Abstract

Introduction: Chronic hepatitis B virus (HBV) infection is difficult to cure, due to the presence of covalently-closed-circular DNA and virus-mediated blunting of host immune response. Existing therapies with nucleos(t)ide analogue or pegylated-interferon are not sufficient to achieve a high rate of HBV surface antigen seroclearance, a more desirable treatment outcome. Novel therapeutic agents targeting alternative viral replication steps are being developed. In this review, we will discuss the hepatitis B core antigen (HBcAg) as a therapeutic target.

Areas covered: The basic structure and fundamental functions of HBcAg including nucleocapsid assembly, pre-genomic RNA encapsidation, reverse transcription, virion formation, cccDNA amplification, immune response regulation, and HBx protein interaction will be reviewed. Most of these are identified as therapeutic targets and tested in *in vitro* and *in vivo* studies, although clinical trials are scanty. Among the different components, the core protein allosteric modulators (CpAM) have been most widely investigated and appear promising in clinical trials.

Expert opinion: The multiple and essential functions of HBcAg for HBV life cycle are important and attractive targets for HBV therapeutic interventions. Controlled trials involving CpAM are awaited. Apart from CpAM, drugs directed against different functions of HBcAg may be further explored to maximize the chance of cure.

Keywords: cccDNA, chronic hepatitis B, core antigen, core protein, core protein allosteric modulator, cure, HBx protein, immune response, nucleocapsid, surface antigen, translation, transcription, virology

Article Highlight Box:

- Hepatitis B core antigen (HBcAg) is a 183-residue polypeptide chain transcribed and translated from the core gene.
- HBcAg is responsible for multiple functions in viral replication and also contributes to viral persistence and hepatocarcinogenesis.
- The most widely studied HBcAg-targeting agents are the core protein assembly modulators (CpAMs) that inhibit capsid assembly or pgRNA encapsidation.
- Another HBcAg target, which is still in pre-clinical stage of development, involves interference with cccDNA synthesis.
- Alternative cocktail therapies incorporating HBcAg-targeting agents may be the next step of research.

1. Introduction

Hepatitis B virus (HBV) chronically infected 257 million people worldwide. In 2015, HBV resulted in 887,000 deaths from HBV-related complications, including cirrhosis and hepatocellular carcinoma (HCC) [1]. This generally non-cytopathic virus is very difficult to eliminate, owing to the stable viral genome pool, i.e. covalently closed circular DNA (cccDNA) inside the host nucleus. Blunting of host immune reactions by viral proteins also contributes to persistence of infection. Currently, only two classes of drug are approved for control of chronic HBV, namely the nucleos(t)ide analogues (NUC) and pegylated-interferon (PEG-IFN)-α. The former class of drug targets the formation of relaxed-circular DNA (rcDNA) from pre-genomic RNA (pgRNA) in the nucleocapsid, and has no actions on the other steps of viral replication such as cccDNA synthesis, messenger RNA transcription, nucleocapsid assembly or virion production. Therefore, NUC can at most lead to the suppression of HBV DNA synthesis, while the cccDNA pool and blunted immune response are not directly tackled. Although the latter class of drug (PEG-IFN α) affects multiple steps of viral replication, its efficacy is modest [2] and the drug is poorly tolerated. Complete cure with cccDNA elimination is not yet achievable with the current therapies. Clearance of HBV surface antigen (HBsAg), without or with anti-HBs seroconversion (preferable), is currently proposed as the best surrogate endpoint, i.e. the so-called 'functional cure', in which the cccDNA is still present but silenced into a transcription-inactive state, as opposed to 'complete cure' with cccDNA elimination. However liver decompensation and HCC can still develop despite achieving this surrogate endpoint especially if HBsAg seroclearance occurs after age 50 years [3]. Moreover, HBsAg seroclearance is still rare with PEG-IFN-α and/ or NUC. Novel therapeutic approaches are therefore needed to reach this surrogate endpoint, preferably before cirrhosis develops. HBcAg is one of the newer therapeutic targets. In the

following sections, the basic structure and fundamental functions of HBcAg in the viral life cycle will be reviewed. The role of HBcAg as therapeutic targets will be addressed and important clinical trials will be discussed.

