REVIEW ARTICLE

Comparative Overview of Hepatitis B Virus and Domestic Cat Hepadnavirus From the Perspective of Viral Biology to Capsid Protein and Its Applications

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ABSTRACT

Hepadnaviruses are a family of partially double-stranded DNA viruses that primarily infect vertebrates, including mammals, birds, amphibians, and fish. These viruses are clinically significant because they cause acute and chronic liver diseases. Among them, hepatitis B virus (HBV) is the most extensively studied member due to its profound impacts on human health, with more than 250 million people chronically infected worldwide despite the availability of effective vaccines. Domestic cat hepadnavirus (DCH), on the other hand, is a recently identified hepadnavirus infecting domestic cats. Although the full extent of its clinical relevance is not yet established, DCH has been detected in both healthy cats and those with liver disease, raising concerns about its potential role as a feline pathogen. This review summarizes the similarities and differences between HBV and DCH, focusing on their taxonomy, epidemiology, pathogenesis, genome organization, protein functions, and current research status. Particular attention is given to the capsid protein, emphasizing its function and potential applications in biotechnology and medicine. As more molecular and structural data on DCH emerge in the future, comparisons with HBV may reveal new insights into hepadnaviral evolution, host specificity, and cross-species transmission.
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INTRODUCTION

Hepadnaviruses are small, enveloped DNA viruses with a unique replication cycle that involves reverse transcription of a pregenomic RNA (pgRNA) intermediate. Hepatitis B virus (HBV), the prototype human hepadnavirus, has been extensively studied for decades due to its significant burden on global public health. HBV is responsible for a range of liver diseases, from acute self-limiting infections to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) (1). In 2018, an HBV-like virus was identified in a domestic cat with lymphoma in Australia, marking the first report of cat hepadnavirus (2). The virus was named domestic cat hepadnavirus (DCH), a novel member of the Hepadnaviridae family, specifically infecting domestic cats (2). The discovery of a novel hepadnavirus has raised questions regarding its biological behavior and potential role in feline hepatic pathology.

After its initial detection in Australia, DCH was subsequently reported in various regions worldwide, including Oceania, Asia, Europe, the Americas, and the Middle East. Quantitative PCR (qPCR) screening has revealed notable variation in DCH prevalence, with rates ranging from 10% to 18% in studies conducted across Asia and parts of Europe, although markedly lower rates of below 2% have been observed in North America and some other European cohorts (2-11). Notably, DCH strains from different countries show considerable genetic divergence, suggesting regional lineages. This global diversity and potential association with feline liver pathology underscore the importance of understanding DCH biology.

Pairwise amino acid sequence comparison between DCH capsid protein (CP) shares ~65% identity and

~76% similarity with the HBV core antigen (HBcAg). Given this amino acid homology, DCH-CP alone could self-assemble into icosahedral virus-like particles (VLPs) resembling the authentic viral nucleocapsid. Such VLPs could serve as innovative platforms for feline vaccine development in veterinary applications. Additionally, the VLPs could be genetically engineered as nano-vehicles for the purpose of packaging and delivering therapeutic cargo.

Despite genetic similarities to HBV, DCH is still in the early stages of research. Future studies exploring the parallels and distinctions between HBV and DCH may provide a framework for understanding not only their pathogenic mechanisms but also their evolutionary trajectories. This review discusses the similarities and differences between HBV and DCH, with emphasis on their taxonomy, epidemiology, pathogenesis, genome organization, and protein functions. A special focus is given to the capsid protein's structure and functions as well as its biotechnological applications, highlighting the potential utility of DCH capsid proteins.

