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BRIEF REPORT

Interaction of hepatitis B virus core protein with human GIPC1

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Abstract Up to now, little is known about hepatitis B virus core protein (HBc) interactions with host-cell proteins, although such interactions might be essential for virus propagation and pathogenicity. In this work, a human liver cDNA library was screened for proteins interacting with HBc. Among several HBc-interacting partners selected, it interacted most strongly with the human protein GIPC1. A common protein interaction domain, PDZ, was identified as the region that is sufficient for the interaction with HBc. The core protein has a putative C-terminal PDZ-interacting motif, and this sequence proved to be important for the interaction with GIPC1.

Hepatitis B virus (HBV) remains a major health problem, causing various clinical manifestations from asymptomatic to fulminant and acute hepatitis. Chronic infection can develop to cirrhosis or hepatocellular carcinoma [2]. Although HBV is relatively well studied, little is known about its core protein interactions with host proteins. There is emerging evidence, however, that structural proteins can play a significant role in viral pathogenesis. For example, human hepatitis C virus core protein is involved in apoptosis, immunomodulation, oxidative stress, carcinogenesis, and other processes [10]. In order to clarify the role of viral structural proteins in the pathogenesis of HBV-infected hepatocytes, it is necessary to investigate their interactions with host-cell proteins. The discovery of interactions might be helpful in identification of human proteins participating in important stages of the virus life cycle, such as virus entry and transport of nucleocapsids to the nucleus [13, 22]. Established protein contacts could serve as targets for antiviral chemotherapy.

In the present work, the yeast two-hybrid system was employed to search a human liver cDNA library for human proteins interacting with HBV core protein (HBc). The full-length core gene was amplified by PCR from the HBV ayw genome [3], kindly provided by Prof. P. Pumpens (Biomedical Research and Study Center, University of Latvia, Riga) and inserted into plasmids pLexA and pB42AD (Clontech). Yeast strain EGY48 (p8op-lacZ) transformed with pLexA-HBc expressed the fusion protein of expected size. Activation of reporter genes was detected by plating transformed yeast on SD/Gal/X-gal (galactose instead of glucose, 0.1 mg/ml of X-gal added) as well as on SD/Gal/−Leu (lacking leucine) medium. As expected, the interaction between HBV core hybrids was strong, whereas negative control SV40 virus T-antigen did not interact with HBc.

Interacting human proteins were screened by transforming LexA-HBc yeast cells with a human adult liver cDNA library inserted into plasmid pB42AD (obtained from Clontech). More than two million transformed cells were plated on medium lacking leucine, and nearly 300 resulting colonies were tested for β-galactosidase activity. False-positive and weakly positive clones were discarded, and 22 selected plasmids were sequenced. BLAST analysis identified the selected sequences as human cDNAs for GIPC1, GIPC2 [14], ribosomal protein L5, and fibrinogen polypeptides alpha and gamma. The interaction specificity of each selected protein was evaluated by measuring interactions between various proteins selected during the two-hybrid screening. Core interaction with three sequenced GIPC1 clones was strongest (Table 1) and specific, and this interaction was analyzed further.

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Table 1 HBV core interaction with human proteins in the yeast two-hybrid system

Human protein	β-galactosidase activity (%)
GIPC1	100
GIPC2	64
Ribosomal L5	37
Fibrinogen alpha chain	28
Fibrinogen gamma chain	42

Results were obtained by β-galactosidase liquid assay. All β-galactosidase activity data are relative to the data of HBV core interaction with GIPC1

GIPC1 (also known as GIPC, TIP-2, GLUT1CBP, RGS19IP1, synectin, SEMCAP, NIP, and IIP-1) is a human protein that interacts with several cellular proteins [5–7, 25] as well as with viral proteins [8, 21]. In most cases, these interactions occur at the PDZ domain, which initially was identified by uniform sequence repeats in PSD-95, Dlg, and a tight junction protein ZO-1 [15]. PDZ domains are built of 80–100 amino acid residues specialized for binding the C-termini of partner proteins, most often transmembrane receptors and channel proteins, or other PDZ domains [12]. The two-hybrid screen resulted in nearly full-length GIPC1 cDNA fragments with an intact PDZ domain. To test whether the PDZ domain is involved in the interaction with HBc, two-hybrid plasmids containing the PDZ domain only (residues 124–228), the PDZ domain with the adjacent C-terminal region, and the C-terminal region only were constructed. GIPC1 61–333, GIPC1 124–333, and GIPC1 124–228 fusions with LexA were able to interact with the core protein-AD domain fusion, whereas GIPC1 260–333 did not interact (Fig. 1a). These results imply that the PDZ domain is responsible for the interaction with the HBV core protein.

