Methodologic research on TIMP-1, TIMP-2 detection as a new diagnostic index for hepatic fibrosis and its significance

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AIM: To set up a new method to detect tissue inhibitors of metalloproteinase-1 and -2 (TIMP-1 and TIMP-2) in sera of patients with hepatic cirrhosis, and to investigate the expression and location of TIMP-1 and TIMP-2 in liver tissue of patients with hepatic cirrhosis, and the correlation between TIMPs in liver and those in sera so as to discuss whether TIMPs can be used as a diagnosis index of hepatic fibrosis.

METHODS: The monoclonal antibodies (McAbs) of TIMP-1 and TIMP-2 were used to sensitize erythrocytes, and solid-phase absorption to sensitized erythrocytes (SPASE) was used to detect TIMP-1 and TIMP-2 in the sera of patients with hepatic cirrhosis. Meanwhile, with the method of in situ hybridization and immunohistochemistry, we studied the mRNA expression and antigen location of TIMP-1 and TIMP-2 in the livers of 40 hepatic cirrhosis patients with pathologic diagnosis.

RESULTS: With SPASE, they were 16.4% higher in the acute hepatitis group, 33.3% higher in the chronic hepatitis group, and the positive rates were 73.6% and 61.2% respectively in sera of hepatic cirrhosis patients, which were remarkably higher than those in chronic hepatitis and acute hepatitis group (P<0.001). In 40 samples of hepatic cirrhosis tissues, all of them showed positive expression of TIMP-1 and TIMP-2 mRNA detected with immunohistochemistry or in situ hybridization (positive rate was 100%). Expression of TIMPs in different degrees could be found in liver tissue with cirrhosis. TIMPs were located in cytoplasm of liver cells of patients with hepatic cirrhosis. There was a significant correlation between serum TIMPS level and liver TIMPs level.

CONCLUSION: SPASE is a useful method to detect the TIMP-1 and TIMP-2 in sera of patients with hepatic cirrhosis, and TIMP-1 and TIMP-2 can be considered as a useful diagnostic index of hepatic fibrosis, especially TIMP-1.

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temperature, fixed at 56°C, washed with PBS 3-4 times, to get rid of the unfixed McAb molecule. 100µL per well of the prepared serum sample (diluted at the ratio volume of 1:10) was added, reacted at 37°C for 1h, washed with PBS 3-4 times, and then 50µL per well of McAb sensitized erythrocytes were added. After shaking mixed, they were standing for 1h at 37°C or 2h at room temperature. The results were determined according to the absorbed condition of erythrocytes (like RPHA). The erythrocytes depositing on the bottom of the well was considered as negative result, showing a spot or a little circle in the center of the well without scattered erythrocytes around. The positive result was determined when the erythrocytes were absorbed and a layer spread on the bottom of the well.

**Immunohistochemical staining** The laboratory procedure referred to references 6-12.SP immunostaining was performed as described by streptomyacin avidin-peroxidase immunochrometry kit (purchased from Maxim Biological Technology Company). Briefly, the liver samples were embedded with paraffin, and serial sections at 4µm thickness were prepared. Paraffin was removed from the sections with xylene and rehydrated with graded ethanol. After the antigens were repaired, unspecific immunoglobulin-binding sites were blocked by a 20min preincubation with 100mL·L⁻¹ normal human sera. The sections were then incubated with monoclonal antibody against TIMP-1 or TIMP-2 at 4°C overnight, and then secondary antibody was added at 37°C for 30-40min, avidin-peroxidase at 37°C for 20min, and finally DAB was added to be stained. After the sections were washed several times, they were counterstained with hematoxylin, dehydrated with ethanol, rinsed in xylene, and the sections were mounted with gum for microscopic examination and photography. To make sure of the reliability and specificity of the result of immunohistochemical staining, rabbit sera and PBS were used to replace the first antibody in our control test. 10 normal liver tissues were also used as the normal control samples.