COMPARATIVE OVERVIEW OF HEPATITIS B VIRUS AND DOMESTIC CAT HEPADNAVIRUS

Taxonomy and Phylogenetics

According to the International Committee on Taxonomy of Viruses (ICTV), the Hepadnaviridae family comprises small, enveloped viruses with a partially double-stranded circular DNA genome of 3.0-3.4 kb, enclosed within an icosahedral nucleocapsid core and surrounded by a lipid bilayer membrane (12). The viral particles are spherical, with diameters ranging from 42 to 50 nm (12). The Hepadnaviridae family comprises five genera: Parahepadnavirus, Metahepadnavirus, Herpetohepadnavirus, Avihepadnavirus, and Orthohepadnavirus. Among them, members Metahepadnavirus and Parahepadnavirus primarily infect teleost fish, often identified via metagenomic approaches. The Herpetohepadnavirus genus targets reptiles and amphibians, while Avihepadnavirus consists of three species, primarily infecting birds. The Orthohepadnavirus comprises of 12 species, infecting mammals, including woodchucks, ground squirrels, arctic squirrels, bats, and primates such as humans, gorillas, gibbons, orangutans, woolly monkeys, and chimpanzees (13).

Both HBV and DCH are classified under the genus Orthohepadnavirus within the family Hepadnaviridae. Host range is a defining feature of the Orthohepadnavirus species. HBV is highly specific to humans, although it is closely related to viruses that infect non-human primates. DCH, meanwhile, is the first hepadnavirus confirmed to infect felines (2). Phylogenetic analyses show that DCH forms a distinct clade within Orthohepadnavirus, separate from primate-associated hepadnaviruses. To date, DCH has not been detected in humans or other animal species, indicating a possible host restriction to domestic cats. However, further surveillance in wildlife and companion animals may reveal a broader host range.

Comparative phylogenetic studies reveal substantial geographical genetic diversity among DCH strains. Italian strains cluster closely with the Australian reference strain, while Thai isolates fall within the Asiatic lineage and include a reported recombinant strain (7, 8). Japanese strains include one that aligns with the Australian lineage and another forming a novel clade distinct from all previously reported strains (11). Hong Kong strains belong to the Asiatic lineage (14), while Turkish strains share conserved elements with Australian viruses but do not fall into any established lineage (3). Taiwanese strains form a Taiwan-specific clade linked to Turkish viruses, while Chilean strains occupy a distinct position outside the main DCH cluster in phylogenetic trees (5, 15). Brazilian isolates belong to the rare genotype B, previously described only in Japan (10). These analyses highlight both conserved evolutionary patterns and region-specific lineages within Orthohepadnavirus.

Epidemiology

HBV remains a major global health burden, with an estimated more than 250 million individuals living with chronic infection worldwide (16). Transmission occurs primarily through exposure to infected blood or body fluids, including perinatal transmission from mother to child, sexual contact, and unsafe medical or dental procedures involving contaminated instruments. Despite its high burden, widespread immunization programs have significantly reduced the incidence of new infections, particularly in countries with robust birth-dose and childhood vaccination policies. Table 1 shows the epidemiological comparison between HBV and DCH.

Table I: Epidemiological comparison between hepatitis B virus and domestic cat hepadnavirus

Feature	HBV	DCH
Natural host	Humans	Domestic cats
Global distribution	Worldwide	Detected in multiple countries
First discovery	1965	2018
Prevalence	~3 - 5% globally	~6 - 12% in tested cats
Transmission route	Blood, body fluids, vertical	Suspected blood-borne
Vaccine available	Yes	No

DCH was first identified in 2018 in Australia through transcriptomic analysis on a domestic cat with multicentric large B-cell lymphoma, with 6.5% of samples tested positive (2). Since then, its presence has been confirmed across Oceania, Asia, Europe, the Americas, and the Middle East, with prevalence varying significantly between regions and studies. Reported DCH positivity rates range from ~10-18% in parts of Asia and Europe to lower than 2% in North America and the UK (2-11, 14, 15). Table 2 summarizes the global distribution and prevalence of DCH. Higher prevalence rates are often observed in cats with hepatic pathology or co-infections such as feline immunodeficiency virus (FIV) or feline leukemia virus (FeLV), suggesting that host immune status may influence susceptibility (4, 14).

