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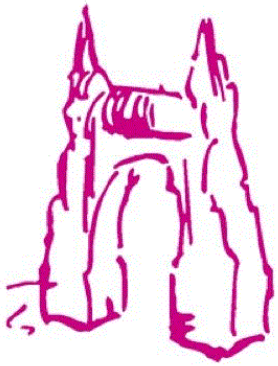

FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

Final project degree

Hepatitis B virus: novel therapies and induction of hepatocellular carcinoma

Main field: Cellular Biology

Secondary fields: Microbiology, Immunology, and Molecular Biology

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1. ABSTRACT

Nowadays the virus of hepatitis B is one of the most insidious viruses that affect chronically nearly 350 Million people worldwide, but only 8% know about their condition. It is estimated that 15-40 % of the infected patients could develop cirrhosis, hepatocellular carcinoma or liver failure, between 0,5-1,2 million die each year due to the complications. Since governments started the immunization of newborn with the HBV vaccine program, the incidence of new infections had plummeted. The remaining population who had already been infected remain in a chronic state, most of them without knowing their conditions, it is an important issue because there are treatments when the virus starts to replicate and nutritional strategies that could render the patients in an inactive state for a longer period of time, those approaches could potentially reduce the incidence of the complications such as HCC and subsequent high rate of mortality. This project is summarizing information on the recent bibliography in research of new approaches on the therapy and treatment of hepatitis B currently on research.

RESUM

Actualment, el virus de l'hepatitis B és un dels virus més insidiosos que afecta de manera crònica gairebé 350 milions de persones a tot el món, però només el 8% sap de la seva condició. S'estima que el 15-40% dels pacients infectats podrien desenvolupar cirrosi, carcinoma hepatocel·lular o insuficiència hepàtica, entre 0,5 i 1,2 milions moren cada any a causa de les complicacions. Atès que els governs van iniciar la vacunació del nounat amb programes de vacunes de l'hepatitis B, la incidència de noves infeccions s'ha desplomat. La població romanent que ja havia estat infectada queden en un estat crònic, la majoria sense conèixer les seves condicions, és un assumpte important perquè hi ha tractaments quan el virus comença a replicar-se i les estratègies nutricionals que podrien fer que els pacients estiguessin inactius durant un període de temps més llarg, aquests enfocaments podrien reduir potencialment la incidència de les complicacions com HCC i la seva elevada taxa de la mortalitat. Aquest projecte resumeix la informació sobre la bibliografia recent en recerca de nous enfocaments sobre la teràpia i el tractament de l'hepatitis B que s'estan investigant actualment.

2. INTEGRATION OF THE DIFFERENTS AREAS

Cellular biology is used as tools to understand the cell on a molecular level and how the signaling pathways, the organelles, the cycle of the cell and metabolic processes interact within the cell and the surrounding environment. This discipline is essential for the study of cancer, a heterogeneous disease that affects different tissues and each of them with their unique characteristics, that needs to approach with distinct strategies dependent on numerous factors as the location, the type or the stage of cancer and other factors, in this case, the HCC is the subject of the topic.

Molecular biology concerns about the molecular mechanism of the cell, especially the interactions of macromolecules as DNA, RNA, transcription factors and proteins.

Microbiology is the study of the microorganisms, and comprise of virology, where lies the HBV, study of the structure of the virus, the interactions with the host immunity, the pathogenesis that causes disease such cirrhosis and HCC.

Immunology is the study of the immune system and the interactions with different pathogens such as HBV, that infects hepatocytes and the responses of immune cells on the clearance of this virus.

3. INTRODUCTION

3.1 History of Hepatitis B

Hepatitis B virus (HBV) is classified as a virus from *Orthohepadnavirus* genus and *Hepadnavirus* family, also as dsDNA-RT group VII in Baltimore classification. The nomenclature of Hepatitis B was designated with hepatitis A in 1947 by F.O. MacCallum, a British hepatologist, Hepatitis B was regarded as ¨serum¨ hepatitis, took it from the transmission route and Hepatitis A as ¨infectious or epidemic¨. Later, WHO adopted those terminologies. It wasn't until 1963 when hepatitis B surface antigen (HBsAg) also known as Australia antigen (AuAg) was discovered from an Australian aborigine (1). Then in 1968 Prince, Murakami, and Okochi (2), through independent studies, confirm that the AuAg is found specifically in patients who had serum hepatitis, but was unclear what the AuAg was. Two years later, Dane et al. (3) elucidated the AuAg was a surface antigen, designated as HBsAg. The first specific vaccine targeting the virus was developed during the mid-1970s and was a made using HBsAg from plasma donors, and various other steps to ensure the vaccine was virus-free (4).

3.2 Epidemiology and Genotype

It is estimated at around 2 billion people with the serological signal from past or present infection. And there is more than 350 Million of chronic hepatitis b (CHB) carriers (5), approximately 75% of those chronically infected lives in that area of Asia and Western Pacific.

The Global Burden of Disease Study 2010 carried by Lozano et al. (7) estimated on 786,000 deaths: 341,000 from liver cancers and 312,000 from cirrhosis. And HBV was the 15th cause of mortality in humankind.

There is a disparate prevalence on the CHB infection worldwide classified with the positive HBsAg amount the population in high (>8%), intermediate (between 2%-7%) and low (<2%) as shown in figure 1.

- High prevalence: It is more common in areas where population density is high and still developing such the areas of sub-Saharan Africa, South East Asia, Amazon Basin, and China. The vast majority has evidence of the populace has serological marks of infection (70-95%), and most of the infections occur during infancy or childhood when the chance to chronic infection is high. Therefore, the subjacent hepatocellular carcinoma (HCC) and liver diseases are high as well (8).

- Intermediate prevalence: The areas where is moderately rooted are Japan, Russia and Eastern and Southern Europe, the strip from India to Turkey and a region of South

America. Here there is 10 to 60 % of the population showing serological of past or present infection. In these areas, the infection is more frequently transmitted in the adolescence and adulthood, and the prevalence of chronic infected is maintained from the infancy and childhood transmission (9).

- Low prevalence: the areas of well-developed countries as Australia, the region of North America, Western Europe, Chile and Argentina the HBV only shows marks of infection on 5-7 % of the population (10). Childhood and infancy infection are rare and the main source of transmission are high-risk groups as health care workers, homosexual males, injection drug users and patients receiving a transfusion of blood or plasma.

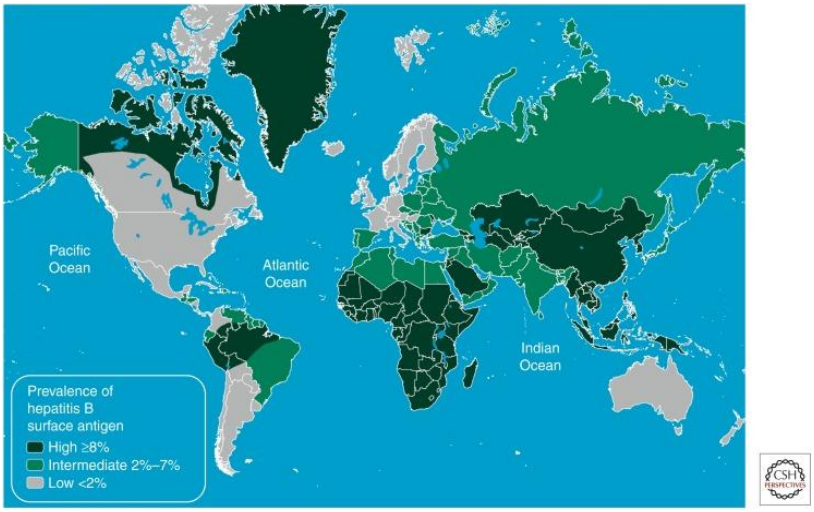

Figure 1. Geographic prevalence of Hepatitis B infection (6).

Serotype

Serotypes of HBV was based on the antigenic determinants of the HBsAg and recognized by Mazzur et al. (11) and classified into 4 subtypes: adr, adw, ayr and ayw, with constant determinant “a” and 2 variables, determinants d/y and w/r with other determinants, currently using antibodies against HBsAg there are classified 9 serological subtypes: ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+ and adrq− (12). The relationship between serotypes and genotype isn’t clear and one serotype could be fit in on several genotypes of HBV. The vaccine is made from adw serotype strains and may reduce the efficacy in the given population if their serotype is different due to a mismatch between antigen and antibody (18).

Genotype

HBV classified as different genotype when there is >8% on the divergence of the DNA sequence, and subgenotype when the genetic difference is 4%-7,5% (14), when the difference between subgenotypes are less than 4% it is named as clades (15). There are

8 genotypes from A to H, and at least 40 subgenotypes, the table of figure 2 shows the distribution of those genotypes.

The evidence shows that depending on the genotype the severity, rates of HBeAg seroconversion, chances of chronicity or induction of HCC may be differing. Genotype C has a higher viral load on serum, more histological inflammation and a higher risk of HCC and cirrhosis compared to Genotype B patients (13), but the later has a correlation of HCC developing at a younger age than Genotype C (16). Genotype A has a higher alanine transaminase (ALT) levels, the risk of progression into CHB from acute infection and the resolution time often is prolonged. Genotype D shows a higher viral load than genotype A and is more associated with acute liver failure than other genotypes (17).

Genotype	**Distribution**	**Subgenotype**	**Distribution**
A	Pandemic, but most prevalent in the USA and Northwest Europe	Aa/A1	Asia and Africa
		Ae/A2	Europe and the USA
B	Northern and Southeast Asia	Bj/B1	Japan
		Ba/B2	China, Taiwan, and Vietnam
		B3	Indonesia
		B4	Vietnam
		B5	Philippines
C	Asia and Pacific region	Ce/C1	East Asia
		Cs/C2	South-east Asia
		C3	Polynesia, Solomon Islands
		C4	Northeast Australia
D	Mediterranean, the Middle East, North America, and India	D1	India, Pakistan, Iran
		D2	India, Russia, and the Baltic region
		D3	India, Pakistan
		D4	Solomon Islands, Oceania
		D5	India
E	Africa and Tunisia	NA	NA
F	Central and South America	F1	Central America, Peru, Venezuela
		F2	Venezuela
		F3	Venezuela
		F4	Bolivia
G	France, Germany, and USA	NA	NA
H	Central and South America and Mexico	NA	NA
NA: data not available			

Figure 2. Geographical distribution of HBV genotype and subgenotype (14).