**Liver tissue in situ hybridization** The investigation procedure referred to related references[13-17]. The in situ hybridization kit was purchased from Boshide Biological Technology Limited Company (Wuhan, China, No. MK1549). In situ hybridization was performed according to the manufacturer’s direction. Briefly, the paraffin embedded serial sections(thickness 4µm), were dried at 80°C, and their paraffin was removed by xylene and rehydrated with graded ethanol. The sections were acidified in HCl for 30min, and blocked in 3mL 300mL·L⁻¹H₂O₂ for 10min before digestion in proteinase K for 30min, and then dehydrated with graded ethanol. After prehybridization at 37-40°C for 2h, the labeled cDNA probes of TIMP-1 and TIMP-2 were denatured in hybridization buffer at 95°C for 10min, then -20°C for 10min, added on tissues which had been prehybridized at 37°C overnight. Sections were washed in turn with 2×SSC, 1×SSC, 0.2×SSC, and Buffer I, blocking water was added at room temperature for 20min, and then rabbit anti-digoxin serum at 37°C for 60min, biotinylated goat anti-rabbit serum at 37°C for 30min, SABC at 37°C for 30min, finally DAB was added to be stained. After several times of washing, the sections were counterstained with hematoxylin, dehydrated with ethanol, rinsed in xylene, and mounted with gum for microscopic examination and photography. (1) Blank control: prehybridization solution was replaced by the cDNA probes of TIMP-1 and TIMP-2 to be hybridized with the positive liver sections; (2) Negative control: the in situ hybridization was performed with 10 normal liver sections.

Semi-quantitative index was used to determine the results of immunohistochemistry and in situ hybridization: no positive cells (-); positive cells occupied hepatocytes of hepatic lobulef less than than1/3(+); 1/3-2/3(++); more than 2/3(+++).

**RESULTS**

**Methodologic Optimization**

The best laboratory condition and influence factors To study the related factor of sensitivity and specificity of this technique, the simulated positive samples were used, and we concluded that there were two main factors might directly influence the experiment condition. One was the concentration of coated antibodies and the other the density of sensitized erythrocytes. Much higher or less concentration of coated antibodies would decrease the sensitivity, while 50-100mg·L⁻¹ might be the best (Table 1). There was a close relation between the density of sensitized erythrocytes, which is the indicator, and at 2.5·L⁻¹ the sensitivity appeared to be the best (Table 2).

**Degree of accuracy and repetitive test** The coefficient of variation (CV) in one lot of samples in the same plate was 6.06%. The coefficient of variation (CV) in different lot of samples in different plate was 7.65%. Both of them were less than 10% (Table 3), which showed that this technique was provided with good degree of accuracy and repetition.

**Clinical Application**

**Serum samples** The 408 serum samples of patients with liver disease were used to detect TIMP-1 and TIMP-2 with SPASE. The
positive rates were 73.6% and 61.2% respectively, which were remarkably higher than those in chronic hepatitis and acute hepatitis group. There was a significant statistical difference ($P<0.001$, Table 4).

**Immunohistochemistry** By immunohistochemistry detection, the positive signal as brown particles were scattered or diffused only in cytoplasm other than nuclei in liver cells. Table 5 represented the result of detecting TIMP-1 and TIMP-2 of 40 liver samples of hepatic cirrhosis, in which, for TIMP-1, 28 samples were (+++), 70.0% of cytoplasm; 4 were (++), 10.0%; 8 were (+), accounted for 20.0%. For TIMP-2, 22 samples were (+++), 55.0% of cytoplasm; 10 were (++), 25.0%; 8 were (+), 20.0%. 10 normal liver tissues were negative. There was no positive signal after abridging the first antibody or the second one, and there was no positive signal when the first antibody replaced by rabbit serum or PBS, which proved that the results of immunohistochemistry detecting were specific.

