Screening of hepatocyte proteins binding to F protein of hepatitis C virus by yeast two-hybrid system

Yan-Ping Huang, Jun Cheng, Shu-Lin Zhang, Lin Wang, Jiang Guo, Yan Liu, Yuan Yang, Li-Ying Zhang, Gui-Qin Bai, Xue-Song Gao, Dong Ji, Shu-Mei Lin, Qing Shao

Aim: To investigate the biological function of F protein by yeast two-hybrid system.

Methods: We constructed F protein bait plasmid by cloning the gene of F protein into pGBKTK7, then recombinant plasmid DNA was transformed into yeast AH109 (a type). The transformed yeast AH109 was mated with plasmid DNA containing liver cDNA library in 2×YPDA medium. Diploid yeast was plated on synthetic dropout nutrient medium (SD/-Trp-Leu-His-Ade) containing X-gal for selection and screening. After extracting and sequencing plasmids from positive colonies, we underwent sequence analysis by bioinformatics.

Results: Thirty-six colonies were selected and sequenced. Among them, 11 colonies were zymogen granule protein, 5 colonies were zinc finger protein, 4 colonies were zinc-α2-glycoprotein, 1 colony was sialyltransferase, 1 colony was complement control protein factor I, 1 colony was vitronectin, and 2 colonies were new genes with unknown function.

Conclusion: The yeast two-hybrid system is an effective method for identifying hepatocyte proteins interacting with F protein of hepatitis C virus. F protein may bind to different proteins.

Key words: Hepatitis C virus; F protein; Yeast two-hybrid system

Hepatitis C virus (HCV) is the major etiologic agent of parenterally transmitted non-A, non-B hepatitis worldwide[1-3]. Most infected individuals are unable to eliminate the virus, resulting in a persistent infection in about 80% of cases. Chronically infected patients often develop progressive liver disease, cirrhosis, hepatic failure, and hepatocellular carcinoma (HCC)[4,5]. Because development of a robust cell culture system for HCV infection has remained elusive, little is known about HCV-host cell interactions and how they influence cell physiology or viral replication. There is no vaccine against HCV.

HCV, discovered by cDNA cloning in 1989, is a member of the Hepacivirus genus within the Flaviviridae family, and a 9.6-kb positive single-stranded RNA virus. The HCV genome carries a single open reading frame (ORF) flanked by untranslated regions and encodes a single polyprotein of about 3 010-3 033 amino acids. HCV polyprotein is cleaved by both host cell and viral proteases into at least 10 distinct structural and nonstructural protein products. The major structural proteins are a core (C) protein and two envelope proteins called E1 and E2 and a short hydrophobic peptide p7. Four major nonstructural proteins called NS2, NS3, NS4, and NS5 are also generated, two of which, NS4 and NS5 are further processed into smaller polypeptides called NS4A, NS4B, NS5A, and NS5B. Most nonstructural proteins have enzymatic activities critical for viral replication. An additional HCV protein, F (for “frameshift protein”) or ARFP (for “alternate reading frame protein”), encoded by the ORF that overlaps the core gene in the +1 frame (core+1 ORF), is synthesized in vitro from the initiator codon of the polyprotein sequence followed by a +1 ribosomal frameshift operating in the region of core codons 8-14[6-9]. Antibodies to F protein have been detected in HCV patients, indicating the production of F protein during natural HCV infection[10,11]. The function of F protein in the life cycle of HCV remains unknown. To investigate the biological
importance of F protein, we screened and identified the proteins interacting with HCV F protein by yeast two-hybrid system. One of the positive interacting proteins, named HCV F-interacting protein (HCV FBP2), was without known function.

**Bacterial yeast strains and plasmids**

All yeast strains and plasmids for yeast two-hybrid experiments were obtained from Clontech Co. (Palo Alto, CA, USA) as components of the MATCHMAKER two-hybrid system 3. Yeast strain AH109 (MATa, trpl-901, leu2-3, 112, ural3-52, his3-200, gal4 Δ, gal80 Δ, LYS2::GAL1 UAS-GAL1 TATA-HIS3, GAL2 UAS-GAL2 TATA-AD E2, URA3: MEL1 UAS-MEL1 TATA- LacZ) containing pGBK7T-53, coding for DNA-BD/ mouse p53 fusing protein was used for cloning of bait plasmid. Yeast strain Y187 (MATa ura3-52, his3-200, A de2-101, trpl-901, leu2-3, 112, gal4 Δ, gal80 Δ, mα, URA3:GAL1 UAS-GAL1 TATA-lacZ MEL1) containing pTD1-1 in which pACT2 coding for AD/ SV40 large T antigen fusing protein was used for cloning of library plasmids. Pretransformed human cDNA liver cell library Y187 and bacterial strain DH5α were used for cloning of each shuttle plasmid. Yeast-E.cherichia coli shuttle plasmids pGBK7D NA-BD, pGAD7T AD, pGBK7T-53, pGAD7T, pGBK7-Lam, pCL1 were from Clontech Co. (K1612-1). pGEM T vector was from Promega Co., USA.