3.3 Diagnosis and Clinical Manifestations

HBV has 3 major antigens and their respect antibody used for the diagnosis:

HBsAg: the most essential serological marker, measures the surface protein of HBV and indicates present infection and capacity of transmission, on acute infections appears on serum for the 4-6th week. HBsAg measures the immune control over HBV infection. **Hepatitis B surface antibody** (**Anti-HBs)** are detected 3 months later from initial infection also positive from the vaccinated population. HBsAg is cleared from serum and acute infection ends granting immune protection, its presence on serum for more than 6 months indicates the patient becomes CHB carrier (20). Recently quantitative HBsAg was proposed as a diagnostic marker for the indication of CHB carrier (28).

Hepatitis B core antigen (HBcAg): it's not found on serum, **hepatitis B core antibody** (**anti-HBc) (total or IgG**) otherwise appears during acute infection and lasts lifelong making a marker of a presence or past infection. **IgM anti-HBc** shows up at a time with anti-HBc and lasts long as the acute infection (22). When IgM anti-HBc plummets anti-HBc starts to peak.

Hepatitis B core antigen (HBeAg): is a secretory protein and a marker of high level both in replication and serum HBV DNA with high rates of transmission, on acute infection the seroconversion from positive HBeAg to **hepatitis B e antibody** (**anti-HBe)** befalls quickly**,** but the seroconversion on CHB carriers varies and happens years later (23).

Other markers of viral infection are:

HBV DNA: also called viral load, measured as IU/mL, the higher the number the higher the replication, it is used as a marker for CHB status and monitorization of the effectiveness of antiviral treatments (20).

Recently was proposed another antigen for the diagnosis of CHB: **HBcrAg,** a core-related antigen, is a serological marker with good correlation with intrahepatic covalently closed circular DNA (cccDNA), a stable form of HBV genome residing inside of the nucleus, total and serum HBV DNA, and lesser correlation with HBsAg. HBcrAg can still be detected even when HBV DNA becomes undetectable and HBsAg is lost. It may be useful to differentiate states of CHB infection, reactivation of occult HBV infection, the efficacy of treatments with interferon or analogs of nucleos(t)ides (NUC) or the risk of HCC and cirrhosis (19).

ALT: alanine transaminase, also known as alanine aminotransferase (ALAT) or serum glutamate-pyruvate transaminase (SGPT) is an enzyme that is presence in majority within hepatocytes, smaller amounts are also found in the kidney, heart, and muscle. Damaged hepatocytes leak their intracellular content into the bloodstream and ALT is

used as a sensible nonspecific indicator of hepatocellular damage, may also increase momentarily when exhausting exercise is done (21).

The 1st case on figure 3, the person has no marker of contact with the virus so vaccination is recommended. The 2nd case, + anti-HBc means the person has or had an infection and – HBsAg with + anti-HBs confirms it's past and has immune protection. The 3rd only anti-HBs is positive due to the vaccine, which is composed of the surface antigen of HBV. The 4th case shows the case of acute infection on course with the + IgM anti-HBc, also + anti-HBc, the later could be negative if the infection is detected early and the body hasn't started to produce the IgG. The 4th case is from CHB, with a marker of current infection with + HBsAg, past the acute phase: + anti-HBc with - IgM anti-HBc. The last case may have different interpretations: negative on both HBsAg and anti-HBs could indicate the body got rid of the virus or almost and the antibody has yet to appear or it's on undetectable levels. The false positive of anti-HBc leads to the 1st case. another possibility is seroconversion of HBsAg to anti-HBs from CHB carriers from a reduction of HBV DNA with good prognosis.

HBsAg **anti-HBc** **anti-HBs**	negative negative negative	Susceptible
HBsAg **anti-HBc** **anti-HBs**	negative positive positive	Immune due to natural infection
HBsAg **anti-HBc** **anti-HBs**	negative negative positive	Immune due to hepatitis B vaccination
HBsAg **anti-HBc** **IgM anti-HBc** **anti-HBs**	positive positive positive negative	Acutely infected
HBsAg **anti-HBc** **IgM anti-HBc** **anti-HBs**	positive positive negative negative	Chronically infected
HBsAg **anti-HBc** **anti-HBs**	negative positive negative	Interpretation unclear; four possibilities: 1. Resolved infection (most common) 2. False-positive anti-HBc, thus susceptible 3. "Low level" chronic infection 4. Resolving acute infection

Figure 3. Hepatitis B Serologic Test Results from Centers for Disease Control and Prevention (20).

3.4 Natural history of acute HBV infection and manifestations

First, the **incubation period** is 75 days average and varies from 30 to 180 until the symptom begins, this period is an **immune tolerant** stage and the virus replicates and there is a weak or no activation from immune system characteristic with normal ALT levels (23). Clinical signs and symptoms sometimes do not appear during the acute infection, the majority of children infected do not show any manifestation and half of the adult population are asymptomatic (24).

Then the **preicteric**, or **prodromal phase** lasts from first signs of symptoms to the beginning of jaundice, lasts 3 to 10 days and is characterized by abdominal pain, headache, fever, myalgia, skin rashes, arthritis and dark urine (24)

HBsAg starts to appear during incubation period just before the onset of symptoms and ALT elevation, reflection the lysis of hepatocytes characterized by the **immune active** stage, when the anti-HBc starts to appear both IgG and IgM. Also, HBeAg, a marker of high both infection and level of HBV DNA, is cleared during this stage and anti-HBe is created even before the seroconversion of HBsAg as we see on figure 4. Immune active phase lasts 3 to 4 weeks and is when the **icteric phase** and characteristic clinical symptoms appear as jaundice, white or light stools, hepatic tenderness and hepatomegaly (24). There is a windows period between the seroconversion from + HBsAg to anti-HBs.

The last acute stage is the **convalescence phase** where tiredness persists during weeks or months and jaundice and other symptoms cease then the person has immunity for future infections and is **recovered** (24). Between 0,1 to 0,5 % will develop fulminant hepatitis, massive necrosis of hepatocytes that ends with liver failure. It is believed the cause is a high immune response and intense lysis of hepatocytes, and absence markers of HBV infection in various patients (26).

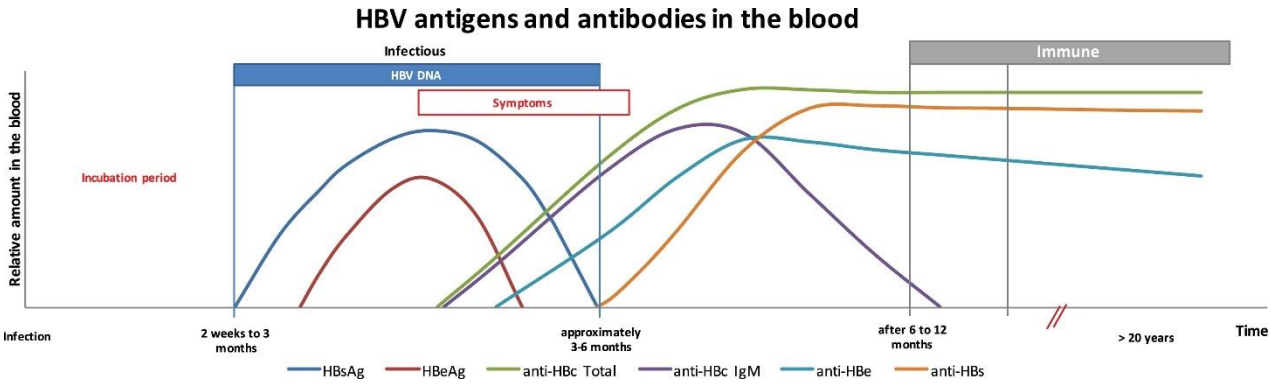

Figure 4. Antigens and antibodies profile during acute infection (23)

3.5 Natural history of CHB infection and complications

There are four phases of chronic HBV infection: immune tolerance, immune clearance, immune control, and immune escape show in figure 5, based on perinatal or infant infection and going through different stages through the ages:

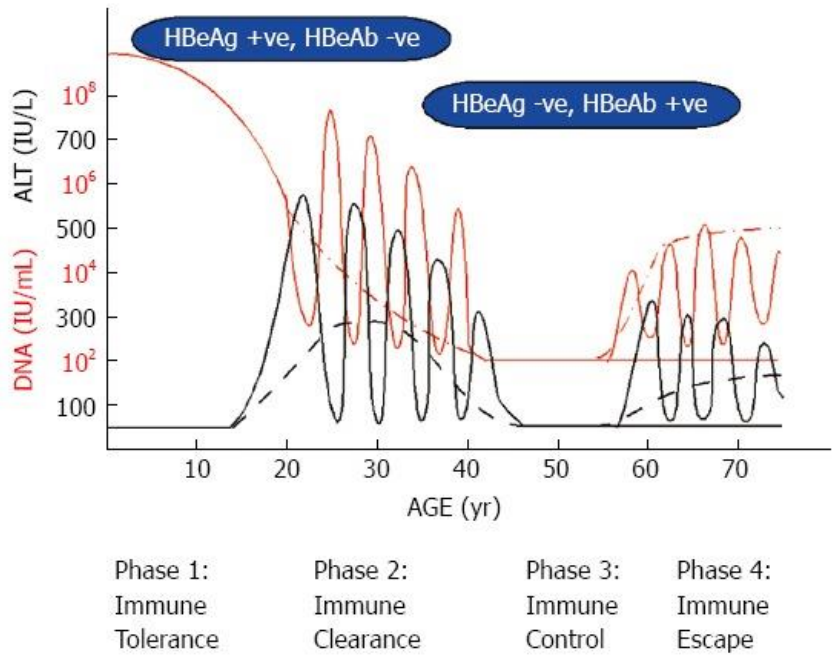

Figure 5. Natural history of CHB (31).