2. Structure of HBcAg

HBcAg is a 183-residue polypeptide chain. During HBV replication, messenger RNA (mRNA) is transcribed from the HBV core gene, which is located on one of the four open-reading frames of HBV. Translation starts with the second AUG on the core gene. The first 149 amino acid residues form the α helix-rich assembly domain (also known as globular N-terminal domain, NTD), while the last 34 amino acid residues form the arginine-rich C-terminal domain (CTD) [4]. Each protein monomer weighs 21.5 kDa. After folding and stabilization by the hydrophobic core, two individual folded HBcAg polypeptide chains will be joined, resulting in a dimer structure with a four-helix bundle [5, 6] (Figure 1). The CTDs are located at the ends of the four-dimer arms.

3. Functions of HBcAg

3.1 Capsid assembly

The most well known function of HBcAg is the formation of the HBV nucleocapsid. Each dimer is tetravalent and binds to four neighboring subunits via weak hydrophobic interactions [7]. The resultant nucleocapsid is an icosahedral lattice (Figure 1) made from the assembly of 240 HBcAg (i.e. 120 dimers) with a triangulation number of 4 (T=4) measuring 35nm,

although a minority of the nucleocapsids are smaller in size (measuring 31nm) containing 180 HBcAg (i.e. 90 dimers) with a triangulation number of 3 (T=3). This dimer clustering (either T=3 or T=4 nucleocapsid) produces radial arrangements of spikes, with bundles of 4-helix chains, on the surface of the lattice [4, 8]. The NTD (assembly domain) is essential for assembly between dimers as shown by *in vitro* studies. Nucleocapsid assembly is not affected even if the CTD is deleted from HBcAg [9]. Although the CTD is not required for capsid assembly, it is involved in many other functions as described in the following sections.

3.2 Encapsidation and reverse transcription of pre-genomic RNA

The pgRNA transcribed from cccDNA will leave the host nucleus and join the reverse transcriptase at the cytoplasm for packaging into the nucleocapsid, i.e. encapsidation. The CTD of the HBcAg inside the nucleocapsid is essential for pgRNA encapsidation. As shown in *in vitro* studies HBcAg lacking CTD does not encapsidate pgRNA [10, 11]. Only specific RNA containing the polymerase-binding epsilon loop will be packaged in studies with human cells [12-14].

After encapsidation, pgRNA undergoes reverse transcription to rcDNA. This multi-step process includes template switch (minus and plus strand DNA), primer translocation and DNA elongation (minus and plus strand DNA), all occurring inside the nucleocapsid. The CTD on HBcAg is involved during rcDNA synthesis [15]. When this arginine rich domain is mutated, rcDNA cannot be synthesized although pgRNA encapsidation can still occur as demonstrated by *in vitro* studies using cell line models [16, 17]. The underlying mechanisms involve HBcAg-polymerase interaction via CTD phosphorylation and RNA chaperoning for base-pair interactions [4, 18].

3.3 Virion formation and secretion

Nucleocapsids are responsible for intracellular trafficking of viral genome. rcDNA containing nucleocapsids are regarded as ‘mature’ and will be enveloped by surface antigens at the endoplasmic reticulum and Golgi apparatus to form complete virions which are transported outside the hepatocytes. HBcAg is responsible for the selection of mature nucleocapsids, i.e. rcDNA containing, to be enveloped, while immature ones will not be secreted until rcDNA synthesis is achieved. This selective ability of the nucleocapsid is thought to be due to destabilization of the nucleocapsid when the rcDNA synthesis approaches full length. These maturation-associated structural changes on the nucleocapsid will signal to the surface proteins for enveloping [19, 20]. However, genome-free virions (enveloped nucleocapsids containing no RNA or DNA) are also secreted in a larger amount than complete virions [19, 21]. The underlying mechanism for this is not clear, although it has been proposed that an alternative pgRNA-dependent blocking signal prevents nucleocapsid envelopment [22], so that even without rcDNA, empty nucleocapsids lacking pgRNA can still be transported outside the hepatocytes.

