HBV Infection and Apoptosis: Molecular Mechanisms and Clinical Implications

Saif Jabbar Yasir
Department of Medical Microbiology, College of Medicine, University of Kufa, Najaf, Iraq. saif.alshehmani@uokufa.edu.iq

Abstract

Hepatitis B virus (HBV) infection is a significant global health burden, with over 250 million people estimated to be living with chronic HBV infection worldwide. The long-term occurrence of HBV infection is directly related to the efficiency that the virus accomplishes in modulation of the host cellular pathways, especially apoptosis. Apoptosis is involved in disposal of infected hepatocytes and control of spreading virus, whereas its disregulation results in chronic infection, hepatitis, fibrosis/cirrhosis, HCC. This review outlines our current understanding of the molecular pathways of apoptosis and apoptotic regulatory networks that are targeted by HBV or its viral proteins, as well as discusses clinical relevance of HBV-induced cell death in liver disease progression.

Hepatitis B virus (HBV) infection is still a worldwide health challenge and it causes chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC). Virus-mediated regulation of host apoptotic pathways is a key contributor to the pathogenesis of HBV. Apoptosis serves as a double-edged sword in HBV infection: It is critical for immunological clearance of infected hepatocytes, but its long-term perturbation leads to chronic inflammation, liver damage, fibrosis and ultimately to carcinogenesis. This article provides an overview of the basic apoptotic pathways (both intrinsic and extrinsic) and discusses how HBV promotes these processes to establish chronic infection. Special attention is paid to the dual pro- and anti-apoptotic functions provided by the mitochondrial-targeting factor of HBx, including multi-step influences on mitochondrial control, redox regulation, tumor suppression signaling, and pro-survival processes such as NF-κB-regulated pathways and PI3K/Akt. Moreover, the participation of HBV-enveloped and core proteins in ER stress, mitochondrial apoptosis, and immune-mediated apoptosis induced by cytotoxic T lymphocytes

(CTLs) are also reviewed. Finally, the clinical relevance of apoptosis dysregulation during chronic HBV infection and hepatocarcinogenesis is emphasized, and novel therapeutic approaches aimed at targeting HBV–apoptosis crosstalk is discussed. Understanding these mechanisms in greater detail could aid toward developing new therapies that strive to reach a functional cure without damaging the liver. HBV continues to replicate in part by modifying the course of hepatocyte apoptosis; controlled cell death pathways can eliminate infected cells, while chronic or erratic apoptosis promotes inflammation/fibrosis/cirrhosis and enhances HCC risk.

Keywords: hepatitis B virus, apoptosis, hepatocytes, HBx, liver disease

1. Introduction

Despite the availability of highly effective vaccines against hepatitis B virus (HBV) infection, this virus continues to be a global public health problem. The causative agent, hepatitis B virus (HBV; genus Orthohepadnavirus), chronically infects over 250 million people globally and HBV-associated liver disease causes hundreds of thousands of deaths each year from cirrhosis and hepatocellular carcinoma (HCC) [1]. Although most cases of acute HBV infection in immune competent adults are self-limiting, failure of the host immune response to clear the virus leads to a chronic phase of infection, characterized by prolonged viral replication, sustained hepatic inflammation and progressive liver damage. HBV is DNA virus belonging to Hepadnaviridae family and has considerable affinity for hepatocyte. Infection by the virus is a multifaceted process that dependents on host cellular machinery with covalently closed circular DNA (cccDNA) formation and maintenance being one of the crucial steps in this replicative cycle progression. This persistent viral minichromosome is the transcriptional template for viral gene expression and one of the primary obstacles to virus clearance. To sustain this chronic state, HBV has developed sophisticated tactics to interfere with host signaling cascades such as those involved in apoptosis, cell survival, immune-responses and cellular metabolism.

Apoptosis, a programmed cell death process, is an intrinsic and basic biological phenomenon which plays a critical role in tissue homeostasis, ontology and immune responses to viral infections. Apoptosis is an effective host defense strategy using the

liver to remove infected or damaged hepatocytes without 2 excessive inflammations. Nevertheless, despite these protective effects, apoptosis plays a dual role on HBV infection. Immune-related apoptosis of infected hepatocytes is also necessary for viral clearance in acute infection. Alternatively, persistent or dysregulated apoptotic signaling in the setting of chronic infection leads to increased hepatocyte turnover and death, cytokine release, fibrosis and cirrhosis, creating a permissive microenvironment for oncogenic transformation.

Evidence is gathering, suggesting that HBV causes not direct highly cytotoxic effects on hepatocytes, and liver damage mainly derives from the host immune system response as well as by virus dependent manipulation of apoptotic pathways. Proteins encoded by hepatitis B virus (HBV), especially the HBV X protein (HBx) interact with effectors of both intrinsic and extrinsic pathways. By doing so, HBV may either enhance or restrict hepatocytes survival in favor of viral persistence or apoptotic cells for a certain condition and thus connect between virus replication/immune resistance/viral pathogenesis. These dynamic apoptosis regulations are increasingly being accepted as a key pathogenic mechanism in chronic hepatitis B and HBV-related hepatocarcinogenesis.

Here, we provide an overview on our current understanding of molecular mechanisms of apoptosis and how HBX and other HBV viral proteins perturb apoptotic signaling. We also consider the role of immune-mediated apoptosis in HBV infection and clinical aspects of imbalance between apoptosis and proliferation in chronic liver disease and HCC. Finally, we discuss emerging therapeutic concepts focusing on HBV–apoptosis interactions which might complement novel approaches for a functional cure of chronic hepatitis B with minimal liver injury. Hepatitis B virus is a small, partly double-strand DNA virus, which belongs to the Hepadnaviridae family and possesses high tropism for hepatocytes. Acute HBV infection is typically self-limited if controlled by an appropriate immune response; yet clearance of the virus does not occur in 5% to 10% of infected individuals, resulting instead in chronic infection and eventual liver disease. Apoptosis, or programmed cell death, is highly regulated and plays a critical role in tissue homeostasis and immune response. During HBV infection, apoptosis acts as a double-edged sword that can remove infected cells while

causing excessive liver damage if abnormally activated. Accordingly, it is important to understand the interplay of HBV with apoptotic pathways for understanding disease pathogenesis and for designing therapeutic options [1,2].

Apoptosis is a "double-edged sword" in HBV: on the one hand, it aids immune clearance of infected hepatocytes, but if sustained it exacerbates liver injury and disease course—hence HBV–apoptosis crosstalk is pivotal for pathogenesis and therapeutic concept.

Overview of Apoptosis

Apoptosis features cellular shrinkage, chromatin condensation, DNA cleavage and cell membrane blebbing without triggering inflammation. Apoptosis can be triggered by two main routes, namely the extrinsic (death receptor-mediated) pathway and the intrinsic (mitochondrial) pathway. The extrinsic pathway is prompted by the ligands, including Fas ligand (FasL) or tumor necrosis factor-α (TNF-α), binding to their corresponding receptors and activating caspase-8. The intrisic pathway is controlled by mitochondrial function and Bcl-2 family members, leading to release of cytochrome c and activation of caspase-9. Both pathways lead to executioner caspases, notably caspase-3, and result in cell death [3,4]. Apoptosis is executed primarily via extrinsic (death receptor /caspase-8) and intrinsic pathways (mitochondrial/caspase-9) that ultimately converge on executioner caspases such as caspase-3; these common end-points are major points of intervention by HBV to promote persistence or injury.

