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TOPIC HIGHLIGHT

2016 Hepatitis B virus: Global view

Precore/core region mutations of hepatitis B virus related to clinical severity

Hong Kim, Seoung-Ae Lee, Seung Yeon Do, Bum-Joon Kim

Hong Kim, Seoung-Ae Lee, Seung Yeon Do, Bum-Joon Kim, Department of Biomedical Sciences, Microbiology and Immunology, Liver Research Institute and Cancer Research Institute, College of Medicine, Seoul National University, Seoul 110-799, South Korea

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Correspondence to: Bum-Joon Kim, PhD, Department of Biomedical Sciences, Microbiology and Immunology, Liver Research Institute and Cancer Research Institute, College of Medicine, Seoul National University, 28 Yongon-dong, Chongno-gu, Seoul 110-799, South Korea. kbumjoon@snu.ac.kr
Telephone: +82-2-7408316
Fax: +82-2-7430881

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Abstract

Despite the availability of an effective vaccine, hepatitis B virus (HBV) infection remains a major health problem, with more than 350 million chronically infected people worldwide and over 1 million annual deaths due to cirrhosis and liver cancer. HBV mutations are primarily generated due both to a lack of proofreading capacity by HBV polymerase and to host immune pressure, which is a very important factor for predicting disease progression and therapeutic outcomes. Several types of HBV precore/core (preC/C) mutations have been described to date. The host immune response against T cells drives mutation in the preC/C region. Specifically, preC/C mutations in the MHC class Ⅱ restricted region are more common than in other regions and are significantly related to hepatocellular carcinoma. Certain mutations, including preC G1896A, are also significantly related to HBeAg-negative chronic infection. This review article mainly focuses on the HBV preC/C mutations that are related to disease severity and on the HBeAg serostatus of chronically infected patients.

Key words: Hepatitis B virus infection; Precore/core mutations; Hepatocellular carcinoma; HBeAg serostatus; Disease severity

Core tip: The presence of several distinct types of mutations in HBV infections has been shown to contribute to the progression of liver disease in chronically infected patients. Although the relationships between single mutation types in the preC/C region and clinical severity have rarely been studied to date,

it was recently reported that some preC/C mutations, particularly in the MHC class Ⅱ restricted region, are significantly correlated with hepatocellular carcinoma. Several preC/C mutations, including preC G1896A, are also related to HBeAg sero-negative status, which can affect the disease progression of chronic patients. Mutations such as I97F/L or P135Q, which inhibit core nucleocapsid formation, also contribute to disease progression by evading host innate immunity. In addition, the P5H/L/T mutation may lead to hepatocarcinogenesis by inducing the ER stress-ROS axis. In this review, we mainly focus on the clinical implications of the reported preC/C mutations.

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INTRODUCTION

Despite the availability of an effective vaccine, hepatitis B virus (HBV) infection remains a major public health concern in most countries, but particularly in endemic areas such as China and South Korea. Globally, there are more than 350 million people who are chronically infected with HBV, which annually causes over 1 million deaths due to serious liver diseases, such as cirrhosis and hepatocellular carcinoma (HCC)[1].

HBV is an enveloped Hepadnavirus belonging to the *Hepadnaviridae* family. HBV has an incomplete double-stranded DNA genome that is approximately 3.2 kb in length and contains 4 overlapping open reading frames (ORFs) encoding the polymerase (P), core (C), surface antigen (S), and X protein[2,3]. Based on an intergroup divergence of > 8% in its complete genome sequence, HBV strains are classified into 8 genotypes, designated A-H, which correlate strongly with the ethnicity of infected patients[4-8]. There is increasing evidence that specific HBV genotypes may play significant roles in causing different disease profiles during chronic hepatitis B (CHB) infection[9-12]. Notably, an extraordinary prevalence of the C2 genotype has been reported in South Korea[13-15]. This genotype is more prone to mutations and is associated with more severe liver disease and poorer antiviral responses compared to genotype B[16,17]. In actuality, several types of HBV mutations that are rarely, if ever, encountered in other areas have been found in South Korea. These mutations were demonstrated *via* molecular epidemiologic and functional studies to be related to the disease progression of chronic patients[14,15,18-34].