3.4 Intracellular cccDNA amplification and regulation

Mature rcDNA containing nucleocapsids can also be transferred back to the host nucleus for nuclear recycling to replenish the cccDNA pool, i.e. the ultimate therapeutic target for achieving complete cure of HBV. The rcDNA released from the nucleocapsid is converted to cccDNA by host polymerase and decorated with chromatin [23]. Apart from providing transport of the rcDNA to the host nucleus, the nucleocapsid plays extra roles in regulating cccDNA. Genome-free empty nucleocapsids (only the capsid layer) are also transported to the host nuclei. HBcAg is found on cccDNA and is a component of the cccDNA minichromosome [24]. It is believed that HBcAg confers regulatory effects on cccDNA

activity via epigenetic mechanisms [25]. HBcAg alters the nucleosome spacing of cccDNA-histone complex. It has been shown to correlate with changes in the number of nucleosomes in *in vivo* and *in vitro* studies [24, 26, 27]. Another mechanism is HBcAg-induced hypomethylation of CpG island 2 in cccDNA. This region is associated with increased activity of cccDNA, and methylation will lead to reduction of cccDNA activity [28, 29]. HBcAg is preferentially bound to this region as shown by Chromatin Immuno-Precipitation (CHip) [28]. When HBcAg binds to CpG island 2 in cccDNA, it is hypomethylated. This leads to increased binding of cyclic AMP-responsive enhancer binding protein (CREB) binding protein (CBP) and enhanced histone-acetylation status, resulting in increased cccDNA transcription [25, 28]. The responsible domain is found to be the CTD, while NTD does not affect HBV replication [30]. Specifically, the arginine clusters III and IV on CTD are involved in HBV transcription [31].

3.5 Interaction with host immune response

Apart from enhancing cccDNA transcription by the above mechanisms, HBcAg is also involved in IFN-α mediated destruction of cccDNA [32]. IFN-α activates APOBEC3A, a nuclear cytidine deaminase which renders cccDNA prone to degradation by nucleases, via direct interaction with cccDNA-bound HBcAg [32]. Moreover, the interactions between HBcAg and the interferon-stimulated genes (ISG) also play a part. In cell line studies, it has been found that HBcAg inhibits double-stranded DNA mediated IFN response. The pre-requisite for this inhibition is a nuclear location of HBcAg, which inhibits the transcription of ISG [33]. HBcAg also interacts with tumour necrosis factor related apoptosis inducing ligand (TRAIL), which is implicated in HBV-infected hepatocyte death. Overexpression of HBcAg decreases TRAIL-induced apoptosis of human hepatoma cells, associated with reduction in death receptor 5 expression [34].

HBcAg is also a target for immune mediated viral clearance by B cells, T helper cells and cytotoxic T lymphocytes (CTL) [35]. Both the NTD and CTD represent CTL and T-cell epitopes, while B-cell epitopes are mainly found on the NTD. Mutations in these epitopes have been shown to influence host immune response [36-38]. In human case series, mutations in the CTL epitopes in the CTD phosphorylation sites are associated with higher stage of liver fibrosis [39]. HBcAg exerts complex regulatory effects on viral activity and hepatocyte destruction via interactions with the host immune factors.

3.6 HBx regulatory function and HCC

HBx is a protein product from the X gene that carries carcinogenic ability [40, 41]. In transgenic mice harboring HBx gene, they succumbed to progressive histopathological changes specifically in the liver, beginning with multifocal areas of altered hepatocytes, followed by benign hepatic adenomas and subsequently malignant carcinomas [40]. It also increases the expression of all HBV proteins including HBcAg by trans-activating the core promoter [42, 43]. In transgenic mice carrying the HBV core gene in cis arrangement with the X gene, the core gene expression was consistently high, compared to absence of core gene expression in transgenic mice without the X gene. Moreover, by providing an intact X gene in the latter group, core gene expression was induced [42]. Conversely, HBcAg down-regulates HBx expression in human cell experiments [44]. It is postulated to be a negative feedback system by HBcAg-induced proteasome-mediated degradation of HBx. Indirectly, HBcAg also blocks the pro-apoptotic effect of HBx when both proteins are present in the same hepatocyte [34].