**Chemical agents and culture media**

Taq DNA polymerase was purchased from MBI Co. T4 DNA ligase, E. coli, and BamHI restriction endonuclease were from Takara Co. c-myc mAb secreted by 1-9E10.2 hybridoma (ATCC), goat anti-mouse IgG conjugated with horseradish peroxidase were from Zhongshan Co., China. Lithium acetate, semi-sulfate adenine, acrylamide and horseradish peroxidase were from Zhongshan Co., China. c-myc mAb secreted by 1-9E10.2 hybridoma (ATCC), goat anti-mouse IgG conjugated with horseradish peroxidase were from Zhongshan Co., China. Lithium acetate, semi-sulfate adenine, acrylamide and horseradish peroxidase were from Zhongshan Co., China. Lithium acetate, semi-sulfate adenine, acrylamide and horseradish peroxidase were from Zhongshan Co., China. Lithium acetate, semi-sulfate adenine, acrylamide and horseradish peroxidase were from Zhongshan Co., China.

**Construction of "bait" plasmid and expression of HCV F protein**

HCV-F sequences were generated by PCR amplification of HCV plasmid (HCV strain 1b) containing coding sequences for all the structural and nonstructural proteins. The sequences of the primers, containing the EcoRI and BamHI restriction enzyme sites, are sense primer: 5'-GAA TTC ATG GCA CGA ATC CTA AAC C-3', and antisense primer: 5'-GGA TCC CTG CGG CTT CCA GAA C-3'. The PCR conditions were at 94 °C for 30 s, at 60 °C for 30 s, at 72 °C for 30 s. Ten nanograms of PCR product was cloned with pGEM-T vector. The primary structure of insert was confirmed by direct sequencing. The fragment of encoding F protein was released from pGEM-T-F by digestion with EcoRI and BamHI, and ligated to pGKT7. Vector pGKT7 expressing proteins were fused with amino acids 1-147 of the GAL4 DNA binding domain (DNA-BD), pGADT7 expressing proteins were fused with amino acids 768-881 of the GAL4 activation domain (AD). Plasmid pGBK7T-F (Figure 1) containing full-length HCV F gene could directly express DNA binding domain, c-myc and F protein. The plasmid was transformed into yeast strain AH109 by lithium acetate method[12]. Western blotting was performed to confirm the expression of the fusion protein using c-myc monoclonal antibody. Transformed AH109 (bait) was cultured on quadruple dropout media to exclude the auto-activation activity.

![Figure 1](image)

**Yeast two-hybrid library screening using yeast mating**

One large (2-3 mm) fresh (<2 mo old) colony of AH109 [bait] was inoculated into 50 mL of SD/-Trp and incubated at 30 °C overnight (16-24 h) with shaking at 250-270 r/min. Then the cells were spun down by centrifuging the entire 50 mL culture at 1 000 r/min for 5 min. After the supernatant was decanted, the cell pellet was resuspended in the residual liquid by vortexing. A human liver cDNA library was cloned into pACT2 and yeast reporter strain Y187 (Clontech Co., USA). The entire AH109 [bait] culture and 1 mL human liver cDNA library (1×10⁶ cfu/mL) were combined and cultured in a 2-L sterile flask and 45 mL of 2×YPDA/Kan was added and swirled gently. After 20 h of mating, the cells were spun down and resuspended, and then spread on 50 large (150-mm) plates containing 100 mL of SD/-Ade/-His/-Leu/-Trp (QDO). After growth for 6-15 d, the yeast colonies were transferred onto the plates containing X-α-gal to check for expression of the MEL1 reporter gene (blue colonies).