PDZ domains usually interact with three or four C-terminal amino acids of their protein partners; therefore, it was presumed that GIPC1 interacts with the C-terminus of the core protein. To test this hypothesis, a two-hybrid plasmid containing amino acids 141–183 of HBc was constructed. Experiments indicated that the C-terminus of the core protein is sufficient for the interaction with GIPC1 (Fig. 1b). To determine whether terminal amino acids of HBc are indeed important for the interaction with GIPC1, four C-terminal residues were removed. The altered C-terminus (Ser-Gln-Ala-Arg-COOH) is not similar to any known PDZ-binding motif (PBM), and this modified core protein was unable to interact with GIPC1 (Fig. 1a).

PDZ domains have been placed into three classes according to their binding selectivity. Class I domains recognize the consensus sequence Ser/Thr-X-ψ-COOH (where X is any amino acid and ψ is hydrophobic); class II domains prefer ψ-X-ψ-COOH; class III domains prefer Asp/Glu-X-ψ-COOH [17]. Initially, the specificity of the GIPC1 PDZ domain was thought to be class I [25]. In some cases, substitution of serine or threonine with another amino acid in the -2 position of PBM interfered with GIPC1 binding [23] (by convention, the C-terminus of PDZ ligands is designated as residue 0, and subsequent residues toward the N-terminus are numbered as residue-1, residue-2, and so forth [1]). But replacement of the -2 serine with alanine did not prevent HBc from interacting with GIPC1 (Fig. 1b). The C-terminus of the modified core protein (Glu-Ala-Gln-Cys-COOH) is similar to the termini of other GIPC1-interacting proteins–dopamine receptors D2 (Ile-Leu-His-Cys-COOH) and D3 (Ile-Leu-Ser-Cys-COOH) [11]—both of which contain leucine instead of serine or threonine in the -2 position. When more data accumulated, it became clear that the PDZ domain of GIPC1 binds sequences of all three classes [20]. But in a slightly altered classification, the class III binding motif is designated as X-X-Cys-COOH. Both the C-terminal sequence of HBc, Glu-Ser-Gln-Cys-COOH, and the modified sequence Glu-Ala-Gln-Cys-COOH resemble this

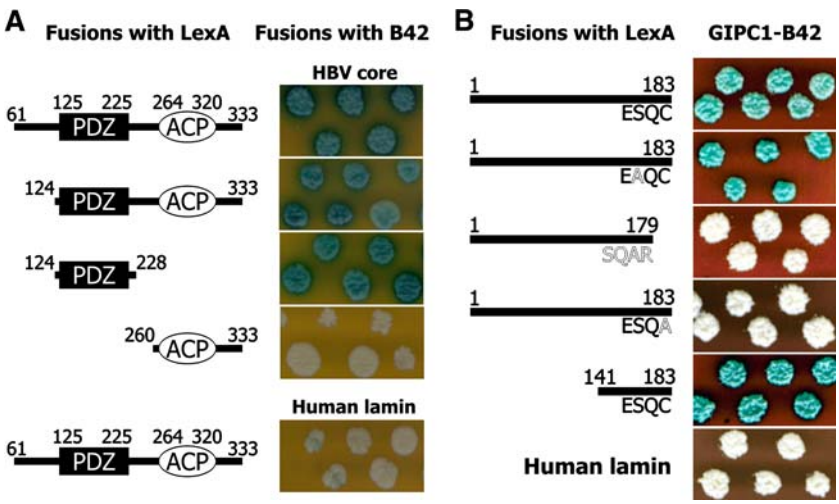

Fig. 1 Interaction of GIPC1 with the HBV core in the yeast two-hybrid system. **a** Mapping of the GIPC1-interacting region. Truncated GIPC1 variants and human lamin C (negative control) probed for interaction with HBc. **b** Mapping of the HBc-interacting region. Nearly full-length GIPC1 (61–333) was probed for interaction with various modifications of HBc and human lamin C

motif. Modified HBc with alanine instead of cysteine in the C-terminal position was unable to interact with GIPC1 (Fig. 1b).

The GST pull-down in vitro assay was used to confirm the determined protein interactions. Various fragments of HBV core and GIPC1 were inserted into a bacterial expression vector containing the GST gene. To enable uniform detection of interacting proteins, the same sequences were fused with the short coding sequence of an influenza virus hemagglutinin HA epitope. Proteins containing GST or HA on their N-terminus were synthesized in bacteria in different quantities, and their solubility also varied. Native GST protein, a typical negative control in this type of experiment, was more soluble and bound more efficiently to glutathione beads than any of the fusion proteins. GST and its fusions with full-length HBc or HBc 141–183 were immobilized on glutathione beads and tested for their ability to interact with HA-tagged GIPC1 61–333. After washing, the same amounts of beads were analyzed for the presence of the tagged proteins, and GIPC1 was found only on the beads with core-containing fusion proteins. Similarly, GST fusions with GIPC1 124–228 and GIPC1 124–333, but not GST alone, were able to bind HA-tagged full-length core protein (Fig. 2). These results confirm that the PDZ domain of GIPC1 interacts with the C-terminus of the HBV core protein.