HBV replicative Cycle and Host Interaction

The replicative cycle of HBV includes viral entry through the sodium taurocholate co-transporting polypeptide (NTCP), nucleocapsid transport to the nucleus, and cccDNA formation. HBV is dependent on the host cell machinery for transcription and replication. Viral proteins co-opt host signaling networks during infection, such as those involved in programmed cell death (apoptosis), cell cycle regulation and immune responses. These interactions enable HBV to sustain and evade premature death of the infected hepatocytes [5]. Hepatitis B virus (HBV) has a relatively convoluted replicative cycle that tightly couples to the host cellular

machinery leading to persistent infections which present significant global health concerns [6]. Though successful vaccines to prevent HBV are available, chronic HBV infection continues to affect around 296 million people globally and is still one of the most common fatal viral related cancers [7]. A complex repertoire of interactions between this enveloped DNA virus and host factors results in a persistent infection leading to oncogenesis [8,9]. The cccDNA molecule— a key intermediate, generated from the rcDNA genome, is the nuclear viral minichromosome that remains central to determining chronicity of infection and can escape existing treatment strategies [10]. This cccDNA transcriptional template inside the infected hepatocyte nucleus is responsible for generating both pgRNA and subgenomic RNAs that, in turn, will be translated to viral proteins required for replication and virion production [11,12]. These virus-host protein interactions are complex, modulating cellular machinery that promotes viral replication and persistence [13]. Following its entry into hepatocytes, which is mediated by binding to the sodium taurocholate co-transporting polypeptide, the viral nucleocapsid is delivered to the nucleus and relaxed circular DNA is converted to cccDNA, a stable episomal minichromosome directing viral transcription [14,15]. This intricate differentiation includes complicated priming and extension events that culminate with the assembly of a viral chromatin-like structure, by which all viral gene products are transcribed [16,17].

As HBV replication is dependent on host factors, and stable nuclear cccDNA, HBV benefits from modifying host signaling (including apoptosis) to avoid premature death of infected hepatocytes—leading to sustained persistence/chronicity.

HBV Proteins and Regulation of Apoptosis

HBx Protein

The hepatitis B virus (HBV) X protein (HBx) has been the best-characterized HBV protein with respect to apoptosis. HBx has been shown to exert pro- and anti-apoptotic effects, which often depend on the cellular context and stage of the disease. It was found that HBx can render the hepatocytes apoptotic via inducing mitochondrial pathway signaling, up-regulating the levels of reactive oxygen species (ROS), and modulating p53 pathways. On the other hand, HBx might also promote cell

survival and viral persistence by triggering survival pathways such as NF-κB, PI3K/Akt and MAPK signaling to suppres apoptosis [18–20]. regulating cell apoptosis that plays in both pro-apoptotic and anti-apoptotic responses in response to its expression levels, cellular localization as well as interaction with different host factors [21-23]. This dual function makes it difficult to design a specific therapeutic that targets HBx, as HBx could prevent apoptosis by NF-κB activation which is an inducer of anti-apoptosis signals but HBx also induces apoptosis when NF-κB is knocked out [24]. The protein's capability to preferentially rescue the targeted infected cells from cell death paradoxically nitroducts a critical link with cancer biology enabling HBx positive hepatocytes to become regenerated more efficiently than surrounding uninfected ones [25]. In addition, HBx also activates important survival signaling pathways such as PI3K and Wnt/β-catenin signaling cascades, both of which contribute to increased cell survival and proliferation [25].

In addition to these direct effects, HBx modifies global cellular regulation through control of transcription and epigenetic activity, creating a cellular environment conducive to viral persistence and oncogenesis [26]. This delicate cross talk between HBx and cellular machinery has a major role in the development of the chronicity of hepatitis B virus infection, which affects 296 million individuals worldwide with nearly one million deaths per year caused by liver cirrhosis and cancer [27]. The pleiotropic effects of HBx, which among its many attributes has regulatory protein properties, are key to the development of HCC as a signature of chronic sustained HBV infection [23]. In particular, HBx can interact with and influence different signaling pathways as mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), resulting in increased cell proliferation and inhibition of apoptosis [21].

Additionally, it has been reported that HBx disrupts essential cellular functions, including DNA repair and autophagy [and] is involved in genomic instability and hepatocyte survival, which play a role in viral persistence and oncogenesis [21]. In addition, to these complicated cellular interactions, HBx also impairs mitochondrial homeostasis by inducing the reduction of enzymes and corresponding accumulation of ROS and lipid peroxides, which could cause intracellular harm, metastasis, cell death

inhibition [28]. These changes cumulatively result in perturbation of cellular homeostasis, and finally create a favorable context for hepatocarcinogenesis [25,29]. Moreover, HBx is known to modulate expression of microRNAs, which are important regulators of post-transcriptional gene regulation [30], and thus may play roles in the control of hepatic responses related to viral pathogenesis and liver disease evolution. These additive effects of HBx highlight the potential importance for viral-mediated liver disease that leads to chronic hepatitis B and in turn hepatocarcinogenic progression and cirrhosis, a health problem attributable to 2 billion people [30].

The key apoptosis "switch" in HBV is HBx: it can induce apoptosis through mitochondrial stress/ROS and p53 effects, or suppress apoptosis by induction of pro-survival pathways (NF-κB, PI3K/Akt, MAPK), thus permitting persistence and resulting eventually in oncogenic transformation.

Surface and Core Proteins

Apoptosis is also influenced by HBV surface antigens (HBsAg) and core protein (HBcAg). Retention of HBsAg in ER that result in ER stress, can trigger apoptotic signaling pathways. It has been reported that HBcAg can bind to mitochondrial membranes and could be involved in the intrinsic apoptosis (cas 3-independent) in certain setting [31,32].

Apoptosis is a highly perceptive process of programmed cell death, which is essential for embryonic development, maintenance of tissue homeostasis and effective functioning of the immune system [33]. This elaborate process requires a coordinated cross-talk of both intrinsic and extrinsic signaling pathways and ultimately results in an organized deconstruction of cellular elements [33]. This self-destructing cascade is accompanied by typical morphological features such as membrane blebbing, chromatin condensation, DNA fragmentation and apoptotic body formation all programmed to preclude inflammation [34,35]. The processes of apoptosis "are programmed so that the cells are eliminated when they are no longer useful and no damage is imposed on neighboring cells." [35] The process of Apoptosis consists in a succession of precisely orchestrated biochemical events that inevitably result in death (suicide) for damaged or redundant tissue with 'no insult nor wound to neighbor tissues'. Apoptosis can be

initiated via the 'intrinsic apoptotic pathway', which is a consequence of internal cellular stresses such as DNA damage, or by the 'extrinsic apoptotic pathway' in response to these death ligands that are external factors whose binding their specific surface receptors activate signalling [36]. Both pathways ultimately lead to the activation of a family of cysteine proteases, the caspases, which carry out the death program of the cell [37]. These caspases act in a cascade, with initiator caspases for instance caspase-8 and -9 activating downstream effector caspases like caspase-3, -6 and -7 that cleave different substrates of the cell to cause the typical apoptotic morphology [33, 38].

