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# Screening of hepatocyte proteins binding to complete S protein of hepatitis B virus by yeast-two hybrid system

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## Abstract

**AIM:** To investigate the biological function of complete S protein and to look for proteins interacting with complete S protein in hepatocytes.

**METHODS:** We constructed bait plasmid expressing complete S protein of HBV by cloning the gene of complete S protein into pGBKT7, then the recombinant plasmid DNA was transformed into yeast AH109 (a type). The transformed yeast AH109 was mated with yeast Y187 (α type) containing liver cDNA library plasmid in 2×YPDA medium. Diploid yeast was plated on synthetic dropout nutrient medium (SD/-Trp-Leu-His-Ade) containing X-α-gal for selection and screening. After extracting and sequencing of plasmids from positive (blue) colonies, we underwent sequence analysis by bioinformatics.

**RESULTS:** Nineteen colonies were selected and sequenced. Among them, five colonies were *Homo sapiens* solute carrier family 25, member 23 (SLC25A23), one was *Homo sapiens* calreticulin, one was human serum albumin (ALB) gene, one was *Homo sapiens* metallothionein 2A, two were *Homo sapiens* betaine-homocysteine methyltransferase, three were *Homo sapiens* $Na^+$ and $H^+$ coupled amino acid transport system N, one was *Homo sapiens* CD81 antigen (target of anti-proliferative antibody 1) (CD81), three were *Homo sapiens* diazepam binding inhibitor, two colonies were new genes with unknown function.

**CONCLUSION:** The yeast-two hybrid system is an effective method for identifying hepatocyte proteins interacting with complete S protein of HBV. The complete S protein may bind to different proteins i.e., its multiple functions *in vivo*.



**Key words:** Complete S protein; Yeast-two hybrid system; Hepatitis B virus

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## INTRODUCTION

Hepatitis B virus (HBV) causes acute and chronic infections of the liver. Acute infections may produce serious diseases, and approximately 0.5% of the diseases will develop into fatal, fulminant hepatitis. Chronic infections may also have remarkable consequences[1]. Thus HBV is considered to be a major etiological factor in the development of human hepatocellular carcinoma (HCC), one of the most frequent fatal malignancies worldwide, and worldwide deaths of HCC exceed one million per year[2-7]. Epidemiological studies have demonstrated an approximately 10-fold increase in the relative risk of HCC among HBV carriers compared to non-carrier.

The precise role of HBV in the etiology of HCC is not well understood. Only occasionally, genes controlling cell growth and differentiation are disturbed by integration of HBV DNA sequences. An alternative mechanism of chronic infections and hepatocarcinogenesis may be the key steps to mutual interaction between viral proteins and hepatocellular proteins, this action may mediate virus to enter into the liver cells and affect the activities and function of these proteins. Moreover, the protein from hepatocytes infected with HBV inversely disturbs virus replication and reduces immunity of the host, resulting in chronic liver diseases and HCC. Elucidating this interaction between these proteins may help to bring some new clues for discovering the pathogenesis of viral hepatitis.

The first full length nucleotide sequence of HBV was published in 1979. Its length is 3 182 nt, and the serum type is ayw. The four open read frames (ORF) defined in HBV genome at that time, are named as the regions of S, C, P and X. The region of S is divided into the sub-regions of pre-S1, pre-S2 and S according to different initial code ATG in frame. Dong *et al*.[8], have shown that there is an

open read frame (ORF) before pre-S1 region in the genome of HBV, amplified from serum of patients infected with HBV by long and accurate polymerase chain reaction (LA-PCR). This region is 135 bp, and named temporarily as pre-pre-S and its promoter activities have been confirmed in 277 bp upstream nucleotide sequences before pre-S1 gene[9]. Pre-pre-S, pre-S1, pre-S2 and S genes are translated in frame according to the same ORF, complete S of HBV, including pre-pre-S, pre-S1, pre-S2 and S regions.

The function of the complete S protein in the life cycle of HBV remains unknown. To investigate the biological importance of the complete S protein, we screened and identified the proteins interacting with HBV complete S protein by yeast-two hybrid system to elucidate the biological functions of complete S protein of HBV genome.