**Plasmid isolation from yeast and transformation of E.coli with yeast plasmid**

Approximately 1×10⁶ colonies were screened and positive clones were identified. Yeast plasmid was isolated from positive yeast colonies by the lyticase method (Clontech Co.), and transformed into super-competence E. coli DH5α by chemical method. Transformants were plated on ampicillin SOB selection media and grown under selection. Subsequently, pACT2-cDNA constructs were re-isolated, and analyzed by...
restriction digests and sequencing.

Bioinformatic analysis
After the positive colonies were sequenced, the sequences were blasted with GenBank to analyze the function of the genes (http://www.ncbi.nlm.nih.gov.blast).

Cell culture and new gene cloning
The hepatoblastoma cell line HepG2 was propagated in DMEM supplemented with 10% FBS, 200 μmol/L L-glutamine, penicillin, and streptomycin. HepG2 cells were plated at a density of 1×10⁶/well in 35-mm dishes. Total cellular RNA was isolated using TRIzol (Invitrogen Co., USA) according to the manufacturer’s instructions. cDNAs were reverse-transcribed from total RNA.

On the basis of liver cDNA library of genes of proteins interacting with HCV-F protein, the coding sequence of a new gene with unknown function, named HCV FBP2, was reverse-transcribed from total RNA. The full-length sequences of HCV F were generated by PCR amplification of HCV plasmid (HCV strain 1b), and a 840-bp fragment of HCV FBP2 was amplified by RT-PCR after total RNA was isolated from HepG2 cells, sequenced and analyzed by comparing to Vector NTI 6 and BLAST database homology search (http://www.ncbi.nlm.nih.gov/blast). After being cut by EcoRI/HindIII, the fragments were in-frame ligated respectively into pGBKT7 and pGADT7.

Identification of recombinant plasmid
The full-length sequences of HCV F were generated by PCR amplification of HCV plasmid (HCV strain 1b), and a 840-bp fragment of HCV FBP2 was amplified by RT-PCR after total RNA was isolated from HepG2 cells, sequenced and analyzed by comparing to Vector NTI 6 and BLAST database homology search (http://www.ncbi.nlm.nih.gov/blast). After being cut by EcoRI/BamHI, the fragments were in-frame ligated respectively into pGBKT7 and pGADT7/EcoRI/BamHI sites. Restriction enzyme analysis of pGBKT7-F, pGADT7-FBP2, and pGADT7-FBP2 plasmids with EcoRI/BamHI yielded two bands respectively: 7 300 bp empty pGBKT7 and 486 bp HCV F, 7 300 bp empty pGBKT7 and 840 bp HCV FBP2. The products of plasmid were amplified by PCR. Analysis of the PCR products by agarose gel electrophoresis showed the clear bands with the expected size (486 bp of F, 840 bp of FBP2). Sequences of the PCR products were correct (Figures 2A-D).

Bioinformatic analysis
After the positive colonies were sequenced, the sequences were blasted with GenBank to analyze the function of the genes (http://www.ncbi.nlm.nih.gov.blast).

Cell culture and new gene cloning
The hepatoblastoma cell line HepG2 was propagated in DMEM supplemented with 10% FBS, 200 μmol/L L-glutamine, penicillin, and streptomycin. HepG2 cells were plated at a density of 1×10⁶/well in 35-mm dishes. Total cellular RNA was isolated using TRIzol (Invitrogen Co., USA) according to the manufacturer’s instructions. cDNAs were reverse-transcribed from total RNA.

On the basis of liver cDNA library of genes of proteins interacting with HCV-F protein, the coding sequence of a new gene with unknown function, named HCV FBP2, was reverse-transcribed from total RNA. The full-length sequences of HCV F were generated by PCR amplification of HCV plasmid (HCV strain 1b), and a 840-bp fragment of HCV FBP2 was amplified by RT-PCR after total RNA was isolated from HepG2 cells, sequenced and analyzed by comparing to Vector NTI 6 and BLAST database homology search (http://www.ncbi.nlm.nih.gov/blast). After being cut by EcoRI/HindIII, the fragments were in-frame ligated respectively into pGBKT7 and pGADT7.