The intrinsic pathway is mainly controlled by the Bcl-2 family of proteins, and its pro-apoptotic members cause the mitochondrial outer membrane permeabilization leading to cytochrome c and other pro-apoptotic factors release in to the cytoplasm [39]. This MOMP constitutes a key "point of no return" in the apoptotic pathway [40]. The extrinsic pathway, on the other hand, is triggered by death ligands (Fas ligand in particular) that bind their cognate death receptors present at the surface of the cell (like Fas), resulting in activation and recruitment of caspase-8 [41]. This caspase-8 activation results in the induction of further effector caspases, including both caspase -3 and -7, which degrade cellular components that ultimately lead to dismantling of tissue structure via proteolytic cleavage of multiple substrates [42].

HBsAg and HBcAg may drive hepatocytes towards intrinsic/ER-stress–associated apoptosis (eg, HBsAg accumulation → ER stress), contributing to liver damage and directing fibrosis progression—especially in the presence of persistently accumulated viral proteins.

Immune-Mediated Apoptosis in HBV Infection

Apoptosis of hepatocytes following HBV infection is predominantly due towards the host immune response, not direct viral cell killing. Cytotoxic T cells (CTLs) may engage in apoptosis of infected hepatocytes by recognizing HBV antigens that are presented on those cells and by the induction of cell death signaling pathways involving interaction between Fas and its ligand, granzyme/perforin. Although this mechanism is crucial for viral clearance in an acute infection, it appears that chronic

HBV-induced T cell-mediated apoptosis plays a central role in continued liver injury and inflammation [43,44].

Hepatitis B virus (HBV) is a major global concern and may lead to chronic infection which ultimately results in severe liver diseases, including cirrhosis and hepatocellular carcinoma [45]. It is estimated that there are around 257 million HBV carriers worldwide and the chronic infection with HBV leads to approximately 0.8 million deaths each year, secondary to complications developed by this form of persistent infection [46,47]. Although there has been an efficacious HBV vaccine for decades, HBV-related liver disease still stands as one of the top causes of death in the world [48]. Non-cytopathic nature of HBV, which implies that immune response plays major role in the pathogenesis of liver damage seen in infection cases [49]. Specifically, although there is no direct cytopathic effect of HBV on infected hepatocytes, the eradication of infected cells by host immunity, particularly CD8+ T cells, results in hepatic inflammation and injury [45,50]. This complex interplay of viral persistence and immune-mediated clearance mechanisms often implicates programmed cell death pathways, specifically apoptosis, which are critical on the one hand in terminating viral infection and cutting liver damage repair but also promoting the progression of liver injury [50,51].

The intricate immunological context of HBV infections requires further insight into which apoptotic pathways are utilized in response to different immune effector cells and how they influence viral control versus immunopathology [52]. In this review, we will try to summarize the current knowledge of immune-mediated apoptosis in HBV infection with an emphasis on both innate and adaptive immune cells which contribute to hepatocyte destruction. The knowledge of these apoptotic pathways is critical for devising new therapeutic interventions that would address chronic hepatitis B by boosting antiviral immunity concomitantly with reduced bystander liver injury [53]. This information is critical for the design of immunotherapy to restore strong HBV-specific adaptive immune responses and ensure long-term immunosurveillance in the presence of persistent infection, with the aim of increasing patient survival [54]. The innate and adaptive immunity of the host is essential for the control of HBV infection, where innate immunity plays an important role in early viral control and also

influences adaptive responses [55]. Acute HBV infection is efficiently controlled by the host immune system, with >90–95% of immunocompetent adults completely clearing the virus and developing life-long immunity due to vigorous polyclonal Mult specific adaptive immune response involving CD8+ and CD4+ T cells that are specific for HBV proteins [56, 57]. These strong responses contribute to viral clearance and protection against chronic infection caused by killing of infected hepatocytes and restriction of virus replication [58].

In HBV, most hepatocyte apoptosis is immune driven (CTL Fas/FasL and perforin/granzyme): this does the host good by clearing cells in acute infections but if persisting in chronic HBV becomes a major driver of current necroinflammation and liver injury.

Apoptosis, Chronic HBV Infection, and Hepatocarcinogenesis

Long-term imbalance of apoptosis is important in HBV-induced hepatocarcinogenesis. Suppression of apoptosis allows the survival of hepatic cell with genetic abnormalities and compensatory proliferation results in increased chances to accumulate mutations. The HBx-mediated regulation of apoptotic and cell cycle pathways is strongly indicated in HCC tumorigenesis. Continued inflammation and apoptosis further drive fibrosis and cirrhosis, form a microenvironment that is favourable for the induction of cancer [59–61]. The HBV infection continues to be an important global health burden, particularly due to its close association with chronic liver disease, cirrhosis and the development of hepatocellular carcinoma [62]. This ubiquitous viral infection in 2019 was estimated to have infected the world population of 296 million people and has contributed to around 820,000 deaths per year mainly due to liver cirrhosis and hepatocellular carcinoma [63]. Long-term presence of HBV causes chronic inflammation which is a critical driver in the process of liver damage and associated with an increased risk for serious endpoints such as hepatocellular carcinoma [64,65].

A minority of those infected with HBV will remain asymptomatic carriers for life; however, a significant number progress to liver disease, which can lead to cirrhosis and HCC, even without preceding cirrhosis in 10–20% of cases [66,67]. HBV

accounts for 50–80% of virus-mediated hepatocellular carcinomas and the risk of HCC in those with HBV infection is 20 times greater than in non-infected individuals [68]. This increased risk further emphasizes the necessity to clarify the complex mechanisms by which chronic HBV infection contributes to carcinogenesis but also it came into attention that HCC can develop without cirrhosis in a substantial part of the population [69]. The oncogenic potential of the virus is accomplished by various factors such as DNA integrations, genetic dysregulation, chromosomal translocations, chronic inflammation and activation influence of oncogene pathways mediated through some HBV proteins [70]. Within these, the Hepatitis B virus X protein is especially involved in viral persistence and tumor formation and dysregulation of cellular host processes takes place [71]. The HBx protein, which is expressed from the HBV X open reading frame, functions as a multifunctional regulatory protein to regulate viral replication and perturb cellular signaling networks directly or indirectly during virus-associated HCC pathogenesis [72,73].

In particular, HBx has been demonstrated to affect host gene expression through disruption of cell-cycle control, abrogation of apoptosis, and stimulation of cell growth and survival, thereby favoring oncogenic transformation of hepatocytes [73]. Its pleiotropic effects are essential for the viral replication process and hepatocellular carcinoma (HCC) formation, contributing to pathogenesis in chronic HBV infections [74,75]. In addition, HBV infection creates an inflammatory microenvironment and mediates the regenerative response of the liver, which results in cycles of cell death and proliferation which provide time for genetic mutation to accumulate to promote carcinogenesis [76.77]. This chronic inflammation and oxidative damage have contributed to genomic instability, ultimately resulting in the dysregulation of key cellular pathways (e.g., p53, TERT and WNT), the mutations of which are common genetic alterations in HCC [78]. This is further exacerbated by the HBx protein, which epigenetically modifies host gene expression that in a paradoxical manner providing some protection to infected hepatocytes against immuno-mediate foraying and thereby permitting continued viral replication and oncogenetic insult [79].

Antitumor chronic apoptosis imbalance facilitates cancer development by providing damaged hepatocytes to escape (anti-apoptosis) and augment the burden of

mutation with cycles of injury-regeneration, combined with HBx-associated disruption of apoptosis and cell-cycle control, along with persistent inflammation/fibrosis contributes creating a pro-cancer liver microenvironment.