Over the past decade, increasing attention has been focused on variant HBV strains that contribute to the clinical severity of liver diseases, especially HCC. To date, certain mutation patterns of HBV, such as the precore (preC) mutation at nucleotide 1896 (G1896A) or the double mutation in the basal core promoter (BCP) region at nucleotides 1762 (A→T) and 1764 (G→A), have been widely studied in the context of clinical severity[35-42]. Recently, several types of naturally occurring mutations in the pre-surface antigen (preS), S and X regions (*i.e.*, the preS1 start codon deletion[28,43], the preS2 deletion[28], W4P/R in preS1[29,32], sW182* in S[28,31,44], and V5M in X[23]), which are related to clinical severity, have also been described.

HBcAg AND HBeAg STRUCTURE AND THEIR VARIANTS

The HBV C protein antigen (HBcAg), the major structural protein of the nucleocapsid, is 183 residues long, of which the N-terminal 149 residues are the assembly domain[45-47]. The preC/C ORF is transcribed and translated into a precore/core fusion protein. During entry into the endoplasmic reticulum (ER), 19 residues of the 29-residue preC region are cleaved off by a signal peptidase, generating a 22-kDa protein. When transported into the Golgi compartment, additional amino acids are removed from the C-terminus by intra-Golgi proteases to form the HBe antigen, leading to a final, heterogeneous secreted protein of 15-18 kDa[48-50]. The secreted HBeAg is regarded as a marker of productive infection in clinical practice. Although the biological function of the HBeAg remains unsolved, it has been suggested that the HBeAg may contribute to HBV replication and modulate the host immune system as a type of tolerogen[51-53].

Most preC/C mutations are generated during HBeAg seroconversion in chronic HBV infections[54-56]. Such mutations can affect HBeAg serostatus and antigenicity, HBV nucleocapsid structure and stability, and the packaging of pregenomic RNA into the nucleocapsid[57]. HBcAg is the principal target of the host immune response and especially of CD4 and CD8 T cell attack[58,59]. Thus, mutations in the preC/C region are mainly distributed in the MHC-restricted region and can induce persistent HBV infections[27,60,61].

Several mutations in the preC/C region have been described, and the most frequently reported mutations in the literature are listed in Table 1[27,61-80]. Except in cases of nucleotide deletions, most mutations in the preC/C region are point mutations[65,80]. Most of the reported mutations in the preC region are associated with reduced HBeAg levels or reduced HBV replication in patient sera. In the C region, mutations are mainly located in immuno-active regions (MHC class Ⅰ + Ⅱ) rather than in immuno-inactive regions.

preC/C MUTATIONS RELATED TO CLINICAL SEVERITY OR HBeAg SEROSTATUS

Certain preC/C mutations have been associated with significant virological or clinical events, such as the

Table 1 Mutations in the hepatitis B virus preC/C region reported by previous literatures