Table II: Current geographical distribution and prevalence of domestic cat hepadnavirus

Region	Country	Prevalence	References
Oceania	Australia	6.5%	Aghazadeh (2)
	Thailand	18.5%	Piewbang (8)
	Malaysia	12.3%	Anpuanandam (4)
Asia	Japan	0.8%	Takahashi (11)
	Hong Kong	11.1%	Capozza (14)
	Taiwan	11.3%	Silva (15)
	Italy	10.8%	Lanave (7)
Europe	United Kingdom (UK)	1.9%	Jeanes (6)
Americas	United States (U.S.)	0.2%	Stone (9)
	Chile	1.7%	Choi (5)
	Brazil	1.7%	Tessmann (10)
Middle East	Turkey	4%	Adigüzel (3)

The mode of transmission is not yet established, but parallels with HBV suggest that blood-borne or vertical transmission may play a role. The detection of DCH DNA in both healthy and clinically ill cats underscores its potential as an emerging feline pathogen and highlights the importance of continued epidemiological surveillance.

Pathogenesis and Clinical Relevance

In humans, HBV can establish chronic infections in 5–10% of virally-infected adults, particularly when acquired perinatally or in early childhood (1). Chronic HBV infection leads to progressive liver damage and poses a major risk factor for hepatocellular carcinoma. The pathogenic mechanisms include persistent immune-mediated liver injury, viral protein-induced cellular stress, and integration of the viral DNA into the host genome, which can disrupt normal cellular regulation (18).

DCH has been detected in both healthy and clinically ill cats, including those with chronic hepatitis and hepatocellular carcinoma (4, 8, 17). However, its role in disease causation remains under investigation. In some studies, DCH DNA was significantly more prevalent in cats with liver disease than in healthy controls, suggesting a possible association between the virus and disease (4, 8). Histopathological analyses of infected livers have revealed chronic inflammatory changes similar to those observed in HBV infection (17). Longitudinal studies and experimental infections will be required to determine whether DCH is a primary pathogen or a secondary opportunist.

Genome Organization

Both HBV and DCH possess a ~3.2 kb partially double-stranded, circular DNA genome, encoding four major overlapping open reading frames (ORFs): surface (S), core (C), polymerase (P), and X protein (Figure 1). These ORFs are transcribed into several sub-genomic RNAs through host-driven transcription from covalently closed circular DNA (cccDNA) in the nucleus (1). The overall genome structure is conserved, but there are notable differences in sequence identity. DCH shares ~53, 67, 53, and 37% amino acid identity with HBV across major proteins, P, C, S, and X. The conservation of genome organization suggests functional parallels in replication strategy, yet the lower sequence homology regions may imply potential divergence in host interaction and immune evasion mechanisms.

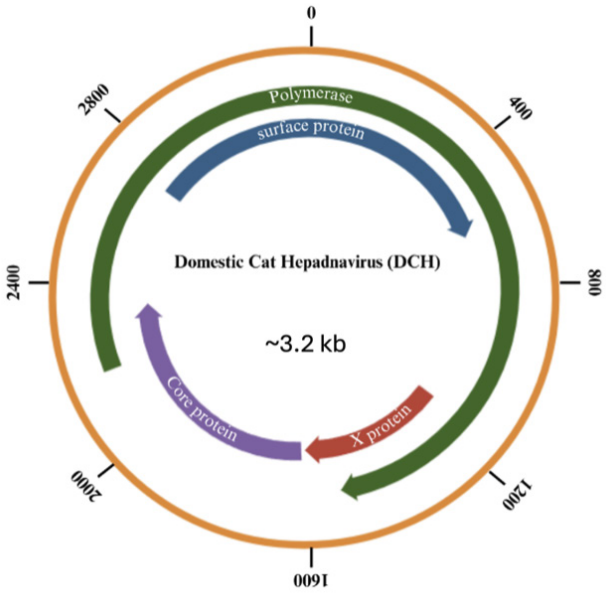

Figure 1: Genomic structure of domestic cat hepadnavirus (DCH). The DCH comprises an approximately 3.2 kb genome. The genome contains four overlapping open reading frames (ORFs) that encode different proteins: polymerase (indicated by a green arrow), surface (indicated by a blue arrow), core (indicated by a purple arrow), and X proteins (indicated by a red arrow). The polymerase gene (green) spans nucleotides 2155-3184 and 1-1484, while the S gene (blue) spans nucleotides 2720-3184 and 1-681. The core protein (purple) ORF is located between nucleotides 1665 and 2321, and the X protein (red) spans nucleotides 1226-1663.

