Interaction between hepatitis C virus core protein and translin protein- a possible molecular mechanism for hepatocellular carcinoma and lymphoma caused by hepatitis C virus

Ke Li, Lin Wang, Jun Cheng, Yin-Ying Lu, Ling-Xin Zhang, Jin-Song Mu, Yuan Hong, Yan Liu, Hui-Juan Duan, Gang Wang, Li Li, Ju-Mei Chen

Abstract

AIM: To investigate the interaction between hepatitis C virus core protein and translin protein and its role in the pathogenesis of hepatocellular carcinoma and lymphoma.

METHODS: With the components of the yeast two hybrid system 3, “bait” plasmids of HCV core the gene was constructed. After proving that hepatitis C virus core protein could be firmly expressed in AH109 yeast strains, yeast two-hybrid screening was performed by mating AH109 with Y187 that transformed with liver cDNA library plasmids - pACT2 and then plated on quadrople dropout (QDO) medium and then assayed for α-gal activity. Sequencing analysis of the genes of library plasmids in yeast colonies that could grow on QDO with α-gal activity was performed. The interaction between HCV core protein and the protein we obtained from positive colony was further confirmed by repeating yeast two - hybrid analysis and coimmunoprecipitation in vitro.

RESULTS: A gene from a positive colony was the gene of translin, a recombinant hotspot binding protein. The interaction between HCV core protein and translin protein could be proved not only in yeast, but also in vitro.

CONCLUSION: The core protein of HCV can interact with translin protein. This can partly explain the molecular mechanism for hepatocellular carcinoma and lymphoma caused by HCV.

Li K, Wang L, Cheng J, Lu YY, Zhang LX, Mu J S, Hong Y, Liu Y, Duan HJ, Wang G, Li Li, Chen J M. Interaction between hepatitis C virus core protein and translin protein- a possible molecular mechanism for hepatocellular carcinoma and lymphoma caused by hepatitis C virus. World J Gastroenterol 2003; 9(2): 300-303. http://www.wjgnet.com/1007-9327/9/300.htm

INTRODUCTION

The core protein of hepatitis C virus (HCV) is the structural protein of the virus[1-4]. However, some evidences suggested that this protein has a pleiotropic nature. In addition to having a packaging function, the core protein has been shown to act in trans on the viral and cellular promoters and it is also capable of transformation of rat embryonic fibroblasts through cooperation with the ras oncogene. Previous studies showed that the core protein could interact with several proteins such as lymphotoxin-β Receptor, heterogeneous nuclear ribonucleoprotein K, RNA helicase[5-8]. In order to understand the pathogenesis of HCV infection we examined the possibility that the HCV core protein interacts with cellular proteins.

MATERIALS AND METHODS

Material

Bacterial, yeast strains and Plasmids All yeast strains and plasmids for yeast two-hybrid experiments were obtained from Clontech (Palo Alto, Calif., USA) as components of the MATCHMAKER Two Hybrid System 3. Yeast strain AH109 (MATα, trp1-901, leu2-3,112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1::GAL1 TATA-HIS3, GAL2::GAL1 TATA-ADE2 URA3::MEL1 TATA-lacZ MEL1) containing pGBK7-T3, coding for DNA-BD/mouse p53 fusing protein and AH109 used for cloning of bait plasmids, yeast strain Y187(MATα ura3-52, his3-200, Ade2-101, trp1-901, leu2-3, 112, gal4Δ, gal80Δ, met-, URA3::GAL1::GAL1 TATA-lacZ MEL1) containing pTD1-1, in which pACT2 coding for AD/SV40 large T antigen fused protein and Y187 used for cloning of library plasmids. Pretransformed cDNA liver cell library Y187. Bacterial strain DH5α used for cloning of every shuttle plasmid. Yeast- Escherichia coli shuttle plasmids pGBK7 T7 DNA-BD cloning plasmid, pGADT7 AD cloning plasmid, pGBK7-T3 control plasmid, pGADT7, pGBK7-Lam control plasmid, pCL1 plasmid from Clontech L.T.D Company(K1612-1). pGEM T vector from Promega Company, USA.