Regions	Type of mutation	Mutations				HBV genotype	MHC class	Ref.
		Amino acid	Changes	Nucelotide	Changes			
preCore	AS	1	M1L/T/I	1814-1816	A1814T/C, T1815C, G1816T/A	A		[69-72]
	AS	2	Q2Stop	1817	C1817T	A		[71, 73]
	Ins		Insertion TT	1821	1821-1825			[74]
	Ins		Insertion TT	1825	1825-1826			[71]
	Del		T deletion	1839	1839del			[72]
	Ins		Insertion T	1839	1839-1840			[71]
	AS	15	P15S	1856	C1856T			[75]
			NC	1858	T1858C	A/B/E		[76]
	AS	17	V17F	1862	G1862T	A1		[95]
	AS	26	W26R	1889	T1889A	B/C		[77]
	Ins	36	Insertion 36nt	1895	1895-1896			[78]
	AS	28	W28Stop	1896	G1896A	B/C/C2/D		[29, 61, 77, 79, 80]
	AS	29	G29D	1899	G1899A	C2		[29]
Core	AS	5	P5T/L/H	1913-1915	C1913A, C1914A/T, G1915A/C	B/C2	Ⅱ	[29, 61, 81]
			P5R	1914	C1914G	A/D		[79]
			NC	1915	G1915A/C, A1915T	B/C		[77, 82]
	AS	12	T12S	1934	A1934T	A/C/D	Ⅱ	[83]
	AS	21	S21H	1961-1962	T1961C, C1962A	A/C/D	Ⅰ	[68, 83]
	AS	27	V/I27I/V	1979	G1979A, A1979G	A/B/C/D	Ⅰ	[68, 77, 83]
			NC	1981	C1981A	A/D		[68]
	AS	32	D32N/H	1994	G1994A/C	C2	Immuno-inactive	[29]
	AS	34	NC	2002	C2002A	A/D	Immuno-inactive	[68]
	AS	35	S35L	2004	C2004T	A/D	Immuno-inactive	[68]
	AS	43	E43K	2027	G2027A	C2	Immuno-inactive	[29]
			NC	2029	G2029A	A/D		[68]
	AS	45	NC	2035	T2035A/G	A/D	Immuno-inactive	[68]
	AS	48	NC	2044	T2044C	B/C	Immuno-inactive	[77]
	AS	49	NC	2047	A2047C	A/D	Immuno-inactive	[68]
	AS	50	P50Y/H/A	2048-2049	C2048T/G, C2049A	B/C/C2	Ⅱ	[29, 84]
	AS	55	L55I	2063	C2063A	A/D	Ⅱ	[68]
	AS	59	I59F	2075	A2075T	A/C/D	Ⅱ	[83]
			NC	2077	T2077A/C	B/C		[77]
	AS	60	L60V	2078	C2078G		Ⅱ	[85]
			NC	2080	C2080A	A/D		[68]
Core	AS	64	E/K64D	2090-2092	A2090G, A2092T/C	A/C/D	Ⅱ	[68, 83]
	AS	65	L65V	2093	C2093G	A/D	Ⅱ	[68]
	AS	67	T67N	2100	C2100A	A/C/D	Ⅱ	[68, 83]
	AS	77	E77Q	2129	G2129C	B/C	Immuno-inactive	[84]
			NC	2131	A2131G	A/D	inactive	[68]
	AS	78	NC	2134	C2134T	B/C	Immuno-inactive	[77]
	Del		105nt deletion	2134-2238	2134-2238			[86]
	AS	79	NC	2137	A2137G/T/C	B/C	Immuno-inactive	[77]
	AS	83	E83D	2149	A2149T/C	C2	Ⅱ	[29]
	Del		105nt deletion	2150-2254	2150-2254			[86]
	AS	87	S87R	2161	C2161G	B/C	Ⅱ	[77]
	AS	89	NC	2167	T2167C	A/D	Ⅰ and Ⅱ	[68]
	AS	92	NC	2176	T2176C	B/C	Ⅰ and Ⅱ	[77]
	AS	95	L95I	2183	C2183A	A/D	Ⅰ and Ⅱ	[68]
	AS	97	I97F/L	2189-2191	A2189T/C, C2191T	A/B/C/C2/D	Ⅱ	[29, 77, 83, 85]
			NC	2191	C2191A/T	A/D		[68]
	AS	100	L100I	2198	C2198A	A/C/C2/D	Ⅱ	[29, 83]
	AS	101	NC	2201	T2201C	B/C	Ⅱ	[77]
	Del		130nt deletion	2204-2333	2204-2333			[86]
	AS	107	NC	2221	C2221T	B/C	Immuno-inactive	[77]