Therapeutic Implications HBV-apoptosis interactions

At present, antiviral treatments have a well-documented inhibition of HBV replication, but it does not target the apoptotic pathways. A deeper knowledge of HBV-apoptosis interconnections might result in innovative therapeutic strategies directed to reconstitute a proper apoptosis, improve immune mediated clearance of infected cells or to stop the exaggerated loss of hepatocytes. Observer of HBx related signaling pathways may be a future direction of promising drugs development. [80]

Apoptosis is an important process for the maintenance of cellular homeostasis, whose disturbance during HBV infection may drive immune escape and continuous viral replication that culminates in Hepatocellular carcinoma [81]. As current antiviral therapies frequently are not capable of eliminating the HBV DNA, we expect that apoptosis-targeted therapy will emerge as a potent strategy for treatment of chronic hepatitis B [82]. The rationale for this approach is to circumvent the drawbacks of nucleos(t)ide analogs, which although are effective in suppression of viral replication via reverse transcription inhibition, do not eradicate the intrahepatic viral reservoir requiring lifelong treatment and carrying the risk of resistance [83,84]. Also, the short half-life of cccDNA (believed to be a few months) adds urgency for development of direct-acting anti-c-DNA therapeutics [85]. Accordingly, knowledge of the complex relationship between HBV and mitochondria functions (including apoptosis), a main regulator of cell deletion, is essential for such curative approaches [86]. Mitochondrial impairment is also a typical event associated with viral infection, and it often causes a rise in reactive oxygen species and the induction of apoptotic signaling pathways [84]. In fact, increasing data have come to light recently which demonstrated that mitochondria are important for host responses to HBV infection and involved in the viral persistence as well as liver disease pathogenesis [97,98]. For example, disturbances in mitochondrial function are associated with the alternation of adenosine triphosphate levels that can be a possible diagnostic biomarker for chronic HBV infection and its progression to severer forms of liver diseases [84]. In addition, HBV

can also modulate mitochondrial metabolism by facilitating oxidative phosphorylation and repressing glycolysis in macrophages, which reduces the generation of interleukin-1β leading to a suppression of antiviral responses [86]. This metabolic reprogramming by HeAg is also advantageous for HBV in terms of viral persistence as it regulates macrophage phenotype and inhibits their antiviral responses [89].

Such exploitation of host immunometabolism is not only an immune evasion mechanism, it also has direct effects on epigenetic pathways within the infected hepatocyte, contributing to the chronicity of infection [90]. Thus, therapeutic regimens of targeting mitochondrial function or directly causing mitochondria-dependent apoptosis in HBV-infected cells are promising for functional cure [91]. Active studies are aimed at finding the best therapeutic combination, offering great hope for achieving a functional cure for HBV [92]. Such novel strategies include long-term formulations and new direct-acting antivirals developed against different steps in the life cycle of the virus, overcoming limitations of existing therapies, that only control (and do not cure) infection [93],94]. Specifically, strategies to deplete cellular inhibitors of apoptosis proteins (cIAPs) are under investigation, as cIAPs inhibit TNF-signaling and apoptosis during HBV infection leading to survival of infected hepatocytes [95]. Accordingly, inhibiting cIAP function might sensitize TNF-induced cell death in HBV-infected hepatocytes and therefore help to eliminate the viruses [96].

Nevertheless, the specific stimuli responsible for transition from immune-tolerant phase of chronic HBV infection liver to immune-activated one causing inflammation and damage are poorly understood [97]. This continued challenge emphasises the necessity for forward thinking studies that target the precise pathways by which immune cell dysfunction and T-cell exhaustion – key features in HBV persistence ([98]) are controlled.

Current antivirals inhibit replication, but do not restore the dysregulation of apoptosis or cccDNA removal; approaches to realign apoptosis—enhancing selective clearance of infected cells while minimizing too much loss of hepatocytes (especially through HBx/survival pathways or IAP/TNF linked nodes) are good candidates for a functional cure.

Conclusion

Apoptosis is a central process in the pathogenesis of HBV infection, influencing viral persistence, immune response, and disease progression. HBV has evolved sophisticated mechanisms to manipulate apoptotic pathways, particularly through the multifunctional HBx protein. Continued research into HBV-induced apoptosis will provide critical insights into chronic hepatitis B and hepatocellular carcinoma and may open new avenues for therapeutic intervention. Apoptosis is a core determinant of HBV outcomes: HBV (particularly HBx) strategically modulates apoptotic pathways to persist, but this same manipulation fuels chronic liver injury and HCC risk—making apoptosis-targeted approaches a key future direction.

Apoptosis plays a central and multifaceted role in the pathogenesis of hepatitis B virus (HBV) infection, influencing viral persistence, immune-mediated liver injury, and long-term disease outcomes. HBV has evolved sophisticated strategies to modulate both intrinsic and extrinsic apoptotic pathways, allowing infected hepatocytes to evade premature cell death while maintaining conditions favorable for viral replication. Among HBV proteins, the hepatitis B virus X protein (HBx) emerges as a key regulator of apoptotic signaling, exerting context-dependent pro- and anti-apoptotic effects through its interactions with mitochondrial function, oxidative stress, tumor suppressor pathways, and multiple pro-survival signaling cascades.

While apoptosis is essential for immune clearance of HBV during acute infection, its chronic dysregulation contributes significantly to sustained inflammation, hepatocyte loss, fibrosis, cirrhosis, and the development of hepatocellular carcinoma. Immune-mediated apoptosis, primarily driven by cytotoxic T lymphocytes, represents a major source of liver injury in chronic hepatitis B. Persistent imbalance between hepatocyte death and regeneration promotes genomic instability and creates a microenvironment that favors malignant transformation. Collectively, these findings underscore apoptosis as a pivotal mechanism linking HBV infection to progressive liver disease and hepatocarcinogenesis.

Recommendations

1. **Future research priorities**: Further studies are needed to delineate the precise molecular switches that determine whether HBV-infected hepatocytes undergo apoptosis or survive. Special emphasis should be placed on context-specific functions of HBx, interactions between viral proteins and host apoptotic regulators, and the role of mitochondrial dysfunction in HBV persistence.
2. **Therapeutic development**: Novel therapeutic strategies should aim to restore balanced apoptotic signaling by selectively eliminating infected hepatocytes while preserving overall liver integrity. Targeting HBx-associated signaling pathways, inhibitor of apoptosis proteins (IAPs), and mitochondrial-mediated apoptosis represents a promising avenue toward achieving a functional cure for HBV.
3. **Integration with immunotherapy**: Combining apoptosis-modulating agents with immune-based therapies may enhance HBV-specific immune responses and promote effective viral clearance while minimizing immune-mediated liver damage.
4. **Clinical translation and biomarkers**: Identification of apoptosis-related biomarkers could improve disease monitoring, risk stratification for hepatocellular carcinoma, and evaluation of therapeutic response in patients with chronic hepatitis B.
5. **Long-term disease prevention**: In addition to antiviral suppression, strategies aimed at correcting apoptosis dysregulation should be incorporated into long-term management plans to reduce progression to cirrhosis and hepatocellular carcinoma.

Prognosis

The prognosis of hepatitis B virus (HBV) infection is closely linked to the balance between viral persistence, host immune responses, and the regulation of apoptotic pathways within hepatocytes. Individuals who achieve effective immune control, either spontaneously or through antiviral therapy, generally have a favorable prognosis with reduced risk of progressive liver disease. In contrast, chronic HBV infection characterized by sustained dysregulation of apoptosis is associated with

ongoing hepatocyte injury, chronic inflammation, fibrosis, and an increased likelihood of cirrhosis and hepatocellular carcinoma (HCC).

