selenium treatment reduced NF-kB expression

Positive staining of NF-κB was not found on sections of normal control group. In model control group, NF-κB was extensively expressed in nuclei of hepatocellular cells. The cells positive for NF-κB were diffusely distributed. After treatment with anti-fibrosis herbs and anti-fibrosis herbs plus selenium, the level of NF-κB staining decreased markedly.

DISCUSSION

Hepatic fibrosis is a common pathological process of chronic hepatic disease, resulting in development of irreversible cirrhosis in patients. In recent years, the mechanism of development of hepatic fibrosis has been partly disclosed[2,4,5]. If treated properly at fibrosis stage, cirrhosis could be prevented. The present study demonstrated that administration of anti-fibrosis herbs and selenium was effective in treating hepatic fibrosis in rats based on both histological examination and functional analysis. The underlying therapeutic mechanism may involve enhanced immunity and down regulation of the expression of NF-κB and TGF-β_1.

There are various kinds of chronic liver injuries all over the world, causing great affliction to patients. The incidence of hepatitis in China is high. Searches for effective ways to inhibit fibrogenesis and to prevent the development of cirrhosis are of great significance. Although many agents were tested, there have been no satisfactory agents with ascertained effectiveness and few side effects. Colchicine has been commonly used for anti-fibrosis[21], but its side effect is high and its clinical use is, therefore, limited. Chinese herbs, well known for their wide range of effectiveness and low prices and few side effects, have particular potentials in the treatment of hepatic fibrosis. In this study anti-fibrosis herb treatment for chronic liver injury in rats, prevented hepatic fibrosis from developing of cirrhosis was shown by histological grading. HA and LN have been found to be good serum markers of hepatic fibrogenesis[22]. We showed that the serum content of HA and LN in AF group and AS group dropped markedly when compared with colchicine group, indicating that anti-fibrosis herb could prevent hepatic fibrogenesis. Anti-fibrosis herb could also enhance the immunity of the body by increasing the percentage of CD_4 and the ratio of CD_4 to CD_8 in AF group, especially in AS group, compared with that in colchicine group.

To understand the mechanism, we evaluated the effect of anti-fibrosis herb treatment on the expression of TGFβ_1 at both the protein and mRNA levels as TGFβ1 has been considered to be the key cytokine in acceleration of the cirrhotic procession and over expression of this cytokine was closely associated with fibrogenesis in many ways[23-27]. Our results showed that

both TGFβ_1 and its mRNA expression decreased significantly in AF group and AS group compared with those in control groups, indicating that anti-fibrosis herb down-regulated the expression of this cytokine, which may have contributed to the reduction of fibrosis.

NF-κB is known to be a family of dimeric transcription factors. It was ubiquitously expressed in non-B cells as an inactive form sequestered in cytoplasm by binding to specific inhibitory proteins termed I-κB[28-32]. When cells were stimulated by inducing agents, the I-κB became phosphorylated, ubiquitinated, and degraded. Degradation of I-κB could free NF-κB, which was then translocated into the nucleus, where it activate transcription[33-35]. NF-κB/Rel has been shown to be implicated in the inflammatory response and synthesis of adhesion molecules. Furthermore, NF-κB has been found to be related to cell proliferation and transformation[36]. Down-regulation of NF-κB/Rel activity could decrease the transcription of TGFβ_1 to reduce the liver injury.

