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ORIGINAL ARTICLE

Asialoglycoprotein receptor interacts with the preS1 domain of hepatitis B virus *in vivo* and *in vitro*

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Abstract
Background The preS1 domain of the large envelope protein has been identified as an essential viral structure involved in hepatitis B virus (HBV) attachment. However, the cellular receptor(s) for HBV has not yet been identified.
Aims To identify a cell-surface receptor for HBV, which could elucidate the molecular mechanism of HBV infection.
Methods A novel yeast two-hybrid system was used to screen proteins interacting with the preS1 region of HBV. Their interaction was verified by yeast cotransformation, coimmunoprecipitation and mammalian two-hybrid assay, while their intracellular and tissue localization was analyzed by confocal microscopy and immunohistochemistry, respectively.
Results Asialoglycoprotein receptor (ASGPR) interacted specifically and directly with the preS1 domain of HBV *in vivo* and *in vitro*. The levels of expression of preS1 and ASGPR in the liver were similar and correlated with each other.
Conclusions ASGPR is a candidate receptor for HBV that mediates further steps of HBV entry.

Introduction

HBV infection is a multistep process that begins with an energy-independent attachment of the viral particle to the carbohydrate side chains of hepatocyte-associated heparan sulphate proteoglycans [1, 2]. This primary attachment, often characterized by low affinity and reversibility, is usually followed by the passage of the virion to a more specific receptor, which mediates further steps of entry. Thus, identification of the "HBV receptor" or HBV binding partners is one of the challenging open questions in the field of HBV biology.

While the cellular structures that mediate viral binding and entry are less understood, more is known about the viral structures involved in this process. The S open reading frame in the HBV genome is comprised of preS1, preS2, and S regions, which encode three related envelope proteins: LHBs, MHBs and SHBs [3, 4]. LHBs, which includes all three regions, is the most abundant form found on the surface of infectious viral particles. As the preS1 region in LHBs is the outermost part of HBV particles, it is believed to play the most important role in the binding of HBV to hepatocytes. The putative attachment site of HBV, located at amino acids 21–47 of preS1, was first reported by Neurath and his colleagues using anti-preS1 antibody [5, 6]. Consistent with this finding, it has been observed that preS1(aa 3-77) and a QLDAPF sequence motif within this domain (corresponding to aa 18-25) are crucial for infectivity [7, 8]. Although it contains the major heptatocyte-binding site, there are reports that further epitopes outside of preS1(aa 21-47) are involved in HBV-cell attachment. Ishikawa et al. [9] showed that the preS1 1-47 peptide was not sufficient for a high-affinity interaction with the HepG2 receptor, whereas elements downstream of this region might be important in receptor interaction. Barrera et al. [10] showed that the preS1 1-40 peptide was insufficient to provide woolly monkey HBV (WMHBV) envelope with the capacity to infect human hepatocytes, suggesting that sequences downstream of residue 40 of preS1 could influence infectivity. So far, several proteins have been proposed to interact with these different regions of the preS1 domain. However, none of them have

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appeared to be generally accepted as a true receptor for the virus.

In an attempt to identify a specfic HBV receptor on human hepatocytes, a novel yeast two-hybrid system (Sos recruitment system, i.e., the CytoTrap system) was used to screen preS1-interactive proteins in this study. Regarding the role of epitopes outside of preS1 (21-47) in the binding step, a fusion protein of the entire preS1 domain with hSos was generated and used as bait. Using this approach, a clone encoding a preS1-interactive protein that is homologous to ASGPR was detected. The interaction between preS1 and ASGPR was verified by yeast cotransformation, coimmunoprecipotation, and mammalian two-hybrid assay. Intracellular and tissue localization of preS1 and ASGPR was further analyzed by confocal microscopy and immunohistochemistry, respectively.

Materials and methods

Yeast two-hybrid screening

The CytoTrap system (Stratagene), a novel yeast two-hybrid technique that detects protein-protein interactions in the cytoplasm as opposed to in the nucleus [11–13], was used to carry out yeast two-hybrid screening (the conventional two-hybrid constructs using intact preS1 fused to GAL4 activation or DNA binding domains resulted in autoactivation; data was not shown). The entire preS1 domain of HBV (subtype ayw) L protein was cloned into the pSos vector (Stratagene) and used as the bait. A stable pSos-preS1 cdc25H yeast strain was first established. The expression of the bait protein was analyzed by western blotting using a monoclonal antibody against preS1 (Santa Cruz). Autoactivation of the bait protein was verified by cotransformation with the pSos-preS1 plasmid and the pMyr Lamin C plasmid and was assayed for growth on galactose-containing medium at 37°C. Subsequently, cdc25H cells were cotransformed with the pooled bait plasmids and the commercially available liver cDNA library. About 10^4 transformants/plate grew on minimal glucose plates after 4 to 6 days of incubation at 25°C. Colonies were replica plated onto minimal galactose plates and incubated at 37°C for 5 to 7 days. Positive colonies exhibiting efficient growth on galactose plates at 37°C were isolated and tested for galactose-dependent growth at 37°C. Library plasmids were recovered and analyzed by DNA sequencing.