Chemical agents and cultural media Taq DNA polymerase purchased from MBI Company, T4 DNA ligase, EcoRI and BamHI restriction endonuclease from Takara. [35S]-coupled ATP from Zongshan Company, China. Lithium Acetate, semi-sulfate adenine, Acrylamide and N, N’-Bis-acrylamide from Sigma, TEMED from Boehringer Mannheim. Tryptone and yeast extracts from OXOID. X-α-Gal and Cultural media: YPDA, SD/Trp SD/-Leu, SD/-Trp/-Leu, SD/Trp/-Leu/-His, SD-Trp/-Leu/-His-Ade from Clontech L.T.D Company. Protein-G agarose from Roche. RT-PCR kit and TNT® Coupled Reticulocyte Lysates Systems from Promega. [35S]-methionine (.1000 Ci/mmol; 10 mCi/ml) from Isotope company of china. Amplify Fluorographic Reagent (#NAMP100) from Amershams Life Sciences. Others from Sigma company.

Methods

Construction of “bait” plasmid and expression of HCV core protein Plasmid pGBK7-core (Figure 1) containing full-
length HCV core gene was constructed by insertion of HCV core gene in-frame into EcoRI/BamHI site, which could direct expression of DNA binding domain, c-myc and core fusion protein. After the plasmid was transformed into yeast strain AH109 by using Lithium Acetate method[9]. Western blotting was performed to confirm the expression of the fusion protein by using c-myc monoclonal antibody. Transformed AH109 was cultured on quadruplo dropout media to exclude the auto-activity.

RT-PCR In order to clone the full-length gene, RT-PCRs were conducted by using PCR primers the designing based on the information of GenBank. The genes amplified by RT-PCR were ligated into yeast plasmid pGADT7.

In vitro translation Mixture of TNT reticulocyte 25 μl, TNT reactio buffer 2 μl, T7 TNT RNA polymerase 1μl, amino acids mixture (minus methionine, 1 mM) 1μl, [35S]methionine 2 μl, RNasin® nuclease inhibitor (40μl/μl) 1μl, DNA template (pGBK7-core or pGADT7-library gene) (0.5 μg/μl) 2 μl, ddH2O 50 μl, 30 °C incubated 90 minutes.

Comimmunoprecipitation The following reactants were combined in a 1.5-ml microcentrifuge tube on ice: Five μl in vitro translated bait protein, 5 μl in vitro translated library protein. The control only added 10 μl pGBK7-core plasmid. The mixtures were incubated at 30 °C for 1 hr. Then, the following reagents added into the reaction tubes: 470 μl commounprecipitation buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM DTT, 5 μg/ml aprotinin, 0.5 mM PMSF, 0.1 % Tween 20), 10 μl Protein-G Agarose Beads, 10 μl c-Myc Monoclonal Antibody. Incubated at 4 for 2 hr with continuous rocking. The tubes were centrifuged at 14 000 rpm for 1-2 min. The supernatants were removed. 0.5 ml TBST added to the tubes. Rinse steps were repeated three times. 15 μl SDS-loading buffer were added. The samples was heated at 80 °C for 5 min. The tubes were placed on ice. Briefly centrifuged, and 10 μl loaded onto an SDS-PAGE minigel to begin the electrophoretic separation. After electrophoresis, the gel was transferred to a tray containing Gel Fixation Solution, and placed on a rotary shaker for 10 min at room temperature. Rinsed the gel with H2O, then Amplify Fluorographic Reagent was added shaked for 20 min at room, then dried at 80 °C under constant vacuum. The gel was exposed to a X-ray film overnight at room temperature. The film was developed by using standard techniques.

RESULTS

Expression of the “bait” fusion protein Yeast strain AH109 transformed with pGBK7-core could stably express the fusion protein at high level (Figure 3) and could only grow on SD/-Trp medium and could not grow on QDO medium. Thus, the transformed yeast could be used for yeast hybrid analysis.

Figure 1 “bait” plasmid pGBK7-core.

Figure 2 To confirm the interaction.

Bioinformatic analysis After sequencing the positive colonies, the sequences blasted with GenBank to analogize the function of the genes.

Figure 3 Western blotting showed the expression of HCV core protein in yeast (arrow indicated). lane 1 is HCV core protein and lane 2 is negative control.