Although current nucleus(t)ide analog therapies successfully suppress viral replication and significantly improve clinical outcomes, they rarely eliminate covalently closed circular DNA (cccDNA) or fully normalize apoptotic signaling. As a result, patients remain at long-term risk for disease progression and HCC, even under effective viral suppression. Prognosis is further influenced by host factors such as age at infection, immune status, viral genotype, and coexisting metabolic or inflammatory liver conditions. Continued advances in understanding HBV–apoptosis interactions are therefore essential to improving long-term outcomes and reducing HBV-related morbidity and mortality.

Future Research Directions

1. **Molecular mechanisms of apoptosis modulation**: Future research should focus on defining the precise molecular determinants that govern the dual pro- and anti-apoptotic roles of HBV proteins, particularly HBx. Elucidating how cellular context, viral load, and disease stage influence apoptotic outcomes will be critical for targeted intervention.
2. **cccDNA and apoptosis interplay**: Investigating the relationship between apoptotic signaling and cccDNA stability, turnover, and silencing may reveal novel strategies to eliminate or functionally inactivate the viral reservoir, a major obstacle to cure.
3. **Mitochondrial dysfunction and cell death pathways**" Greater attention should be given to mitochondrial-mediated apoptosis and its integration with other regulated cell death pathways, including autophagy and necroptosis, in the setting of chronic HBV infection.
4. **Immune–apoptosis crosstalk**: Understanding how immune exhaustion, immune checkpoint pathways, and immunometabolic reprogramming affect apoptosis in HBV-infected hepatocytes may guide the development of combined immunomodulatory and apoptosis-targeted therapies.
5. **Therapeutic targeting and combination strategies**: Preclinical and clinical studies are needed to evaluate agents that modulate apoptotic pathways—such

as inhibitors of apoptosis proteins (IAP) antagonists or mitochondrial-targeted compounds—in combination with existing antivirals and emerging immunotherapies.

6. **Biomarkers and personalized medicine**: Identification of apoptosis-related biomarkers could enable early prediction of disease progression, stratification of HCC risk, and personalization of treatment strategies for patients with chronic hepatitis B.

References

1. World Health Organization. Global hepatitis report 2017. Geneva: WHO; 2017.
2. Seeger C, Mason WS. Molecular biology of hepatitis B virus infection. *Virology*. 2015;479-480:672-686.
3. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol*. 2007;35(4):495-516.
4. Green DR, Llambi F. Cell death signaling. *Cold Spring Harb Perspect Biol*. 2015;7(12): a006080.
5. Levrero M, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. *J Hepatol*. 2016;64(1 Suppl): S84-S101.
6. Dandri M. Epigenetic modulation in chronic hepatitis B virus infection. Seminars in Immunopathology. Springer Science+Business Media; 2020 Mar 17;42(2):173. Available from: https://doi.org/10.1007/s00281-020-00780-6
7. Hwangbo S, Kim G, Choi Y, Park YK, Bae S, Ryu JY, et al. Analysis of Host Factor Networks during Hepatitis B Virus Infection in Primary Human Hepatocytes. Research Square (Research Square). 2024 May 6; Available from: https://doi.org/10.21203/rs.3.rs-4321520/v1
8. Nevola R, Beccia D, Rosato V, Ruocco R, Mastrocinque D, Villani A, et al. HBV Infection and Host Interactions: The Role in Viral Persistence and Oncogenesis. International Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute; 2023 Apr 21 [cited 2025 Oct];24(8):7651. Available from: https://doi.org/10.3390/ijms24087651
9. Hwangbo S, Kim G, Choi Y, Park YK, Bae S, Ryu JY, et al. Analysis of host factor networks during hepatitis B virus infection in primary human hepatocytes. Virology

Journal. 2024 Aug 1 [cited 2025 Oct];21(1). Available from: https://doi.org/10.1186/s12985-024-02446-3

10. Li Y, Luo G. Human low-density lipoprotein receptor plays an important role in hepatitis B virus infection. PLoS Pathogens. 2021 Jul 22 [cited 2025 Oct];17(7). Available from: https://doi.org/10.1371/journal.ppat.1009722

11. Piracha ZZ, Saeed U, Piracha IE, Noor S, Noor E. Decoding the multifaceted interventions between human sirtuin 2 and dynamic hepatitis B viral proteins to confirm their roles in HBV replication. Frontiers in Cellular and Infection Microbiology. 2024 Jan 4;13. Available from: https://doi.org/10.3389/fcimb.2023.1234903

12. Peng T, Ma Q, Li J, Wang X, Zhang C, Ma J, et al. HBV promotes its replication by up-regulating RAD51C gene expression. Scientific Reports. 2024 Jan 31;14(1). Available from: https://doi.org/10.1038/s41598-024-53047-7

13. Damme EV, Vanhove J, Severyn B, Verschueren L, Pauwels F. The Hepatitis B Virus Interactome: A Comprehensive Overview. Frontiers in Microbiology. Frontiers Media; 2021 Sep 16;12. Available from: https://doi.org/10.3389/fmicb.2021.724877

14. Guo H, Urban S, Wang W. In vitro cell culture models to study hepatitis B and D virus infection. Frontiers in Microbiology. Frontiers Media; 2023 Apr 5;14. Available from: https://doi.org/10.3389/fmicb.2023.1169770

15. Xiao P, Liu C, Mitra B, Kim ES, Sun N, Jurado A, et al. HBV p22-interacting protein C1QBP inhibits viral replication through impeding nucleocapsid formation and nuclear import. PLoS Pathogens. 2025 Oct 17;21(10). Available from: https://doi.org/10.1371/journal.ppat.1013581

16. Ligat G, Goto K, Verrier ÉR, Baumert TF. Targeting Viral cccDNA for Cure of Chronic Hepatitis B. Current Hepatology Reports. 2020 Jul 9;19(3):235. Available from: https://doi.org/10.1007/s11901-020-00534-w

17. Soltani S, Shenagari M, Emadi MS. Exploring the Replication Mechanisms of DNA and RNA Viruses. In: IntechOpen eBooks [Internet]. IntechOpen; 2023. Available from: https://doi.org/10.5772/intechopen.1003767

18. Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature*. 1991;351(6324):317-320.

19. Clippinger AJ, Bouchard MJ. Hepatitis B virus HBx protein modulates apoptosis. *J Virol*. 2008;82(6):2806-2816.

20. Benhenda S, Cougot D, Buendia MA, Neuveut C. Hepatitis B virus X protein molecular functions and its role in virus life cycle and pathogenesis. *Adv Cancer Res*. 2009; 103:75-109.