Immune tolerance/tolerant: this phase assembles with acute immune tolerant stage, in perinatal and infant infection may remain until adolescence or lasts for decades, with a high level of virus replication: HBV DNA levels of > 20000 IU/mL and normal to mild ALT levels (26) (31).

Immune active/clearance: this phase may last for months to years, the host loses his immunotolerance towards the virus and starts the clearance, therefore, leads to high levels of necroinflammation, hepatocellular lysis with an increase of ALT and finally ends with a decrease of viral title and seroconversion from HBeAg to anti-HBe. When the immune reaction is virulent or prolonged may form fibrosis and can progress into the development of cirrhosis (29).

Immune control, inactive carrier state or low replication phase: on this state the host has already undergone seroconversion of HBeAg to anti-HBe and has normally less than 2000 IU/mL of HBV DNA or undetectable, some authors states may also apply HVB DNA levels up to 20.000 if accompanies with normal range ALT levels, normal liver functions and low or no necroinflammation (32). Thus, the clinical prognosis is well on those carriers and risk of cirrhosis or HCC are low.

Immune escape, active carrier or HBeAg-negative CHB: patients are normally older**,** this stage there is fluctuating both HBV DNA levels ≥2000 IU/mL and high ALT levels with and moderate to severe necroinflammation with strong correlation from fibrosis, cirrhosis, and HCC. The cause is due to the virus evades the immune system with mutations on precore and/or basal core promoter region of HBV (31).

There are more phases that may not undergo in a linear way:

Resolved Hepatitis B: 1%-2% per year may even undergo HBsAg seroclearance annually in Western countries, where infection is usually acquired in adulthood or 0.05 to 0.8% per year in endemic areas, where HBV infection is mostly acquired perinatally or in early childhood (30), Ungtrakul et al. (27) define - HBsAg as HBsAg <0.05 IU/mL this is the closest point to a cure for CHB carriers, but cccDNA can still persist within the hepatocyte and serving as template for viral replication.

Occult HBV infection (OBI) occurs when HBsAg is undetectable due to mutations on the surface antigen that allow escaping from the detection screenings and undetectable or <200 UI/mL HBV DNA (23).

Reactivation: is an abrupt increase in HBV replication with ALT elevation and the symptomatology assembles to acute hepatitis. The reactivation can occur spontaneously but is more common as a consequence of immunosuppression due to chemotherapy, solid tumors or neoplasia (29).

Cirrhosis

Cirrhosis is a process in which the liver forms fibrotic nodule and tissue becomes scarred due to long-term destruction of hepatocytes and inflammation. This condition alters the lobular organization of liver, whirls early fibrosis does not impact on the normal liver development, chronic fibrosis leads to lose function and cirrhosis. We call early cirrhosis with a small amount of scarring and normally asymptomatic or non-specific symptoms as weakness, weight loss or fatigue **compensated cirrhosis,** then if damage continues and extends the fibrosis symptoms as jaundice, ascites hepatic encephalopathy appears and we have **decompensated cirrhosis** which is generally irreversible (34).

Fibrosis is mediated by hepatic stellate cells, ubicated in the perisinusoidal space, between the hepatocytes of bile conduct and sinusoids, while they remain quiescent function as vitamin A storage, once are activated by cytokines and growth factors released by Kupffer cells loses vitamin A and transform into myofibroblasts and start to produce collagen, the main ingredient for fibrotic and scar tissue. The prolonged inflammation builds up pressure into the sinusoids known as intrasinusoidal or portal hypertension and fluids leak to peritoneal cavity causing ascites and enlarging the liver, the high pressure difficult the entry of blood into the liver through portal veins and arterial blood compromising the hepatic outflow (34).

Common causes of cirrhosis are Hepatitis B and C viruses, alcohol liver disease, nonalcoholic steatohepatitis, hemochromatosis, autoimmune hepatitis, etc. Current treatment is based on preventing further damage by treating the underlying causes, in this case, control the HBV replication (33), but in cases of decompensated cirrhosis liver transplantation may be the only current solution. Patients who don't undergo HBeAg seroconversion are more prone to cirrhosis due to the incapacity of the immune system to tamper down and control viral replication with subsequent necroinflammation (29).

Hepatocellular Carcinoma

HCC is the most common primary liver cancer, named after the primary liver cells: the hepatocytes. It's considered one of the most common malign tumors in the world. Principal risk factors for HCC are HVB and HCV, both accounts for the 50% and 25% of total cases, also from toxins like aflatoxin, alcohol abuse, non-alcoholic fatty liver disease (NAFLD), hemochromatosis, diabetes, obesity, etc. As we see the risk factors closely resemble to cirrhosis, people with the cirrhotic condition have high chances to develop HCC, although HCC can be developed in the absence of cirrhosis. Some symptoms are abdominal pain, lack of appetite, tiredness, ascites, jaundice, weight loss, also resembles cirrhosis. Surveillance every 6 months in CHB patients should be done in order to early detection, especially on cirrhosis, including hepatic ultrasound, tomography, magnetic resonance for the physical assessment and alpha-fetoprotein, a glycoprotein produced by fetal liver and levels above 500 g/L may indicate HCC development (35).

Pathogenesis is further explained in point 6.4

3.6 Transmission and Prevention

The transmission of the virus could be horizontal and vertical from fluids of an either acute or chronic person with + HBsAg. The virus remains infectious at least 7 days on environmental surfaces even without the visible trail. The concentration of virus on fluids:

- High: Blood, serous fluids.
- Moderate: saliva, tears, urine and semen.
- Not clearly or no detectable: breast milk, tears, sweat, urine, stool, or droplet nuclei (20).

Horizontal: transmitted from person to person via parenteral or no parenteral.

Via parenteral: transmission through direct contact with the blood or serum of the infected person by percutaneous exposure from tattoos, acupuncture, needle-drug users.

Via no parenteral: semen and vaginal secretions serve as a vehicle of transmission on sexual intercourse. Saliva could infect though bite, scratches, abrasions or burns.

Vertical: **Via perinatal**: from infected mother to the newborn and the transmission is due to the contact with the vaginal secretions and blood infected with HBV. Without postexposure prophylaxis and the mother is + for HBsAg and HBeAg 70%-90% of infants get infected and 90% of infected infants become chronically infected. If it's HBsAg + only the 10% of infants are infected and 90% of infected infants become chronically infected (20).

The probability of becoming chronic depends on the age of the infection:

- Children: 80-90% infected during the first year becomes CHB carrier and 30-50% before the age of 6. The likelihood diminishes with age.
- Adults: less than 5% becomes chronic from infection of HBV (20).

Currently, there are prevention strategies made an effort to eliminate the transmission, are basically vaccinate the risk group of the population, like children and newborn, as we state above the rate of chronification staggers at 90% if infection occurs. Mothers positive in HBV could past the virus to their child and prophylaxis postpartum be required (20).

Vaccine

Currently, HBV vaccine is recombinant, made from *Saccharomyces cerevisiae,* contains more than 95% of the HBsAg protein, which determinant "a" is the target the antibodies, and 5% from yeast product and adsorbed to aluminum hydroxide or aluminum hydroxyphosphate sulfate. HBV vaccine is produced by Merck (Recombivax HB) and GlaxoSmithKline Pharmaceuticals (Engerix-B), both available for the pediatric and adult population. The 3 intramuscular doses are effective in the development of antibodies in 95% effective on pediatric population up to 19 years, 90% in healthy adults, but declines to 75% at the age of 65. Immunocompromised and hemodialysis patients may need more doses in order to confer protection (20).

The 3 intramuscular doses are the primary vaccination, in children normally is administrated first when the newborn is discharged from the hospital, then 1 to 2 months old and the last 6 to 18 months. the third dose must be administered at least 8 weeks after the second dose, and at least 16 weeks after the first dose. In adolescence are also 3 shoots, the first two doses separated by no less than 4 weeks, and the third 4 to 6 months after the second dose. In adults over 20 years have the same schedule than adolescence (20).

Anti-HBs indicates immunity against HBV, titles above 10 mIU/mL 1-2 months after receiving the whole primary vaccine series are considered seroprotected and classified as vaccine responders. Neither routine serologic testing nor boosted doses are not recommended for healthy children or adults responders according to United States Centers for Disease Control and Prevention guidelines and WHO considers people with title of antibody anti-HBs below 10 IU/ mL also protected by the strong response of cellular immunity against HBV. Challenge or booster dose of HVB vaccine were administrated years later after the vaccine series for testing the anamnestic response of the vaccine responders and shows over 60% to 96% boosted the >anti-HBs 10 IU/ mL, so immunocompetent person with >anti-HBs 10 IU/ mL after a challenge dose are considered boosted, with or without the decline of anti-HBs title over the years after the primary vaccination (20) (36). But the reduction in anamnestic response has been observed after 20 years of primary vaccine series also the response to challenge doses decrease with age and the duration of fully vaccinated people is unknown (37).

3.4 Prophylaxis and Current treatments

Prophylaxis after exposure

Birth dose: on newborns from infected mothers postexposure prophylaxis is needed in order to minimize the risk of infection, within 12 hours of birth the vaccine and HBIG are provided, given alone the efficacy is 75% and 71% respectively but combined the efficacy rises to 94% (24). Also, women who have a high title of DNA HBV, >200,000 IU/mL, antiviral therapy is recommended.

There are people who don't respond well to the Hepatitis B vaccine and provides temporary protection after exposure through passive anti-HBs and it may last 3-6 months (24).

The vaccine is useful and can attenuate or even halt the progression to CHB even if the person is already infected and going through the incubation period (25). Figure 6 shows procedures followed depending on the status of the patient.

