Deciphering the mystery of hepatitis B virus receptors: A historical perspective

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Abstract Hepatitis B virus is one of the major reasons of viral hepatitis with an estimated 350 million infected patients worldwide. Although, the virus was discovered and cloned more than three decades ago, its entry mechanism has still been in investigation. Numerous potential candidates have been proposed and investigated rigorously to reveal HBV entry mechanism and to unveil the first door of viral entry to hepatocytes. This review provides a short account of role of receptors for entry of HBV into hepatocytes. The viral preS1 region of large surface protein is involved in the attachment of HBV to hepatocytes. The putative attachment site of HBV is located at amino acids 21–47 of preS1. So far, several proteins have been proposed to interact with these different regions of the preS1 domain which includes human immunoglobulin A receptor, glyceraldehyde-3-phosphate dehydrogenase, interleukin-6, a 31-kDa protein, HBV binding factor, asialoglycoprotein receptor, nascent polypeptide-associated complex α polypeptide, lipoprotein lipase, hepatocyte-associated heparan sulfate proteoglycans, glucose-regulated protein 75. However, none of them have appeared to be generally accepted as a true receptor for the virus until recently when sodium taurocholate cotransporting polypeptide identified as HBV entry receptor. Current review provides scientific historical perspective of various candidates known to be interacting with preS1 of HBV for their possible role in viral entry.

Keywords Hepatitis B virus · preS1 · Asialoglycoprotein receptor · Hepatitis B virus binding protein

Introduction

Viruses use specific proteins, glycoproteins, or carbohydrate residues on the cell surface of host for their attachment and subsequent entry into the host cell. These proteins, glycoproteins, or carbohydrate residues, present on cell surface, termed as receptors. They are mainly responsible for the site of initiation of the infection and also bear immunological significance in term of signature epitopes and antiviral vaccines.

The first step in hepatitis B virus (HBV) infection is the successful entry of virus into the host cell which is aided by the binding of one-or more-viral envelope protein(s) to cell membrane structures. The viral proteins that are involved in attachment are well studied but a conclusive cellular receptor for HBV and the resulting biochemical role of that receptor leading to infection is not well determined. Hepatocytes are the main target of HBV, Neurath et al. identified that 21–47 (10–36 amino acids in genotype D) residues of preS1 are liable for the attachment of HBV to hepatocytes [47]. Further studies localized the binding site of preS1 to 21–36 or 16–38 residues of preS1 [45, 53]. Mainly HBV binds to receptors on the surface of hepatocytes but there are studies showing the receptors of HBV (10–36 residues of preS1) on the cells of non-hepatic origin like, hematopoietic cells of the B lymphocyte lineage and peripheral blood lymphocytes [46].

The mechanism by which HBV-particles gain access into target cells and its uptake and all the entities involved, in particular into hepatocytes are yet to be identified. Past studies on viral uptake mechanism suggest one of these

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Fig. 1 Different interacting proteins that binds to preS1 region of HBV. *HBV-BF* hepatitis B virus binding factor, *HBV-BP* hepatitis B virus binding protein, *NACA* nascent polypeptide-associated complex

pathways, either phagocytotic or endocytotic pathways, but which one is more dominant in HBV, this remains a mystery till now [32]. Studies report the binding of preS1 and preS2 viral envelope proteins with hepatocellular membrane. Several receptors on these hepatocellular membranes have been proposed which could bind either to wild-type HBV particles or to genetically engineered virus.

The search for of HBV entry receptor has kept the scientist awaited for quite some time. Although different studies targeting the area of research lead to identification of more than ten different HBV preS1 interacting proteins, until recently, NTCP gained the privilege of stating to be the bona fide receptor of HBV. Most of the candidate receptor's tried, have underlying molecular and cellular interactions, largely unknown [10].

The list includes interleukin-6 because it contains recognition site for the earlier reported residues on preS1-10–36 amino acids [48]. Then there was a 31-kDa protein binding to the same residues identified in HepG2 cell lysate. The same HepG2 cells were later reported to carry human squamous cell carcinoma antigen 1 [11], a membrane protein, interacting with the same infamous residues preS1 21–47 amino acids [47]. The list gets populated by past studies reporting immunoglobulin A receptor [54], asialoglycoprotein receptor [67], hepatitis B virus binding factor [5], Glyceraldehyde 3 phosphate

α polypeptide, *IL6* interleukin-6, *GAPDH* glyceraldehyde 3 phosphate dehydrogenase, *ASGPR* asialoglycoprotein receptor, *NTCP* sodium taurocholate cotransporting polypeptide

dehydrogenase [52], P80 [56], nascent polypeptide-associated complex α polypeptide (NACA) [30], GRP75 [7], lipoprotein lipase [70], heparin sulfate [57] and sodium taurocholate cotransporting polypeptide (NTCP) (Fig. 1).