RT-PCR experiments The yeast two hybrid analysis showed 30 blue colonies grew on QDO plates containing X-α-Gal. After confirming the true interaction in yeast, we isolated the plasmids from the blue colonies containing only pGBK7-core and one library plasmid other than other plasmids. Sequencing the gene and blasted with the data from GenBank, a gene is translin. To further prove the interaction between HCV core protein and translin protein (Translin), a pair of primer were designed based on the gene of translin (Forward: 5’-GAA TTC ATG TCT GTG AGC GAG ATC TTC GTG G -3’, down: 5’-GGA TCC CTA TTT TTC AAC ACA AGC
TSGC C-3'), up and down primers containing EcoRI and BamHI restriction endonuclease site, respectively. Total RNAs were prepared from HepG2. A 687bp fragment was amplified by using RT-PCR (Figure 4A). After cut by EcoRI/BamHI, the fragment was in-frame ligated into pGADT7 EcoRI/BamHI site (Figure 4B).

Thus, the molecular mechanism by which the HCV core protein and HCV induce hepatocellular carcinoma is not clear. Recent epidemiological research indicated that a significant increase in the prevalence of HCV infection in a group of B-cell non-Hodgkin’s lymphoma (NHL) [22-29].

In this study, yeast two-hybrid system was used to clone oncogenic gene. Yeast two-hybrid system 3 based on the system originally designed by Fields and Song [30] is developed by Bendixen [31], which is commercially available from Clontech Company Ltd. In this system, the promoters controlling HIS3, ADE2, and MEL1 expression in AH109 have significantly fewer false positives and the simple mating protocol significantly reduces the labor and time involved in performing a two-hybrid library screening and improves the chances of finding rare protein-protein interactions and leads to more reproducible results.

The "bait" plasmid pGBKTT7-core was transformed into yeast strain. After mating with liver cDNA library yeast strain Y187, the diploid yeast cells were plated on QDO media containing X-α-gal, 30 true positives were obtained. Sequencing analysis of isolated library plasmids, we find one of the genes is translin [32] - a recombination hotspot binding protein. In order to further confirm the interaction between the expressed protein and HCV core protein, we performed the experiment of communoprecipitation of both proteins. A strong interaction between the HCV core protein and Translin protein in vitro was observed.

A number of studies have shown that chromosomal translocations either result in the activation of proto-oncogenes by joining them to immunoglobulin (Ig) or T-cell receptor genes or lead to the creation of tumor-specific fusion proteins. In man, such translocations consistently occur at particular sites in the genome. Translin protein, which specifically binds to the consensus sequences ATGCAG and GCCC (A/T) (G/C) (G/C) (A/T) found at the breakpoint junctions in many cases of chromosomal translocations, is a unique DNA binding protein [33]. The nuclear translocation of translin protein only happened in the time when the cells were treated with mutagen. translin protein may be a typical DNA end binding protein, which is in contrast with one of the other DNA binding proteins, the Ku antigen, that initially binds to DNA ends and then moves to internal positions within the DNA molecule [34]. Previous report showed that translin protein was not found in liver tumors. But in this study, we found the gene expressed in liver tumor cell HepG2 and in liver cDNA library. And the interaction between translin protein and HCV core protein not only existed in yeast, but also in vitro. The results suggested that translin protein may play a role in hepatocellular carcinoma. But there has had no report giving the evidence that the patients infected with HCV other than HBV have the chromosomal translocation, whether the hepatocellular carcinomas caused by infection of HCV have chromosomal translocation worthy of further studying.

The effects of translin protein on normal lymphocytes need the induction by some factors such as mutagens or biological factors (HCV infection). In lymphoproliferative disorder patients infected with HCV, some reports showed chromosomal translocation happened in B-cell [35,36]. Therefore, our report indicated a molecular mechanism that the interaction between HCV core protein and translin protein may trigger the B-cell progressing into lymphoma in patients infected with HCV. How the interaction between the HCV core protein and translin protein causes chromosomal translocation or rather, causes lymphoma, more experiments are necessary to elucidate it.