Plasmid construction

The entire coding region of HBV preS1 was cloned into four vectors: (i) the pSos vector (N-terminal Sos tag; cloning site, *Nco*I and *Mlu*I; Stratagene) for the yeast two-hybrid experiments; (ii) the pGBKT7 vector (N-terminal c-Myc tag; cloning site, *EcoR*I and *Sal*I; Clontech) for coimmunoprecipitation; (iii) the pM vector (N-terminal GAL4 BD tag; cloning site, *EcoR*I and *Sal*I; Clontech) for the mammalian two-hybrid assay; and (iv) the pcDNA3.1 (-) vector (cloning site, *EcoR*I and HindIII; Invitrogen) for the colocalization assay. Additionally, the entire coding region of ASGPR was cloned into three vectors: (i) the pMyr vector (N-terminal Myr tag; cloning site, *EcoR*I and *Sal*I; Stratagene) for the yeast two-hybrid experiments; (ii) the pGADT7 vector (N-terminal HA tag; cloning site, *EcoR*I and *BamHl*I; Clontech) for coimmunoprecipitation; and (iii) the pVP16 vector (N-terminal VP16 AD tag; cloning site, *EcoR*I and *Sal*I; Clontech) for the mammalian two-hybrid assay. All of the recombinant plasmids were verified by restriction enzyme digestion and DNA sequencing.

Yeast cotransformation

cdc25H cells were transformed with constructs encoding HBV preS1 and ASPGR (pSos-preS1, pMyr-ASPGR; generated as described above) by a slightly modified lithium acetate transformation procedure as described in the kit, and the growth of the cotransformants was compared to that of the positive and negative controls provided by Stratagene.

Coimmunoprecipitation (*Co-IP*)

To confirm the preS1-ASGPR interaction, *in vitro* Co-IP experiments were carried out using a Matchmaker™ Co-IP kit (Clontech). pGBKT7-c-Myc-preS1 and pGADT7-HA-ASGPR were transcribed and translated *in vitro* using a T_NT T7-coupled reticulocyte lysate system (Promega) according to the manufacturer's instructions. Before Co-IP, the *in vitro*-translated proteins were confirmed by western blot analysis, and thereafter, 10 μL c-Myc-preS1 protein was mixed gently with 10 μL HA-ASGPR protein, and the mixture was incubated at room temperature for 1 h. Ten μL of c-Myc monoclonal antibody or HA-Tag polyclonal antibody was added to the mixture, which was then incubated at room temperature for 1 h. Three μL of prepared protein A beads was added to the reaction tube, which was then rotated at room temperature for 1 h. After collection by centrifugation, the beads were washed five times with wash buffer. After resuspension in 20 μL of 2 × SDS loading buffer, samples were boiled and centrifuged briefly. Twenty μL of each sample was then subjected to SDS-PAGE, followed by immunoblotting with specific antibody according to the standard procedure. As a negative control, either the *in vitro*-translated c-Myc-preS1

protein or HA-ASGPR protein alone was immunoprecipitated with the specific antibody.

Mammalian two-hybrid assay

The preS1-ASGPR interaction was examined in a mammalian two-hybrid system (Clontech), strictly following the manufacturer's instructions. One day before transfection, 8×10^5 COS-7 cells were seeded in a 60-mm culture dish. COS-7 cells were cotransfected with purified plasmids (4 μg pM-preS1, 4 μg pVP16-ASGPR and 0.8 μg pG5CAT) using 22 μL Lipofectamine 2000 (Invitrogen) per dish. After incubation for 48 h, the interaction between preS1 and ASGPR was assayed by measuring chloramphenicol acetyltransferase (CAT) gene expression with a CAT detection ELISA kit (Roche Diagnostics GmbH). Three biological replicates were conducted for each assay, and the assay was repeated three times. A 3.5-fold or higher activation of CAT expression compared to the basal control was considered significant according to the supplier's protocol.