AS	113	E113Q	2237	G2237C	A/D	Immuno-inactive	[68]
AS	115	NC	2245	C2245T	B/C	Immuno-inactive	[77]
AS	117	NC	2251	G2251A	B/C	Ⅱ	[77]
AS	119	NC	2257	G2257A	A/D	Ⅱ	[68]
AS	120	NC	2260	G2260A	B/C	Ⅱ	[77]
AS	126	NC	2278	T2278A	A/D	Ⅱ	[68]
AS	130	P130S/T	2288	C2288T/A	B/C	Ⅱ	[74, 77]
		NC	2290	C2290T	B/C		[77]
AS	131	A131P/N/G	2291-2293	G2291C/A, C2292A/G, T2293C	A/C/C2/D	Ⅰ and Ⅱ	[29, 83]
		NC	2293	C2293T	A/D		[68]
AS	134	NC	2302	A2302G	A/D	Ⅰ	[68]
AS	135	P135Q/S/A	2303-2304	C2303T/G, C2304A	A/B/C/D	Ⅰ	[61, 68, 83]
AS	142	NC	2326	A2326T	A/D	Ⅰ and Ⅱ	[68]
AS	145	NC	2335	A2335G	A/D	Ⅰ and Ⅱ	[68]
AS	149	V149I	2345	G2345A	B/C	Ⅰ and Ⅱ	[84]
AS	181	S181P/H	2441-2442	T2441C, C2442A	C2	Immuno-inactive	[29]
AS	182	Q182K/Stop	2444-2445	C2444A/T, A2445G	C2	Immuno-inactive	[29]

AS: Amino acid substitution; Del: Deletion; Ins: Insertion; MHC: Major histocompatibility complex; NC: No change.

failure to form a nucleocapsid, liver disease progression, or HBeAg seroconversion.

preC/C mutations related to HBeAg serostatus

As patients with HBeAg-negative CHB respond poorly to conventional interferon-alpha therapy, they should be treated differently from those with HBeAg-positive CHB[81-83]. Thus, preC/C mutation analysis can provide valuable information for the management of patients with HBeAg-negative CHB. Basal core promoter mutations (BCP, nt1742-1849) that suppress the production of preC mRNA at the transcriptional level may contribute to the defective synthesis of HBeAg *in vivo*[35]. However, the most frequently occurring mutations that are responsible for an HBeAg-negative hepatitis B profile are mutations that occur within the preC region and that inhibit translation of the protein due to frameshift mutations or premature stop codons (Table 1). Among the mutations reported thus far (Table 1), the mutation that is most often responsible for defective HBeAg secretion is a point mutation, namely a G to A transition at nucleotide 1896 (G1896A) that changes the 28th codon of preC from tryptophan (UGG) into a translational stop codon (UAG). The reduced HBeAg level can also have an important effect on HBV replication and can thereby influence liver disease progression, particularly in fulminant hepatitis and acute exacerbation of CHB[66]. Several studies have reported a positive association between the severity of liver disease and the occurrence of G1896A mutations[27,41,70]. However, it has also been reported that there is no correlation between this mutation and liver disease[72-74]. The discrepancy between different findings may be due to various factors, including HBV genotype, the geographical location or race/ethnicity of patients, host immune competence, and co-infection with other viruses, such as HIV or HCV[5,30,84-86].

Most published studies have reported that HBeAg-negative CHB due to the preC G1896A mutation is only common in non-A genotypes. In patients with genotype A, preC start codon mutations (A1814C/T, T1815C/A) that lead to a failure of HBeAg production have been frequently found[87], but such mutations are very rare in those infected with non-A genotypes. Recently, Mayaphi *et al*[61] reported that 24% of patients with sub-genotype A1 had preC start codon mutations, suggesting that this mutation, rather than the G1896A mutation, may contribute to HBeAg-negative CHB infection in patients with sub-genotype A1.

The G1862T mutation, which leads to a valine-to-phenylalanine amino acid substitution at residue 17 of the preC region, can affect the expression of HBeAg by interfering with signal peptidase cleavage[88]. This mutation prevails in HBV genotype A and particularly in sub-genotype A1; together with preC start codon mutations, G1862T is responsible for the HBeAg-negative serostatus and much lower viremia titers in patients infected with HBV genotype A. Moreover, Saha *et al*[77] recently reported that all tested HBV/A1 isolates from Eastern India harbored the G1862T mutation irrespective of HBeAg status, supporting the idea that this mutation might represent a natural variation in HBV/A1 rather than an adaptive mutation.