## MATERIALS AND METHODS

### Bacterials, 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, *trp1-901*, *leu2-3,112*, *ura3-52*, *his3-200*, *gal4*△, *gal80*△, *LYS2*: $GAL1_{UAS}$-$GAL1_{TATA}$-*HIS3*, $GAL2_{UAS}$-$GAL2_{TATA}$-*ADE2*, *URA3*: $MEL1_{UAS}$-$MEL_{1TATA}$-*LacZ*) containing pGBKT7-53, coding for DNA-BD/mouse p53 fusing protein was used for cloning of bait plasmid. Yeast strain Y187 (MATa *ura3-52, his3-200, Ade2-101, trp1-901, leu2-3, 112, gal4*△*, gal80*△*, met-, URA3:* $GAL1_{UAS}$-$GAL1_{TATA}$-*lacZ MEL1*) containing pTD1-1, coding for AD/SV40 large T antigen fusing protein was used for cloning of library plasmids. Pre-transformed human cDNA liver cell library Y187 and bacterial strain DH5a were used for cloning of shuttle plasmids. Yeast-*Escherichia coli* shuttle plasmids, pGBKT7 DNA-BD cloning plasmid, pGADT7 AD cloning plasmid, pGBKT7-53 control plasmid, pGADT7, pGBKT7-Lam control plasmid, pCL1 plasmid were all 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, *Eco*RI and *BamH*I restriction endonuclease were from Takara Co., Japan. Anti-c-myc monoclonal antibody secreted by 1-9E10.2 hybridoma (ATCC) was prepared in our laboratory. Goat anti-mouse IgG conjugated with horseradish peroxidase was from Zhongshan Co., China. Lithium acetate, semi-sulfate adenine, acrylamide and N, N'-bis-acrylamide were from Sigma Co., and TEMED was from Boehringer Mannheim Co. Tryptone and yeast extracts were from OXOID Co. X-α-gal and culture media: YPDA, SD/-Trp SD/-Leu, SD/-Trp/-Leu, SD/-Trp/-Leu/-His, SD/-Trp/-Leu/-His/-Ade were from Clontech C., protein-G agarose was from Roche C., and pGEM-T vector was from Promega Co.

RT-PCR kit and TNT® coupled reticulocyte lysate systems were from Promega Co. [$^{35}$S]-methionine (1 000 Ci/mmol, 10 mCi/mL) was from Isotope Company of China. Amplification fluorographic reagent (#NAMP100) was from Amersham Life Sciences Co. Others reagents were from Sigma Co., USA.

### Construction of "bait" plasmid and expression of HBV complete S protein

HBV-complete S sequences were generated by PCR amplification using the plasmid G376 A7 (GenBank number: AF384371[8,10-13]) as template. The sequences of the primers containing the *Eco*RI and *BamH*I restriction enzyme sites were: sense primer (*Eco*RI): 5'-GAA TTC ATG CAG TTA ATC ATT ACT TCC-3'; antisense primer (*BamH*I): 5'-GGA TCC TCA AAT GTA TAC CCA AAG AC-3'. The PCR conditions were at 94 ℃ for 60 s, at 55 ℃ for 60 s, at 72 ℃ for 90 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 complete S was released from the pGEM-T- complete S by digestion with *Eco*RI and *BamH*I, and ligated to pGBKT7. Vector pGBKT7 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 pGBKT7-complete S (Figure 1) containing full-length HBV complete S gene could express DNA binding domain, c-myc and complete S fusion protein. The plasmid was transformed into yeast strain AH109 by lithium acetate method[14]. Western blotting was performed to confirm the expression of the fusion protein using anti-c-myc monoclonal antibody. Transformed AH109 (bait) was cultured on quadruple dropout media to exclude the auto-activation activity.

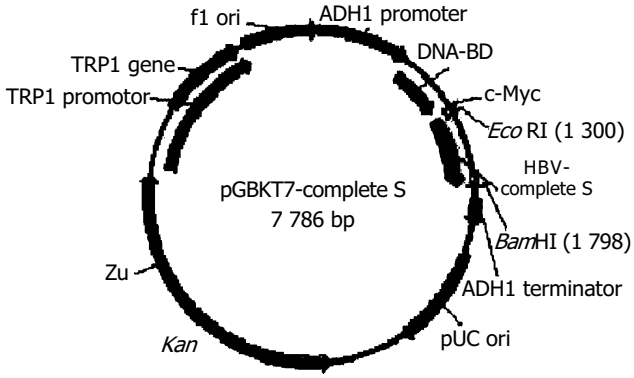


**Figure 1** Structure of "bait" plasmid pGBKT7-complete S.

### 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 ℃ overnight (16-24 h) and shaken at 250-270 r/min. Then the cells were spanned 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 cloned into pACT2 and yeast reporter strain Y187 (Clontech Co., USA) were co-cultured. The entire AH109 [bait] culture and the 1 mL human liver cDNA library ($1\times10^6$ cfu/mL) were combined and cultured in a 2-L sterile flask and 45 mL of 2X YPDA/Kan was added and swirled gently. After 20 h of mating, the cells were spanned down and resuspended, and then spread on 50 large (150 mm) plates containing 100 mL of SD/-Ade/-His/-

