Human proteins interacting with human hepatitis B core antigen

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By employing a yeast two-hybrid system and human hepatocyte cDNA library, we searched for human proteins interacting with human hepatitis B core protein. After screening of ~2.5 million transformants we isolated several human cDNA clones specifically interacting with HBV core. Five unique clones were sequenced and aligned against the sequences currently available at public DNA databanks. Isolated cDNAs were identified as encoding human fibrinogen gamma and alpha polypeptides, human ribosomal protein L5 and two proteins, GIPC and GIPC2, of a similar domain structure.

Key words: yeast, two-hybrid system, human proteins, HBcAg

INTRODUCTION

The hepatitis B virus may cause various clinical manifestations, from asymptomatic to fulminant, and acute hepatitis. Chronic infection can develop, up to cirrhosis or hepatocellular carcinoma [1]. Although HBV is relatively well-studied, major attention so far has been devoted to the regulatory X protein function in the host cell [2, 3]. However, there is evidence that structural proteins can play a significant role in viral pathogenesis as in case of HCV core protein [4, 5]. In order to elucidate the role of viral structural proteins in the pathogenesis of HBV-infected hepatocytes, it is necessary to investigate their interactions with proteins of the host cell. These interactions might also be helpful in identification of human proteins participating in the virus life cycle, *e.g.*, in the transport of viral nucleocapsids to the nucleus [6]. The established protein contacts can serve as targets for antiviral chemotherapy. To identify human proteins interacting with HBV core, we employed a yeast two-hybrid system [7] and the human hepatocyte cDNA library.

MATERIALS AND METHODS

Strains. *Escherichia coli* strain DH5α (F^- *gyrA*96(NaI^r) *recA1 relA1 endA1 thi*-1 *hsdR*17(r^-_k m^+_k) *glnV44 deoR* Δ(*lacZYA-argF)U169*[ϕ80dΔ*(lacZ)*M15]) was used for plasmid construction. *S. cerevisiae* strain EGY48 (MATα, his 3, trp1, ura3, LexAop. – LEU2) was purchased from Clontech Inc.

Media. *E. coli* strain was grown in Luria–Bertani broth. The growth media YNB (0.67% yeast nitrogen base, 1% casamine acid, 3% galactose) supplemented with the amino acid mix and x-gal or a YNB lacking leucine was used for assaying the reporter gene's activity.

DNA methods. Most of recombinant DNA experiments were performed as described in [8]. All enzymes were obtained from Fermentas AB (Vilnius, Lithuania) and used according to the manufacturer's recommendations.

The human hepatocyte cDNA library in plasmid pB42AD as well as all other plasmids used in two-hybrid assay were purchased from Clontech Inc [9].

The primers:

2H1 5'-GCAATTCGACATTGATCCTTATAAAGAA-3'

2H2 5'-GCCTCGAGCTAACATTGAGATTCCCGAG-3'

were used for fusion of the HBV core gene with the AD domain in plasmid pB42AD.

ADdir-5 5'-CCAGCCTCTTGCTGAGTGGAGATG-3'

ADrev-3 5'-CGTATCTACCAACGATTTGACCC-3'

were used for PCR analysis and the sequencing of human cDNA library clones.

RESULTS AND DISCUSSION

Searching for proteins interacting with HBV core, we used a Matchmaker LexA yeast two-hybrid system and the human LexA hepatocyte cDNA library from Clontech Inc. To construct the bait for a two-hybrid assay, we linked the EcoRI and XhoI sites to the ends of the HBV core gene and fused it with the LexA DNA-binding domain in plasmid