In HBV, the P protein is a multifunctional enzyme that plays a central role in viral replication, including protein priming, nucleic acid synthesis, and ribonuclease H activity (18). The S protein, also known as HBsAg, is expressed in three forms known as the large (L-HBsAg), medium (M-HBsAg), and small (S-HBsAg) surface proteins based on domain structure and their glycosylation status, mediating virus-host cell attachment and modulating the host immune responses (19). The C protein, also known as hepatitis B core antigen (HBcAg), on the other hand, is a relatively small protein comprising ~183 amino acid residues and multiple subunits of HBcAg capable of self-assembling to form the viral icosahedral nucleocapsids (20). In addition to nucleocapsid formation, it also plays critical roles in the HBV life cycle and host epigenetic regulation (21). Meanwhile, HBx protein is a multifunctional regulatory protein, comprising 154 amino acid residues that enhances HBV replication by modulating transcription, signalling pathways, and epigenetic regulation, capable to impair host immunity and DNA repair. Its pleiotropic functions contribute to viral persistence and play a central role in HBV-associated hepatocarcinogenesis (22, 23).

Among the four proteins, the C protein (also known as capsid protein) is of particular interest because it forms the protective shell that encapsidates the viral genome and is capable of forming recombinant VLPs resembling the native virus. Although DCH proteins likely share functional similarities with HBV proteins, their specific roles remain poorly characterized.

Structural and Functional Insights of Hepadnaviral Capsid Proteins

HBV structural biology has advanced significantly, with high-resolution structures available for the capsid (24, 25), polymerase (26), and envelope proteins (27). Cryo-electron microscopy and X-ray crystallography have elucidated the assembly dynamics and conformational flexibility of the HBV capsid (25, 28). These insights have been instrumental in antiviral drug and vaccine development. In contrast, structural data on DCH are limited. Recent efforts to produce the chimeric VLPs of DCH-CP using the baculovirus expression system showed that DCH-CP could form VLPs (29), thus highlighting its potential for structural studies. Amino acid comparison suggests that although DCH-CP shares structural similarities with HBcAg, it also reveals potential differences in surface topology and antigenicity. Structural data for other viral proteins, such as the polymerase or X-like protein, are also lacking.

Functionally, the capsid proteins of HBV encapsidate pgRNA together with the viral polymerase during assembly of the viral nucleocapsid (29). Additionally, capsid phosphorylation regulates genome maturation and intracellular trafficking (30, 31). Similar mechanisms are presumed for DCH, but they remain to be experimentally validated. Immunologically, HBcAg is a dominant antigen in HBV infection, while DCH-CP has shown immunoreactivity in seropositive cats (32, 33), though its T-cell epitopes and role in immune modulation remain uncharacterized.

Structural Conservation and Divergence of HBV and DCH Capsid Proteins

HBV capsid protein (HBcAg) consists of an N-terminal assembly domain (NTD), which forms the spherical shells, and a C-terminal Arg-rich domain that interacts with nucleic acids. Multiple HBcAg subunits assemble into icosahedral capsids with T=3 and T=4 symmetry, as revealed by cryo-electron microscopy (cryo-EM) (24) and X-ray crystallography (25). The capsid-forming core proteins of hepadnaviruses share highly conserved structural motifs essential for capsid assembly and stability, as revealed by structural studies across multiple species (36). In HBV, the NTD of HBcAg adopt an all-α-helical fold, with central helices, α3 and α4, forming an antiparallel hairpin. Two HBcAg monomers associate through this motif to form a four-helix bundle motif, which serves as a stable dimer and represents the fundamental building block of the icosahedral capsid (Figure 2). Upon dimerization, these bundles protrude from the capsid surface to form a prominent spike, while assembly into the full capsid is stabilized by inter-dimer contacts mediated by α5 helices and a proline-rich "hand region" ensuring capsid integrity (34).