Status of exposed person	Status of source		
	HBsAg positive	HBsAg negative	Unknown
Unvaccinated	HBIG (1 dose) and HBV vaccination	HBV vaccination	HBV vaccination
Vaccinated			
Responders*	No treatment	No treatment	No treatment
Nonresponders	HBIG (1 dose) and HBV revaccination (3 doses)	No treatment Revaccination (3 doses) for future protection	If high-risk source, HBIG (1 dose) and HBV revaccination (3 doses)
Response unknown	Test anti-HBs - if positive, no treatment - if negative, HBIG (1 dose) and HBV vaccination (3 doses)	No treatment	Test anti-HBs - if positive, no treatment - if negative, HBIG (1 dose) and HBV revaccination (3 doses)

* Responders is a person with an anti-HBs titer > 10 mIU after the completion of primary vaccine series.

Figure 6. Postexposure Immunoprophylaxis (25)

Treatment of acute HBV

There is no specific treatment for acute HBV infection, just ensure adequate nutrition and healthy lifestyle (5). There is a not clearly understanding the progression of acute to chronic hepatitis but it's likely to a weak immune response, therefore interferon-mediated treatment may improve the HBsAg clearance (38).

Treatment of Severe Acute Hepatitis B

Lamivudine: a nucleoside analog, was positive in reducing the mortality vs placebo in cases of severe Hepatitis B, with 2 of the 3 conditions: hepatic encephalopathy, serum bilirubin >10.0 mg/dl, and international normal ratio (INR) of coagulopathy >1.6, use of antiviral treatment show useful and should continue until HBsAg clearance (38). For fulminant hepatitis, there is no agreement whether treatment or not (38).

Treatment for chronic hepatitis B infection

Current therapies consist of oral antiviral, as nucleotide or nucleotide analogs (NUC) or interferon α (IFN-α) and derivates. NUC inhibit HBV polymerase activity and INF-α inhibits viral protein synthesis and activates immune system cells: macrophages, natural killer and CD8+ T-cell (39).

Drugs approved by the Food and Drug Administration and also by European Medicines Agency and other Asian countries for the CHB treatment illustrated on figure 7: interferon-a2b (INF- a2b), pegylated interferon-a2α (peg-IFN-a2α), lamivudine (LAM), adefovir dipivoxil, entecavir (ETV), telbivudine, tenofovir (TDF) and tenofovir alafenamide (TAF) (46). The most commonly used first-line agents are peg-IFN-a2α, TAF, and ETV (41).

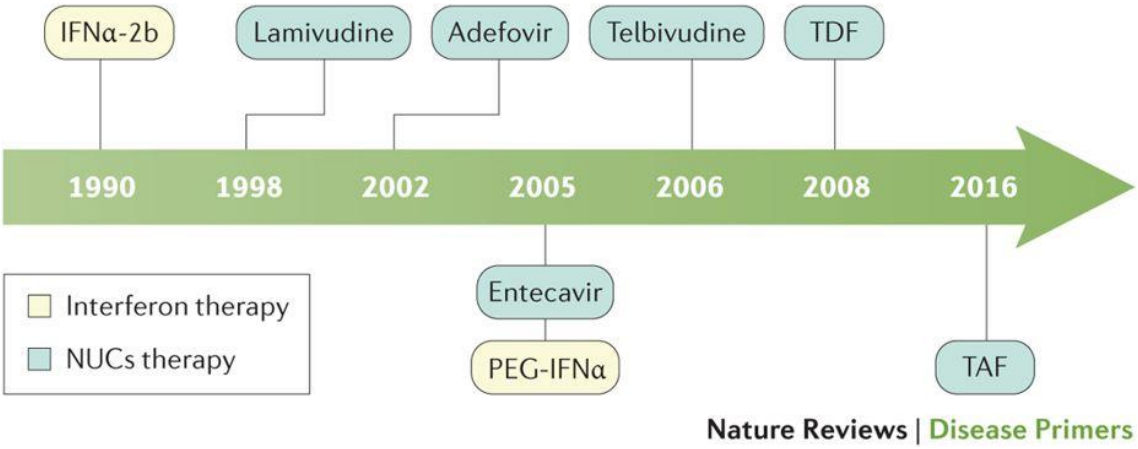

Figure 7. Approved drugs for HBV treatment (46).

TDF and ETV are the recommended oral antiviral drugs for WHO, due to its low rate of drug-resistance HBV mutation and high potency. PEG–IFN-α shows better performance than IFN-α, the polyethylene glycol covalently attached to IFN increases half-life by reduction of renal clearance and reduce immunogenicity, it is used for HBeAg-positive CHB, and the duration of treatment is shorter than NUC and have long durability effect at the end of administration, though official guidelines for HBV treatment are unavailable (39). Genotype may also influence the effectiveness of PEG–IFN-α, Genotype A, B, and F showed better HBeAg loss rate than C, D or E (42).

There are secondary effects though, HBV develops resistance to NUC as the treatment prolongs, only a few numbers of patients achieve sustained viral suppression and the responders should follow a lifelong treatment due the impossibility for the current drugs to target cccDNA located inside nucleus, integrated HBV DNA into the host DNA and extracellular reservoir of HBV (39) (40). Patient under IFN-α treatment shows symptoms of tiredness, fatigue, depression, bone-marrow suppression among others, but without a sign of resistant mutant (39). The goal of current treatment for any CHB patient is to prevent the development of cirrhosis, hepatic failure, and HCC.

4. OBJECTIVES

The aim for this project is to elucidate how the Hepatitis B virus works, the underlining molecular mechanism, in order to understand the pathogenesis of one of the most insidious virus worldwide, a major risk factor of hepatocellular carcinoma. Also, summarize the state of the art in the research of new approaches for the treatments and therapies for chronic hepatitis B virus, dig inside of the mechanism of HBV-related hepatocarcinogenesis of the chronically infected population that could give some clues of the causes.

Summarizing, the main objectives are:

- Understand the structure and mechanism of action of the HBV.
- Summarize the new approaches, treatments, and drugs that are currently developing for the HBV treatment.
- Understand the mechanism on the HBV-induced HCC.

5. MATERIAL AND METHODS

This project degree is based eminently a bibliographic synthesis and analysis of reviews and papers throughout the scientific literature, searching keywords such as: hepatitis B, HBV, HCC, Hepatocellular carcinoma, mechanism of action HBV, treatment of hepatitis B, pathogenesis of HCC, serology HBV, hepatitis B vaccine, autophagy HBV, chronic HBV and so on. Generic information was gathered from OMS, Mayo Clinic, Medline, etc.

The papers and reviews where found on:

- Webpage of the main journals as Nature, Science, Cell, Plos One, Clinical Gastroenterology and Hepatology, and their sub-journals.
- Scientific search engines: NCBI, Google Scholar, and Scopus
- Database and editorials: ScienceDirect, Scielo, , and Elsevier.

The work of this synthesis is to summarize all the data from the articles that could contribute to enlarge and share the knowledge of this topic in a comprised project.

6. RESULTS

6.1 Genome and Structure

HBV contains DNA virus, icosahedral capsid and surface envelope. Hepatitis B genome is of approximately 3,2 kilobases length and formed by a relaxed circular DNA (RC-DNA) and it's only partially double-stranded, the minus/L strand is complete and covalently attached with the viral DNA polymerase at the 5' -end, the plus/S strand is shorter and incomplete (45). As showed in figure 8 it is composed of 4 open reading frames (ORF) overlapped with each other, which encodes for 7 proteins (34) (41) (46):

- Gene P/polymerase: coding for the RNA-dependent DNA polymerase and serves as HVB reverse transcriptase.
- Genes PreS1/L, PreS2/M, and Surface/S: coding for the respective envelope proteins large, middle and major, the 3 of them making HBsAg. Large and middle are the most immunogenic part of the envelope.
- Gene precore/core: translation for respectively HBeAg, a secretory protein and nucleocapsid core protein that encapsulates HBV nucleic acids.
- Gene X: coding for the HBx protein, enhancing the viral replication.

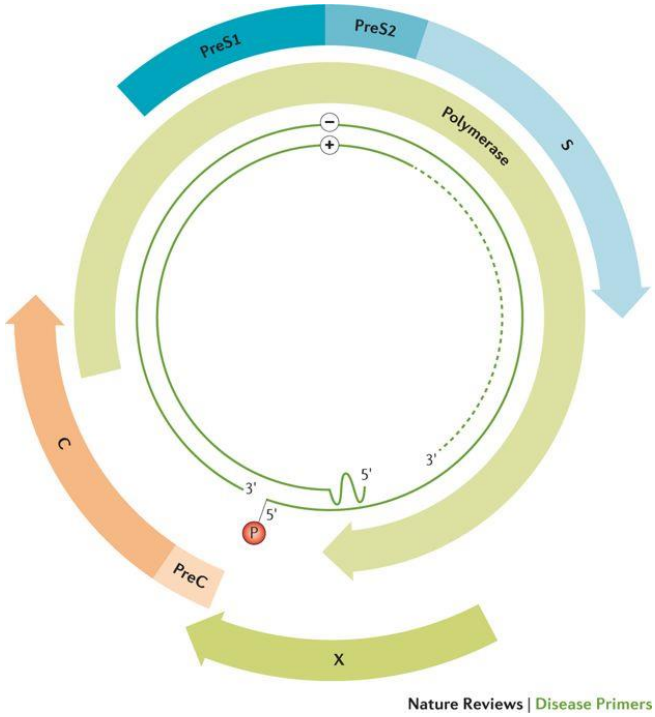

Figure 8. HBV genome: four ORF and double-stranded DNA (46).

The virus produces 3 types of particles out of hepatocytes depicted in figure 9: Dane particle, the real infectious particle with envelope enclosing the nucleocapsid and two forms with only the envelope and its proteins without any nucleic acid nor infecting capacity.

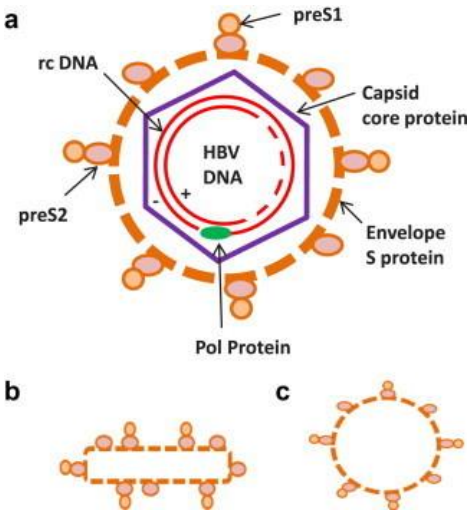

figure 9. HBV particles (43):

a: Dane particle: complete infectious particle made of HBV DNA and double-shelled capsid and surface envelope.