HBcAg also has direct impact on hepatocarcinogenesis. It up-regulates the human telomerase

reverse transcriptase expression and therefore promotes HCC cell proliferation in cell line experiments [45]. In transgenic mice implanted with HepG2.2.15 cells, inhibition of core gene transcription led to lower tumor volumes and weights [45]. The relationship between HBcAg and HCC has been further studied in human subjects who developed HCC. Certain amino acid polymorphisms, mostly located in the NTD, are present in the tumor cells. Examples include P130T (38.8%), I97L (37.8%) and S87G (23.5%), as well as internal deletions of amino acids of up to 59 residues [46]. In contrast, certain mutations are associated with decreased risk of HCC. These are HBcAg variants with amino acid changes residing in the region within or flanking the known immune epitopes [47].

Figure 1 is a diaphragmatic representation of the structure of HBcAg and summarizes its fundamental functions in hepatitis B virus.

4. Therapeutic targets

Since HBcAg has no human homolog, it is a good therapeutic target with viral specificity.
The following antiviral mechanisms have been examined in both *in vitro* and *in vivo* studies.

4.1 Targeting nucleocapsid assembly and pgRNA encapsidation

The first class of nucleocapsid-targeting agent is the core protein allosteric modulators (CpAM), which are small molecules that interfere with nucleocapsid assembly by allosteric mechanisms. These small molecules alter the subunit of each HBcAg dimer and/ or interfere with inter-subunit interactions, and results in defective nucleocapsid formation [48, 49]. Mechanistically, the action of CpAM can be divided into two classes: formation of aberrantly

assembled nucleocapsid (instead of the icosahedral lattice), and formation of 'normal shaped' nucleocapsid which cannot encapsidate pgRNA, i.e. empty capsids.

The fluorescent dye 5.5'-bis[8-(phenylamino)-1-naphthalenesulfonate] (Bis-ANS) is the first CpAM identified and its action is mediated through the formation of aberrantly assembled nucleocapsid [49]. *In vitro* studies show that Bis-ANS inhibited capsid assembly induced by ionic strength, where the binding energy of Bis-ANS for capsid protein was -5.9 kcal/mol, compared to -3 kcal/mol of inter-dimer hydrophobic interaction between HBcAg dimers [4, 49]. However, no *in vivo* studies have been performed for Bis-ANS. The heteroaryldihydropyrimidines (HAPs) is another type of CpAM and have been more extensively investigated. *In vitro* studies show that HAPs enhance HBcAg assembly into non-functional capsid polymers over a broad concentration range [50, 51]. For lower concentrations, HAPs induce assembly-active states of HBcAg in a dose-dependent manner, whilst in higher HAPs concentrations, they stabilize non-capsid polymers with an end result of non-functional nucleocapsids [51]. Different HAPs lead to different forms of HBcAg polymers [50]. In transgenic mice treated with Bay 41-4109 and GLS4 (both are HAPs), the reduction of intrahepatic viral DNA was dose-dependent and the efficacy is comparable to lamivudine. In addition, HAPs lead to significantly lower cytoplasmic HBcAg levels, in contrast to lamivudine-treated mice that have static amount of cytoplasmic HBcAg [52, 53]. Apart from interfering with capsid assembly, HAPs show viral suppressive activity at a concentration much lower than that required for affecting capsid assembly, which indicates a kinetic effect of HAPs on antiviral activity independent of aberrant capsid structure [54]. No severe toxicities have been reported including liver enzymes, body weights and liver masses in mice. Moreover, the severity of viral rebound was lower than lamivudine 2 weeks after completion of treatment (6.77 fold increase in viral DNA for 15mg/kg/day GLS4 dosing vs.

166.9 fold increase in viral DNA for lamivudine), and this was comparable to BAY 41-4109 (2.1 fold increase in viral DNA) [53]. Internal fragments of HBcAg consisting of amino acids 78-117 were also shown to inhibit nucleocapsid assembly in HepG2.2.15 cell line models [55].