**In situ hybridization** The positive signal of *in situ* hybridization showed brown particles, and distributed in the cytoplasm, scattered or diffused. There was no positive signal in nuclei. All the 40 samples of hepatic cirrhosis tissues showed positive expression of TIMP-1 and TIMP-2 mRNA, and the positive rate was 100%. Table 6 showed the intensity of TIMP-1 and TIMP-2 mRNA expression in the liver samples. For TIMP-1 mRNA, 32 samples were (+++), 80.0% of cytoplasm; 6 were (++), 15.0%; 2 were (+), 5.0%. Of TIMP-2 mRNA, 22 samples were (+++), in which 55.0% of cytoplasm; 16 were (++), accounted for 40.0%; 2 were (+), 5.0%. The expression intensity of TIMP-1 mRNA was stronger than that of TIMP-2 mRNA. There was no positive signal when TIMP-1 cDNA probes or TIMP-2 cDNA probes were replaced by the prehybridization mRNA. There was no positive signal when TIMP-1 cDNA probes or TIMP-2 cDNA probes, all were negative. These proved that the results of *in situ* hybridization were specific.

| Table 5 Expressing of TIMP-1 and TIMP-2 in liver |
|-----------------------------------------------|
| Group                  | n   | TIMP-1 | TIMP-2 |
|------------------------|-----|--------|--------|
| Normal group           | 10  | 0      | 0      |
| Hepatic cirrhosis      | 40  | 8      | 28     |

| Table 6 Expressing of TIMP-1 mRNA and TIMP-2 mRNA in liver of hepatic cirrhosis |
|-----------------------------------|
| Group                        | n   | TIMP-1 | TIMP-2 |
| Hepatic cirrhosis             | 40  | 32     | 6      |
| Normal liver                  | 10  | 0      | 0      |

**DISCUSSION**

SPASE is a new method used to detect the TIMP-1 and TIMP-2 in sera of patient with hepatic cirrhosis. During the setting up of this technique, we preliminarily tried to find out how to treat the influence factors and choose the condition. We consider that this method can not only possess both the advantages of SPA and IHT (indirect hemagglutination test), but also avoid the radiation pollution of RIA or cancer-causing danger of ELISA. Meanwhile, this method possesses many other advantages, such as wider range of usage, less influence factors, saving McAb, being easy to operate and judge detecting results. In a word, this is a specific, sensitive, rapid and economic method. There are two main factors that may influence the laboratory condition. Firstly, lower concentration of coated antibodies may result in fewer antibodies combined on the surface of support, which won’t be able to catch antigens, while higher density may result in overlap of antibodies on the surface of support, which will have effect on the space position of conjunction of antigens and antibodies, and then antigens can’t be effectively caught. Secondly, acting as the indicator system, a reverse correlation exists between the density of sensitized erythrocyte and sensitivity of detection. When the density of sensitized erythrocyte is higher than 3L$^{-1}$, the detection sensitivity will be significantly decreased, while clarity of the result would be impaired if the density is lower than 1L$^{-1}$. Because the erythrocytes can only react with one layer of the agent on the surface of solid support, excessive erythrocytes didn’t combine with agents and would deposit at the bottom of wells, then the judgment of the results would be affected. For this reason, a layer of the sensitized erythrocytes with adequate density is better. The density we used in practice is about 2.5L$^{-1}$, which is lower than that used in RPHA. In present study, the way of coating the antibodies was improved, and proved that the method of heating-fixed antibodies was feasible. Sheep erythrocytes and chicken erythrocytes sensitized by formaldehyde were detected with sensitization test, and no specific difference was observed except a little faster sedimentation rate of the former. This insured the source of erythrocytes.