REFERENCES

1 **Missale G,** Ferrari C, Fiaccadori F. Cytokine mediators in acute inflammation and chronic course of viral hepatitis. *Ann Ital Med Int* 1995; **10**: 14-18
2 **Wang YJ,** Sun ZQ. Advance in cytology and molecular biology investigation in liver fibrosis. *Xin Xiaohua Bingxue Zazhi* 1994; **2**: 244-246
3 **Wang FS,** Wu ZZ. Current situation in studies of gene therapy for liver cirrhosis and liver fibrosis. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 371-373
4 **Zhu YH**, Hu DR, Nie QH, Liu GD, Tan ZX. Study on activation and c-fos, c-jun expression of *in vitro* cultured human hepatic stellate cells. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 299-302
5 **Du WD**, Zhang YE, Zhai WR, Zhou XM. Dynamic changes of type I, III and IV collagen synthesis and distribution of collagen-producing cells in carbon tetrachloride-induced rat liver fibrosis. *World J Gastroenterol* 1999; **5**: 397-403
6 **Huang ZG**, Zhai WR, Zhang YE, Zhang XR. Study of heteroserum-induced rat liver fibrosis model and its mechanism. *World J Gastroenterol* 1998; **4**: 206-209
7 **Jia JB**, Han DW, Xu RL, Gao F, Zhao LF, Zhao YC, Yan JP, Ma XH. Effect of endotoxin on fibronectin synthesis of rat primary cultured hepatocytes. *World J Gastroenterol* 1998; **4**: 329-331
8 **Cheng ML**, Wu YY, Huang KF, Luo TY, Ding YS, Lu YY, Liu RC, Wu J. Clinical study on the treatment of liver fibrosis due to hepatitis B by IFN-α_1 and traditional medicine preparation. *World J Gastroenterol* 1999; **5**: 267-269
9 **Riley TR 3rd**, Bhatti AM. Preventive strategies in chronic liver disease: part II. Cirrhosis. *Am Fam Physician* 2001; **64**: 1735-1740
10 **Liu YK**, Shen W. Inhibitive effect of cordyceps sinensis on experimental hepatic fibrosis and its possible mechanism. *World J Gastroenterol* 2003; **9**: 529-533
11 **Ma X**, Qiu DK, Xu J, Zeng MD. Effects of Cordyceps polysaccharides in patients with chronic hepatitis C. *Huaren Xiaohua Zazhi* 1998; **6**: 582-584
12 **Yang Q**, Yan YC, Gao YX. Inhibitory effect of Quxianruangan Capsulae on liver fibrosis in rats and chronic hepatitis patients. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 1246-1249
13 **You H**, Wang B, Wang T. Proliferation and apoptosis of hepatic stellate cells and effects of compound 861 on liver fibrosis. *Zhonghua Ganzangbing Zazhi* 2000; **8**: 78-80
14 **Nan JX**, Park EJ, Kim YC, Ko G, Sohn DH. Scutellaria baicalensis inhibits liver fibrosis induced by bile duct ligation or carbon tetrachloride in rats. *J Pharm Pharmacol* 2002; **54**: 555-563
15 **Wang QC**, Shen DL, Zhang CD, Xu LZ, Nie QH, Xie YM, Zhou YX. Effect of Rangansuopiwan in expression of tissue inhibitor of metalloproteinase-1 / 2 in rat liver fibrosis. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 379-382
16 **Shen M**, Qiu DK, Chen Y, Xiong WJ. Effects of recombinant augmenter of liver regeneration protein, danshen and oxymatrine on rat fibroblasts. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 1129-1133
17 **Wang XL**, Liu P, Liu CH, Liu C. Effects of coordination of FZHY decoction on functions of hepatocytes and hepatic satellite cells. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 663-665
18 **Yao XX**, Tang YW, Yao DM, Xiu HM. Effect of yigan decoction on the expression of type I, III collagen proteins in experimental hepatic fibrosis in rats. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 263-267
19 **Hang M**, Song G, Minuk GY. Effects of hepatic stimulator substance, herbal medicine, Selenium / Vitamin E, and ciproloxacin on cirrhosis in the rat. *Gastroenterology* 1996; **110**: 1150-1155
20 **Buljcbac M**, Roimic Z, Vucclic B. Serum selenium concentration in patients with liver cirrhosis hepatocellular carcinoma. *Acta Med Crearice* 1996; **50**: 11-15
21 **Weng HL**, Cai WM, Liu RH. Animal experiment and clinical study of effect of gamma interferon on hepatic fibrosis. *World J Gastroenterol* 2001; **7**: 42-48
22 **Li BS**, Wang J, Zhen YJ, Liu JX, Wei MX, Sun SQ, Wang SQ. Experimental study on serum fibrosis markers and liver tissue pathology and hepatic fibrosis in immuno-damaged rats. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 1031-1034
23 **Bissell DM**. Chronic liver injury, TGF-beta, and cancer. *Exp Mol Med* 2001; **33**: 179-190
24 **Friedman SL**. Cytokines and fibrogenesis. *Semin Liver Dis* 1999; **19**: 129-140
25 **Chen WX**, Li YM, Yu CH, Cai WM, Zheng M, Chen F. Quantitative analysis of transforming growth factor beta 1 mRNA in patients with alcoholic liver disease. *World J Gastroenterol* 2002; **8**: 379-381
26 **Gressner AM**, Weiskirchen R, Breitkopf K, Dooley S. Roles of TGF-beta in hepatic fibrosis. *Front Biosci* 2002; **7**: d793-807
27 **Lewindon PJ**, Pereira TN, Hoskins AC, Bridle KR, Williamson RM, Shepherd RW, Ramm GA. The role of hepatic stellate cells and transforming growth factor-beta (1) in cystic fibrosis liver disease. *Am J Pathol* 2002; **160**: 1705-1715
28 **Beraud C**, Henzel WJ, Baeuerle PA. Involvement of regulatory and catalytic subunits of phosphoinositide 3-kinase in NF-κB activation. *Proc Natl Acad Sci U S A* 1999; **96**: 429-434
29 **Regnier CH**, Song HY, Gao X, Goeddel DV, Cao Z, Rothe M. Identification and characterization of an IκB kinase. *Cell* 1997; **90**: 373-383
30 **Zandi E**, Karin M. Bridging the gap: composition, regulation, and physiological function of the IκB kinase complex. *Mol Cell Biom* 1999; **19**: 4547-4551
31 **May MJ**, Ghosh S. IκB kinases: Kinsmen with different crafts. *Science* 1999; **284**: 271-273
32 **Baeuerle PA**. IκB-NF-κB structures: at the interface of inflammation control. *Cell* 1999; **95**: 729-731
33 **Baeuerle PA**, Henkel T. Function and activation of NF-κB in the immune system. *Annu Rev Immunol* 1994; **12**: 141-179
34 **Ozes ON**, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NF-κB activation by tumour necrosis factor requires the Akt serine -threonine kinase. *Nature* 1999; **401**: 83-85
35 **Romashkova JA**, Makarov SS. NF-κB is a target of AKT in anti-apoptotic PDGF signalling. *Nature* 1999; **401**: 86-90
36 **Hinz M**, Krappmann D, Eichten A, Scheidereit C, Strauss M. NF-κB function in growth control: regulation of cyclin D1 expression and G0-G1-to-S-phase transition. *Mol Cell Biol* 1999; **19**: 2690-2698

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