Leu/-Trp (QDO). After 6-15 d of growth, 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 $1\times10^6$ colonies were screened and positive clones were identified. Yeast plasmid was isolated from positive yeast colonies with lyticase method (Clontech Co., USA), and transformed into super-competence *E.coli* DH5α using chemical method. Transformants were plated on ampicillin SOB selection media and grown under selection. Subsequently, pACT2-cDNA constructs were re-isolated, analyzed by restriction digestion 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*

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 $1\times10^6$/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 HBV-complete S protein, the coding sequence of a new gene without known function, HBV CSBP1, was obtained by bioinformatics methods. The standard PCR cloning technique was used to amplify HBV CSBP1 gene. Total cell RNA was isolated from HepG2 cells. RNA was used for RT-PCR amplification. The PCR conditions were: 94 ℃ for 60 s, 58 ℃ for 60 s, and 72 ℃ for 60 s, for 35 cycles. The PCR product was cloned with pGEM-T vector (Promega Co., USA). The primary structure of insert was confirmed by direct sequencing. The gene fragment was cloned into yeast plasmids pGBKT7 and pGADT7.

### *Confirmation of the true interaction in yeast*

To confirm the true protein-protein interaction and exclude false positives, the plasmids of positive colonies were transformed into yeast strain Y187, and then mating experiments were carried out by mating with yeast strain AH109 containing pGBKT7-complete S or pGBKT7-Lam. After mating, the diploids yeast was plated on SD/-Ade-His-Leu-Trp (QDO) covered with X-α-gal to test the specificity of interactions.

### *In vitro translation*

Twenty-five microliter mixture of TNT®reticulocytes, 2 μL TNT® reaction buffer, 1 μL T7 TNT® RNA polymerase, 1 μL amino acids mixture (minus methionine, 1 mol/L), 1 μL [$^{35}$S]-methionine 2 μL, RNasin RNase inhibitor (40 u/mL), 2 μL DNA template (pGBKT7-complete S or pGADT7-library gene) (0.5 μg/mL), 16 μL dd$H_2O$, were incubated at 30 ℃ for 90 min.

### *Coimmunoprecipitation*

The following reactants were combined in a 1.5 mL microcentrifugation tube on ice: 5 μL *in vitro*-translated bait protein, 5 μL *in vitro*-translated library protein. The only control added was 10 μL pGBKT7-complete S plasmid. The mixtures were incubated at 30 ℃ for 1 h. Then, the following reagents were added into the reaction tubes: 470 μL coimmunoprecipitation buffer (20 mol/L Tris-HCl (pH 7.5), 150 mol/L NaCl, 1 mol/L DTT, 5 μg/mL aprotinin, 0.5 mol/L PMSF, 0.1 % Tween 20, 10 μL protein-G agarose beads, 10 μL anti-c-myc monoclonal antibody. Incubation was done at 4 ℃ for 2 h with continuous shaking. The tubes were centrifuged at 14 000 *g* for 1-2 min. The supernatants were removed. Half a milliliter of TBST was added into the tubes. Rinse steps were repeated thrice. Fifteen microliters of SDS-loading buffer was added. The samples were heated at 80 ℃ for 5 min. The tubes were placed on ice and then briefly centrifuged, and 10 μL was loaded onto a SDS-PAGE mini-gel to begin the electrophoretic separation. After electrophoresis, the gel was transferred onto a tray containing gel fixation solution, and placed on a rotary shaker for 10 min at room temperature. The gel was rinsed with $H_2O$, then amplification fluorographic reagent was added and shaken for 20 min at room temperature, then dried at 80 ℃ under constant vacuum. The gel was exposed to an X-ray film overnight at room temperature. The film was developed by standard techniques.