REFERENCES

1. Harada S, Watanabe Y, La Monic N, Suzuki T, Katayama T,
Takebe Y, Saito I, Miyamura T. Expression of processed core protein of hepatitis C virus in mammalian cells. J Virol 1991; 65: 3015-3021

2 Santolini E, Migliaccio G, La Monica N. Biosynthesis and biochemical properties of the hepatitis C virus core protein. J Virol 1994; 68: 3631-3641

3 Lo SY, Selby M, Tong M, Ou JH. Comparative studies of the core gene products of two different hepatitis C virus isolates: Two alternative forms determined by a single amino acid substitution. Virology 1994; 199: 124-131

4 Liu Q, Tackney C, Bhat RA, Prince AM, Zhang P. Regulated processing of hepatitis C virus core protein is linked to subcellular localization. J Virol 1997; 71: 657-662

5 Yasui K, Wakita T, Tsukiyama-Kohara K, Funahashi S, Ichikawa M, Kajita T, Moradpour D, Wands JR, Kohara M. The native form and maturation process of hepatitis C virus core protein. J Virol 1998; 72: 6048-6055

6 Mamiya N, Woodman HJ. Hepatitis C virus core protein binds to a DEAD box RNA helicase. J Biol Chem 1999; 274: 15753-15756

7 You LR, Chen CM, Yeh TS, Tsai TY, Mai RT, Lin CH, Lee YH. Hepatitis C virus core protein interacts with cellular putative RNA helicase. J Virol 1999; 73: 2841-2853

8 Chen CM, You LR, Hwang LM, Lee YH. Direct interaction of hepatitis C virus core protein with the cellular lymphotxin-beta receptor modulates the signal pathway of the lymphotxin-beta receptor. J Virol 1997; 71: 9417-9426

9 Matsumoto M, Hsieh TY, Zhu N, VanArsdale T, Hwang SB, Jeng KS, Gorbalenya AE, Lo SY, Ou JH, Ware CF, Lal MM. Hepatitis C virus core protein interacts with the cytoplasmic tail of lymphotxin-beta receptor. J Virol 1997; 71: 1303-1309

10 Gietz D, St Jean A, Woods RA, Schiestl RH. Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res 1992; 20: 1425

11 Yan ZY, Wang HL. Short protocols in molecular biology. Beijing: Scientific press 1998: 23-24

12 HeYW, Liu W, Zen L, Xiong KJ, Luo DD. Effect of interferon therapy in chronic hepatitis C. China Natl J New Gastroenterol 1996; 2: 179-181

13 Wei L, Wang Y, Chen HS, Tao QM. Sequencing of hepatitis C virus cDNA with polymerase chain reaction directed sequencing. China Natl J New Gastroenterol 1997; 3: 12-15

14 Tang ZY, Qi JY, Shen HX, Yang DL, Hao LJ. Short- and long-term effect of interferon therapy in chronic hepatitis C. China Natl J New Gastroenterol 1997; 3: 77

15 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

16 Assy N, Minuk GY. A comparison between previous and present histologic assessments of chronic hepatitis C viral infections in humans. World J Gastroenterol 1999; 5: 107-110

17 Huaang F, Zhao GZ, Li Y. HCV genotypes in hepatitis C patients and their clinical significances. World J Gastroenterol 1999; 5: 547-549

18 Yan FM, Chen AS, Hao F, Zhao XP, Gu CH, Zhao LB, Yang DL, Hao LJ. Hepatitis C virus may infect extrahepatic tissues in patients with hepatitis C. World J Gastroenterol 2000; 6: 805-811

19 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

20 Shih CM, Chen CM, Chen SY, Lee YH. Modulation of the trans-suppression activity of hepatitis C virus core protein by phosphorylation. J Virol 1995; 69: 1160-1171

21 Cho JW, Baek WK, Suh SI, Yang SH, Chang J, Sung YC, Suh MH. Hepatitis C virus core protein promotes cell proliferation through the upregulation of cyclin E expression levels. Liver 2001; 21: 137-142

22 Hahn CS, Cho YG, Kang BS, Lester IM, Hahn YS. The HCV core protein acts as a positive regulator of fas-mediated apoptosis in a human lymphoblastoid T cell line. Virology 2000; 276: 127-137