This review provides a comprehensive knowledge of potential candidates, considered as an entry receptor to HBV. Knowledge of these candidate receptors can help in better understanding the viral re-uptake mechanism of HBV, which has been in shadow, even though the virus has been cloned more than three decades ago. This account will also further enhance the understanding of other host related factors, in order to come up with more effective candidates to inhibit HBV entry.

Hepatitis B virus binding protein (HBV-BP)

The exploration of attachment site for HBV in HepG2 cells revealed trimeric synthetic peptides (preS1 residue 10–36) having molecular weight of 44 kDa. It was subsequently named HBV-BP and bears sequence homology with squamous cell carcinoma antigen 1 (SCCA1) [11]. Hao et al. reported that Ferritin light chain (FTL) act as a coreceptor along with SCCA1 in entry of HBV into hepatocytes (preS-FTL-SCCA1) [14]. The FTL and SCCA1 both act as coreceptor in entry of virus into cells.

lineage and peripheral blood lymphocytes also infected with HBV [46].

In an attempt to identify the receptors of HBV on different cells other than hepatocytes, Neurath et al. identified IL6 as the HBV receptor on cells of non-hepatic origin [48]. IL6 is produced by a variety of cells [19], which suggest the diversity of target cells for HBV. Many cells of extrahepatic origin like, T cell lines, PBMC and promonocytic, did not constitutively expressed IL6, HBV could not bind to these cells, these cells express the binding site of IL6 and HBV only after activation. The replication of HBV in PBMC [78] and peripheral T lymphocytes [22] is due to the expression of IL6 in variety of cells. As IL6 expressed on variety of cells, this also explain the reactivation of latent HBV infection of lymphoid cells after lipopolysaccharide activation [23].

As HBV envelope protein have binding site for IL6, after binding with IL6, the HBV binds to cell surface through another anchor protein and this protein is reported to be a proteoglycan in nature [48].

However, these non-hepatic cells require activation by lectins and lipopolysaccharides, in order to interact with HBV-preS1. This finding leads to the conclusion that preS1 of HBV interacts with IL6 after its activation.

Glyceraldehyde 3 phosphate dehydrogenase (GAPDH)

Studies have shown that preS1 (amino acid 21–47) of HBV binds to 35 KDa protein which is homologous to glyceraldehyde 3 phosphate dehydrogenase (GAPDH). It is a glycoprotein has high molecular weight mannose side chain [52]. GAPDH is a soluble protein that performs diverse intracellular functions like apoptosis induction, receptor-associated kinase, tRNA export or DNA repair [66].

Recent studies have shown that GAPDH involved in the posttranscriptional regulation of hepatitis B virus gene expression through binding with PRE. The hepatitis B virus posttranscriptional regulatory element (PRE), is an RNA *cis*-element that functions by activating mRNA and exports it to the cytoplasm thus essential for high-level expression of HBV surface gene [31].

P80

Another receptor investigated to be used by HBV, is an 80-kDa protein (p80) on human hepatocytes binds specifically to the preS1 domain of HBV. The preS1 12–20 and 82–90 amino acids (ady/adw) are involved in attachment with p80. The binding domain of p80 lies outside from the receptor binding domain, 21–47 residues. The expression of p80 is not only in hepatocytes, but also in all human tissues and cell lines (human oral epidermal cells, PBMC,

HEK cells, HeLa cells, and rat hepatocytes) except fibroblasts. Human hepatocytes may also be expressing some proteins and specifically p80, which is reported to be crucial for viral entry machinery [56].

Nascent polypeptide-associated complex α polypeptide (NACA)

Nascent polypeptide-associated complex α polypeptide (NACA) also explored for HBV entry. Mainly the NACA present in the cytoplasm but there are evidences of its presence close to the plasma membrane. Its presence close to the plasma membrane is explained by the fact that it binds to other cellular proteins and are recruited to cell surface as protein complex. There are several examples of proteins that use same strategy and act as viral receptor. The localization of NACA provides evidence that it binds with preS1 of HBV and act as entry receptor [30].

NACA also have sequence similarity with many transcription-regulating proteins [30]. This similarity leads to some hypothetical deductions which are, NACA may be acting as a transcriptional coactivator enhancing the c-Jun/c-Jun homodimer and c-Fos [42]. It might be interacting with the TATA box-binding protein and four-way DNA junction. Several members of the TNF-receptor gene superfamily has reported to be intracellularly regulated by NACA [61]. Reported literature showed that modulating the ability of NACA to act as a transcriptional coactivator may enable the virus to inhibit transcriptional activation of host genes. It might be a merely possibility that for regulating the transcriptional function of preS1 NACA may act as a cellular protein and that its binding to preS1 may prevent interaction of NACA with other host proteins important for its function. The interaction between preS1 and NACA and the resulting pathophysiological significance is largely unknown [30].