The occurrence and progress of hepatic cirrhosis were the result of the interaction between hepatocytes and extracellular matrixes (ECM$^{[18-25]}$). The increase of ECM synthesis and decrease of ECM degradation will result in excess deposition of ECM in liver. More important reason of the excess deposition of ECM is the decrease of ECM degradation in the late stage. The metrical metalloproteinases played a leading role during the degradation of ECM$^{[26-30]}$. MMPs were a group of zinc-ion dependent enzymes, which created conditions for further degradation of other proteinases though reducing the stability of helical structure of collagen and changing the secondary structure of substrates. TIMPs were a group of polypeptides with the ability of inhibiting the function of MMPs. TIMPs would inhibit the degradation of ECM through two main ways, which is non-covalent modification or conjugated with proenzyme. Research work showed that TIMP could be divided into four classes: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. However, only TIMP-1 and TIMP-2 could be detected in liver$^{[37-44]}$. All the 40 samples of hepatic cirrhosis tissues, showed positive expression of TIMP-1 and TIMP-2, and the positive rate was 100%. TIMP-1 and TIMP-2 were expressed at the same time, and were located in the liver cytoplasm, not in the nuclei. The expression of TIMP-1 was more obvious than that of TIMP-2. No expression of related antigens of TIMP-1 and TIMP-2 were detected. All these indicated that TIMP-1 play an important role in the development of liver fibrosis and liver cirrhosis. The inhibitory effect of MMPs was enhanced with the high level expressing of TIMP-1 and TIMP-2 which resulted in the decrease of degradation of ECM, the deposit of ECM and the development of liver fibrosis and liver cirrhosis. The cause of the higher expression of TIMP-1 probably lay in two main reasons. First, the different classes of MMP resulted in the different inhibition activity of TIMP to MMP. TIMP-1 to procollagenase (MMP1) and TIMP-2 to gelatinase-A (MMP2), and gelatinase-B (MMP9) had assumed stronger inhibition activity. During the degradation of ECM, the main one was MMP1, and collagen I, III were the main object of MMP1. Second, the promotion of TIMP-1 was 10 times more than TIMP-2 to the proliferation of cells (including fibroblast, epithelial cells, endothelial cells, and smooth muscle cells). Furthermore, this action had no relation with the inhibition action of TIMP to MMP$^{[45-59]}$.

*In situ* hybridization was mainly used to observe the characteristics and accurate location of gene expression. The high sensitivity and strong specificity of this technique will be preferable to
study the pathogenesis of hepatic fibrosis and to demonstrate the diagnosis. Recently, digoxin has become a widely used non-isotope labeled compound characterized by its perfect specific and stability. Its sensitivity is almost the same as isotope, but without pollution. So it is easily accepted and used to label TIMP-1 and TIMP-2 cDNA probe to detect the paraffin sections of liver. The results showed that this technique was high sensitive and specific. The rates of positive expressions of TIMP-1 and TIMP-2 were 80.0% and 55.0% respectively. The location of TIMPs expression was in cytoplasm of hepatocyte, except nuclei, and the mRNA expression of TIMP-1 was stronger than that of TIMP-2, which was in accordance with the results of immunohistochemistry, and further proved that the TIMPs played a key role in the development of hepatic fibrosis and hepatic cirrhosis.

It is now known that there is a noticeable increase of TIMP-1 in the injured liver, which takes place earlier and increases faster, therefore, more and more researchers has regarded it as the diagnostic index of hepatic fibrosis. Detecting TIMP-1 and TIMP-2 with SPASE was used as a quick laboratory diagnosis of hepatic fibrosis. The sera from hepatic cirrhosis patients pathologically confirmed and normal people served as positive and negative control respectively. The results of detecting TIMP-1 and TIMP-2 in the sera of 408 patients with hepatic disease showed that positive rate of TIMPs was higher in sera of hepatic cirrhosis patients than that of acute or chronic hepatitis (P<0.001). TIMPs in sera of chronic hepatitis patients were apparently higher than those of acute hepatitis patients. This conclusion enumerated above was Supported by the detecting results of TIMP-1 and TIMP-2 by using immunohistochemistry and in situ hybridization.