Mutations in the C-terminus of the preC/C region alter the biosynthesis, transportation, and secretion of HBeAg[73,79,89]. Such mutations lead to the cytoplasmic accumulation of the HBeAg proprotein (p22) in hepatocytes, resulting in decreased HBV replication in patient sera due to down-regulated HBV DNA replication and HBcAg capsid polymerization[73]. Recently, Wu *et al*[73] reported that, together with the G1896A mutation, the C2304A mutation, which causes a glutamine-to-proline substitution at residue 135 of HBcAg (P135Q), is a predictor of spontaneous HBeAg seroconversion following long-term immune-tolerance development

Table 2 Mutations in the hepatitis B virus preC/C region leading to change of HBeAg serostatus in chronic patients

Regions	Mutations				HBV genotype	MHC class	HBeAg serostatus (*P* value)	Ref.
	Amino acid	Changes	Nucleotide	Changes				
preCore	28	W28Stop	1896	G1896A	A/B/C/C2/D		N (n = 14) *vs* P (n = 3) (P = 0.004)	[27]
							eAg Seroconverters (n = 14 of 29, 48.3%)	[73]
							N (43.4%) *vs* P (11.9%) (P = 0.001)	[72]
							N (61.5%) *vs* P (10.9%) with HIV/HBV ($P \leq 0.0001$)	[77]
Core	5	P5R	1914	C1914G	A/D	Ⅱ	N (23.4%) *vs* P (4.7%) (P = 0.001)	[72]
	32	D32N/H	1994	G1994A/C	C2	Immuno-inactive	N (n = 2) *vs* P (n = 7) (P = 0.074)	[27]
	43	E43K	2027	G2027A	C2	Immuno-inactive	N (n = 1) *vs* P (n = 7) (P = 0.024)	[27]
	50	P50Y/H/A	2048-2049	C2048T/G, C2049A	C2	Ⅱ	N (n = 5) *vs* P (n = 0) (P = 0.020)	[27]
	131	A131P/N/G	2291-2293	G2291C/A, C2292A/G, T2293C	C2	Ⅱ	N (n = 4) *vs* P (n = 0) (P = 0.039)	[27]
	135	P135Q	2304	C2304A	B	Ⅰ	eAg Seroconverters (n = 18 of 29, 62.1%)	[73]
	181	S181P/H	2441-2442	T2441C, C2442A	C2	Immuno-inactive	N (n = 4) *vs* P (n = 0) (P = 0.039)	[27]

MHC: Major histocompatibility complex; N: Negative; P: Positive.

within chronic HBV genotype B-infected subjects. Based on a functional study, this mutation was significantly associated with increased cytoplasmic accumulation of the 22-kDa HBeAg proprotein (p22), decreased mature 17-kDa HBeAg (p17) secretion, and a decreased number of HBV capsid particles in Huh7 hepatoma cells, suggesting that this mutation may be associated with spontaneous HBeAg seroconversion in chronic HBV genotype B-infected patients *via* the decreased secretion of the 17-kDa mature HBeAg (p17)[57,90-92].

Recently, we reported that a total of 5 preC/C mutations (G1896A in preC plus 4 in the C region, namely E43K, P50A/H/Y, A131G/N/P and S181H/P) were found to be significantly related to HBeAg serostatus in chronic patients infected with sub-genotype C2[27]. Of those, interestingly, 2 mutations (D32N/H, and E43K) were related to the HBeAg-positive serostatus that was first introduced in the report. These 2 mutations were not located in the regions encoding T or B cell epitopes, suggesting that they may be induced by mechanisms other than immune evasion. Notably, none of the above-described 5 preC/C mutations that were related to HBeAg-negative serostatus was significantly related to HCC, although the G1896A mutation tended towards association with HCC (P = 0.093). These findings raise questions regarding the actual pathogenetic implications of HBeAg-defective mutants. Prospective studies on mutations in the preC/C region and their molecular mechanisms as they relate to the progression of liver disease would provide a better understanding of the clinical relevance of preC/C mutations in relation to HBeAg-negative serostatus. The preC/C mutations that have been reported to cause a change in the HBeAg serostatus of CHB patients are summarized in Table 2.