Identification of recombinant plasmid
The full-length sequences of HCV F were generated by PCR amplification of HCV plasmid (HCV strain 1b), and a 840-bp fragment of HCV FBP2 was amplified by RT-PCR after total RNA was isolated from HepG2 cells, sequenced and analyzed by comparing to Vector NTI 6 and BLAST database homology search (http://www.ncbi.nlm.nih.gov/blast). After being cut by EcoRI/BamHI, the fragments were in-frame ligated respectively into pGBKT7 and pGADT7/EcoRI/BamHI sites. Restriction enzyme analysis of pGBKT7-F, pGADT7-FBP2, and pGADT7-FBP2 plasmids with EcoRI/BamHI yielded two bands respectively: 7 300 bp empty pGBKT7 and 486 bp HCV F, 7 300 bp empty pGBKT7 and 840 bp HCV FBP2. The products of plasmid were amplified by PCR. Analysis of the PCR products by agarose gel electrophoresis showed the clear bands with the expected size (486 bp of F, 840 bp of FBP2). Sequences of the PCR products were correct (Figures 2A-D).
Expression of "bait" fusion protein
Yeast strain AH109 transformed with pGBK7-F and pGBK7-FBP2 stably expressed the fusion protein at high level (Figure 3) and only grew on SD/-Trp medium but not on QDO medium. Thus, the transformed yeast could be used for yeast hybrid analysis.

Figure 3  Expression of HCV F and HCV FBP2 proteins in yeast. Lane 1: HCV F protein; lane 2: positive control; lane 3: HCV FBP2 protein.

Screening of liver cell cDNA library
We isolated plasmids from the blue colonies containing only pGBK7-F and one library plasmid other than other plasmids. Because plasmid pACT2- cDNA contains two restriction endonuclease sites of BglII, the gene fragments of the liver cell cDNA library (pACT2- cDNA) were released by BglII digestion (Figure 4). The gene fragments with different lengths proved that these screened clones were positive colonies but not false positive colonies growing on SD/-Trp/-Leu/-His/-Ade culture medium after mating.

Figure 4  Identification of different colonies by BglII digestion.

Analysis of cDNA sequence and homology
A total of 36 positive colonies were grown on the selective SD/-Trp/-leu/-his/-ade/ X-α-gal medium. These colonies were prescreened by BglII digesting to make sure that only colonies with different inserts were subjected to sequencing. Thirty-six colonies from cDNA library were sequenced. Using the BLAST program from the National Center for Biotechnology Information, two of the sequences were unknown genes and one of them was named as HCV FBP2. The full-length sequences accepted by GenBank were obtained by bioinformatics method. The other 34 sequences had a high similarity to known genes. The data are presented in Table 1.

| Similar number | Similar Homolog (%) |
|----------------|---------------------|
| Homo sapiens zymogen granule protein 16 | 11 99 |
| Homo sapiens zinc-alpha-2-glycoprotein | 4 99 |
| Highly similar to Homo sapiens SL15 protein | 5 100 |
| Homo sapiens RAB14, member RAS oncogene family | 1 98 |
| Human mRNA for complement control protein factor I | 1 98 |
| Homo sapiens angiotensinogen (serine or cysteine) proteinase inhibitor, clade A (alpha-1 proteinase, antiproteinase), member B | 1 97–99 |
| Homo sapiens sialyltransferase 1 | 1 97 |
| Similar to Homo sapiens zinc finger protein 83 | 5 100 |
| Homo sapiens mRNA for peroxisomal ion protease | 2 100 |
| Homo sapiens cathepsin B mRNA | 1 99 |
| Homo sapiens vitronectin (serum spreading factor, somatomedin B, complement 5-protein) | 1 100 |
| Homo sapiens serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member B | 1 99 |
| Homo sapiens genes of unknown functions | 2 |

Back-across testing
We confirmed the protein-protein interaction between HCV F and a new gene named as HCV FBP2 by back-across in yeast. Expressive vector pGADT7-HCV FBP2 was constructed and transformed into yeast Y187. This Y187 containing pGADT7-HCV FBP2 was mated with the yeast AH109 containing pGBK7-F. The mated yeast was plated on the dropout medium. The positive colonies emerged and became blue.

In vitro co-immunoprecipitation
HCV F protein containing 161 aa, Mr = 17 710, being smaller than FBP2 containing 280 aa, Mr = 30 800 is shown in Figure 5.

Figure 5  Interaction between HCV F protein and FBP2 protein identified by co-immunoprecipitation. Lane 1: HCV F protein; lane 2: interaction between HCV F and FBP2 proteins.