Recently, Murawaki et al[60-62] has detected TIMP-1 and TIMP-2 in sera of patients with chronic liver disease by means of ELISA, and found a good relation between TIMP-1 and TIMP-2 in sera and in liver. The sensitivity and specificity of TIMP-1 were higher than those of TIMP-2. In injured liver, especially in fibrotic liver, TIMP-1 predominated, and the degree of TIMP-1 was remarkably related to the severity of hepatic fibrosis. Compared with TIMP-1, the specificity and sensitivity of TIMP-2 were inadequate for diagnosis of hepatic fibrosis. So, TIMP-1 was more important than TIMP-2 in the determination of histological change of hepatic fibrosis[60-70]. This was proved by our study of the expression of related antigens and the location of mRNA of TIMP-1 and TIMP-2. The expression of related antigens in liver could be reflected through detecting TIMPs in sera. So, TIMP-1 and TIMP-2 could be considered as useful diagnostic index of hepatic fibrosis, especially TIMP-1. Because viral hepatitis is common in China[71-99], liver fibrosis is the focus of diagnostic index of hepatic fibrosis, especially TIMP-1. Because of related antigens in liver could be reflected through detecting TIMPs in the determination of histological change of hepatic fibrosis[63-70]. The specificity and sensitivity of TIMP-2 were inadvisable for diagnosis to the severity of hepatic fibrosis. Compared with TIMP-1, the predominated, and the degree of TIMP-1 was remarkably related and found a good relation between TIMP-1 and TIMP-2 in sera and in sera of patients with chronic liver disease by means of ELISA, in practice in the future.