B: Tubular particle: no infectious, lacking DNA.

C: Small spherical particle: no infectious, lacking DNA.

6.2 Life cycle

Attachment and internalization: HBV enters hepatocyte by interacting with heparan sulphate proteoglycans (HSPGs) and promoting the specific biding to sodium taurocolate cotransporting polypeptide (NTCP) receptor at the cell membrane by preS1-domain of HBsAg. Then endocytosis is mediated by caveolin and clathrin (47).

Once inside hepatocyte nucleocapsid is transported mediated by microtubule (47), RC-DNA is released from capsid and translocated into nucleus. The relaxed circular DNA is not suitable for replication template, plus strand must be completed by host enzymes converting to cccDNA (46). cccDNA is associated with histones H3, H4 and no-histone proteins forming a minichromosome and serves as the real template for transcription of both viral mRNA and pregenomic RNA (pgRNA) by host RNA polymerase II (45) (46).

Replication, assembly, and release: mRNAs are exported to the endoplasmic reticulum (RE) for their translation. PgRNA is exported to the cytoplasm and encapsulated with the viral polymerase synthesizing the relaxed circular DNA: first the minus strand, which serves as the template for the plus strand. Then, nucleocapsid can be either translocated in the RE acquiring envelope and thus creating a new virion exiting through the cellular secretory pathway or recycled by nucleases back to the nucleus forming cccDNA for maintaining viral infection (45).

Viral DNA polymerase lacks 3' to 5' exonuclease proofreading activity causing mutations on the virus genome, the rate of mutation is between a real retrovirus and DNA viruses, if the mutation is not lethal it may lead to genetically different species infecting one host named quasispecies and competing with each other if an advantage is acquired.

The viral DNA can also be integrated into the host genome but loses the ability to transcribe pgRNA, leading to a replicative dead end. But nevertheless, it can still transcribe the HBsAg and the HBx mRNAs, HBx protein may lose some amino acids but still retaining some of its functions (49).

6.3 Immunopathogenesis

HBV is not cytopathic to hepatocytes, patients infected in early life don't have any tissue damage in the immunotolerance phase. It's the host cytotoxic immune response to small epitopes of virus the cause of cytolysis, especially the HBeAg on hepatocytes surface. Thus, the liver necroinflammation and subsequent fibrosis are caused mainly by host immune response (46).

Kupffer cells act as intrahepatic macrophage with the capacity of phagocytosis, acting as an antigen-presenting cell (APC) and secretor of proinflammatory cytokines (48).

Hepatocytes leverage **class I major histocompatibility complex (HLA-I)** for presenting peptide fragments from intracellular processing of HBV particles to T-cell receptors (TCR) of CD8 + cells or cytotoxic T cells (CTL) triggering a response of enzymes leading the apoptosis of the infected cell.

Class II major histocompatibility complex (HLA-II) expressed from Kupffer cells and other APC present antigens to the TCR of CD4 + cells or T helper cells, transforming into T helper 1 (Th1) cells and enhancing cytokine signal response of CTL or transforming to T helper 2 (Th2) cells stimulating lymphocytes B cells specific antibody production (47). Dendritic cells have impaired function in HBV infection by lowering HLA-DR (type of HLA-II) expression (48).

The difference between immune clearance of virus or the chronic infection resides into the host immune response: sufficient antigen recognition from TCR generates a complete virus elimination and adequate immunoglobulin production halting reinfections of hepatocytes.

The virus may have the capacity to suppress immune response by suppressing recruitment of natural killer cells, natural killer T cells, and macrophages; blocks Toll-like receptors (TLR) signaling; decrease cytokine secretion (interferon-γ (IFNγ), tumor necrosis factor (TNF), IL-6, IL-8 and IL-1β) from Kupffer cells and blocking retinoic-acid-inducible gene I protein (RIG-I) (46).

RIG-I (retinoic acid-inducible gene-I)-like receptors (RLRs) is part of the innate immune system of pattern recognition receptors (PRRs), RIG-I functions sensing viral acid nucleic then enhancing type I IFN production (50).

HBeAg especially acts as a T cell tolerogen suppressing the immune response towards to HBcAg by stimulating Interleukin 10 secretion, thus raising the susceptibility to chronification (48), HBeAg also generates viral tolerance by downregulating the TLR2 on hepatocytes, Kupffer cells and peripheral monocytes in the Immunotolerant HBeAg-positive phase, thus halting the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) production (46). The loss of HBeAg production in CHB carrier due to mutation, exhibits higher levels of serum HBV DNA, due to the decrease of Th2 stimulation, and inversely increase of Th1 response subsequently resulting with higher inflammation (48). HBeAg and HBcAg induce regulatory T cells to down-regulate the T cells activation, Th1 is more susceptibility to down-regulated by HBeAg than Th2.

The excessive of serum HBsAg sub-viral particles act as a decoy for the humoral immunity promoting exhaustion of T cell by constant triggering its antigen receptor pathways (46). HBV polymerase suppresses the production of myeloid differentiation primary response protein (MYD88), a universal adapter protein of almost all TLR necessary for NF-κB activating (46).

HBx is an unstructured protein, it appears to acquire secondary structure by interacting with different proteins in both cytoplasm and nucleus, this characteristic may explain the vast diverse pleiotropic functions (54). HBx acts as a multi-functional transcriptional modulator of cellular and viral genes, inhibitor or enhancer of transduction pathways and binding of proteins, aiding the viral replication and liver carcinogenesis by (54):

- Enhancing reactive oxygen species (ROS) production, contributing higher rates of mutation, both in viral and host DNA (48).

- Blocking host viral restriction factors as structural maintenance of chromosome 5/6 complex (Smc5/6) which function is silencing and repress cccDNA translation, among other viruses. HBx directs DNA-damage binding protein 1 (DDB1) E3 ubiquitin ligase complex to degrade Smc5/6 (52). Depletion of smc5/6 in dividing cells induces genomic instability and are more prone to genetic errors under conditions that affect DNA stability as HBV induced necroinflammation, therefore a plausible mechanism of HCC induction.

- Forming a complex with p53 protein and sequestering into the cytoplasm (43) thus preventing its nuclear entry and therefore hinder the signaling for apoptosis, p21-related cell arrest and DNA repairing.

- Activating the phosphatidylinositol 3-kinase (PI3K/AKT) signaling pathway, and modulating the transcription factor hepatocyte nuclear factor 4α (HNF4α), this way may decrease in some degree the HBV replication and transcription but can activate oncogenic AKT-related pathways promoting cell survival (55).

- Inhibiting transforming growth factor-β (TGF-β) induced apoptosis by activating PI3K/AKT (57).

- Decreasing virus presentation to lymphocytes via HLA-I by Inhibiting the proteasome degradation of viral particles (46).

- Negative regulation of type I IFN by blocking RIG-I signaling and mitochondrial antiviral signaling (MAVS) (51) (56).

- Increasing telomerase reverse transcriptase (TERT) transactivation thus telomerase activity and increasing of hepatocyte survival and tumorigenesis (60).

6.4 Hepatocellular Carcinoma: HBV hepatocarcinogenesis

HCC is like any other cancers a dysregulation of the cellular machinery that drives the continuous cell division avoiding apoptosis signals. The etiology of cancers may due to mutation of DNA, changes of chromosomes or DNA methylation with subsequent hyper acting oncogenes or inhibiting DNA repair genes, leading to more rate of mutation and proliferation until a cell or group of cells gain characteristics of immortal cells. HBV is without a doubt a driver of HCC, here we evaluate the main mechanisms which the virus leads to HCC.

Oxidative stress

Cells are continuously exposed to the DNA damage from different sources, one in particular, reactive oxygen species (ROS) play crucial a role in HCC. Chronic infection with HBV promotes persistent liver inflammation and produces ROS and oxidative stress leading to DNA damage (59). Levels of oxygen radicals in liver from CHB patients were 1.4-4 times more pronounced than healthy people and levels of 8-Oxoguanine, a marker of DNA lesions was elevated (58), also serum elevation on ROS was observed, and total glutathione levels were found lower and higher its oxidized form. Oxidative stress was much higher in patients with cirrhosis than both CHB carrier or healthy people (58).

More than 80% of the HCC is developed in a cirrhotic liver, the more developed the cirrhosis the more the risk of HCC (53). Activated hepatic stellate cells secrete platelet-derived growth factor (PDGF) and TGF-β modulate a decompensated and upregulated inflammatory immune response. PDGF can activate mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) and PI3K/AKT inhibiting apoptosis (53).

In HBV-protein-mediated ROS production participates HBx, HBsAg, and HBcAg.

Some HBV mutants accumulate the HBsAg and HBcAg in the ER activating the unfolded protein response (UPR) and releasing calcium into the cytoplasm with increasing of ROS production (58). HBx can reduce the respiratory complexes and create pores in mitochondrial thus decreasing membrane potential with enhancing of ROS production (58) (59).

HBx modulation

HBx protein may play a major role on the HCC carcinogenesis by its multiple mechanisms of action that may explain the malignant transformation of hepatocytes, a brief diagram is shown in figure 10 with some of the mechanism of HBx-induced carcinogenesis. Some of the oncogenic mechanisms of HBx protein are stated above at point 6.3.

HBx has interactions with nuclear transcription factors: RNA polymerase binding protein (RBP5), transcriptional factor IIB (TFIIB), transcriptional factor IIH (TFIIH), a subunit of RNA polymerase, cAMP response element-binding protein (CREB), CREB1-binding protein (CBP)/p300, activating transcription factor 2 (-2), activating protein (AP)-2, AP-1, and NF-κB (61).