pLexA, thus obtaining the plasmid pLexA-core. To embrace the whole variability of cDNA library, we screened about 2.5 million colonies by transforming pB42AD-cDNA plasmids into yeast *S.cerevisiae* strain EGY48, which had been already pretransformed with the reporter plasmid p8op-lacZ and pLexA-core. These transformants were spread on a synthetic yeast medium lacking leucine and colonies capable of growing on this medium and then transferred to the media containing components necessary for β-galactosidase expression. Thus, several transformants able to activate both reporter genes were selected for further analysis. From several yeast clones, inducing expression of the reporter genes at various levels, we isolated pB42AD plasmids with cDNA inserts. These plasmids were analyzed and transformed back to the yeast *S.cerevisiae* strain EGY48 containing plasmids p8pop-lacZ and pLexA-core, to confirm that plasmids with a cDNA fragment are really necessary for inducing expression of the reporter genes. Isolated pB42AD-cDNA plasmids were also transformed into yeast EGY48 strain containing plasmids p8op-lacZ and pLexA-Lam, to prove that protein encoded by cDNA fragment specifically interacts with the core protein but not with the control protein. Only five unique cDNA clones, which appeared to meet the requirements described above (see Figure and Table 1) were sequenced. The resulting DNA sequences were aligned against the sequences available at public databanks by employing the BLASTA2 algorithm [10]. Data on proteins encoded by isolated human cDNA clones are presented in Table 2.

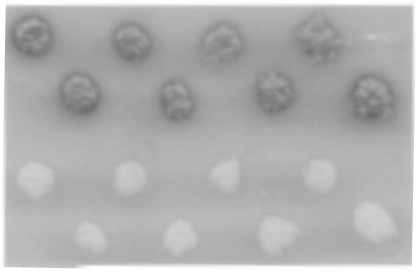

Figure. Specific human cDNA clones activate the reporter gene's expression, if they are cotransformed with HBV core gene, but not with the gene for control protein lamin

Table 1. **Activation of two-hybrid reporter genes by cDNA – HBV core or cDNA-human lamin interactions**

cDNA clone	β-galactosidase activation		Leu2 activation	
	HBV core	Human lamin	HBV core	Human lamin
55	++	–	+	–
19	++	–	+	–
53	+–	–	+	–
A21	++	– –+	+	–
A13	++	–	+	–

Table 2. **Data on proteins encoded by isolated human cDNAs**

cDNA clone	cDNA length	Official name in the databanks	5'-end completeness	3'-end completeness
55	800 bp	Homo sapiens fibrinogen, gamma polypeptide	Lacks 500 bp	Complete
19	1.6 kb	Homo sapiens fibrinogen, A alpha polypeptide	Lacks 280 bp	Complete
53	900 bp	Human ribosomal protein L5	Lacks 350 bp	Complete
A21	1.5 kb	Homo sapiens RGS-GAIP interacting protein GIPC	Lacks 350 bp	Complete
A13	1.7 kb	Homo sapiens PDZ domain protein GIPC2	Lacks 120 bp	Complete

Although ribosomal protein L5 specifically interacted with the HBV core in our tests, some ribosomal proteins are known to be false-positive in the two-hybrid system. Hence we must consider this interaction with care. Fibrinogen is a human blood protein, but e antigen, the product of HBV core gene alternative translation, is also secreted into blood, so under physiological conditions this interaction is quite possible, especially in the light of clinical observations evidencing that HBV frequently associates with fibrinogen. The HBV core interaction with GIPC and GIPC2 proteins [11, 12], which have been recently identified, is quite interesting, because these proteins participate in intracellular vesicular transport and can be involved in carrying HBV nucleocapsids to the cell nucleus.

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SU HEPATITO B ŠERDIES ANTIGENU SĄVEIKAUJANTYS ŽMOGAUS BALTYMAI

S a n t r a u k a

Pasitelkę mielių dviejų hibridų sistemą ir žmogaus hepatocitų kDNR duomenų bazę, ieškojome žmogaus baltymų, sąveikaujančių su žmogaus hepatito B šerdies antigenu. Iš ~2,5 mln. transformantų atrinkome keletą žmogaus kDNR klonų, specifiškai sąveikaujančių su HBV šerdimi. Buvo nustatytos penkių unikalių klonų pirminės nukleotidų sekos ir palygintos su sekomis, esančiomis viešuose DNR duomenų bankuose. Nustatyta, kad iškirtos kDNR koduoja žmogaus fibrinogeno alfa ir gama polipeptidus, žmogaus ribosominį baltymą L5 ir du baltymus, turinčius panašią domeninę struktūrą – GIPC ir GIPC2.