Glucose-regulated protein 75 (GRP75)

Glucose-regulated protein 78 (GRP78) and glucose-regulated protein 75 (GRP75) are the members of HSP70 family (HSP-70s), a molecular chaperone that plays critical roles in protein folding and trafficking [18]. Studies have reported the interactions of preS1 of HBV with GRP75 and GRP78 [7]. HBV virion employs GRP78 and GRP75 as an adjunctive carrier for cellular entry during viral infection. GRP78 and GRP75 have ATPase domain (N-terminal 44-kDa), peptide-binding domain (N-terminal 18-kDa domain) and C-terminal 10-kDa fragment [18]. As both have peptide binding domain, they bind to preS1 through N-terminal 18-kDa domain. The 12–20 and 82–90 residues of preS1 involved in binding with GRP78 [6, 12] while the exact binding site for GRP75 is not yet known [7].

GRP78 and GRP75 have different cellular localization. GRP78 localized in endoplasmic reticulum (ER) [18], while GRP75 localized in mitochondria and both of these protein are reported expressing in cell surface [55, 60].

There are two possible roles of GRP78 and GRP75, either they help in HBV binding to hepatocyte and subsequent internalization or they help in proper folding of the large envelope protein [7]. As HSP-70s prevents translocation of abnormally structured proteins from the ER [6, 17, 18], it can be inferred that GRP78 and GRP75 involved in the retention of L protein into the ER through binding with partially assembled L protein.

Lipoprotein lipase (LPL)

Lipoprotein lipase (LPL) is another candidate receptor reported to bind to HBV preS1 (10–36 residues). Deng and colleagues identified that preS1 binding motif is W/FT/SXWW/F where X can be any amino acid. Through BLAST search and molecular modeling studies, they have identified that this binding motif is present in carboxy terminal domain (amino acid 313–448) of LPL [10]. It has been reported that synthesis of LPL is done in extrahepatic tissues [38]. On the basis of its synthesis Deng and colleagues inferred that during HBV infection LPL may act as extrahepatic carrier in bridging HBV to cell surface. LPL is distributed throughout the body, present on many cells of body despite of hepatocytes. HBV can only interact to those cells that express LPL [10].

Heparin sulfate

The extracellular matrix and the plasma membrane contains glycoproteins, glycolipids, and proteoglycans and these can be used as attachment site by different pathogens, including protozoae (e.g., leishmania), bacteria (e.g., *Bordetella pertussis*), and viruses (such as herpes simplex viruses or human immunodeficiency virus) [13, 33, 50, 75]. Studies have shown that heparin sulfate proteoglycans (HSPGs) provides an initial binding site to HBV on the surface of hepatocytes [57]. Studies have reported that the preS1 binds to HSPG through myristoylated N-terminal domain [57].

Although many cells expressed HSPGs but the proteoglycan content of one cell is different from the other. Liver heparan sulfates differs from other in the way that they have large number of N-sulfated glucosamine residues at distal end of core protein and low sulfation density in proximal end relative to core protein [34, 71].

Asialoglycoprotein

Asialoglycoprotein is a hepatic lectin, also known as the hepatic galactose/N-acetylglucosamine (GlcNAc) receptor [3], the Ashwell receptor or the Ashwell–Morell receptor. Asialoglycoprotein receptor (ASGPR) participating role in the hepatocyte entry has been elucidated in case of HAV, HBV and Marburg Virus. ASGPR is expressed primarily on the sinusoidal surface of the hepatocytes [2].

In 1988, Wu and colleagues, for the first time reported that HBV DNA transfer into hepatocytes can be done via ASGPR. This finding further intensified the competition of finding the entry receptor and participating factors involved in HBV infection especially with relation to ASGPR [74].

Later Triechel and colleagues showed that plasma samples of HBV carriers with high viremia can recognize human ASGPR. They also showed that an ASGPR-specific ligand, asialofetuin, could inhibit secretion of hepatitis B surface antigen into cell culture supernatant at a level of approximately 50 % [68].

After recognizing the importance of ASGPR role, several studies targeted their efforts in targeting the exact residues involved in hepatocyte entry in HBV genome. Several studies have provided evidence that HBV binds to ASGPR through 10–36 residues of preS1. The binding of ASGPR to glycoproteins is through the carbohydrate binding site, comprising the sequence Q239–N264 (specifically Q239, D241, W243, E252, D253 and N264) [20, 21, 59]. As ASGPR binds to desialylated ligands, one of the studies had showed that attachment of HBV to ASGPR is mediated through desialylated serum factors that serve as intermediate of attachment. Only preS1 or peS2 of HBV binds to ASGPR while the glycosylated HBsAg not [67]. Kuroda et al. identified 10 potential N-linked glycosylation sites in preS1 [27] which provides strong evidence that preS1 binds to ASGPR.