The core protein of HCV is a multifunctional protein[13-15]. There is evidence that this protein can directly modulate intracellular signal transduction, gene transcription, cell proliferation, and cell death, interfere with lipid metabolism...
and suppress host immune responses [16-19]. HCV F protein is a newly discovered HCV gene product encoded by the ORF that overlaps the core gene in the +1 frame [20-22]. F protein might have previously escaped attention because it possesses the immunodominant epitopes located in the N-terminus, in common with the core protein. A F protein analog synthesized in vitro reacts with the sera of HCV patients but not with the sera of hepatitis B patients, indicating that F protein is expressed during natural HCV infection. F protein is a very unstable protein with a half-life of <10 min in HuH7 hepatoma cells and in vitro, and is mediated by the proteosome pathway [23,24]. Immunofluorescence staining and subcellular fractionation experiments indicate that the subcellular localization of F protein is in the endoplasmic reticulum, and is similar to those of HCV core and NS3A proteins, suggesting that F protein may participate in HCV morphogenesis or replication. F protein shows more sequence diversity than the core protein [20-22]. Mutations of the core protein help virus to escape host immunologic reaction and may trigger cellular malignancy conversion. F protein might regulate cellular functions that are important for the viral life cycle or might play a role in viral morphogenesis or viral entry.

Because proteins often assemble into large complexes to perform different activities, characterization of the interaction pattern of a protein could contribute to the elucidation of the functions of protein [25]. Interaction between viral and hepatocellular proteins plays an important role in the pathogenesis of the virus and may mediate virus to enter hepatocytes. Their network interaction can change normal biological functions of proteins, influence self-replication of virus, prolong infection and develop diseases. Yeast two-hybrid 3 is an effective gene analysis method which can analyze interactions between protein and protein, protein and DNA, protein and RNA in eukaryotic cells, as well as a new genetics technique for studying interactions of proteins in physiologic condition in vivo.

Yeast two-hybrid system 3 is based on the system originally designed by Fields and Song [26] that takes the advantage of the properties of GAL4 protein of the yeast Saccharomyces cerevisiae. GAL4 yeast two-hybrid assay uses two expression vectors, one uses GAL4-DNA-binding domain (DBD) and the other uses GAL4-activation domain (AD). The GAL4-D-DBD is fused to protein ‘X’ and GAL4-AD is fused to protein ‘Y’ to form the bait and the target of the interaction trap, respectively. A selection of host cells with different reporter genes and different growth selection markers can detect and confirm protein-protein interactions and has fewer false positives [25-28].

In this study, the "bait" plasmid pGBKT7-F was transformed into yeast strain AH109. HCV F gene was expressed in yeast cells. After the "bait" plasmid pGBKT7-F yeast strain AH109 was mated with liver cDNA library yeast strain Y187, the diploid yeast cells were plated on QDO media containing X-α-gal, 36 true positives were obtained. By sequencing analysis of isolated library plasmids, we got the sequences of 34 genes with known functions and the sequences of two genes with unknown function, one of them was named as HCV FBP2. In order to further confirm the interaction between expressed protein and HCV F protein, we performed the experiment of co-immunoprecipitation of both proteins. A strong interaction between HCV F protein and HCV FBP2 protein in vitro was observed.

We screened Homo sapiens zymogen granule (ZG) protein 16 (ZG16p) interacting with F protein from liver cDNA library. ZG protein is highly expressed in the pancreas cells and regulates ATP-sensitive K(+) conductance of ZGs. ZG16p is one of the linker proteins with a submembranous protein matrix on the inner surface of ZG. The submembranous matrix composed of sulfated proteoglycans, glycoproteins and lectin ZG16p is essential for the binding and sorting of aggregated zymogens during granule formation. ZG16p is associated with lipid microdomains ('rafts') and affects the protein synthesis and intracellular transport of secretory proteins and maturation of zymogens in ZGs [29-31]. HCV infection and chronic hepatopathies caused by HCV are significantly associated with diabetes, and reduction of glucose tolerance is related to the severity of chronic hepatitis C [32,33]. The result of our experiment showed that interaction occurred between HCV F and ZG16p, thus providing a new clue for revealing the function of HCV F protein and relationship between pathogenesis of HCV and diabetes mellitus.