## RESULTS

### *Identification of recombinant plasmid*

The full length sequences of HBV complete S were generated by PCR amplification of the plasmid G376 A7 (GenBank number: AF384371)[1,10-13], and a 942-bp fragment of HBV CSBP1 was amplified by RT-PCR after total RNA was prepared from HepG2 cells, sequenced and analyzed by comparing Vector NTI 6 and BLAST database homology search (http://www.ncbi.nlm.nih.gov/blast). After being cut by *Eco*RI/*BamH*I, the fragments were in-frame ligated, respectively into pGBKT7 and pGADT7 at the *Eco*RI/*BamH*I sites. Restriction enzyme analysis of pGBKT7-complete S, pGBKT7-CSBP1 and pGADT7-CSBP1 plasmids with *Eco*RI/*BamH*I yielded respectively two bands: 7 300 bp empty pGBKT7 and 1 338 bp HBV complete S, 7 300 bp empty pGBKT7 and 942 bp of HBV CSBP1, 7 900 bp empty pGADT7 and 942 bp HBV CSBP1. The products of plasmid were amplified by PCR. Analysis of the PCR amplified products by agarose gel electrophoresis showed the clear bands with the expected size (1 338 bp of complete S, 942 bp of CSBP1). Sequences of the PCR products were correct (Figures 2A-D).

### *Expression of "bait" fusion protein*

Yeast strain AH109 transformed with pGBKT7-complete S and pGBKT7-CSBP1 could stably express the fusion protein at high level (Figure 3) and could only grow on SD/-Trp medium but not on QDO medium. Thus, the transformed yeast could be used for yeast hybrid analysis.

### *Screening of liver cell cDNA library*

We isolated plasmids from the blue colonies containing only pGBKT7-complete S and one library plasmid other than other plasmids. Because plasmid pACT2-cDNA contains

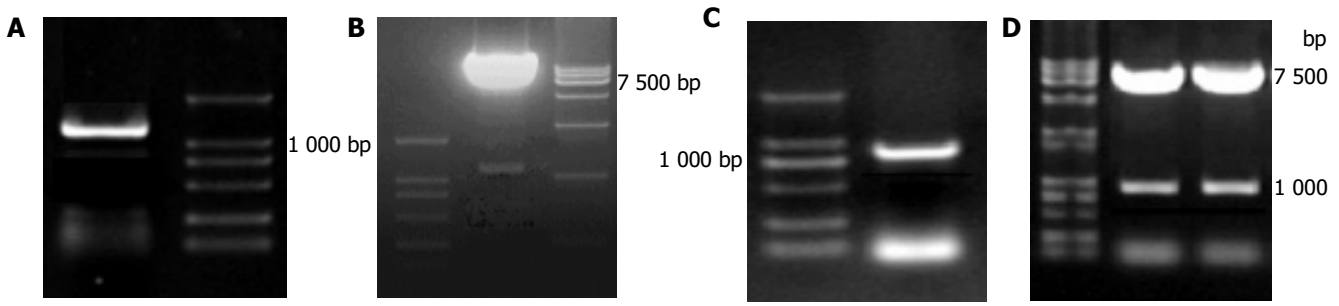


**Figure 2** One thousand three hundred and thirty eight basepair fragment-complete S amplified by RT-PCR (**A**), pGBKT7-complete S cut by *Eco*RI/*BamH*I (**B**), a 945 bp fragment-CSBP1 amplified by RT-PCR (**C**), pGBKT7-CSBP1 and pGADT7-CSBP1 cut by *Eco*RI/*BamH*I (**D**).

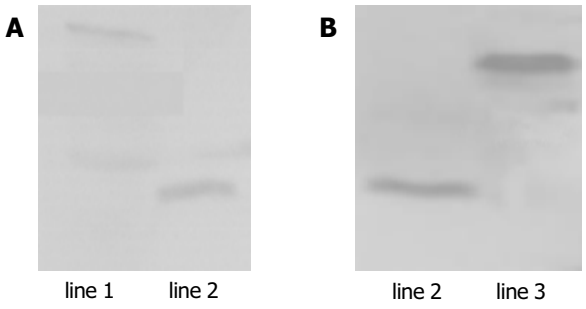


**Figure 3** Expression of HBV complete S and HBV CSBP1 protein in yeast confirmed by Western blotting. Lane 1: HBV complete S protein; lane 2: positive control; lane 3: HBV CSBP1 protein.

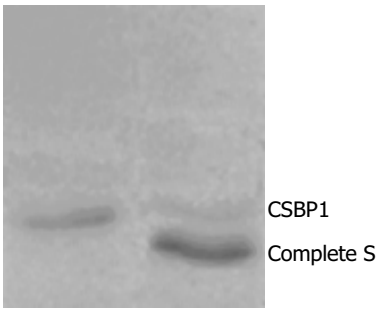


**Figure 5** Interaction between HBV complete S protein and CSBP1 protein identified by coimmunoprecipitation. Lane 1: HBV complete S protein; lane 2: interaction with two proteins.