Confocal microscopy

Indirect immunofluorescence (IIF) was used to detect intracellular localization of HBV preS1 and ASPGR. A total of 8×10^4 HepG2 cells were inoculated on coverslips in a 24-well culture plate one day before transfection. HepG2 cells were transfected with the pcDNA3.1(-)-preS1 (0.8 μg) vectors using 2 μL Lipofectamine 2000 per well, and cells were incubated at 37°C in a 5% CO_2 incubator for 30 h prior to performing IIF with two primary antibodies (mouse monoclonal anti-preS1 [Santa Cruz], dilution 1:100; goat polyclonal anti-ASGPR [Santa Cruz], dilution 1:100) and two fluorescent conjugated secondary antibodies (FITC-conjugated horse anti-mouse, dilution 1:50; TRITC-conjugated rabbit anti-goat, dilution 1:50). Nuclei were stained with DAPI (1 mg/mL; Roche Diagnostics GmbH) before being examined under Olympus FV1000 confocal laser scanning biological microscope. Six to eight adjacent random fields were evaluated for each section.

Immunohistochemistry study

Preserved paraffin-embedded liver tissue samples from 19 HBsAg-positive hepatocellular carcinoma (HCC) patients were obtained from the Department of Pathology, First Affiliated Hospital of the Medical College of Xi'an Jiaotong University. After being dewaxed and hydrated, the tissue sections were treated as follows: blocking for 8 min in 3% H_2O_2, washing with phosphate-buffered NaCl solution (PBS), blocking for 20 min in 5% BSA, incubation with primary antibodies overnight at 4°C, washing with PBS, incubation with secondary antibodies at 37°C for 30 min, and DAB staining. Five fields per section were examined. The expression of pre-S1 and ASGPR in the liver was scored semiquantitatively according to the proportion of positive cells on a 0 to 4^+ scale, corresponding to positivity in 0%, 1-10%, 11-25%, 26-50%, and >50% of hepatocytes examined, as published previously [14].

Results

Yeast two-hybrid interactions

The HBV preS1 ORF was subcloned into the pSos vector to be expressed as the bait protein. The recombinant plasmid, pSos-preS1, verified by DNA sequencing, was introduced into yeast cdc25H by transformation. The properly expressed fusion protein Sos-preS1 was identified by western blotting using a mouse monoclonal antibody against preS1 (Fig. 1), while no autoactivation of the bait protein was detected because cdc25H cells coexpressing pMyr-Lamin C and the pSos-preS1 fusion protein failed to grow at 37°C (Fig. 2). cdc25H cells were cotransformed with pSos-preS1 and a human liver cDNA library. Potential protein interaction partners of preS1 were identified by transferring colonies to galactose-containing media and screening for colony growth. This approach yielded five positive clones. DNA sequence analysis revealed that the five cDNA fragments shared identity with five different genes in the GenBank database (Table 1). Upon considering properties such as cellular location (membrane spanning or extracellular), tissue distribution (liver distribution

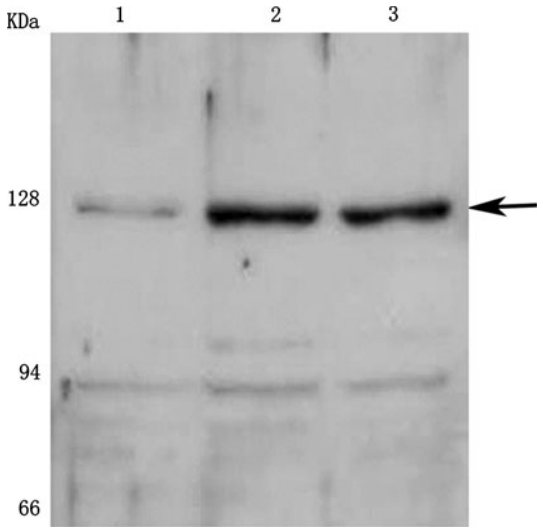

Fig. 1 Western blot analysis of cell extracts from cdc25H cells expressing pSos-preS1, probed with an anti-preS1 monoclonal antibody. Lanes 1-3, cell extracts of cdc25H cells expressing pSos-preS1