21. Agustiningsih A, Rasyak MR, Turyadi T, Jayanti S, Sukowati C. The oncogenic role of hepatitis B virus X gene in hepatocarcinogenesis: recent updates. Exploration of Targeted Anti-tumor Therapy. 2024 Feb 20;5(1):120. Available from: https://doi.org/10.37349/etat.2024.00209

22. Delphin M. Polarization of Hepatic Macrophages by HBsAg : a means to an end for viral maintenance. HAL (Le Centre pour la Communication Scientifique Directe). 2021 Oct 5; Available from: https://theses.hal.science/tel-03623551

23. Schollmeier A, Glitscher M, Hildt E. Relevance of HBx for Hepatitis B Virus-Associated Pathogenesis. International Journal of Molecular Sciences [Internet]. Multidisciplinary Digital Publishing Institute; 2023 Mar 4;24(5):4964. Available from: https://doi.org/10.3390/ijms24054964

24. Kim HJ, Kim O, Hong H, Lee SH, Kim S. Harnessing adipose-derived stem cells to release specialized secretome for the treatment of hepatitis B. International Journal of Molecular Medicine. 2021 Jan 8;47(3). Available from: https://doi.org/10.3892/ijmm.2021.4848

25. Medhat A, Arzumanyan A, Feitelson MA. Hepatitis B x antigen (HBx) is an important therapeutic target in the pathogenesis of hepatocellular carcinoma. Oncotarget. 2021 Sep 16;12(24):2421. Available from: https://doi.org/10.18632/oncotarget.28077

26. Sivasudhan E, Blake N, Lu ZL, Jia M, Rong R. Hepatitis B Viral Protein HBx and the Molecular Mechanisms Modulating the Hallmarks of Hepatocellular Carcinoma: A Comprehensive Review. Cells. Multidisciplinary Digital Publishing Institute; 2022 Feb 21;11(4):741. Available from: https://doi.org/10.3390/cells11040741

27. Li D, Hamadalnil Y, Tu T. Hepatitis B Viral Protein HBx: Roles in Viral Replication and Hepatocarcinogenesis. Viruses. Multidisciplinary Digital Publishing Institute; 2024 Aug 26;16(9):1361. Available from: https://doi.org/10.3390/v16091361

28. Ghosh S, Chakraborty A, Banerjee S. Persistence of Hepatitis B Virus Infection: A Multi-Faceted Player for Hepatocarcinogenesis. Frontiers in Microbiology. Frontiers Media; 2021 Aug 30 ;12. Available from: https://doi.org/10.3389/fmicb.2021.678537

29. Shoraka S, Hosseinian SM, Hasibi A, Ghaemi A, Mohebbi SR. The role of hepatitis B virus genome variations in HBV-related HCC: effects on host signaling pathways. Frontiers in Microbiology. Frontiers Media; 2023 Jul 31;14. Available from: https://doi.org/10.3389/fmicb.2023.1213145

30. Wang F, Song H, Xu F, Xu J, Wang L, Yang F, et al. Role of hepatitis B virus non-structural protein HBx on HBV replication, interferon signaling, and hepatocarcinogenesis. Frontiers in Microbiology. 2023 Dec 21;14. Available from: https://doi.org/10.3389/fmicb.2023.1322892

31. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol*. 1995; 13:29-60.

32. Liang TJ. Hepatitis B: the virus and disease. *Hepatology*. 2009;49(5 Suppl): S13-S21.

33. Mustafa MR, Ahmad R, Tantry IQ, Ahmad W, Siddiqui S, Alam M, et al. Apoptosis: A Comprehensive Overview of Signaling Pathways, Morphological Changes, and Physiological Significance and Therapeutic Implications. Cells [Internet]. Multidisciplinary Digital Publishing Institute; 2024 Nov 6;13(22):1838. Available from: https://doi.org/10.3390/cells13221838

34. Wanner E, Thoppil H, Riabowol K. Senescence and Apoptosis: Architects of Mammalian Development. Frontiers in Cell and Developmental Biology. Frontiers Media; 2021 Jan 18;8. Available from: https://doi.org/10.3389/fcell.2020.620089

35. Bhat IA, Bhat AM, Abdullah ST. Apoptosis-Mechanisms, Regulation in Pathology, and Therapeutic Potential. In: Biochemistry. IntechOpen; 2025. Available from: https://doi.org/10.5772/intechopen.1008890

36. Singhal M, Shaha S, Katsikogianni M. Comparative Analysis of Cytotoxicity Assays, from Traditional to Modern Approaches. In: Biochemistry [Internet]. IntechOpen; 2024. Available from: https://doi.org/10.5772/intechopen.1006842

37. Lossi L. The concept of intrinsic versus extrinsic apoptosis. Biochemical Journal. 2022 Feb 11;479(3):357. Available from: https://doi.org/10.1042/bcj20210854

38. Battistelli M, Falcieri E. Apoptotic Bodies: Particular Extracellular Vesicles Involved in Intercellular Communication. In 2021. p. 473. Available from: https://doi.org/10.1201/9781003180449-20

39. Tutar Y. Regulation and Dysfunction of Apoptosis [Internet]. IntechOpen eBooks. IntechOpen; 2021. Available from: https://doi.org/10.5772/intechopen.92915

40. Wani AK, Akhtar N, Mir T ul G, Singh R, Jha PK, Mallik SK, et al. Targeting Apoptotic Pathway of Cancer Cells with Phytochemicals and Plant-Based Nanomaterials. Carolina Digital Repository (University of North Carolina at Chapel Hill). 2023 Jan 1 [cited 2025 Oct]; Available from: https://doi.org/10.17615/asch-qv66

41. Hu S, Wang Y, Xu Z, Zhou Y, Cao J, Zhang H, et al. Identification of the Bcl-2 and Bax homologs from Rhipicephalus haemaphysaloides and their function in the degeneration of tick salivary glands. Parasites & Vectors. 2021 Aug 4;14(1). Available from: https://doi.org/10.1186/s13071-021-04879-z

42. Dho SH, Cho M, Woo W, Jeong S, Kim LK. Caspases as master regulators of programmed cell death: apoptosis, pyroptosis and beyond. Experimental & Molecular Medicine. Springer Nature; 2025 Jun 24 [cited 2026 Jan];57(6):1121. Available from: https://doi.org/10.1038/s12276-025-01470-9

43. Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis. *Annu Rev Pathol*. 2006; 1:23-61.

44. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol*. 2005;5(3):215-229.

45. Iannacone M, Guidotti LG. Immunobiology and pathogenesis of hepatitis B virus infection. Nature reviews Immunology. Nature Portfolio; 2021 May 17;22(1):19. Available from: https://doi.org/10.1038/s41577-021-00549-4

46. Hillaire MLB, Lawrence P, Lagrange B. IFN-γ: A Crucial Player in the Fight Against HBV Infection? Immune Network. 2023 Jan 1 [cited 2025 Oct];23(4). Available from: https://doi.org/10.4110/in.2023.23.e30

47. Yang G, Wan P, Zhang Y, Tan Q, Qudus MS, Yue Z, et al. Innate Immunity, Inflammation, and Intervention in HBV Infection. Viruses. Multidisciplinary Digital Publishing Institute; 2022 Oct 17;14(10):2275. Available from: https://doi.org/10.3390/v14102275

48. Thomas E, Baumert TF. Hepatitis B Virus–Hepatocyte Interactions and Innate Immune Responses: Experimental Models and Molecular Mechanisms. Seminars in Liver Disease. Thieme Medical Publishers (Germany); 2019 Jul 1;39(3):301. Available from: https://doi.org/10.1055/s-0039-1685518

49. Binder B, Hofmann M, Thimme R. Role of Immunomodulators in Functional Cure Strategies for HBV. Current Hepatology Reports [Internet]. 2020 Aug 27;19(4):337. Available from: https://doi.org/10.1007/s11901-020-00538-6

50. Khanam A, Chua JV, Kottilil S. Immunopathology of Chronic Hepatitis B Infection: Role of Innate and Adaptive Immune Response in Disease Progression. International Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute; 2021 May 23 ;22(11):5497. Available from: https://doi.org/10.3390/ijms22115497