Another important protein interacting with F protein from liver cDNA library is the Homo sapiens zinc-alpha 2-glycoprotein (Zn-α2-GP). It is present at a high concentration in the seminal plasma and in other human body fluids. Hepatocytes are a source of protein in blood plasma and epithelia. Zn-α2-GP is present at carcinoma of prostate, breast cancer and hydroadenoma. Zn-α2-GP production is associated with tumor differentiation, decreased or absent Zn-α2-GP production is found in poorly differentiated (high Gleason grade) tumors. Zn-α2-GP production in tumors can elevate the systemic ZAG concentration and induce lipolysis leading to cachexia. Zn-α2-GP is a potential serum marker of prostate cancer that may elevate early in tumor development. Whether tumors can induce secretion of endogenous Zn-α2-GP by normal prostatic or other secretory ZAG expression can predict the risk of a progressive disease needs further study [34,35].

Vitronectin, also known as a serum spreading factor, is a glycoprotein present in both human plasma and serum. It is also present in different loose connective tissues, often in co-localization of elastic fibrils. Vitronectin binds to cells through interaction of the Arg-Gly-Asp (RGD) sequence in its cell-binding domain with vitronectin-specific cell surface receptors and may modulate blood coagulation and complement-induced cytolysis. Matrix-bound vitronectin plays a role in regulating pericellular proteolysis by binding to plasminogen activator inhibitor type I. Vitronectin may promote cell attachment, spreading, proliferation, and differentiation of normal and neoplastic cells [36,37].

C1 inhibitor (C1 INH) is a highly glucosylated protein. The content of glucose is up to 35-49%. It has 30% homology of amino acids of serine proteinase inhibitor superfamily members including α1 anti-trypsin, α1 anti-chymotrypsin and anti-thrombin III. C1 INH combines activated C1r or C1s to form a stable complex, leading to inactivation of serine proteinase C1. C1 INH may prevent auto-activation of C1. Because C1 INH still inhibits factors XII, XIa,
kallikrein and fibrinolysis, it plays an important role in regulation of blood coagulation, kinin, and fibrinolytic system[40,41].

Sialyltransferase is a member of glycosyltransferase family and presents at Golgi apparatus. Sialyltransferase possesses the functions of catalyzing cellular adhesive molecule-ganglioside polysialic acidification. Ganglioside, a kind of glycosphingolipid containing sialic acid, is an important factor for cellular adhesion and malignancy cell growth in vitro culture.

HCV F protein also interacts with complement control protein factor I, peroxisomal ion protease, cathepsin B, RAS oncogene. These interacting proteins are closely correlated with carbohydrate metabolism, immunoregulation, occurrence and development of tumor. How interactions between HCV F protein and interacting proteins affect the occurrence and development of chronic hepatitis C, hepatic fibrosis and hepatocarcinoma, needs to be further elucidated.

Gastroenterol 2004; 10: 1746-1749

14 O Kamoto K, Morish K, Miyamura T, Matsuya Y. Intramembrane proteolysis and endoplasmic reticulum retention of hepatitis C virus core protein. J Virol 2004; 78: 6370-6380

Majeau N, Gagne V, Boivin A, Bolduc M, Majeau JA, Ouellet D, Leclerc D. The N-terminal half of the core protein of hepatitis

15 C virus is sufficient for nucleocapsid formation. J Gen Virol 2004; 85(Pt 4): 971-981

Carlos MP, Yamamura Y,Vu Q, Conzen K, Anderson DE, Torres JV. Humoral immunity to immunodominant epitopes of Hepatitis C virus in individuals infected with genotypes 1a or 1b. Clin Immunol 2004; 111: 22-27

Kunkel M, Watsowich SJ. Biophysical characterization of hepa-

16 titis C virus core protein: implications for interactions within the virus and host. FEBS Lett. 2004; 557: 174-180

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. Virol 2000; 276: 127-137

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

Ogata S, Nagano-Fujii M, Ku Y, Yoon S, Hotta H. Comparative sequence analysis of the core protein and its frameshift product, the F protein, of hepatitis C virus subtype 1b strains obtained from patients with and without hepatocellular carcinoma. J Clin Med (cobiol 2002; 40: 3625-3630

18 Xu Z, Choi J, Lu W, Ou JH. Hepatitis C virus core protein is a short-lived protein associated with the endoplasmic reticulum. J Virol 2003; 77: 1578-1583

Brass V, Bieck E, Montserret R, Walk B, Hellinga JA, Blum HE, Penin F, Moradpour D. An amino-terminal amphipathic alpha-helix mediates membrane association of the hepatitis C virus nonstructural protein 5A. J Biol Chem 2002; 277: 8130-8139