HBc promotes oncogene transactivation and cell cycle deregulation, thus hepatocyte proliferation by modulating cytoplasmic transduction signal pathways as: MAPK/ERK pathway, Janus kinase/STAT (JNK/STAT), Wnt/β-catenin, focal adhesion kinase (FAK), proline-rich tyrosine kinase 2 (Pyk2), protein kinase C (PKC) and Src-dependent PI3K/Akt (61) (53).

HBx changes TGF-β signaling from the pSmad3C pathway (tumor-suppressive) to the pSmad3L pathway (oncogenic) in early carcinogenic process (53)

HBx can modulate tumor suppressor genes with epigenetic changes in methylation, it seems HBx can upregulate methylation and hypermethylated by transactivation of genes related to DNA methylation as DNA methyltransferase (DNMT) thus repressing *E-cadherin, a* tumor suppressor gene. Also, the $p16^{INK4A}$ gene is hypermethylated on its promotor and have a high correlation with HCC and HBx levels (60).

HBx interferes with nucleotide excision repair (NER) pathway, a cellular mechanism for DNA lesion repairing that comprises more than 30 genes. Including the series XPA to XPG, TFIIH, and P53 to name a few (60).

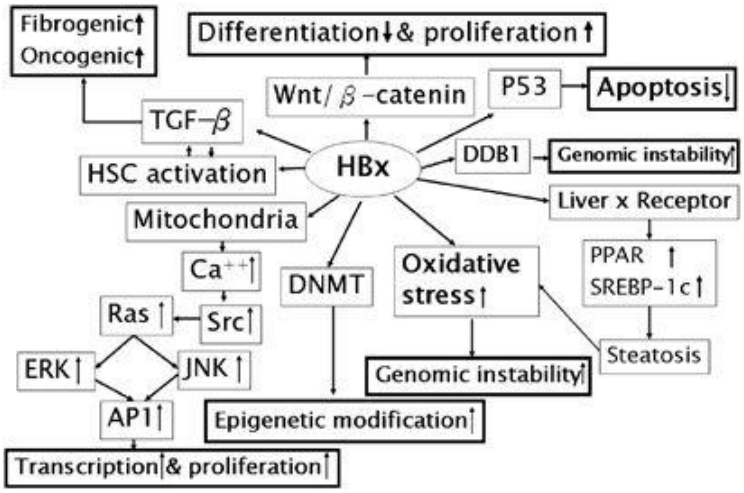

Figure 10. Cellular signaling pathways on HBx-related hepatocarcinogenesis (53).

Virus integration

Integration of virus DNA into the host genome can elucidate its importance on HCC pathogenesis, as genomic integration is present over 85% of HBV related HCCs (53). Exposure to oxidative stress increases the rate of integration, also the chronic inflammation promotes DNA integration by increased replication and damage on DNA (53).

HBV integration can cause genetic alterations such as chromosomal deletions, translocations, fusion transcripts, DNA amplification, and generalized chromosomal and genomic instability. There is no specific site or region on chromosomes where HBV integrates (62). The integration into the host genome can alter the expression of genes related to proliferation, tumor suppressor and microRNA. HBx and pre-S2/S genes are more prone to integrations with their promotor active and can still be coded into functional proteins, some amino acid deletion can occur and their function may vary but certainly contributing to the hepatocarcinogenesis (53).

Truncated pre-S2/S protein may have transactivation activities affecting: c-myc, c-fos, and c-Haras, also active MAPK/ERK signaling and upregulating the TERT activity (53). The ER accumulation of pre-S mutants can increase ROS production and produce genomic instability. Another way pre-S mutant promote carcinogenesis is upregulating cyclooxygenase-2 and cyclin A production thus enhancing cell cycle progression and proliferation (53).

6.5 New treatments and therapies

A summary of treatment for CHB carriers was seen in point 3.5, the currently approved drugs fight the virus but do not target cccDNA, and that is one of the main reasons therapy fails to archive a durable virus clearance. Another reason for the failing of the current treatments is the inability of the immune response of CHB carriers to recognize and eliminate the virus in infected hepatocytes (64). Tang et al. (64) stated 3 viral stages with immunotherapy program could archive a functional cure for HBV by:

- Complete suppression of HBV replication to prevent the spread of HBV infection to susceptible host cells and the amplification of cccDNA pool in infected cells.

- Reducing viral antigen load and induce HBsAg seroclearance through eradication or inactivation of cccDNA and RNA interference-mediated degradation of viral mRNA.

- Activating a functional antiviral immune response against HBV through therapeutic immunization or immunotherapy restoring adaptive immune responses versus HBV.

A **functional cure** is durable control of infection with the “durable HBsAg loss (with or without HBsAg seroconversion), undetectable serum HBV DNA, persistence of cccDNA in a transcriptionally inactive status, and the absence of spontaneous relapse after the cessation of treatment” (65).

Inhibition of HBV entry

HBV enters the hepatocyte by the interaction of its large envelope protein (HBL) with HSPG then specific binding of the pre-S1 region of HBL to NTCP. **Myrcludex B**, a synthetic HBV preS1 domain-derived lipopeptide, binds to NTCP and inhibits HBV infections of hepatocytes in vitro and in vivo on a humanized mouse model. Now phase 2 of a clinical trial has been finished (65).

Another novel HBV entry inhibitor is **troglitazone**, Kento et al (65) observed that NTCP was oligomerized in the presence of HBV preS1, then treatment with troglitazone cancels this oligomerization and seems to inhibit HBV entry and infection.

Inhibition of HBV polymerase

HBV polymerase facilitates the pgRNA and polymerase itself being packaged into nucleocapsid. Tavis et al. (65) (67) discovery of HBV ribonuclease H (RNase H) inhibitors like: **napthyridinone derivative**, **β-thujaplicinol**, **2-hydroxyisoquinoline-1,3(2H,4H)-dione (HID) derivatives and α-hydroxytropolones (αHT**), were active against HBV RNase H and inhibited HBV DNA replication by chelating the divalent cations in the RNase H active site.

Inhibition of nucleocapsid assembly

Competent nucleocapsid needs cellular HSP90 chaperone to fold HBV DNA. inhibition of HSP90 ATPase activity can efficiently inhibit pgRNA encapsidation into capsids.

Figure 11 represents chemotypes of nucleocapsid assembly inhibitors: **Bay 41-4109** and **GSL-4** misdirect capsid assembly to form non-capsid polymers of core proteins, **4-methyl HAP 34a, DVR-23, AT-61, NZ-4, and Compound 3711** induce the formation of normal capsids without viral pgRNA and DNA polymerase (65).

All nucleocapsid assembly inhibitors have shown good antiviral activity against NUC resistant strains. **GLS4, NVR 3-778 and AB-423** are now under preclinical o clinical development (65) (68) (69).

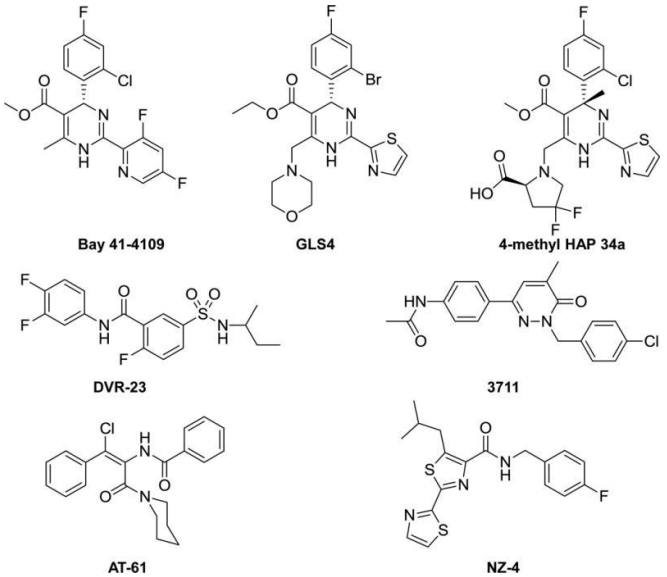

Figure 11. HBV capsid assembly inhibitors. Bay 41-4109, GSL4 and 4-methyl HAP 34a are heteroaryl dihydro pyrimidine derivatives. DVR-23 is a sulfamoyl benzamide. AT-61 is a phenyl propenamide. NZ-4 is an isothiafludine. Compound 3711 is a pyridazinone. (65)

Targeting cccDNA HBV core and X protein

There are been conducting experiments to direct targeting cccDNA with specific DNA targeting technologies as zinc finger nucleases, meganucleases, transcription activator-like effector nuclease (TALENs) and the clustered regularly interspaced short palindromic repeats (CRISPR)/CAS9. However, currently, the administration and security issues need to resolve before digging into the clinic area.

Some molecules as IFN, cytokines or chemokines, that can enhance cccDNA deamination and degradation. Lymphotoxin-β receptor (LT-βR) **agonist monoclonal antibody** (mAb), was used to stimulate LT-βR in HBV-infected cells *in vitro* with good results. LT-βR upregulates APOBEC3B protein and may deaminate cccDNA. Experiments *in vivo* with mice reduced viral load, HBV proteins, and mRNAs (70).

HBV core and X proteins may be recruited to cccDNA to regulate its transcription activity, disruption their interactions could alter cccDNA stability. HBV modifies cccDNA status condensation of cccDNA minichromosomes. **Terbinafine**, an FDA approved drug suppressed HBx-mediated HBV transcription via an unknown mechanism. Further investigation needs to be done in order to clarify this discover (65).

REP2139, in early phase 2, reduces the exit of HBsAg and thus preventing the downregulation of T cells, it has its safety issues: the accumulation of HBsAg may have detrimental effects on hepatocytes (72).

Targeting viral mRNA via RNA interference

The use of RNA interference (iRNA) has been successfully proved to reduce HBV proteins by inhibiting the viral mRNA. Technologies as ARB-1467, ARC-520, AVV/HB-BB-331, and ALN-HBV has been demonstrated in preclinical and clinical phases promising results (65).