REFERENCES

1 Nie QH, Huang C, Zhang KR, Peng BM. SPASE for rapid detection of Shigella flexneri form fecal samples with monoclonal antibody. Zhonghua Chuanranbing Zazhi 1995;13:145-148
2 Nie QH, Huang C, Zhang KR, Peng BM. Preparation of prevalent monoclonal antibodies of Shigella flexneri and their application in rapid diagnosis of shigellosis. Seventh International Congress on Rapid Methods and Automation in Microbiology and Immunology (LONDON). Programme Abstracts 1993:41
3 Li CW. The modern immunohistochemical technique. First ban. Shanghai: Shanghai Sci. & Tech. Publishing House 1992:235-239
4 Han CY. Indirect hemagglutinative Technique. Beijing: Sci Publi House 1979:94-111
5 The prevention and treatment program of viral hepatitis. Zhonghua Neike Zazhi 1995;34:788-791
6 Nie QH, Xie Q, Hu DR, Li MD, Li L. The expression of hepatitis G virus-related antigens in the liver tissue of patients with HGV/GBV-C infection. Di-san Junyi Daxue Xuebao 1997;19:394-396
7 Nie QH, Hu DR, Li MD, Xie Q. The expression of HGV/GBV-C or HCV related antigens in the liver tissue of patients coinfected with hepatitis C and G viruses. Shijie Huanwen Xuebao Zazhi 2000;8:114-115
8 Nie QH, Li MD, Hu DR, Li L. The expression of hepatitis G virus-related antigens in the liver tissues of patients with hepatitis G. Zhonghua Chuanranbing Zazhi 2000;18:173-175
9 Wang D, Shi QJ, Liu FX. Immunochemical detection of proliferating cell nuclear antigen in hepatocellular carcinoma. China Natl J New Gastroenterol 1997;3:101-103
10 Zhang LG, Peng WW, Yao JL, Tang YH. Immunochemical detection of HCV infection in patients with hepatocellular carcinoma and other liver diseases. World J Gastroenterol 1998;4:64-65
11 Yan JP, Liu JC, Ma XH, Jia JB, Zhao YC, Xu RL, Li CM, Han DW. Immunohistochemical O study on basic fibroblast growth factor in experimental liver fibrosis. Xin Xiangoubangxi Zazhi 1997;5:642-644
12 Nie QH, Li L, MD, Hu DR. Clinical and immunopathological study on GB virus B (GBV-B) infection. Shijie Huanwen Xuebao Zazhi 2000;8: 775-781
13 Nie QH, Li MD, Hu DR. Detection of hepatitis G virus RNA in liver tissue using digoxigenin labelled probe by in situ hybridization. J Gastroenterol Hepatol 1999;14:A365
14 Liu YJ, Cong WM, Xie TP, Wang H, Shen F, Guo YJ, Chen H, Wu MC. Detecting the localization of hepatitis B and C virus in hepatocellular carcinoma by in situ hybridization. China Natl J New Gastroenterol 1996;2:187-189
15 Zhao GQ, Xue L, Xu HY, Tang XM, Hu RD, Dong J. In situ hybridization assay of androgen receptor gene in hepatocarcinogenesis. World J Gastroenterol 1998;4:503-505
16 Qian QJ, Xue HB, Ou Q, Kong SG, Cao HF, Wu MC. In situ detection of tumor infiltrating lymphocytes expressing perforin and fas-ligand genes in human HCC. World J Gastroenterol 1999;5:12-14
17 Nie QH, Hu DR, Li MD, Li L, Zhu YH. Detection of hepatitis G virus RNA in liver tissue using digoxigenin labelled probe by in situ hybridization. Shijie Huanwen Xuebao Zazhi 2000;771-774
18 Friedman SL. The cellular basis of hepatic fibrosis: mechanism and treatment strategies. N Engl J Med 1993;328:1828-1835
19 George DK, Ramam GA, Walker NI, Powell LW, Crawford DH. Elevated serum type IV collagen: a sensitive indicator of the presence of cirrhosis in haemochromatosis. J Hepatology 1999; 31: 47-52
20 Kossakowska AE, Edwards DR, Lee SS, Urbanski LS, Stabbler AL, Zhang CL, Phillips BW, Zhang Y, Urbanski SJ. Altered balance between matrix metalloproteinases and their inhibitors in experimental biliary fibrosis. Am J Pathol 1998;153:1895-1902
21 Arthur MJ, Mann DA, Iredale JP. Tissue inhibitors of metalloproteinases, hepatic stellate cells and liver fibrosis. J Gastroenterol Hepatol 1998; 13: S33-38
22 Arthur MJ. Role of Ito cells in the degradation of matrix in liver. J Gastroenterol Hepatol 1995; 10: S57-62
23 Arthur MJ. Collagenases and liver fibrosis. J Hepatology 1995; 22: S43-48
24 Arthur MJ. Degradation of matrix proteins in liver fibrosis. Pathol Res Pract 1994; 190: 825-833
25 Liu XS, Li DG, Lu HM, Xu QF. Effects of tetrandrine and verapamil on fibroblastic growth and proliferation. J Gastroenterol Hepatol 1999; 14: A365