51. Zheng P, Dou Y, Wang Q. Immune response and treatment targets of chronic hepatitis B virus infection: innate and adaptive immunity. Frontiers in Cellular and Infection Microbiology. Frontiers Media; 2023 Jun 22 [cited 2025 Oct];13. Available from: https://doi.org/10.3389/fcimb.2023.1206720

52. Dandri M, Bertoletti A, Lütgehetmann M. Innate immunity in hepatitis B and D virus infection: consequences for viral persistence, inflammation, and T cell recognition. Seminars in Immunopathology. Springer Science+Business Media; 2021 May 21;43(4):535. Available from: https://doi.org/10.1007/s00281-021-00864-x

53. Akbar SMF, Yoshida O, Hiasa Y. Immune therapies against chronic hepatitis B. Journal of Gastroenterology. Springer Science+Business Media; 2022 Jun 16;57(8):517. Available from: https://doi.org/10.1007/s00535-022-01890-8

54. Maini MK, Burton AR. Restoring, releasing or replacing adaptive immunity in chronic hepatitis B. Nature Reviews Gastroenterology & Hepatology. Nature Portfolio; 2019 Sep 23;16(11):662. Available from: https://doi.org/10.1038/s41575-019-0196-9

55. Jin X, Bi J. Prospects for NK-based immunotherapy of chronic HBV infection. Frontiers in Immunology. Frontiers Media; 2022 Dec 15 [cited 2025 Sep];13. Available from: https://doi.org/10.3389/fimmu.2022.1084109

56. Dumolard L, Aspord C, Marche PN, Jílková ZM. Immune checkpoints on T and NK cells in the context of HBV infection: Landscape, pathophysiology and therapeutic exploitation. Frontiers in Immunology. Frontiers Media; 2023 Mar 28; 14:1148111. Available from: https://doi.org/10.3389/fimmu.2023.1148111

57. Ye J, Chen J. Interferon and Hepatitis B: Current and Future Perspectives. Frontiers in Immunology. Frontiers Media; 2021 Sep 7 [cited 2025 Sep];12. Available from: https://doi.org/10.3389/fimmu.2021.733364

58. Zaltron S, Cambianica A, Gregorio MD, Colangelo C, Storti S, Tiecco G, et al. Case report: An occult hepatitis B virus infection reactivation in an HIV/HCV coinfected patient during an immune reconstitution inflammatory syndrome. Frontiers in Cellular and Infection Microbiology. 2023 Apr 14 [cited 2025 Oct];13. Available from: https://doi.org/10.3389/fcimb.2023.1143346

59. Feitelson MA, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. *Cancer Lett*. 2007;252(2):157-170.

60. Block TM, Mehta AS, Fimmel CJ, Jordan R. Molecular viral oncology of hepatocellular carcinoma. *Oncogene*. 2003;22(33):5093-5107.

61. Arzumanyan A, Reis HMGPV, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer*. 2013;13(2):123-135.

62. Agustiningsih A, Rasyak MR, Turyadi T, Jayanti S, Sukowati C. The oncogenic role of hepatitis B virus X gene in hepatocarcinogenesis: recent updates. Exploration of Targeted Anti-tumor Therapy. 2024 Feb 20 [cited 2025 Oct];5(1):120. Available from: https://doi.org/10.37349/etat.2024.00209

63. Lin C, Ou Q. Emerging role of mitochondria in response to HBV infection. Journal of Clinical Laboratory Analysis. 2022 Sep 16 [cited 2025 Oct];36(10). Available from: https://doi.org/10.1002/jcla.24704

64. Park E, Dezhbord M, Lee AR, Park BB, Kim K. Dysregulation of Liver Regeneration by Hepatitis B Virus Infection: Impact on Development of Hepatocellular Carcinoma. Cancers. Multidisciplinary Digital Publishing Institute; 2022 Jul 22; 14(15): 3566. Available from: https://doi.org/10.3390/cancers14153566

65. Yao J, J G, Xie Y. Hepatitis B virus induced cirrhosis and hepatocarcinoma: pathogenesis and therapeutics. Exploration of Digestive Diseases. 2025 Feb 21; Available from: https://doi.org/10.37349/edd.2025.100565

66. Akpınar MY, Şımşek G, Aksoy EK, Sapmaz FP, Kantarci S, Uzman M, et al. Survivin expression starts before hepatocellular cancer development in the liver of chronic hepatitis B patients: a pilot, cross-sectional study. Gastroenterology Review. 2019 Aug 20; 15(2): 138. Available from: https://doi.org/10.5114/pg.2019.87081

67. Liu P, Harris JM, Marchi E, D'Arienzo V, Michler T, Wing PAC, et al. Hypoxic gene expression in chronic hepatitis B virus infected patients is not observed in state-of-

the-art in vitro and mouse infection models. Scientific Reports. 2020 Aug 24;10(1). Available from: https://doi.org/10.1038/s41598-020-70865-7

68. Shoraka S, Hosseinian SM, Hasibi A, Ghaemi A, Mohebbi SR. The role of hepatitis B virus genome variations in HBV-related HCC: effects on host signaling pathways. Frontiers in Microbiology. Frontiers Media; 2023 Jul 31;14. Available from: https://doi.org/10.3389/fmicb.2023.1213145

69. Lim HK, Jeffrey GP, Ramm GA, Soekmadji C. Pathogenesis of Viral Hepatitis-Induced Chronic Liver Disease: Role of Extracellular Vesicles. Frontiers in Cellular and Infection Microbiology. Frontiers Media; 2020 Nov 10;10. Available from: https://doi.org/10.3389/fcimb.2020.587628

70. Tu T, McQuaid T, Jacobson IM. HBV-Induced Carcinogenesis: Mechanisms, Correlation with Viral Suppression, and Implications for Treatment. Liver International. Wiley; 2024 Dec 25;45(1). Available from: https://doi.org/10.1111/liv.16202

71. Siddiqui ZI, Azam SA, Khan WH, Afroz M, Farooqui SR, Amir F, et al. An in vitro Study on the Role of Hepatitis B Virus X Protein C-Terminal Truncation in Liver Disease Development. Frontiers in Genetics. 2021 Mar 12 [cited 2025 Sep];12. Available from: https://doi.org/10.3389/fgene.2021.633341

72. Wang F, Song H, Xu F, Xu J, Wang L, Yang F, et al. Role of hepatitis B virus non-structural protein HBx on HBV replication, interferon signaling, and hepatocarcinogenesis. Frontiers in Microbiology. 2023 Dec 21; 14:1322892. Available from: https://doi.org/10.3389/fmicb.2023.1322892

73. Sivasudhan E, Blake N, Lu ZL, Jia M, Rong R. Hepatitis B Viral Protein HBx and the Molecular Mechanisms Modulating the Hallmarks of Hepatocellular Carcinoma: A Comprehensive Review. Cells. Multidisciplinary Digital Publishing Institute; 2022 Feb 21;11(4):741. Available from: https://doi.org/10.3390/cells11040741

74. Li D, Hamadalnil Y, Tu T. Hepatitis B Viral Protein HBx: Roles in Viral Replication and Hepatocarcinogenesis. Viruses. Multidisciplinary Digital Publishing Institute; 2024 Aug 26;16(9):1361. Available from: https://doi.org/10.3390/v16091361