Induction of HBsAg seroclearance and anti-HBs seroconversion

The resolution of acute HBV infection is related to a strong and polyclonal HBV-specific T-cell response in contrast to CHB infection, which is weak and barely detectable HBV-specific T-cell response. However, some cells still have persistent cccDNA and survive T cell elimination can cause reactivation when the patients experiment immunosuppression (70). The responsible for the undetectable HBsAg-specific CD8+ T-cell is HBsAg, high levels of HBsAg on serum function as tolerogen: depletes and exhausts of HBV-specific T cells thus activating molecules for its downregulation. Prolonged exposure with NUC does not increase the HBsAg seroclearance, on the contrary, pegylated IFN-α therapy does (65). The natural and drug induce seroclearance of HBsAg then anti-HBs seroconversion could due to the restoration of an effective antiviral response to control HBsAg. Immunotherapy may have a role here to accelerate and activate the immune response.

Immune-modulating agents

Inarigivir (formerly SB9200) is a RIG I agonist and activates innate and adaptive immunity with antiviral effect via inhibition of encapsidation of pgRNA and HBV DNA polymerase. Currently on phase II trial and it's postulating as a peg-INF-α alternative without the side-effect (71).

Programmed cell death protein 1 (PD-1) is a receptor expressed on T cell surface, it has regulatory effects: its activation by PD-L1 and PD-L2 downregulates the immune system and suppressing T cell inflammatory activity. Blockade of PD-1/PD-L1 increased HBsAg-specific CD8+ T-cell functions, also in other chronic infections (70). In a mouse HBV-infected model showed the blockade of PD-1/PD-L1 by **anti-PD-1 mAb** reversed the exhaustion of intrahepatic T cells (73). The exhaustion likely produced by the overexcretion of HBsAg.

Immunotherapy

The contact with HBcAg and CD4+ can stimulate both humoral and cellular immune response. CD4+ release of IL21 strongly modulates CD8+ antiviral activity. The ability of HBcAg to activate immune response has led to some groups like Buchmann et. al (63) developing therapeutic vaccines to activate the patient's immune system to fight and control the virus in CHB. They used a combination of HBsAg and HBcAg, in a particulate form with a saponin-based ISCOMATRIX™ adjuvant in C57BL/6 infected mice and transgenic mice model of neonatal infection, archiving anti-HBs seroconversion, obtaining HBcAg-specific T cells in spleen and liver. Therapeutic vaccines are more important the induction of T cell response, this vaccine induces multiple responses to HBsAg and HBcAg epitopes. T cells produced IFNγ considered a key to control HBV replication related to the absence of liver damage, TNFα, and IL-2. Currently, various therapeutic vaccines are being developed.

7. CONCLUSIONS

HBV is a virus with a wide range of modulating effects inside the body, its abilities to hide and suppress immune response allow replication and persistence infection.

The coded protein HBx seems to have a huge variety of functions and plays a major role in the HBV pathogenesis.

HBV induces HCC by increasing ROS thus genomic instability, also HBx plays a huge role in the hepatocarcinogenesis by modulating diverse proteins enhancing oncogenic effects. Also, integration into the host genome has an insidious consequence.

Current treatment for CHB seems to need to be updated by the new approaches that have been contributed by researchers on the field.

Basic research along with clinical trials is currently going hand in hand. To understand the mechanism underlying the HBV is necessary in order to engineer drugs or cell response mechanism to fight against the virus. Then research on the therapeutic area is started, powered by fundamental research.

8. BIBLIOGRAPHY

1. Blumberg BS, Riddell NM 1963. Inherited antigenic differences in human serum β lipoproteins. A second antiserum. J Clin Invest 42: 867–875.
2. Thomas, Emmanuel; Yoneda, Masato; Schiff, Eugene R. (2015). Viral Hepatitis: Past and Future of HBV and HDV. *Cold Spring Harbor Perspectives in Medicine*. **5** (2): a021345.
3. Dane DS, Cameron CH, Briggs M 1970. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. Lancet 1: 695–698.
4. Hilleman MR, Buynak EB, Roehm RR, Tytell AA, Bertland AU, Lampson GP 1975. Purified and inactivated human hepatitis B vaccine: Progress report. Am J Med Sci 270: 401–404.
5. Hepatitis B: World Health Organization Fact Sheet 204. World Health Organization; [2000]. http://www.who.int/mediacentre/factsheets/fs204/en/
6. Centers for Disease Control. 2012. Hepatitis B—2012 Yellow Book. wwwnc.cdc.gov/travel/yellowbook/2014/chapter-3-infectious-diseases-related-to-travel/hepatitis-b.
7. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, et al. 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 380: 2095–2128.
8. Alter M. Epidemiology of hepatitis B in Europe and worldwide. J Hepatol. 2003;39:S64–S69.º
9. Toukan A. Strategy for the control of hepatitis B virus infection in the Middle East and North Africa. Vaccine. 1990;8(suppl):S117–S121.
10. McQuillan GM, Townsend TR, Fields HA, Carroll M, Leahy M, Polk BF. Seroepidemiology of hepatitis B virus infection in the United States. Am J Med. 1989;87(suppl 3A):5S–10S.
11. Mazzur S, Burgert S, Blumberg BS. Geographical distribution of Australia antigen determinants d, y and w. Nature. 1974;247:38–40.
12. S. Laperche, A. Girault, M.J. Beaulieu, F. Bouchardeau, A.M. Couroucé
13. Chan HL, Tsang SW, Liew CT, Tse CH, Wong ML, Ching JY, Leung NW, Tam JS, Sung JJ. Viral genotype and hepatitis B virus DNA levels are correlated with histological liver damage in HBeAg-negative chronic hepatitis B virus infection. Am J Gastroenterol. 2002;97:406–412.
14. Bassem S.S. Guirgis, Radwa O. Abbas, Hassan M.E. Azzazy. Hepatitis B virus genotyping: current methods and clinical implications. International Journal of Infectious Diseases, Volume 14, Issue 11, 2010, pp. e941-e953, ISSN 1201-9712.
15. Kramvis A, Arakawa K, Yu MC, Nogueira R, Stram DO, Kew MC. Relationship of serological subtype, basic core promoter and precore mutations to genotypes/subgenotypes of hepatitis B virus. J Med Virol. 2008;80:27–46.

16. C. Liu, J. Kao. Hepatitis B virus genotypes: epidemiology and therapeutic implications. Hep B Annual, 3 (2006), pp. 54-75.
17. Wai CT, Fontana RJ, Polson J, Hussain M, Shakil AO, Han SH, Davern TJ, Lee WM, Lok AS. Clinical outcome and virological characteristics of hepatitis B-related acute liver failure in the United States. J Viral Hepat. 2005;12:192–198.
18. Viviani S, Carrieri P, Bah E, Hall AJ, Kirk GD, Mendy M, Montesano R, Plymoth A, Sam O, Van der Sande M, et al. 2008. 20 years into the Gambia Hepatitis Intervention Study: Assessment of initial hypotheses and prospects for evaluation of protective effectiveness against liver cancer. Cancer Epidemiol Biomarkers Prev 17: 3216–3223.
19. Mak LY, Wong DK, Cheung KS, Seto WK, Lai CL, Yuen MF. Review article: hepatitis B core-related antigen (HBcrAg): an emerging marker for chronic hepatitis B virus infection. Aliment Pharmacol Ther. 2018 Jan;47(1):43-54. doi: 10.1111/apt.14376. Epub 2017 Oct 16.
20. Hepatitis B. Epidemiology and Prevention of Vaccine-Preventable Diseases, 13th Edition. 2015. https://www.cdc.gov/vaccines/pubs/pinkbook/hepb.html
21. Lee M. Bass, David M. Steinhorn. Chapter 85 - Gastrointestinal Structure and Function. Editor(s): Bradley P. Fuhrman, Jerry J. Zimmerman. Pediatric Critical Care (Fourth Edition), Mosby, 2011, Pages 1207-1220, ISBN 9780323073073.
22. NC Hepatitis B Public Health Program Manual/Hepatitis B Serology Interpretation of Hepatitis B Serology February 2012.
23. Bozza, Claudia & Cinausero, Marika & Donatella, Iacono & Puglisi, Fabio. (2015). Hepatitis B and cancer: A practical guide for the oncologist. Critical Reviews in Oncology/Hematology. 98. 10.1016/j.critrevonc.2015.10.017.
24. Schillie S, Vellozzi C, Reingold A, et al. Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep 2018;67(No. RR-1):1–31. DOI: http://dx.doi.org/10.15585/mmwr.rr6701a1
25. Kwon, So Young and Chang Hong Lee. “Epidemiology and prevention of hepatitis B virus infection” Korean journal of hepatology vol. 17,2 (2011): 87-95.
26. Giovanna Fattovich. “Natural history of hepatitis B”. Journal of Hepatology 39 (2003) S50–S58.
27. Ungtrakul T, Sriprayoon T, Kusuman P, et al. Role of quantitative hepatitis B surface antigen in predicting inactive carriers and HBsAg seroclearance in HBeAg-negative chronic hepatitis B patients. Medicine (Baltimore). 2017;96(13):e6554.
28. Martinot-Peignoux M, Lapalus M, Asselah T, et al. HBsAg quantification: useful for monitoring natural history and treatment outcome. Liver Int 2014;34Suppl 1:97–107.
29. Carretero C., Herráiz M. Infección crónica por el VHB. Anales Sis San Navarra [Internet]. 2004; 27(Suppl 2): 27-32.
30. Huo TI, Wu JC, Lee PC, et al. Sero-clearance of hepatitis B surface antigen in chronic carriers not necessarily implies a good prognosis. Hepatology. 1998;28:231–236. doi: 10.1002/hep.510280130.