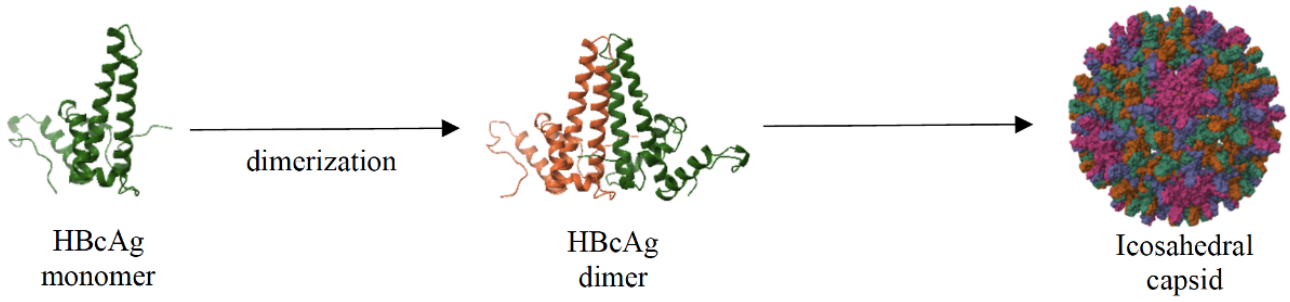

Figure 2: Hepatitis B virus capsid assembly. Two α-helical HBcAg monomers form a dimer through dimerization, and repeating dimers subsequently form an icosahedral capsid via inter-dimer contacts.

Comparative analyses suggest that the DCH capsid protein (DCH-CP) shares these conserved structural motifs and is able to self-assemble into VLPs, although high-resolution structures remain unavailable (8, 14). Pairwise amino acid sequence comparison (Figure 3) shows that DCH-CP shares ~65% identity and 76% similarity with HBcAg, with notable variations in the α1, α2, α3, and α4a helices. In particular, polar amino acids in the immunodominant region of HBcAg (amino acids 74 to 78) are replaced with hydrophobic amino acids in DCH-CP (amino acids 74 to 82). By contrast, the extensive hydrophobic core, principally formed by residues in α1, the α1–α2 loop, α4b, and α5, remains conserved in human variants, underscoring its importance in maintaining the monomer fold (25). This hydrophobic core, except the residues of α1, remains conserved in DCH-CP, suggesting that they play an important role in maintaining the stability of the monomer fold. Other conserved regions include the N-terminus, the N-terminal portion of α3 (amino acids 46–56), and the C-terminal proline- and arginine-rich regions.