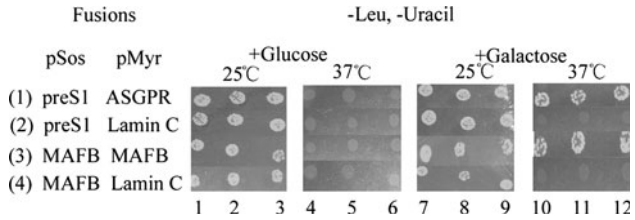

Fig. 2 CytoTrap two-hybrid system identifies interaction between preS1 and ASGPR. Temperature-sensitive cdc25H yeast cells were cotransformed with the indicated constructs: (1) experimental group: pSos-preS1 and pMyr-ASGPR; (2) autoactivation test group: pSos-preS1 and pMyr-Lamic; (3) positive control: pSos-MAFB and pMyr-MAFB; (4) negative control: pSos-MAFB and pMyr-Lamin C. Three independent clones are shown for each interaction. "+ glucose" : minimal glucose plate; "+ galactose": minimal galactose plates; "-leu, -uracil": minimal glucose or galactose plates without leu and uracil

or accessibility), and function of the proteins encoded by the five clones, ASGPR was selected as the most likely cell-surface receptor for HBV.

To confirm the interaction, ASGPR was cloned into pMyr vector as the target construct (pMyr-ASGPR) and used to transform the same yeast strain with the bait construct (pSos-preS1). Although all of the cells grew at 25°C, only cdc25H cells coexpressing the pMyr-ASGPR and the pSos-preS1 fusion protein or pSos-MAFB and pMyr-MAFB (positive control) could grow at 37°C, indicating that the amino terminus was processed and the carboxyl terminus remained intact (Fig. 2). The data indicated that the interaction between HBV preS1 and ASGPR was specific in yeast cells.

Interaction of preS1 with ASGPR in vitro

To further confirm preS1-ASGPR interaction, *in vitro* Co-IP analysis was performed. The *in vitro*- translated pGBKT7-c-Myc-preS1 protein and pGADT7-HA-ASGPR protein were mixed together. Complexes were immunoprecipitated with a tag-specific antibody (c-Myc monoclonal antibody or HA-Tag polyclonal antibody) and subjected to western blot analysis. An approximated 42-kDa protein (the predicted size of HA-ASGPR) was coimmunoprecipitated with c-Myc monoclonal antibody

Table 1 preS1-interactive proteins identified using the CytoTrap system

Protein	Cellular location	Tissue distribution
Homo sapiens potassium channel modulatory factor 1(KCMF1)	Nuclear	Ubiquitous
Cytochrome C (Cyt-C)	Mitochondrion	Ubiquitous
Vitamin D binding protein (VDBP)	Extracellular	Plasma, asctic fluid, cerebrospinal fluid and urine
Homo sapiens albumin (ALB)	Extracellular	Plasma, asctic fluid, cerebrospinal fluid and urine
Homo sapiens asialoglycoprotein receptor (ASGPR)	Membrane	Liver

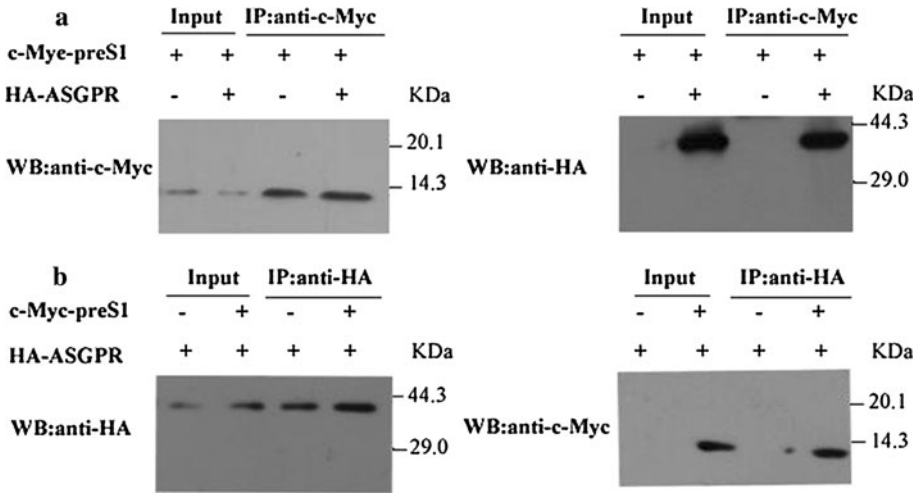

Fig. 3 ASGPR binds to preS1 *in vitro*. (a) Complexes of *in vitro*-translated pGBKT7-preS1 and pGADT7-ASGPR or *in vitro*-translated pGBKT7-preS1 alone were immunoprecipitated with c-Myc monoclonal antibody, and the immunoprecipitates were subjected to western blot analysis using HA-Tag polyclonal antibody. (b) Complexes of *in vitro*-translated pGBKT7-preS1 and pGADT7-ASGPR or *in vitro*-translated pGADT7-ASGPR alone were immunoprecipitated with HA-Tag polyclonal antibody, and the immunoprecipitates were subjected to western blot analysis using c-Myc monoclonal antibody