23 Sabile A, Perlemuter G, Bono F, Kohara K, Demaille F, Kohara M, Matsuura Y, Miyamura T, Brechot C, Barba G. Hepatitis C virus core protein binding to apolipoprotein AI and its secretion is modulated by fibrates. Hepatology 1999; 30: 1064-1076

24 Ray RB, Meyer K, Ray R. Suppression of apoptotic cell death by hepatitis C virus core protein. Virology 1996; 226: 176-182

25 Moriya K, Fujii H, Shintani Y, Totsuyanagi H, Tsutsuji T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med 1998; 4: 1055-1057

26 Moriya K, Totsuyanagi H, Shintani Y, Fujii H, Ishibashi K, Matsuura Y, Miyamura T, Koike K. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 1997; 78: 1527-1531

27 Ferri C, La Civita L, Longombardo G, Cecchetti R, Giannini C, Zignego AL. Type C chronic hepatitis complicated by B-cell lymphoma. Am J Gastroenterol 1995; 90: 2071-2072

28 Ferri C, Monti M, La Civita L, Carecchia G, Mazzaro C, Longombardo G, Lombardini F, Greco F, Passero G, Bombardieri S. Hepatitis C virus infection in non-Hodgkin’s B-cell lymphoma complicating mixed cryoglobulinaemia. Eur J Clin Invest 1994; 24: 781-784

29 Brind AM, Watson JP, Burt A, Kesteven P, Wallis J, Proctor SJ, Bassendine MF. Non-Hodgkin’s lymphoma and hepatitis C virus infection. Leuk Lymphoma 1996; 21: 127-130

30 Pioltelli P, Parganti L, Cassi E, Sani Torelli, Bilioli G, Maglione EM, Morra E. Hepatitis C virus infection and non-Hodgkin’s lymphoma. Leuk Lymphoma 1999; 30: 1532-1534

31 Fields S, Song O. A novel genetic system to detect protein-protein interactions. Nature 1989; 340: 245-246

32 Benedixen C, Gangloff F, Rothstein R. A yeast mating-selection scheme for detection of protein-protein interactions. Nucleic Acids Res 1994; 22: 1778-1779

33 Suriawinata A, Ye MQ, Emre S, Strauchen J, Thung SN. Hepatocellular carcinoma and non-Hodgkin lymphoma in a patient with chronic hepatitis C and cirrhosis. Arch Pathol Lab Med 2000; 124: 1532-1534

34 Zignego AL, Giannini C, Monti M, La Civita L, Carecchia G, Mazzaro C, Longombardo G, Lombardini F, Greco F, Passero G, Bombardieri S. A novel gene, Translin, encodes a recombinase-like protein. J Gen Virol 1996; 77: 3631-3641

35 Mamiya N, Woodman HJ. A reevaluation of hepatitis C virus infection in non-Hodgkin’s lymphoma. J Mol Biol 1997; 265: 67-78

36 Aoki K, Suzuki K, Sugano T, Tsatuka T, Nakahara K, Kuge O, Omori A, Kasai M. A novel gene, Translin, encodes a recombinase-like protein associated with chromosomal translocations. Nat Genet 1995; 10: 167-174

37 Kasai M, Matsuzaki T, Katayanagi K, Omori A, Miaziar RT, Strominger JL, Aoki K, Suzuki K. The translin ring specifically recognizes DNA ends at recombination hot spots in the human genome. J Biol Chem 1997; 272: 11402-11407

38 de Vries E, van Driel WB, Bergsma WG, Arnerberg AC, van der Vliet PC. HeLa nuclear protein recognizing DNA termini and translocating on DNA forming a regular DNA-multimeric protein complex. J Biol 1999; 205: 65-78

39 Zignego AL, Naito M, Katayama K, Zanuzzo ME, Mazzocca A, Ferri C, Giannini C, Monti M, Cani P, Villa GL, Laffi G, Gentilini P, et al. Translocation in chronic hepatitis C virus infection. Hepatology 2000; 31: 474-479

40 Zuckerman E, Zuckerman T, Sahar D, Strachman S, Attias D, Sabo E, Yeshurun D, Rowe J. bcl-2 and immunoglobulin gene rearrangement in patients with hepatitis C virus infection. Br J Haematol 2001; 112: 364-369

Edited by Zhang JZ