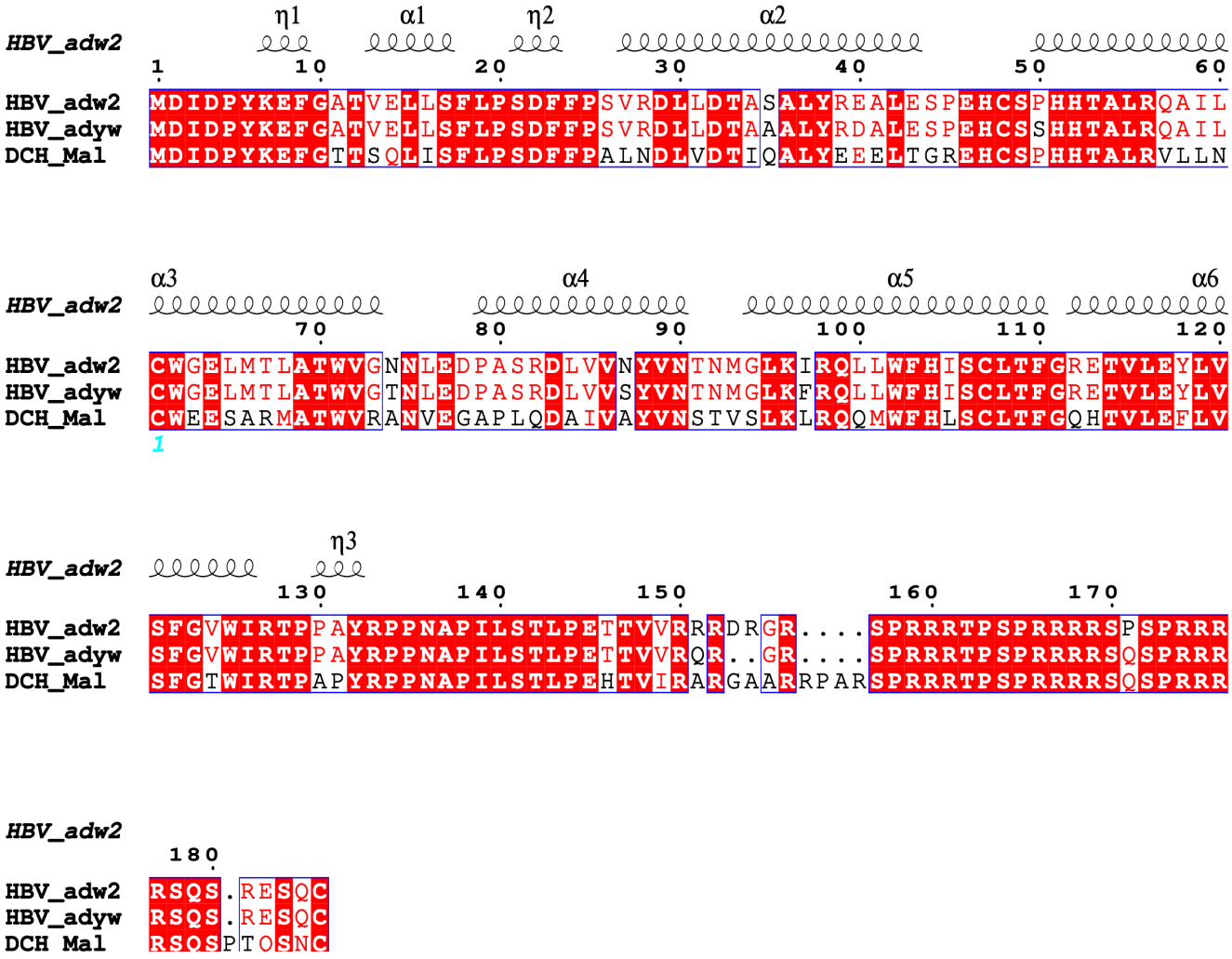

Figure 3: Multiple sequence alignment of hepatitis B virus and domestic cat hepadnavirus capsid proteins. The secondary structures of HBcAg determined with X-ray crystallography (PDB code: 1QGT) are shown on top of the amino acid sequences. Conserved amino acids are highlighted in red. Pairwise amino acid sequence comparison revealed ~65% identity and ~76% similarity between HBcAg of HBV (serotype: adyw) and the DCH capsid protein (GenBank accession no. MK902920) with conserved regions in the hydrophobic core and N-terminal sequences but notable variations in the immunodominant region (residues 74–82).

The conservation of these structural motifs highlights their essential role in capsid assembly and function across hepadnaviruses. In DCH-CP, these elements likely drive self-assembly into particulate structures, providing a framework for understanding capsid stability, immune recognition, and host specificity in felines. Moreover, the ability of DCH-CP to form VLPs opens opportunities for downstream applications in structural studies, vaccine development, and other biomedical platforms, which will be further discussed in the following section.

Potential Applications of DCH Capsid Protein

The DCH-CP, similarly to HBcAg of HBV, has been shown to be able to self-assemble into VLPs (35), making it a promising candidate for biotechnological applications. In HBV, VLPs derived from HBcAg have been widely used in vaccine development, drug delivery, and diagnostic assays due to their stability, high immunogenicity, and structural versatility.

Comparative Insights

HBcAg exhibits a well-defined icosahedral structure that supports both spontaneous self-assembly and foreign epitope insertion at specific sites (e.g., the major immunodominant region or MIR). These properties make it highly suitable for designing chimeric VLPs capable of inducing robust immune responses against heterologous antigens. DCH-CP also exhibits spontaneous self-assembly into VLPs, and preliminary studies suggest it may tolerate surface modification or epitope insertion, although further validation is required. Comparative structural analyses suggest that while both HBcAg and DCH-CP adopt similar icosahedral symmetry, subtle differences in the surface charge distribution and loop flexibility may influence their immunogenic potential and tropism.