26 Wang YF, Li QF, Wang H, Mao Q, Wu Q. Effects of vitamin E on experimental hepatic fibrosis in rats. World J Gastroenterol 1998;4:157
27 Huang ZG, Zhai WR, Zhang Y, Zhang XR. Study of heteroserum induced rat liver fibrosis model and its mechanism. World J Gastroenterol 1998;4:206-209
28 Jia JB, Han DW, Xu RL, Gao F, Zhao LF, Zhao YC, Yan JP, Ma XH. Effect of endotox in on fibronectin synthesis of rat primary cultured hepatocytes. World J Gastroenterol 1998;4:329-331
29 Cheng ML, Wu YY, Huang HK, Luo TY, Ding YS, Lu YY, Liu RC, Wu J. Clinical study on the treatment of liver fibrosis due to hepatitis B by IFNá-1 and traditional medicine preparation. World J Gastroenterol 1999;5:12-14
30 Qian QJ, Xue HB, Ou Q, Cao HF, Wu MC. Detection of hepatitis G virus RNA in liver tissue using digoxigenin labelled probe by in situ hybridization. Shijie Huanwen Xuebao Zazhi 2000;771-774
31 Dong J, Xu HY, Tang XM, Hu RD, Qi MQ. Detection of hepatitis C virus RNA in liver tissue using digoxigenin labelled probe by in situ hybridization. World J Gastroenterol 1998;4:664-666
32 Sun ZQ, Wang YJ, Quan QZ, Liu XF, Pan X, Jiang XL. Prevention and treatment action of tetrandrine on experimental liver fibrosis in rats. Xin Xiangoubangxi Zazhi 1994;2:19-20
33 Sun ZQ, Wang YJ, Quan QZ, Han GY, Jin XH. Change of serum phosphonate esterase in hepatic fibrosis in rats. Xin Xiangoubangxi Zazhi 1994;2:206-207
cDNA with polymerase chain reaction directed sequencing. China Natl J New Gastroenterol 1997;3:12-15
88 Zhou P, Cai Q, Chen YC, Zhang MS, Guan J, Li XJ. Hepatitis C virus RNA detection in serum and peripheral blood mononuclear cells of patients with hepatitis C. China Natl J New Gastroenterol 1997;3:108-110
89 Sun DG, Liu CY, Meng ZD, Sun YD, Wang SC, Yang YQ, Liang ZL, Zhuang H. A prospective study of vertical transmission of hepatitis C virus. China Natl J New Gastroenterol 1997;3:111-113
90 Gao JE, Tao QM, Guo JP, Ji HP, Lang ZW, Ji Y, Feng BF. Preparation and application of monoclonal antibodies against hepatitis C virus nonstructural proteins. China Natl J New Gastroenterol 1997;3:114-116
91 Zhang LF, Peng WW, Yao JL, Tang YH. Immunohistochemical detection of HCV infection in patients with hepatocellular carcinoma and other liver diseases. World J Gastroenterol 1998;4:64-65
92 Zhu PL, Lu HY, Li Z, Qi ZT. Cloning and expression of NS3 cDNA fragment of HCV genome of Hebei isolate in E. coli. World J Gastroenterol 1998;4:165-168
93 Soresi M, Carroccio A, Bonfissuto G, Agate V, Magliarisi C, Aragona F, Levrero M, Notarbartolo A, Montalto M. Ultrasound detection of abdominal lymphadenomegaly in subjects with hepatitis C virus infection and persistently normal transaminases: a predictive index of liver histology severity. World J Gastroenterol 1998;4:270
94 Yang JM, Wang RQ, Bu BG, Zhou ZC, Fang DC, Luo YH. Effect of HCV infection on expression of several cancer associated gene products in HCC. World J Gastroenterol 1999;5:25-27
95 Feng DY, Chen RX, Peng Y, Zheng H, Yan YH. Effect of HCV NS3 protein on p53 protein expression in hepatocarcinogenesis. World J Gastroenterol 1999;5:45-46
96 Huang F, Zhao GZ, Li Y. HCV genotypes in hepatitis C patients and their clinical significances. World J Gastroenterol 1999;5:547-549
97 Dai YM, Shou ZP, Ni CR, Wang NJ, Zhang SP. Localization of HCV RNA and capsid protein in human hepatocellular carcinoma. World J Gastroenterol 2000;6:136-137
98 Dai YM, Shou ZP, Ni CR, Wang NJ, Zhang SP. Localization of HCV RNA and capsid protein in human hepatocellular carcinoma. World J Gastroenterol 2000;6:136-137
99 Cheng JL, Tong WB, Liu BL, Zhang Y, Yan Z, Feng BF. Hepatitis C virus in human B lymphocytes transformed by Epstein-Barr virus in vitro by in situ reverse transcriptase chain reaction. World J Gastroenterol 2001;7:370-375
100 Nie QH, Li MD, Hu DR, Chen GZ. Study on the cause of human protective immunodeficiency after HCV infection. Shijie Huaren Xiaohua Zazhi 2000;8:28-30
101 Nie QH, Cheng YQ, Xie YM, Zhou YX, Cao YZ. Inhibiting effect of antisense oligonucleotides phosphorthioate on gene expression of TIMP-1 in rat liver fibrosis. World J Gastroenterol 2001;7: 363-369

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