31. Croagh CM, Lubel JS. Natural history of chronic hepatitis B: phases in a complex relationship. World J Gastroenterol. 2014;20(30):10395-404.
32. Papatheodoridis GV, Manolakopoulos S, Liaw YF, Lok A. Follow-up and indications for liver biopsy in HBeAg-negative chronic hepatitis B virus infection with persistently normal ALT: a systematic review. J Hepatol. 2012;57:196–202.
33. Sharma B, John S. Hepatic Cirrhosis. [Updated 2018 Oct 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482419/
34. Schuppan, D., & Afdhal, N. H. (2008). Liver cirrhosis. Lancet (London, England), 371(9615), 838-51.
35. Marcos António Gomes, Denise Gonçalves Priolli, José Guilherme Tralhão, Maria Filomena Botelho. Carcinoma hepatocelular: epidemiologia, biologia, diagnóstico e terapias. Revista da Associação Médica Brasileira, Volume 59, Issue 5, 2013, Pages 514-524, ISSN 0104-4230.
36. Williams IT, Goldstein ST, Tufa J, et al. Long term antibody response to hepatitis B vaccination beginning at birth and to subsequent booster vaccination. Pediatr Infect Dis J. 2003;22:157–63.
37. Mahmood, Sajid & Shah, Kifayat Ullah & Khan, Tahir. (2018). Immune Persistence After Infant Hepatitis-B Vaccination: A Systematic Review and Meta-Analysis. Scientific Reports. 8. 10.1038/s41598-018-30512-8.
38. Tillmann H, L, Patel K: Therapy of Acute and Fulminant Hepatitis B. Intervirology 2014;57:181-188. doi: 10.1159/000360939.
39. Smruti R Mohanty, Sonia S Kupfer & Vijay Khiani. Treatment of chronic hepatitis B. Nature Clinical Practice Gastroenterology & Hepatology. vol 3, pag 446–458 (2006).
40. ML. Mateos, M. Tato, V. Moreira. Resistencia del virus de la hepatitis B a lamivudina y adefovir en un paciente con hepatitis crónica B y antígeno e negative. Gastroenterología y Hepatología. Vol. 29. Núm. 2. Pag. 63-111.
41. Rajbhandari R, Chung RT. Treatment of Hepatitis B: A Concise Review. Clin Transl Gastroenterol. 2016;7(9):e190. Published 2016 Sep 15. doi:10.1038/ctg.2016.46
42. Croagh, C. M., Desmond, P. V., & Bell, S. J. (2015). Genotypes and viral variants in chronic hepatitis B: A review of epidemiology and clinical relevance. World journal of hepatology, 7(3), 289-303.
43. Datta, Sibnarayan et al. Molecular biology of the hepatitis B virus for clinicians. Journal of clinical and experimental hepatology vol. 2,4 (2012): 353-65.
44. Samal, Jasmine et al. “Molecular mechanisms underlying occult hepatitis B virus infection” Clinical microbiology reviews vol. 25,1 (2012): 142-63.
45. Alan Kay, Fabien Zoulim. Hepatitis B virus genetic variability and evolution. Virus Research, Vol 127 Issue 2, 2007, Pag 164-176.
46. Yuen, Man-Fung & Chen, Ding-Shinn & M. Dusheiko, Geoffrey & L. A. Janssen, Harry & T. Y. Lau, Daryl & A. Locarnini, Stephen & G. Peters, Marion & Lai, Ching-Lung. (2018). Hepatitis B virus infection. Nature Reviews Disease Primers. 4. 18035. 10.1038/nrdp.2018.35.

47. Zhang, Z. , Zehnder, B. , Damrau, C. and Urban, S. Visualization of hepatitis B virus entry – novel tools and approaches to directly follow virus entry into hepatocytes. (2016). FEBS Lett, 590: 1915-1926. doi:10.1002/1873-3468.12202
48. Balmasova, Irina P et al. “Immunopathogenesis of chronic hepatitis B” World journal of gastroenterology vol. 20,39 (2014): 14156-71.
49. Tu, Thomas et al. “HBV DNA Integration: Molecular Mechanisms and Clinical Implications” Viruses vol. 9,4 75. 10 Apr. 2017, doi:10.3390/v9040075
50. Matsumiya, Tomoh and Diana M Stafforini. “Function and regulation of retinoic acid-inducible gene-I” Critical reviews in immunology vol. 30,6 (2010): 489-513.
51. Zhaohua Hou, Jian Zhang, Qiuju Han, Chenhe Su, Jing Qu, Dongqing Xu, Cai hang & Zhigang Tia. “Hepatitis B virus inhibits intrinsic RIG-I and RIG-G immune signaling via inducing miR146a” (2016) Scientific Reports volume 6, Article number: 26150
52. Livingston, Christine M et al. “Identifying and Characterizing Interplay between Hepatitis B Virus X Protein and Smc5/6” Viruses vol. 9,4 69. 3 Apr. 2017, doi:10.3390/v9040069
53. W-L Tsai, R T Chung. “Viral hepatocarcinogenesis”. (2016) Oncogene volume 29, pages 2309–2324. https://doi.org/10.1038/onc.2010.36
54. Rivière L., Ducroux A., Buendia M.A. The Oncogenic Role of Hepatitis B Virus. In: Chang M., Jeang KT. (2014) (eds) Viruses and Human Cancer. Recent Results in Cancer Research, vol 193. Springer, Berlin, Heidelberg
55. Rawat S, Bouchard MJ. “The hepatitis B virus (HBV) HBx protein activates AKT to simultaneously regulate HBV replication and hepatocyte survival” J Virol. 2015 Jan;89(2) 999-1012. doi:10.1128/JVI.02440-14. PMID: 25355887; PMCID: PMC4300660.
56. Congwen Wei, Caifei Ni, Ting Song, Yu Liu, XiaoLi Yang, Zirui Zheng, Yongxia Jia, Yuan Yuan, Kai Guan, Yang Xu, Xiaozhong Cheng, Yanhong Zhang, Xiao Yang, Youliang Wang, Chaoyang Wen, Qing Wu, Wei Shi, Hui Zhong. The Hepatitis B Virus X Protein Disrupts Innate Immunity by Downregulating Mitochondrial Antiviral Signaling Protein. The Journal of Immunology July 15, 2010, 185 (2) 1158-1168
57. Wen-Ling Shih, Min-Liang Kuo, Shuang-En Chuang, Ann-Lii Cheng, and Shin-Lian Doong. Hepatitis B Virus X Protein Inhibits Transforming Growth Factor-β-induced Apoptosis through the Activation of Phosphatidylinositol 3-Kinase PathwayJ. Biol. Chem. (2000) 275: 25858-. doi:10.1074/jbc.M003578200
58. Ivanov, Alexander V et al. “Oxidative stress, a trigger of hepatitis C and B virus-induced liver carcinogenesis” Oncotarget vol. 8,3 (2016): 3895-3932.
59. Higgs M, Chouteau P, Lerat H. J. ‘Liver let die’: oxidative DNA damage and hepatotropic viruses. (2014) Journal of General Virology 95(5):991-1004 doi:10.1099/vir.0.059485-0
60. Kew, M. C. Hepatitis B virus x protein in the pathogenesis of hepatitis B virus ‐induced hepatocellular carcinoma. (2011), Journal of Gastroenterology and Hepatology, 26: 144-152. doi:10.1111/j.1440-1746.2010.06546.x

61. Ali, Ashraf et al. “Hepatitis B virus, HBx mutants and their role in hepatocellular carcinoma” World journal of gastroenterology vol. 20,30 (2014): 10238-48.
62. Tu, Thomas et al. “HBV DNA Integration: Molecular Mechanisms and Clinical Implications” Viruses vol. 9,4 75. 10 Apr. 2017, doi:10.3390/v9040075
63. Pascale Buchmann, Claudia Dembek, Larissa Kuklick, Clemens Jäger, Raindy Tedjokusumo, Miriam John von Freyend, Uta Drebber, Zbigniew Janowicz, Karl Melber, Ulrike Protzer. A novel therapeutic hepatitis B vaccine induces cellular and humoral immune responses and breaks tolerance in hepatitis B virus (HBV) transgenic mice. Vaccine, Volume 31, Issue 8, 2013, Pages 1197-1203.
64. Tang, Liudi et al. “The current status and future directions of hepatitis B antiviral drug discovery” Expert opinion on drug discovery vol. 12,1 (2016): 5-15.
65. Wang, Xuan-Yi and Yu-Mei Wen. “A "sandwich" strategy for functional cure of chronic hepatitis B” Emerging microbes & infections vol. 7,1 91. 17 May. 2018, doi:10.1038/s41426-018-0092-3
66. Fukano, Kento et al. “Troglitazone Impedes the Oligomerization of Sodium Taurocholate Cotransporting Polypeptide and Entry of Hepatitis B Virus Into Hepatocytes” Frontiers in microbiology vol. 9 3257. 8 Jan. 2019, doi:10.3389/fmicb.2018.03257
67. Tavis JE, Lomonosova E. “The hepatitis B virus ribonuclease H as a drug target” Antiviral Res. 2015 Jun; 118():132-8.
68. Qiu Z et al. “Design and Synthesis of Orally Bioavailable 4-Methyl Heteroaryldihydropyrimidine Based Hepatitis B Virus (HBV) Capsid Inhibitors” Journal of Medicinal Chemistry 2016 59 (16), 7651-7666
69. Wu, Guoyi et al. “Preclinical characterization of GLS4, an inhibitor of hepatitis B virus core particle assembly” Antimicrobial agents and chemotherapy vol. 57,11 (2013): 5344-54.
70. Zhang E, Kosinska A, Lu M, Yan H, Roggendorf M. “Current status of immunomodulatory therapy in chronic hepatitis B, fifty years after discovery of the virus: Search for the "magic bullet" to kill cccDNA” Antiviral Res. 2015 Nov;123:193-203
71. Yuen, M.-F. et al. Dose response and safety of the daily, oral RIG-I agonist Inarigivir (SB 9200) in treatment naïve patients with chronic hepatitis B: results from the 25 mg and 50 mg cohorts in the ACHIEVE trial. J. Hepatol. 68, S509–S510 (2018).
72. Vaillant A. REP 2139: Antiviral Mechanisms and Applications in Achieving Functional Control of HBV and HDV Infection. ACS Infect Dis. 2018 Oct 5
73. H.T. Tzeng, H.F. Tsai, H.J. Liao, Y.J. Lin, L. Chen, P.J. Chen, P.N. Hsu “PD-1 blockage reverses immune dysfunction and hepatitis B viral persistence in a mouse animal model” PLoS One, 6 (2012), p. e39179