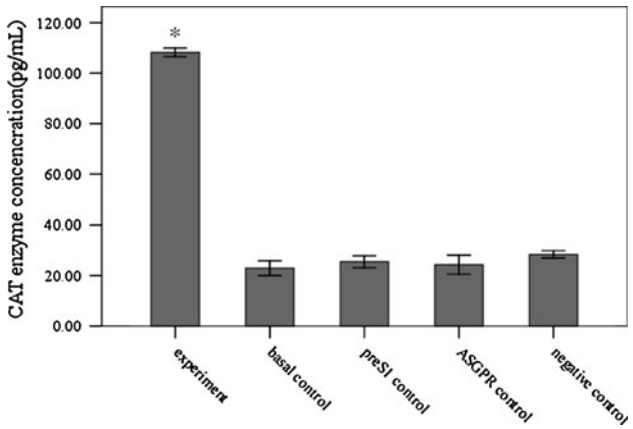

Fig. 4 CAT activities of different cotransfection groups. Experiment: pM-preS1, pVP16-ASGPR and pG5CAT; basal control: pM, pVP16 and pG5CAT; preS1 control: pM-preS1, pVP16 and pG5CAT; ASGPR control: pM, pVP16-ASGPR and pG5CAT; negative control: pM-53, pVP16-CP and pG5CAT. The CAT activity of each group is expressed as the concentration of the CAT enzyme. The "experiment" group showed much higher CAT activity than the basal control group (108.2 pg/mL v.s. 22.9 pg/mL, *P value <0.0001)

from the complexes, while an approximated 13-kDa protein (the predicted size of c-Myc-preS1) was coimmunoprecipitated with HA-Tag polyclonal antibody from the complexes. Therefore, these data confirmed a direct and specific interaction between ASGPR and the preS1 domain of LHBs *in vitro* (Fig. 3).

Interaction of preS1 with ASGPR in mammalian cells

COS-7 cells that were cotransfected with the recombinant plasmids (pM-preS1 and pVP16-ASGPR) together with the reporter-gene-expressing vector (pG5CAT) were lysed, and the quantity of CAT enzyme in the cell extracts was determined. When both pM-preS1 and pVP16-ASGPR were introduced, an about 5-fold increase in CAT activity was observed compared to the basal control, and this difference was statistically significant (Fig. 4, Student's *t*-test, $P < 0.0001$). However, there was no significant difference in CAT activity between the basal control and the preS1 control or the ASGPR control ($P > 0.05$).

Intracellular localization of HBV preS1 and ASPGR

The localization of preS1 and ASGPR in HepG2 cells transfected with pcDNA3.1(-)-preS1 plasmid was analyzed by confocal fluorescence microscopy (Fig. 5). Cells that were positive for HBV preS1 had green fluoresence, while cells positive for ASGPR had red fluorescence, and nuclei counterstained with DAPI were light blue (plate a). These results indicated that preS1 was located on membranes, in the cytoplasm and in the nucleus (plate b), while ASGPR was observed to localize strongly to the cell membrane and weakly to the cytoplasm (plate c). The merge of the datasets analysed by double-channel confocal microscopy showed a yellow color (plate d), which revealed exact co-localization between preS1 and ASGPR on membranes of HepG2 cells and confirmed the preS1-ASGPR interaction.

Location and distribution of preS1 and ASPGR in liver tissues

All of the 19 patients, both HBeAg-positive and -negative, had preS1 and ASGPR detectable in the liver, on the plasma membrane and/or in the cytoplasm (Fig. 6). The expression of preSl was solely membranous in one case, mixed membranous and cytoplasmic in five cases, exclusively cytoplasmic in 11 cases, and mixed membranous, cytoplasmic and nuclear in two cases. The expression of ASGPR was solely membranous in three cases, mixed membranous and cytoplasmic in seven cases, and exclusively cytoplasmic in nine cases. Interestly, the expression of ASGPR was similar to that of preS1 (membranous: 52.6% or 10/19 vs 36.8% or 7/19, $p = 0.515 > 0.05$; cytoplasmic: 94.7% or 18/19 vs 84.2% or 16/19, $p = 0.604 > 0.05$). In addition, the expression of ASGPR and correlated significantly with the expression of preS1 (Pearson corrlection 0.776 [$P < 0.0001$]).