Another class of CpAMs that acts via the second mechanism (i.e. formation of empty capsids which cannot encapsidate pgRNA) is the phenylpropenamides (PPAs). These compounds inhibit HBV infectivity in cell culture and accelerate capsid assembly [56, 57]. In the presence of PPAs, capsid assembly is not aberrant, meaning that icosahedral nucleocapsids with normal morphology are still formed [58]. However these 'normal nucleocapsids' will not encapsidate pgRNA, leading to accumulation of empty capsids which affect subsequent virological replication downstream, as shown by *in vitro* studies using different PPAs: AT-61 and AT-130. In these studies, HBV DNA replication and encapsidated RNA were reduced while total RNA production, viral DNA polymerase activity or core protein translation were unaffected. A newer molecule, NZ-4, carries similar effects of production of genome-free capsids [59]. These findings suggest that the mechanism of action of PPAs is at the level of viral encapsidation and RNA packaging [60]. Sulfamoylbenzamides are another group of CpAMs that lead to empty capsid formation [61, 62]. These molecules are still in the preclinical stages of development.

A few CpAMs have been studied in human subjects. These include NVR3-778, GLS4, JNJ379 and ABI-H0731. Table 1 summarizes the key information of these investigational products in clinical trials. The phase I trial of NVR3-778 has completed. When given for 4 weeks, especially when combined with PEG-IFN-α, NVR3-778 achieved significant

reductions in HBV DNA, HBV RNA and HBV e antigen (HBeAg) levels in HBeAg-positive patients [63].

4.2 Targeting cccDNA amplification pathway

Although the exact mechanisms for cccDNA formation from rcDNA are not known, it is previously shown that HBcAg is an essential protein that takes part in it. The significance of HBcAg at this viral replication step was examined in two types of compounds: HAP12 and sulfaniladmides.

HAP12 is a specific type of HAPs that targets cccDNA formation by inhibiting cccDNA transcription. In cell line models using AD38 cells, HAP12 reduces the cccDNA-bound H3 histone acetylation and decreases HBcAg occupancy on the cccDNA in infected cells. The inhibition is very pronounced when HAP12 was started during initial infection, with >95% reduction of cccDNA formation and thereby leading to hampered viral replication [64].

Sulfanilamides is another group of compound that targets cccDNA formation by inhibiting the conversion of DP-rcDNA into cccDNA. After pgRNA encapsidation and reverse transcription, completion of plus-strand DNA synthesis triggers the rcDNA deproteinization (i.e. removal of the covalently-bound viral polymerase) and structural changes of the nucleocapsid that leads to exposure of nuclear localization signals in the CTD of HBcAg, which mediates the nuclear transportation of the deproteinized-rcDNA (DP-rcDNA) and subsequent ligation of both DNA strands to generate cccDNA [65-67]. Two of these compounds, CCC-0975 and CCC-0346, are structurally related disubstituted sulfonamides. They reduce levels of both DP-rcDNA and cccDNA, while the rate of decay is not enhanced, suggesting that the underlying mechanism is inhibition of cccDNA synthesis as shown in cell

line culture experiments [68]. Nevertheless, direct evidence of HBcAg inhibition by these compounds is not presented in these studies. These molecules are still in the pre-clinical stages of development.

5. Conclusions

HBcAg is a polypeptide chain, which is transcribed and translated from the core gene. It harbors multiple functions in viral replication as well as interaction with the host. Its functions are terminal-specific and location-specific. HBcAg is currently a popular therapeutic target in HBV treatment. Nucleocapsid-targeting agents have been shown to inhibit capsid assembly, pgRNA encapsidation or interfere with cccDNA synthesis in preclinical studies. However, not many compounds have been tested in human subjects in phase I or II clinical trials. Available data in phase I study have already shown efficacies in reduction of viraemia and viral proteins. Further studies are required to examine whether addition of these compounds into the existing treatment can enhance control of viral activities. After all, since patients are already treated with long term NUCs, any new treatment will need to be at least equally safe and well tolerated.

6. Expert opinion

Novel drugs targeting HBcAg include the following two groups of compounds: CpAMs and therapeutic vaccines. The efficacy of the former group is promising, while that of the latter group is doubtful. Moreover, there are yet no HBcAg-directing compounds that can target other unique roles of HBcAg, such as virion formation and interaction with HBx protein,

with direct implications on carcinogenesis. HAP12 and sulfanilamides have been shown to inhibit cccDNA synthesis, but clinical studies are lacking. To knock down the functions of HBcAg is complicated because of the pleiotropic effects on the viral replication cycle, including the complex interaction with HBx protein and its interaction with the host immune system.