Wild-type core proteins usually form homodimers and self-assemble into capsids. To determine whether fusion proteins in two-hybrid experiments form similar particles, yeast lysates with expressed HBc fusion proteins were subjected to electron microscopy, but core-like particles were not detected. It is likely that the addition of the lexA domain or the low concentration of fusion proteins interfered with particle formation. Although it is generally accepted that the arginine-rich C-terminal region of HBc interacts with the viral genome inside the particle, and although we did not detect core-like particle formation by the fusion proteins, it is still possible that GIPC1 might interact with intact HBV capsids. There are some data suggesting that at least a fraction of the C-termini are exposed on the external surface of the particle. For example, the arginine-rich protamine-like region of the core protein is responsible for the attachment of nucleocapsids to cell-surface-expressed heparan sulfate [24], monoclonal antibody directed against this region binds to intact HBV capsids, and trypsin can clip off this domain from recombinant HBV capsids [4]. It has been suggested that the peptide at the boundary between the assembly and arginine-rich domains of the core protein forms a mobile array and may allow an extreme mobility of the C-terminal domain [26].

What actual role the ability of the core protein to interact with GIPC1 might play in the life cycle of HBV remains to be determined. Accumulating data suggest that GIPC1 is an adaptor protein that couples other proteins to myosin VI movement and participates in recycling of membrane receptors [16]. Since cytoplasmic diffusion operates only within very small volumes, active membrane traffic or cytosolic transport of viral proteins and genome-protein complexes is required. It has been determined that HBV capsids are actively transported towards the nucleus with the aid of the cellular microtubule transport system [18]. Whereas the large gap between the cell periphery and the nucleus is usually bridged by microtubule transport, in the cell periphery and possibly in the nucleus, transport is mediated by the actin system [19]. Thus, one possible function of interaction between HBc and GIPC1 might be the short-distance intracellular transport of core proteins or capsids.

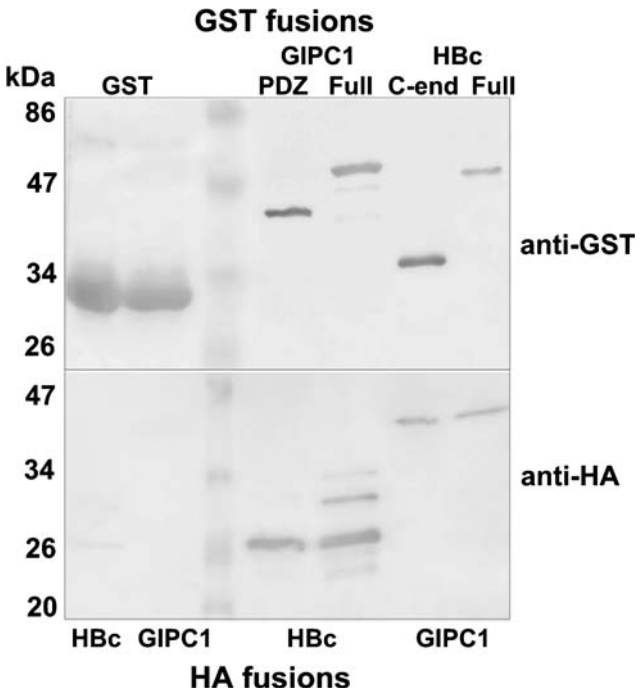

Fig. 2 GST pull-down experiment. GST (negative control) and GST fusion proteins were bound to the same amount of glutathione–agarose beads and allowed to interact with HA-tagged proteins. Samples were washed and analyzed by western blot with anti-GST (*top*) and anti-HA (*bottom*) antibodies. *Lane 1*: GST + HA–HBc; *2*: GST + HA–GIPC1; *3*: Protein molecular weight marker; *4*: GST–GIPC1(124–228) + HA–HBc; *5*: GST–GIPC1(124–333) + HA–HBc; *6*: GST–HBc(141–183) + HA–GIPC1; 7: GST–HBc + HA–GIPC1

GIPC1 interacts at least with two other viral proteins—Tax of T-cell leukemia virus type 1 (HTLV1) [21] and E6 of human papillomavirus type 18 (HPV-18) [8], but the exact functions of these interactions are unknown. It is known that the GIPC1-interacting proteins GLUT1 and NRP1 are involved in HTLV1 entry [9]. Deletion of the Tax PBM in HTLV-1 rendered the mutant virus unable to

establish persistent infections in rabbits. It was noticed also that although HTLV-1 and HTLV-2 are close relatives, only HTLV-1 is firmly associated with adult T-cell leukemia or inflammatory neurologic diseases. Tax1 protein, but not Tax2, contains a PDZ-binding motif at its C-terminus [27]. Similarly, the E6 oncoproteins from high-risk HPV strains contain a PDZ-binding motif, whereas those from low-risk isolates do not. Therefore, it is possible that HBc interactions with PDZ-containing proteins might be important in HBV pathogenicity or entry.

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