Discussion

Despite the fact that the specific preS1 domain for attachment of the LHBs to human liver cells and/or cell lines has been repeatedly verified, the cellular protein that is responsible for this binding has not been found yet. This study shows, by different *in vivo* and *in vitro* methods, that ASGPR on human hepatocytes binds specifically to the pre-S1 domain of HBV (*ayw* subtype). Taken together, the data suggest that ASGPR may be a important and specific binding partner of HBV that mediates viral entry into host cells.

There is a constantly growing list of proteins that have been found to bind to HBV, but for none of these potential binding factors is there convincing evidence of its essential relevance for the infection process. Human immunoglobulin A (IgA) receptor[15], glyceraldehyde-3-phosphate dehydrogenase [16], interleukin-6 [17], a 31-kDa protein [18], HBV binding factor (HBV-BF) [19] and ASGPR [20–22] have been proposed as possible receptors in earlier studies. Ryu et al. [23] identified an 80-kDa protein (p80) using a glutathione S-transferase-preS1 fusion protein, while De Falco et al. [24] identified a 44-kDa protein (HBV-binding protein; HBV-BP) using the preS1 peptide. More recently, lipoprotein lipase (LPL) [25] and

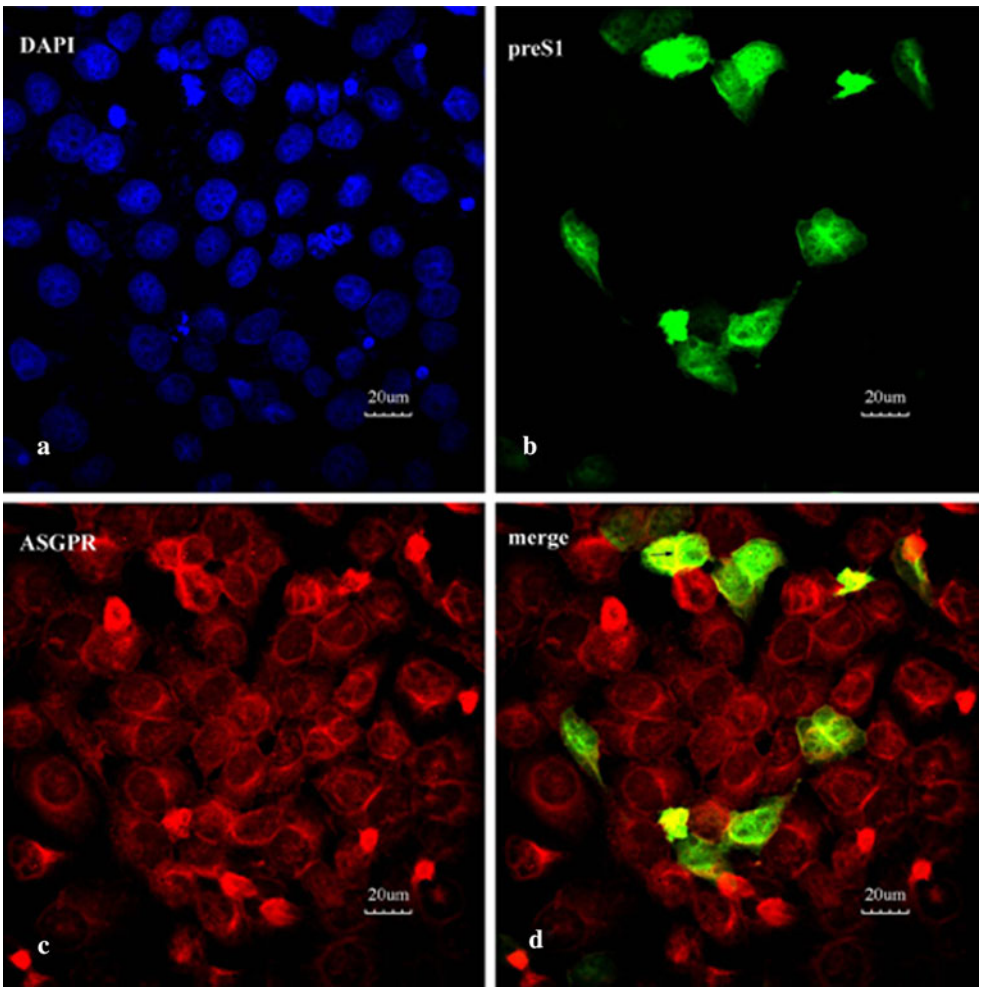

Fig. 5 Intracellular localization of HBV preS1 protein and ASPGR in HepG2 cells (original magnification, 10 × 40). (**a**) DAPI-stained nuclei showed light blue fluorescence; (**b**) preS1 detected indirectly using green fluorescent antibody was observed at the membrane, cytoplasm and nucleus; (**c**) ASGPR detected indirectly using red fluorescent antibody was observed on the plasma membrane and in the cytoplasm; (**d**) cells doubly stained to detect preS1 (green) and ASGPR (red); areas of co-localization appear yellow (arrow)