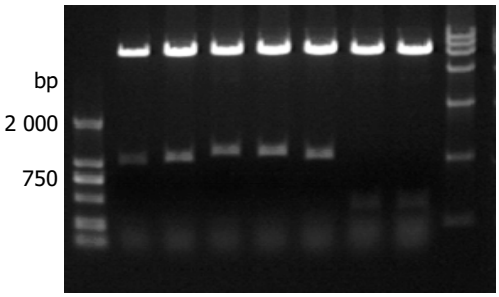


**Figure 4** Identification of different colonies with *Bgl*II digestion.

two restriction endonuclease sites of *Bgl*II on both sides of multiple cloning sites, the gene fragments of the liver cell cDNA library (pACT2-cDNA) screened were released by *Bgl*II digestion (Figure 4). The gene fragments of different lengths in Figure 5 proved that these screened clones were positive colonies growing on SD/-Trp/-Leu/-His/-Ade culture medium after mating.

### *Analysis of cDNA sequencing and homology*

We obtained a total of 19 positive colonies growing on the selective SD/-trp-leu-his-ade/X-α-gal medium. These colonies were prescreened by *Bgl*II digestion to make sure that only colonies with different inserts were subjected to sequencing. Nineteen colonies from cDNA library were sequenced. Using the BLAST program at the National Center for Biotechnology Information, 17 sequences had a high similarity to known genes. The data are presented in Table 1.

**Table 1** Comparison between positive clones and similar sequences in GenBank

| High similarity to known genes | Number of similar (%) | Homology (%) |
|---|---|---|
| *Homo sapiens* calreticulin | 1 | 99 |
| *Homo sapiens* solute carrier family 25, member 23 (SLC25A23) | 5 | 99 |
| Human serum albumin (ALB) gene | 1 | 100 |
| *Homo sapiens* metallothionein 2A | 1 | 98 |
| *Homo sapiens* betaine-homocysteine methyltransferase, mRNA | 2 | 98 |
| *Homo sapiens* diazepam binding inhibitor | 3 | 96 |
| *Homo sapiens* Na+ and H+ coupled amino acid transport system N | 3 | 100 |
| *Homo sapiens* CD81 antigen (CD81) | 1 | 100 |

### *In vitro coimmunoprecipitation*

HBV complete S protein containing 447 aa, was smaller than CSBP1 containing 280 aa (Figure 5).

## DISCUSSION

The open reading frame of HBV complete S gene consists of four coding regions: pre-pre-S, pre-S1, pre-S2 and S, each starting with an ATG codon in frame. Through in frame translational initiation at each of the four ATG codons, complete S (pre-pre-S+pre-S1+pre-S2+S), large (LHBs; pre-S1+pre-S2+S), middle (MHBs; pre-S2+S) and small (SHBs; S) envelope glycoproteins can be synthesized[12,13,15-17]. Interactions between viral and hepatocellular proteins play an important role in the pathogenesis of the virus and may mediate virus

to enter into hepatocytes. Their network interactions can change normal biological functions of proteins, influence self-replication of virus, and result in diseases. Yeast-two hybrid system 3 is an effective gene analysis method to analyze the interactions between protein and protein, protein and DNA, protein and RNA in eukaryotic cells and a new genetics technique for studying interactions of proteins in physiologic conditions *in vivo*.

Yeast-two hybrid system 3 is based on the system originally designed by Fields and Song by taking advantage of the properties of the 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-actibationg domain (AD). The GAL4-DBD fused to protein 'X' and a GAL4-AD 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 provides a means to detect and confirm protein-protein interactions and has significantly fewer false positives[18-21].

In this study, the "bait" plasmid pGBKT7-complete S was transformed into yeast strain AH109. HBV complete S gene was expressed in yeast cells. After the "bait" plasmid pGBKT7-complete S yeast strain AH109s mated with liver cDNA library yeast strain Y187, the diploid yeast cells were plated on QDO media containing X-α-gal, 19 true positives were obtained. By sequencing analysis of isolated library plasmids, we got the sequences of the 17 genes with known functions and two genes with unknown functions, one of them was named as complete S-binding protein 1 (CSBP1). In order to further confirm the interaction between the expressed protein and HBV complete S protein, we performed the experiment of coimmunoprecipitation of both proteins. A strong interaction between HBV complete S protein and CSBP1 protein *in vitro* was observed.