Vaccine Design

HBcAg-based VLPs have served as scaffolds for presenting epitopes from various pathogens. Epitopes such as the domain III of dengue virus envelope protein (EDIII) (36), matric protein 2 (M2e) of influenza A (37), Gag and envelope protein of human immunodeficiency virus (HIV) (38), Mycobacterium tuberculosis epitope (39), foot-and-mouth-disease (FAMD) virus epitope (40), and L1 protein of human papillomavirus (41) have been successfully displayed on the outer surface of the HBcAg-based VLPs. Similarly, DCH-CP VLPs could be engineered to display foreign antigens, with the added advantage of being non-human in origin, potentially not to be neutralized by pre-existing immunity. Their feline origin may also facilitate species-specific vaccines in veterinary applications.

Drug Delivery

Both HBcAg and DCH-CP are potentially to be developed as nano-carriers for delivering therapeutic molecules. HBcAg VLPs have been used to encapsulate nucleic acids, peptides, and small molecules, benefiting from their uniform size and capacity for cell targeting. The VLPs derived from HBcAg were used to deliver therapeutic agents, such as the anti-cancer drug doxorubicin for cancer therapy (42), and short hairpin RNA (shRNA) (43). The DCH-CP-derived VLPs, if shown to be similarly stable and biocompatible, could represent a novel delivery platform, especially in feline medicine or for targeting hepatocytes.

Diagnostic Purposes

HBcAg is a well-established marker in serological assays for HBV infection, and its detection forms a cornerstone of clinical diagnosis. Recombinant HBcAg is used in ELISA to detect anti-HBc antibodies, which serve as indicators of either previous exposure or ongoing HBV infection (44). The presence of anti-HBc antibodies is routinely used in combination with HBsAg and anti-HBs profiles to differentiate between acute, chronic, and resolved infections, underscoring its critical diagnostic value (45). DCH-CP could serve a similar role in veterinary diagnostics, enabling detection of anti-DCH antibodies in cat sera. Moreover, structural homology with HBcAg may allow adaptation of existing HBV assay platforms for DCH with minimal modification.

Emerging VLP-based applications

HBcAg-derived VLPs are increasingly explored as scaffolds for presenting tumor-associated antigens. For example, HBcAg VLPs engineered with MAGE-3 epitope could encapsulate CpG oligodeoxynucleotides to enhance peptide-specific CTL responses (46). Zhang et al. (47) developed an HBcAg VLP-based vaccine for hepatocellular carcinoma incorporating MAGE-1, MAGE-3, AFP1, and AFP2, which induced strong CTL activity, elevated IFN-γ secretion, and inhibited tumor growth. Although not yet applied to DCH-CP, these strategies underscore the versatility of hepadnaviral capsids for immunological applications. Beyond cancer immunotherapy, both HBcAg and DCH-CP VLPs also hold promise in nanotechnology, supporting chemical conjugation, enzyme display, and assembly into higher-order structures for biosensing and nanoreactors.

Future Considerations

Although DCH-CP VLPs hold considerable promise, several aspects need further investigation. These include optimizing expression and purification systems, confirming the immunogenicity of chimeric particles, and characterizing host cell interactions. Determining the 3D structure of the DCH-CP is essential to reveal the molecular details that enable direct structural comparison with HBcAg. Furthermore, complementary functional assays are required to determine the full translational potential of DCH capsid-based platforms.

CONCLUSION

HBV and DCH exemplify the diversity and adaptability of the Hepadnaviridae family. While both viruses

share core genomic and structural features, their differences in host specificity, clinical relevance, and level of characterization are substantial. HBV remains a significant human pathogen with established preventative and therapeutic strategies. DCH, on the other hand, is an emerging virus with uncertain pathogenic potential. Continued comparative studies, particularly of the capsid proteins, will enhance our understanding of hepadnavirus biology and may uncover novel mechanisms of virus-host interaction, immune evasion, and biotechnological application. Future high-resolution structural studies of DCH-CP would be important for validating sequence-based predictions, clarifying conservation and divergence from HBcAg, thus enabling the rational design of vaccines and therapeutic drugs.

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