hepatocyte-associated heparan sulfate proteoglycans [1] were shown to have attachment sites for HBV. In addition, Li et al. [26] speculated that nascent polypeptide-associated complex ɑ. polypeptide (NACA) was a functional target of hepatitis B virus preS1 protein in cells. Cui et al. [27] identified glucose-regulated protein 75 (GRP75) as a novel preS1-binding protein using a proteomics strategy. However, the nature of the interaction of HBV with these putative receptors has not been confirmed. As to the assay systems, the majority of the previous studies used synthetic peptide preS1 (21-47) or HBsAg (or HBV) as a ligand. In contrast, in this study, purified whole preS1 domain was used as a ligand. In addition, our assay system may have several other advantages. First, the whole preS1 domain may exhibit a more specific interaction with the receptor molecule than synthetic peptides because its structural features are much closer in configuration to those of the native protein. In addition, the purified preS1 protein is less likely to react nonspecifically with cellular proteins than HBsAg, which contains a small percentage of the L protein and consists mostly of the major S protein. Finally, glycine 2 of the preS1 protein is modified with a myristate[28], which is required for infectivity [29–31], and so far, none of the proteins described above have been shown to require a preS1 protein containing the amino-terminal myristate for infectivity. In contrast, the yeast two-hybrid system used in this study would allow the preS1 protien to undergo post-translational modification such as glycosylation, phosphorylation and myristylation. Using this assay system, we identified ASGPR as the protein in hepatocytes that interacted specifically with the pre-S1 domain of HBV.

ASGPR is a transmembrane glycoprotein that is highly and exclusively expressed on the surface of the hepatocytes in a polar manner, i.e., it is present on the sinusoidal and lateral plasma membranes but not on the bile canalicular membrane[32–34]. It mediates binding, internalisation and