We screened *Homo sapiens* metallothionein 2A (MT) interacting with complete S protein from liver cDNA library. Metallothionein is a low-molecular weight protein with pleiotropic functions and a family member of metal binding proteins. MT is localized in nuclei and/or cytoplasm of tumor cells[22]. It may be involved in the regulation of carcinogenesis and apoptosis in addition to various physiological processes. Metallothionein is a small stress response protein that can be induced by exposure to heavy metal cations, oxidative stressors, and acute phase cytokines that mediate inflammation[23]. In humans, there are four groups of MT proteins. MT-2A is highly expressed in epithelial cells of breast cancer, and differentially up-regulated in invasive breast cancer cells[24]. Jin *et al*.[25], reported that 26-100% of invasive ductal breast cancers express the MT protein, and are associated with cell proliferation and higher histological grade in invasive ductal breast cancer tissues. Rao *et al*.[26], thought that the mechanism may be completed through interaction between protein kinase C (PKC) signal transduction and MT 2A. Therefore this protein plays an important role in maintaining transition metal ion homoeostasis, redox balance in cells and fundamental cellular processes such as proliferation, apoptosis, and regulation of carcinogenesis[27,28]. MT 2A is also involved in human prostate homeostasis and carcinogenesis[29]. MT-2A is expressed in retinal pigment epithelial (RPE) cells, photoreceptor cells, inner nuclear layer cells and ganglion cells, and can protect cells against oxidative stress and apoptosis[30].

Another important protein interacting with complete S protein from liver cDNA library is *Homo sapiens* calreticulin. Yoon *et al*.[31], have analyzed nuclear matrix proteins in 11 hepatocellular carcinomas and compared them with corresponding non-neoplastic liver tissue by two-dimensional gel electrophoresis. Calreticulin is also found in the nuclear matrices of various carcinoma cell lines. The formation and/or expansion of calreticulin-nuclear matrix may be related to the activated cell growth. Le Naour *et al*.[32], showed that autoantibodies of calreticulin are detectable in patients with HCC (27%), suggesting that a distinct repertoire of autoantibodies is associated with HCC. Therefore it may play a role in early diagnosis of HCC.

HBV complete S protein also interacts with *Homo sapiens* CD81 antigen (CD81). The protein encoded by this gene is a member of the transmembrane four superfamily, also known as the tetraspanin family[33]. Most of these members are cell-surface proteins characterized by the presence of four hydrophobic domains. CD81, a signal transducing molecule, significantly increases in peripheral blood and more dramatic in the liver of HCV-infected individuals[34]. Recently, CD81 has been identified as a hepatitis C virus (HCV) receptor of B lymphocytes, the large extracellular loop of CD81 is a determinant combination position for viral entry[35-39]. These data suggest a functional role for CD81 as a coreceptor for HCV glycoprotein-dependent viral cell entry, providing a mechanism by which B cells are infected with and activated by the virus. It has recently been shown that peripheral B-cell CD81 overexpression is associated with HCV viral load and development of HCV-related autoimmunity. The human CD81 (hCD81) molecule has been identified as a putative receptor in B lymphocytes for hepatitis C virus with murine fibroblast cell line NIH/3T3 model. CD81 chimeras could internalize recombinant E2 protein and E2-enveloped viral particles from serum of HCV-infected patients into Huh7 liver cells. The latter result in persistent positive-strand viral RNA and accumulation of replication, therefore CD81 represents one of the pathways by which HCV can infect hepatocytes. Although CD81 can bind to HCV E2 protein, its role as a receptor for HBV remains controversial. These questions need further study. Whether CD81 could be used as the receptor or co-receptor for hepatocytes, should be studied intensively. CD81 proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth and motility. This protein appears to promote muscle cell fusion and support myotube maintenance. Also it may be involved in signal transduction. This gene is localized in the tumor-suppressor gene region, thus it is a candidate gene for malignancies.

These interacting proteins screened by yeast-two hybrid are closely correlated with carbohydrate metabolism, immunoregulation, occurrence and development of tumor, and may provide a new study clue for revealing biological functions of HBV complete S protein, pathogenesis of HBV and causes of malignancy conversion. How the interactions between HBV complete S protein and the above-mentioned interacting proteins affect the occurrence and development

of chronic hepatitis B, hepatic fibrosis and hepatocarcinoma, needs to be further studied.