Randomized controlled trials for CpAMs are awaited to prove their superior efficacy in viral control. To enhance the efficacies of therapeutic vaccines, one should consider combination therapy with IFN-α instead of NUCs in view of the limited efficacies shown in the phase II trials. Theoretically, one may expect the efficacy of therapeutic vaccines to be more pronounced if they are boosted by IFN injections. Other cocktail treatment should also be explored to tackle the issue of blunted immune response in HBV infection. HBsAg subviral particles lead to impairment of T cell immune response. Traditional therapy with NUCs only reduces rcDNA formation, with no effect on the amount of HBsAg that are directly translated from mRNA transcribed from the S gene in the open reading frame. If viral mRNA transcription can be controlled, viral protein expression will be greatly reduced especially HBsAg and HBcAg, leading to host immune reconstitution and enhanced HBsAg seroclearance [69]. Combining NUC with short-interfering RNA (siRNA) has been shown to profoundly suppress HBsAg [70]. Further exploratory trials may be performed, starting with preclinical studies, to ascertain whether siRNA can be combined with therapeutic vaccines and perhaps PEG-IFN-α to maximize the chance of viral clearance.

For the existing cccDNA pool, no drug yet can act as a direct cccDNA scavenger. Currently, the only possible direct therapeutic way to enhance clearance of cccDNA is via restoration of host immune control, although it is also envisaged that cccDNA total depletion can be

achieved by natural cell death by prolonged NUC monotherapy. IFN-α and lymphotoxin-β, which are naturally occurring cytokine and cytokine receptor agonist, respectively, can enhance degradation of cccDNA through deamination by APOBEC3A and 3B [32]. However, only 23% of HBV patients treated with PEG-IFN-α could achieve low serum HBV DNA (≤ 2000 IU/mL) at 5 years post-treatment [71], with no studies showing elimination of cccDNA. The efficacy of therapeutic vaccines in clinical trials is not convincing. One possible obstacle is that, nuclear-locating HBcAg inhibits ISG transcription and blunts double-stranded DNA mediated IFN response. Theoretically, blocking HBcAg-cccDNA interaction can enhance IFN response and increase the chance of cure. It seems that delivering 'extra' HBcAg by therapeutic vaccines is complicated because of the contradictory effects of HBcAg on the host immunity. Ways to selectively prevent HBcAg-mediated inhibition of cytokine response and at the same time boosting T-cell and humoral response using therapeutic vaccines should be further explored.

A novel therapeutic approach aiming at cccDNA clearance is genome-editing technology which induces viral DNA breaks. These compounds include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats associated system (CRISPR/Cas). One major technical problem is the lack of an appropriate delivery method into target cells. Compared to ZFNs and TALENs, CRISPR/Cas system can be more easily programmed and delivered both *in vitro* and *in vivo* to cleave DNA sequence pre-defined by the guide RNAs (gRNA) [72, 73]. Lentiviral vectors have been studied to deliver CRISPR/Cas into host cells [74]. Studies have shown that these CRISPR/Cas systems effectively mediate gene disruption of any desired viral genome sequences, including HBV cccDNA and integrated HBV DNA [75, 76]. However, CRISPR/Cas systems can lead to off-target cleavage and their *in vivo* delivery efficiency

remains to be improved. Another concern is their integration into the host genome resulting in insertional mutagenesis. Moreover, DNA repair after cccDNA cleavage may lead to mutations too and these could be harmful in terms of risk of malignancy. Therefore, future research using these genome-editing technologies will need to look for ways to improve *in vivo* system delivery, enhance cleavage specificity and maintain genome stability.