75. Schollmeier A, Glitscher M, Hildt E. Relevance of HBx for Hepatitis B Virus-Associated Pathogenesis. International Journal of Molecular Sciences [Internet]. Multidisciplinary Digital Publishing Institute; 2023 Mar 4;24(5):4964. Available from: https://doi.org/10.3390/ijms24054964

76. Shen C, Jiang X, Li M, Luo Y. Hepatitis Virus and Hepatocellular Carcinoma: Recent Advances. Cancers. Multidisciplinary Digital Publishing Institute; 2023 Jan 15 ; 15(2): 533. Available from: https://doi.org/10.3390/cancers15020533

77. Wang S, Yeh S, Chen P. Unique Features of Hepatitis B Virus-Related Hepatocellular Carcinoma in Pathogenesis and Clinical Significance. Cancers [Internet]. Multidisciplinary Digital Publishing Institute; 2021 May 18;13(10):2454. Available from: https://doi.org/10.3390/cancers13102454

78. Gu W. Development of Human Papillomavirus Integration Analysis Technologies for Human Papillomavirus-Associated Cancer Research. Deep Blue (University of Michigan). 2024 Jan 1; Available from: https://hdl.handle.net/2027.42/193372

79. Medhat A, Arzumanyan A, Feitelson MA. Hepatitis B x antigen (HBx) is an important therapeutic target in the pathogenesis of hepatocellular carcinoma. Oncotarget. 2021 Sep 16; 12(24): 2421. Available from: https://doi.org/10.18632/oncotarget.28077

80. Revill PA, Chisari FV, Block JM, et al. A global scientific strategy to cure hepatitis B. *Lancet Gastroenterol Hepatol*. 2019;4(7):545-558.

81. Tepjanta P, Fujiyama K, Misaki R, Kimkong I. The N-linked glycosylation modifications in the hepatitis B surface protein impact cellular autophagy, HBV replication, and HBV secretion. PLoS ONE. 2024 Mar 15 [cited 2025 Oct];19(3). Available from: https://doi.org/10.1371/journal.pone.0299403

82. Clark MP, Huynh T, Rao S, Mackiewicz L, Mason HS, Romal S, et al. Clinical stage drugs targeting inhibitor of apoptosis proteins purge episomal Hepatitis B viral genome in preclinical models. Cell Death and Disease. 2021 Jun 23 [cited 2025 Oct];12(7). Available from: https://doi.org/10.1038/s41419-021-03924-0

83. Nevola R, Beccia D, Rosato V, Ruocco R, Mastrocinque D, Villani A, et al. HBV Infection and Host Interactions: The Role in Viral Persistence and Oncogenesis. International Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute; 2023 Apr 21; 24(8): 7651. Available from: https://doi.org/10.3390/ijms24087651

84. Gao Y, Lu X, Shi Y. Editorial: Hepatitis B virus and host interactions in liver diseases. Frontiers in Cell and Developmental Biology. 2023 May 4;11. Available from: https://doi.org/10.3389/fcell.2023.1204280

85. Song H, Huang Y, Li C, Liu Q, Tan G. Editorial: Interferon and its antiviral effect in response to HBV infection. Frontiers in Immunology. 2023 Feb 2;14. Available from: https://doi.org/10.3389/fimmu.2023.1135649

86. Kaur KK, Allahbadia G, Singh M. Targeting dysfunctional mitochondrial metabolism of hepatocytes caused by hepatitis B virus (HBV) in the treatment of the chronic HBV infection- a narrative review. Journal of Human Virology & Retrovirology. MedCrave Group; 2024 Jan 25;11(1):4. Available from: https://doi.org/10.15406/jhvrv.2024.11.00273

87. Choi YM, Kim B, Lee SA, Lee SY, Kim B. A Telomerase-Derived Peptide Exerts an Anti-Hepatitis B Virus Effect via Mitochondrial DNA Stress-Dependent Type I Interferon Production. Frontiers in Immunology. 2020 May 21 [cited 2025 Oct];11. Available from: https://doi.org/10.3389/fimmu.2020.00652

88. Lin C, Ou Q. Emerging role of mitochondria in response to HBV infection. Journal of Clinical Laboratory Analysis. 2022 Sep 16 [cited 2025 Oct];36(10). Available from: https://doi.org/10.1002/jcla.24704

89. Li Y, Wu C, Lee J, Ning Q, Lim J, Eoh H, et al. Hepatitis B virus e antigen induces atypical metabolism and differentially regulates programmed cell deaths of macrophages. PLoS Pathogens. 2024 Mar 11 [cited 2025 Oct];20(3). Available from: https://doi.org/10.1371/journal.ppat.1012079

90. Wang Z, Liu N, Yang Y, Tu Z. The novel mechanism facilitating chronic hepatitis B infection: immunometabolism and epigenetic modification reprogramming. Frontiers in Immunology. Frontiers Media; 2024 Jan 15;15. Available from: https://doi.org/10.3389/fimmu.2024.1349867

91. Zhang J, Fu X, Jia F, Yu J, Zhang J, Bai A, et al. Mitochondrial orchestration of PANoptosis: mechanisms, disease pathogenesis, and emerging therapeutic frontiers. Cell Death Discovery. Springer Nature; 2025 Oct 21 [cited 2025 Oct];11(1). Available from: https://doi.org/10.1038/s41420-025-02750-z

92. Gopalakrishna H, Ghany MG. Perspective on Emerging Therapies to Achieve Functional Cure of Chronic Hepatitis B. Current Hepatology Reports. 2024 Feb 10;23(2):241. Available from: https://doi.org/10.1007/s11901-024-00652-9

93. Rybicka M, Bielawski KP. Recent Advances in Understanding, Diagnosing, and Treating Hepatitis B Virus Infection. Microorganisms [Internet]. Multidisciplinary

Digital Publishing Institute; 2020 Sep 15;8(9):1416. Available from: https://doi.org/10.3390/microorganisms8091416

94. Ogunnaike M, Das S, Raut SS, Sultana A, Nayan MU, Ganesan M, et al. Chronic Hepatitis B Infection: New Approaches towards Cure. Biomolecules [Internet]. Multidisciplinary Digital Publishing Institute; 2023 Aug 1;13(8):1208. Available from: https://doi.org/10.3390/biom13081208

95. Boortalary T, Shinn B, Halegoua-DeMarzio D, Hann H. Achieving a Cure: The Next Frontier in Hepatitis B Treatment. In: Liver Cancer. Karger Publishers; 2021. p. 109. Available from: https://doi.org/10.36255/exonpublications.livercancer.2021.ch6

96. Torresi J, Tran BM, Christiansen D, Earnest-Silveira L, Schwab R, Vincan E. HBV-related hepatocarcinogenesis: the role of signalling pathways and innovative ex vivo research models. BMC Cancer. BioMed Central; 2019 Jul 18;19(1). Available from: https://doi.org/10.1186/s12885-019-5916-6

97. Wang J, Lu H, Li Q. Hepatic macrophage niche: a bridge between HBV-mediated metabolic changes with intrahepatic inflammation. Frontiers in Immunology. Frontiers Media; 2024 Jul 18;15. Available from: https://doi.org/10.3389/fimmu.2024.1414594

98. Zheng P, Dou Y, Wang Q. Immune response and treatment targets of chronic hepatitis B virus infection: innate and adaptive immunity. Frontiers in Cellular and Infection Microbiology. Frontiers Media; 2023 Jun 22 [cited 2025 Nov];13. Available from: https://doi.org/10.3389/fcimb.2023.1206720