## REFERENCES


1 **Beasley RP.** Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer* 1988; **61**: 1942-1956
2 **Xia X,** Cheng J, Yang J, Zhong Y, Wang G, Fang H, Liu Y, Li K, Dong J. Construction and expression of humanized anti-HBsAg scFv targeting interferon-alpha in *Escherichia coli*. *Zhonghua Ganzangbing Zazhi* 2002; **10**: 28-30
3 **Dong J,** Cheng J, Wang Q, Huang FJ, Shi S, Zhang G, Hong Y, Li L, Si C. The study on heterogeneity of hepatitis B virus DNA. *Zhonghua Yiyue Zazhi* 2002; **82**: 81-85
4 **Dong J,** Cheng J, Wang Q, Wang G, Shi S, Liu Y, Xia X, Li L, Zhang G, Si C. Quasispecies and variations of hepatitis B virus: core promotor region as an example. *Zhonghua Shiyan He Linchuang Bingduxue Zazhi* 2002; **16**: 264-266
5 **Dong J,** Cheng J, Wang Q, Shi S, Wang G, Si C. Cloning and analysis of the genomic DNA sequence of augmenter of liver regeneration from rat. *Chin Med Sci J* 2002; **17**: 63-67
6 **Deng H,** Dong J, Cheng J, Huangfu KJ, Shi SS, Hong Y, Ren XM, Li L. Quasispecies groups in the core promoter region of hepatitis B virus. *Hepatobiliary Pancreat Dis Int* 2002; **1**: 392-396
7 **Parkin DM,** Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. *Int J Cancer* 1999; **80**: 827-841
8 **Dong J,** Cheng J. Study on definition of pre-pre-S region in hepatitis B virus genome. *Shijie Huaren Xiaohua Zazhi* 2003; **8**: 1091-1096
9 **Yang Q,** Dong J, Cheng J, Liu Y, Hong Y, Wang JJ, Zhuang SL. Definition of pre-pre-S promoter sequence from hepatttis B virus genome and identtfication of its transcription activity. *Jiefangjun Yixue Zazhi* 2003; **9**: 761-762
10 **Liu Y,** Cheng J, Shao DZ, Wang L, Zhong YW, Dong J, Li K, Li L. Synergetic transactivating effect of HCV core and HBV X proteins on SV40 early promoter/enhancer. *Zhonghua Shiyan He Linchuang Bingduxue Zazhi* 2003; **17**: 70-72
11 **Huangfu J,** Dong J, Deng H, Cheng J, Shi S, Hong Y, Ren X, Li L. A preliminary study on the heterogeneity of preS2 region in hepatitis B virus. *Zhonghua Neike Zazhi* 2002; **41**: 233-236
12 **Borchani-Chabchoub I,** Mokdad-Gargouri R, Gargouri A. Glucose dependent [correction of dependant] negative translational control of the heterologous expression of the pre-S2 HBV antigen in yeast. *Gene* 2003; **311**: 165-170
13 **Soussan P,** Pol S, Garreau F, Brechot C, Kremsdorf D. Vaccination of chronic hepatitis B virus carriers with pre-S2/S envelope protein is not associated with the emergence of envelope escape mutants. *J Gen Virol* 2001; **82**: 367-371
14 **Matsumoto M,** Hsieh TY, Zhu N, VanArsdale T, Hwang SB, Jeng KS, Gorbalenya AE, Lo SY, Ou JH, Ware CF, Lai MM. Hepatitis C virus core protein interacts with the cytoplasmic tail of lymphotoxin-beta receptor. *J Virol* 1997; **71**: 1301-1309
15 **Park JH,** Lee MK, Kim HS, Kim KL, Cho EW. Targeted destruction of the polymerized human serum albumin binding site within the preS2 region of the HBV surface antigen while retaining full immunogenicity for this epitope. *J Viral Hepat* 2003; **10**: 70-79
16 **Borchani-Chabchoub I,** Gargouri A, Mokdad-Gargouri R. Genotyping of Tunisian hepatitis B virus isolates based on the sequencing of preS2 and S regions. *Microbes Infect* 2000; **2**: 607-612
17 **Tai PC,** Suk FM, Gerlich WH, Neurath AR, Shih C. Hypermodification and immune escape of an internally deleted middle-envelope (M) protein of frequent and predominant hepatitis B virus variants. *Virology* 2002; **292**: 44-58
18 **Fields S,** Song O. A novel genetic system to detect protein-protein interactions. *Nature* 1989; **340**: 245-246
19 **Osman A.** Yeast two-hybrid assay for studying protein-protein interactions. *Methods Mol Biol* 2004; **270**: 403-422