Zhang provide another evidence of ASGPR–preS1 interactions, as there is correlated membranous or mixed membranous and cytoplasmic expression of ASGPR and preS1 in liver tissue, hence it may be inferred that preS1 interact with ASGPR. Zhang also explained the post binding steps resulting in endocytosis of HBV leading to its degradation, which results in the integration of HBV DNA into the host genome leading to chronic viremia and liver inflammation [79]. The internalization of asialoglycoproteins by ASGPR is done through clathrin-coated pit pathway, followed by delivery to lysosomes for degradation [69]. After performing its function, ASGPR is recycled and returned back to the cell surface [58, 64].

Sodium taurocholate cotransporting polypeptide (NTCP)

Sodium taurocholate cotransporting polypeptide (NTCP) is member of *SLC10A* gene family. It is present on basolateral membrane of hepatocytes. It is the only transporter that is involved in uptake of bile salts into hepatocytes. Steroid hormones, thyroid hormones, conjugated drugs also transported along with bile acid through NTCP [1].

Yen et al., 2012 identified NTCP as bonafied HBV receptor using tandem affinity purification. They identified that residue 157–165 (KGIVISLVL) bind to receptor binding domain of preS1 region of HBV [77]. Further studies also elucidated that inducing NTCP expression in HepG2 and Huh7 cell lines make them susceptible to HBV infectivity and down regulation of NTCP expression abolishes the HBV infection [25, 26].

Now the question here is how the virus gains entry into hepatocytes? Physiologically, NTCP is known to transport bile salt so its been hypothesized that virus may be transported along with bile salts into hepatocytes hence its transport is in Na^+ dependent manner.

The other possibility is that after binding of HBV envelope protein with NTCP, the receptor is internalized by endocytosis allowing virus to enter the hepatocyte [72, 73]. If virus enters the cells through endocytosis then what would be the signal for internalization and how is that signal generated? In rat the internalization and retrieval of NTCP done through $\text{PKC}\alpha$ [63, 76], but this remains to be investigated in case of human NTCP.

This recently reported involvement of NTCP has opened new avenues in field of HBV to understand role of NTCP in HBV infection and develop HBV entry inhibitors.

There are some compounds that are known to inhibit HBV infection by binding with NTCP. These includes Myrcludex-B [43], Cyclosporin A (CsA), progesterone, propranolol and bosentan [73]. NTCP substrates, such as taurocholate, tauroursodeoxycholate and bromosulphothalein, also inhibited HBV infection [43, 49, 73].

But if we inhibit viral entry by inhibiting NTCP then there may be a possibility that normal function of NTCP that is bile transport may be impaired.

The reported literature suggests a very strong evidence of NTCP to be HBV entry receptor in hepatocytes. However, the findings related to NTCP are recent and more research requires to elucidate its viral interaction. Moreover, due to interplay of other co-receptors or co-factors for productive HBV infection known to be interacting with pre S1, other host viral factors in addition to NTCP may be involved in infecting hepatocytes and this also raises the question of whether NTCP constitutes the only or major HBV receptor in vivo.

Why many receptors for HBV

One of the reasons of binding of HBV with large number of receptors/interacting proteins is the unstructured nature of preS1 [28, 35]. It is the property of unstructured proteins that when they bind to their target protein they induce few changes in their structure that helps in the binding [29]. Another reason is the use of peptide, almost all studies utilized synthetic peptides or just the preS1 region but none of them uses the whole large surface protein for identification of receptor.

The primary target organ of HBV is hepatocytes, and all of receptors reported so far are not hepatocyte specific as P80 expresses not only in hepatocytes but also in all human tissues [56], HBV-BF is soluble receptor [8], IL6 also expresses on hepatic as well as on cells of non-hepatic origin [48], heparin sulfate is also not a specific hepatic receptor it expresses on many different cells [57], lipoprotein lipase is also not a liver specific receptor [10]. Although studies have reported that these receptors have binding motif for preS1 region of HBV but none of the study elaborating the post binding events. May be HBV binds to these receptors but it cannot be internalized into the cells and thus could not be able to cause infection. Only ASGPR and NTCP are hepatocyte specific but in 2012 NTCP declare as bonafied HBV receptor using primary cell line isolated from *Tupaia belangeri* the only specie that is susceptible to human HBV infection.

Concluding remarks

Understanding the entry process of HBV bears immense importance with respect to viral hepatitis pathogenesis, underpinning immune response, and specie tropism. Substantial progress in recent years with identification of NTCP, has provided new horizon for effective management of Hepatitis B with development of viral entry inhibitors. The advent of inhibitors like Myrcludex B along with conventional management with nucleoside analogues, provides new hope to survive for not only HBV infected hepatocytes but also to prevent infection in healthy hepatocytes.

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