19 Lanford RE, Notvall L, Chavez D, White R, Frenzel G, Simonsen C, Kim J. Analysis of hepatitis C virus capsid, E1, and E2/NS1 proteins expressed in insect cells. Virol 1993; 197: 225-235

Mclauchlan J, Lemberg MK, Hope G, Martoglio B. Intramembrane proteolysis promotes trafficking of hepatitis C virus core protein to lipid droplets. EMBO J 2002; 21: 3980-3988

Shi ST, Poljak SJ, Tu H, Taylor DR, Gretch DR, Lai MM. Hepatitis C virus NS5A colocalizes with the core protein on lipid droplets and interacts with apolipoproteins. Virol 2002; 292: 198-210

17 Emmert-Buck MR, Gillespie JW, Pawletz CP, Ornstein DK, Basur V, Appella E, Wang GH, Huang J, Hu N, Taylor P, Petricoin EE 3rd. An approach to proteomic analysis of human tumors. Mol Carcinog 2000; 27: 158-165

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

18 Osman A. Yeast two-hybrid assay for studying protein-protein interactions. M ethods M ol Biol 2004; 270: 403-422

Gietz RD, Woods RA. Screening for protein-protein interactions in the yeast two-hybrid system. M ethods M ol Biol 2002; 185: 471-486

20 Zhen Z. A suppression subtractive hybridization study. W ord J

19 Kalus J, Hodel A, Koch A, Kloene R, Edwardson JM, Schrader M. Interaction of syncoll with GP-2, the major membrane protein of pancreatic zymogen granules, and association with lipid microdomains. Biochem J 2002; 362(Pt 2): 433-442

Hodel A, An SJ, Hansen NJ, Lawrance J, Walse B, Schrader M, Edwardson JM. Cholesterol-dependent interaction of syncoll with the membrane of the pancreatic zymogen granule. Biochem J 2003; 356(Pt 3): 843-850

Schmidt K, Schrader M, Kern HF, Kloene R. Regulated apical secretion of zymogens in rat pancreas: Involvement of the GPI-anchored glycoprotein GP-2, the lectin ZG16p and cho-
lesterol-glycosphingolipid enriched microdomains. J Biol Chem 2001; 276: 14315-14323

34 Kleene R, Dartsch H, Kern HF. The secretory lectin ZG16p mediates sorting of enzyme proteins to the zymogen granule membrane in pancreatic acinar cells. Eur J Cell Biol 1999; 78: 79-90

35 Arao M, Murase K, Kusakabe A, Yoshioka K, Fukuzawa Y, Ishikawa T, Tagaya T, Yamanouchi K, Ichimiya H, Sameshima Y, Kakumu S. Prevalence of diabetes mellitus in Japanese patients infected chronically with hepatitis C virus. J Gastroenterol 2003; 38: 355-360

36 Paraschiv C, Graur M, Butnariu G, Mihai B, Constantinescu D. The pathological mechanisms of glyceregulation disturbances in chronic B and C. Rev Med Chir Soc Med Nat Iasi 2002; 107: 294-297

37 Hale LP, Price DT, Sanchez LM, Demark-Wahnefried W, Madden JF. Zinc alpha-2-glycoprotein is expressed by malignant prostatic epithelium and may serve as a potential serum marker for prostate cancer. Clin Cancer Res 2001; 7: 846-853

38 Stawowy P, Kallisch H, Veinot JP, Kiliimnik A, Prichett W, Goetz S, Seidah NG, Christen M, Fleck E, Graf K. Endoproteolytic activation of alpha (v) integrin by proprotein convertase PCS is required for vascular smooth muscle cell adhesion to vitronectin and integrin-dependent signaling. Circulation 2004; 109: 770-776

39 Faucheux N, Schweiss R, Lutzow K, Werner C, Groth T. Self-assembled monolayers with different terminating groups as model substrates for cell adhesion studies. Biomaterials 2004; 25: 2721-2730

40 Sim RB, Tsiftsoglou SA. Proteases of the complement system. Biochem Soc Trans 2004; 32(Pt 1): 21-27

41 Toomayan GA, Chen LE, Jiang HX, Qi WN, Seaber AV, Frank MM, Urbaniak JR. C1-esterase inhibitor and a novel peptide inhibitor improve contractile function in reperfused skeletal muscle. Microsurgery 2003; 23: 561-567

**Science Editor** Wang XL  **Language Editor** Elsevier HK