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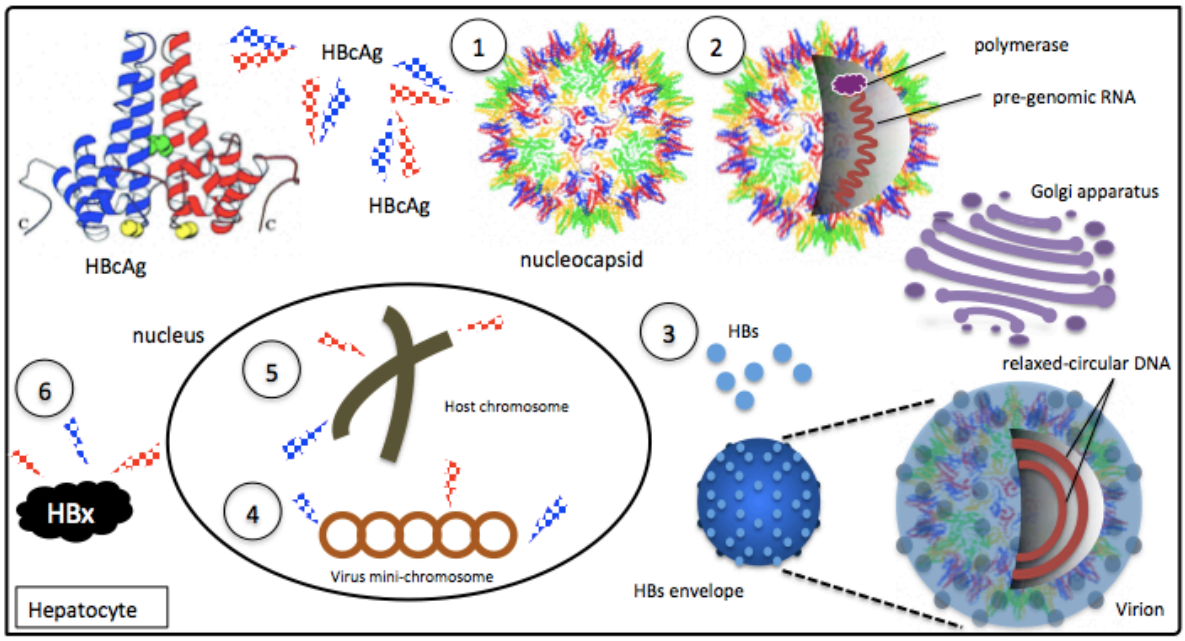
Figure 1. Diaphragmatic illustration of the structure and fundamental functions of HBcAg



Diaphragmatic illustration of the structure and functions of HBcAg. Left upper corner: Two individual HBcAg protein monomers joined to form a dimer structure with a four-helix bundle*. (1) Capsid assembly: Assembly of 120 dimers forms an icosahedral lattice*. (2) Encapsidation and reverse transcription of pre-genomic RNA: The nucleocapsid encapsidates pre-genomic RNA (wavy brown line) which undergoes reverse transcription to relaxed-circular DNA inside the capsid. (3) Virion formation and secretion: Mature relaxed-circular DNA (double curved brown lines) containing capsids will be enveloped by HBs to form virions which are transported outside the hepatocyte. (4) Intracellular cccDNA amplification and regulation: HBcAg confers regulatory effects on cccDNA via intracellular trafficking and other additional mechanisms (see text). (5) Interaction with host immune response: HBcAg is involved in interferon-mediated destruction of cccDNA and are epitopes of lymphocytes (see text). (6) HBx regulatory function and hepatocellular carcinoma: HBcAg interacts with HBx expression and promotes hepatocarcinogenesis. HBcAg=HBV core antigen, HBs=HBV surface antigen, HBx=HBV x protein.

* Structure of HBcAg and nucleocapsid reprinted from Molecular Cell 1999; volume 3, S.A Wynne, R.A Crowther, A.G.W Leslie. The Crystal Structure of the Human Hepatitis B Virus Capsid, pages 771-780, with permission from Elsevier.

Tables

Table 1. Core protein allosteric modulators in clinical trials

Name	Likely mechanism of action		Stage of development	Sponsor	Reference clinical trial number
	aberrant capsid	empty capsid			
GLS4	▯		Phase II	HEC Pharm	not available; refer to company website
NVR3-778		▯	Phase Ib	Johnson & Johnson	NCT02112799
JNJ379		▯	Phase I	Johnson & Johnson	NCT02662712
ABI-H0731		▯	Phase Ib/ IIa	Assembly Biosciences	NCT03109730