20 **Gietz RD,** Woods RA. Screening for protein-protein interactions in the yeast two-hybrid system. *Methods MolBiol* 2002; **185**: 471-486
21 **Zhen Z.** Progress in proteomics. *Shengwu Gongcheng Xuebao* 2001; **17**: 491-493
22 **Yamamura Y,** Ohta Y, Iguchi T, Matsuzawa A. Metallothionein expression and apoptosis in pregnancy-dependent and -independent mouse mammary tumors. *Anticancer Res* 2001; **21**: 1145-1149
23 **Canpolat E,** Lynes MA. *In vivo* manipulation of endogenous metallothionein with a monoclonal antibody enhances a T-dependent humoral immune response. *Toxicol Sci* 2001; **62**: 61-70
24 **Jin R,** Bay BH, Chow VT, Tan PH, Dheen T. Significance of metallothionein expression in breast myoepithelial cells. *Cell Tissue Res* 2001; **303**: 221-226
25 **Jin R,** Chow VT, Tan PH, Dheen ST, Duan W, Bay BH. Metallothionein 2A expression is associated with cell proliferation in breast cancer. *Carcinogenesis* 2002; **23**: 81-86
26 **Rao PS,** Jaggi M, Smith DJ, Hemstreet GP, Balaji KC. Metallothionein 2A interacts with the kinase domain of PKCmu in prostate cancer. *Biochem Biophys Res Commun* 2003; **310**: 1032-1038
27 **Jin R,** Huang J, Tan PH, Bay BH. Clinicopathological significance of metallothioneins in breast cancer. *Pathol Oncol Res* 2004; **10**: 74-79
28 **Tai SK,** Tan OJ, Chow VT, Jin R, Jones JL, Tan PH, Jayasurya A, Bay BH. Differential expression of metallothionein 1 and 2 isoforms in breast cancer lines with different invasive potential: identification of a novel nonsilent metallothionein-1H mutant variant. *Am J Pathol* 2003; **163**: 2009-2019
29 **Hasumi M,** Suzuki K, Matsui H, Koike H, Ito K, Yamanaka H. Regulation of metallothionein and zinc transporter expression in human prostate cancer cells and tissues. *Cancer Lett* 2003; **200**: 187-195
30 **Lu H,** Hunt DM, Ganti R, Davis A, Dutt K, Alam J, Hunt RC. Metallothionein protects retinal pigment epithelial cells against apoptosis and oxidative stress. *Exp Eye Res* 2002; **74**: 83-92
31 **Yoon GS,** Lee H, Jung Y, Yu E, Moon HB, Song K, Lee I. Nuclear matrix of calreticulin in hepatocellular carcinoma. *Cancer Res* 2000; **60**: 1117-1120
32 **Le Naour F,** Brichory F, Misek DE, Bréchot C, Hanash SM, Beretta L. A distinct repertoire of autoantibodies in hepatocellular carcinoma identified by proteomic analysis. *Mol Cell Proteomics* 2004; **1**: 197-203
33 **Levy S,** Todd SC, Maecker HT. CD81 (TAPA-1): a molecule involved in signal transduction and cell adhesion in the immune system. *Annu Rev Immunol* 1998; **16**: 89-109
34 **Curry MP,** Golden-Mason L, Doherty DG, Deignan T, Norris S, Duffy M, Nolan N, Hall W, Hegarty JE, O'Farrelly C. Expansion of innate CD5pos B cells expressing high levels of CD81 in hepatitis C virus infected liver. *J Hepatol* 2003; **38**: 642-650
35 **Van Compernolle SE,** Wiznycia AV, Rush JR, Dhanasekaran M, Baures PW, Todd SC. Small molecule inhibition of hepatitis C virus E2 binding to CD81. *Virology* 2003; **314**: 371-380
36 **Cao J,** Zhao P, Miao XH, Zhao LJ, Xue LJ, Qi Zt Z. Phage display selection on whole cells yields a small peptide specific for HCV receptor human CD81. *Cell Res* 2003; **13**: 473-479
37 **Cao J,** Zhao P, Zhao LJ, Wu SM, Zhu SY, Qi ZT. Identification and expression of human CD81 gene on murine NIH/3T3 cell membrane. *J Microbiol Methods* 2003; **54**: 81-85
38 **Zhang J,** Randall G, Higginbottom A, Monk P, Rice CM, McKeating JA. CD81 is required for hepatitis C virus glycoprotein-mediated viral infection. *J Virol* 2004; **78**: 1448-1455
39 **Bartosch B,** Vitelli A, Granier C, Goujon C, Dubuisson J, Pascale S, Scarselli E, Cortese R, Nicosia A, Cosset FL. Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. *J Biol Chem* 2003; **278**: 41624-41630


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