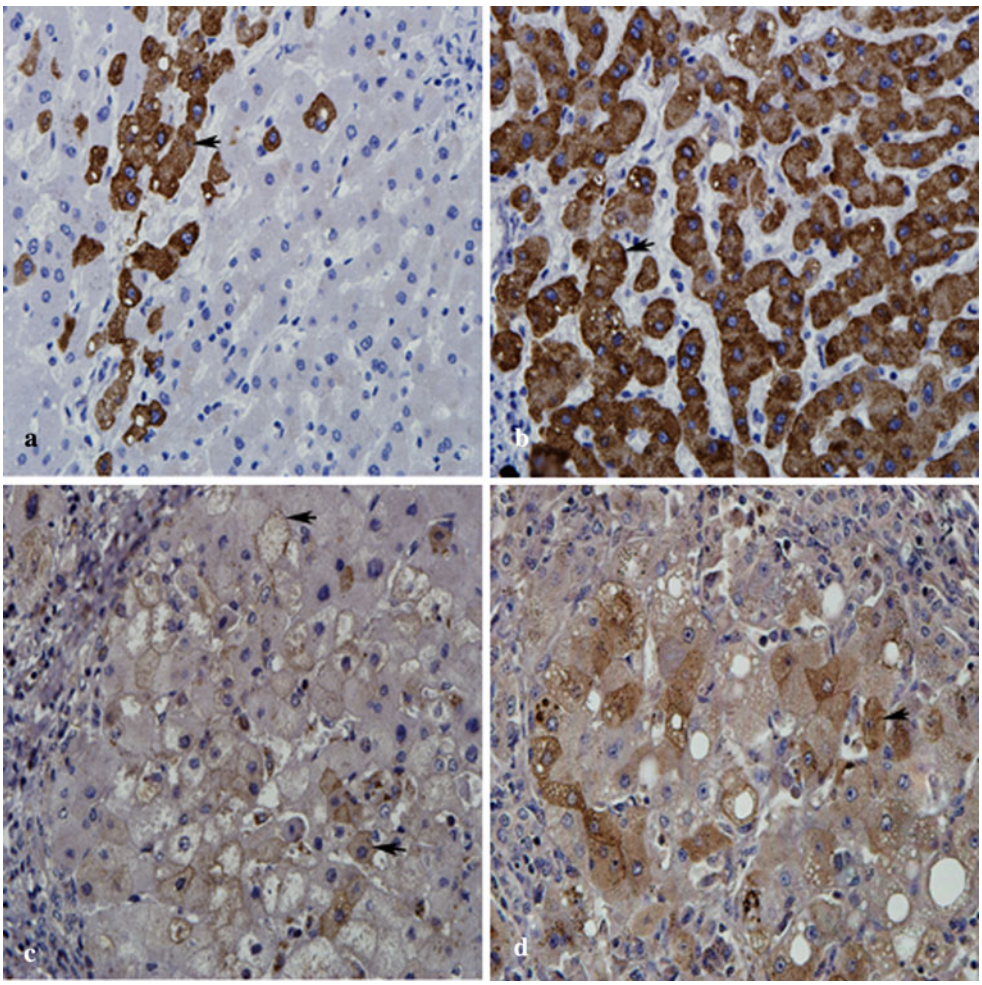

Fig. 6 Immunohistochemical detection of preS1 and ASGPR in hepatocellular carcinoma tissue (original magnification, 10 × 40). (**a** and **b**): preS1 was strongly expressed in the cytoplasm of hepatocytes and partially expressed on the membrane (arrows); (**c** and **d**): The expression of ASGPR on the plasma membrane was usually diffuse, while that in the cytoplasm was either focal or in clusters (arrows)

degradation of extracellular glycoproteins that have exposed terminal galactose residues, and previous reports have demonstrated its capability to bind specifically to HBV within the preS1 domain [20–22]. However, there are still controversial issues that have to be addressed. First, in a previous study, HBV particles isolated from HBV carriers with high-titre viraemi served as the source of HBV. However, it has been shown that the interaction between ASGPR and desialylated glycoprotein or glycolipid on the surface of HBV particles is critical for infection by HBV. Hence, it must be considered that desialylated serum factors attached to HBV may serve as mediators of interaction with the binding sites of receptors. In addition, the effect of an anti-preS1 MAb (MA18/7) remains unclear with regard to a putative attachment site on the HBV. As O- and N-linked glycosylation of the preS1 polypeptide have been described [35], the binding of the MAb to the preS1 epitopes may hinder attachment by interfering with a D-galactose-bearing glycoside on the virus, although the essential sequence element needed for infection is elsewhere. These points led us to refocus on ASGPR as a candidate cellular receptor for HBV infection. Therefore, in this study, the authenticity of the specific interaction between preS1 and ASGPR was further identified using coimmunoprecipitation, mammalian two-hybrid and co-localization assays. In support of and consistent with previous work,

the data demonstrated that ASGPR could interact with the preS1 domain of HBV specifically and directly *in vitro* and *in vivo*. More importantly, the mammalian two-hybrid system and co-localization assay used in this study may allow characterization of the protein:protein interactions within a cellular environment that more closely mimics the native protein environment than the *in vitro* binding assay used in the previous study, due to protein folding, post-translational modification and cellular localization.

However, more detailed studies are needed to determine the minimal binding domain on both preS1 as well as ASGPR by analysis of deletion mutants.

Previous studies have shown successful detection of preS1 polypeptides in liver tissue, on the plasma membrane and/or in the cytoplasm [14, 36]. Almost consistently, in this study, the preS1 domain was found to be localized generally on the plasma membrane, in the cytoplasm and in the nucleus of hepatocytes, by both immunofluorescence and immunohistochemistry assays. Interestly, ASGPR showed membranous expression or mixed membranous and cytoplasmic expression in liver tissue, which is similar to and correlated with the distribution of preS1. Since intracellular localization influences protein function by controlling access to and availability of all types of molecular interaction partners, the study of protein intracellular localization is important for elucidating protein function. Thus, the intracellular localization of the preS1 region may demonstrate its role in viral entry, intracellular localization, replication and extracellular appearance of HBV, as described previously [37]. In this process, ASGPR may play an important role by interacting with the preS1 region because of their similar and correlated distribution in the liver. Accordingly, we speculate that the preS1-ASGPR interaction described here may be functionally important in events that guide entry, fusion and intracellular transport of the virus. To our knowledge, there have been no similar reports on this basis until now.

In conclusion, the findings of this study show that ASGPR on human hepatocytes can interact specifically and directly with the preS1 domain of LHBs (ayw subtype) and is a candidate receptor for HBV, mediating further steps of HBV entry. This leads to the hypothesis that, at least in part, inhibitors of ASGPR, such as anti-ASGPR antibody, may be useful in preventing HBV infection. Further studies are needed to define the role of preS1-ASGPR interactions in the life cycle of HBV and determine whether ASGPR-mediated entry is the major route of HBV infection.

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