HBcAg mutations related to clinical severance

As HBcAg is the principal target of the host immune response and particularly of CD4 and CD8 T cell attack, the mutations in this region are mainly distributed in the MHC restricted region and may induce persistent HBV infection[58,59,78]. Indeed, a positive relationship between the frequency of HBcAg and the progression of liver disease has been reported[93-96]. We recently reported that the mutation rate in the MHC class Ⅱ restricted region (M2RR) (2.7% *vs* 1.9%, P = 0.024), but not in the MHC class I restricted region (M1RR) (2.4% *vs* 1.8%, P = 0.3), was significantly higher in HCC patients infected with sub-genotype C2 than in non-HCC patients with the same HBV genotype[27]. Furthermore, the difference between HCC and non-HCC patients with respect to the hotspot region in the M2RR (residues 81-105) was also pronounced (5.6% *vs* 2.6%, P = 0.002). In genotype A1-infected patients, HBcAg mutations were also most commonly found in the M2RR, suggesting that mutations in the M2RR of HBcAg that aid in immune escape can contribute to persistent HBV infection and influence disease progression[97].

We recently introduced 5 mutations in the HBcAg region (P5H/L/T, E83D, I97F/L, L100I and Q182K/Stop) that have been significantly associated with HCC patients compared to patients at other stages of the disease, such as liver cirrhosis (LC) and chronic hepatitis (CH)[27]. Notably, 4 of the 5 HCC-related HBcAg mutations, namely P5H/L/T, E83D, I97F/L and L100I, were located in the M2RR. I97F/L, which was previously known to lead to defects in HBcAg assembly[98,99], was most frequently found in HCC patients (13/35 HCC patients, 37.1%). The frequency of this mutation (16%) was also higher among sub-genotype A1 variants in South Africa[61]. Intrahepatic

Table 3 Mutations in the hepatitis B virus preC/C region related to clinical severance

Regions	Mutations				HBV genotype	MHC class	Clinical significance (*P* value)	Ref.
	Amino acid	Changes	Nucleotide	Changes				
preCore	28	W28Stop	1896	G1896A	C2		HCC (n = 12) *vs* Mild (n = 5) (P = 0.093)	[27]
					A/D		HCC (P < 0.05), FHF (45.0%) *vs* AVH (17.4%) (P = 0.038)	[72]
Core	5	P5T/L/H	1913-1915	C1913A, C1914A/T, G1915A/C	C2	Ⅱ	HCC (n = 5) *vs* Mild (n = 0) (P = 0.02)	[27]
		NC	1915	G1915A/C	C3		HCC (P = 0.020)	[70]
		P5R	1914	C1914G	C4		HCC (32.2%) *vs* ASC (5.0%)/ CHB (7.8%)/LC (10.3%) (P < 0.05)	[72]
	78	NC	2134	C2134T	C5	Immuno-inactive	HCC (P = 0.001)	[70]
	83	E83D	2149	A2149T/C	C6	Ⅱ	HCC (n = 4) *vs* Mild (n = 0) (P = 0.039)	[27]
	92	NC	2176	T2176C	C7	Ⅰ and Ⅱ	HCC (P = 0.139)	[70]
	97	I97F/L	2189-2191	A2189T/C, C2191T	C8	Ⅱ	HCC (n = 13) *vs* Mild (n = 4) (P = 0.024)	[27, 73, 93]
	100	L100I	2198	C2198A	C9	Ⅱ	HCC (n = 6) *vs* Mild (n = 1) (P = 0.046)	[27]
	107	NC	2221	C2221T	C10	Immuno-inactive	HCC (P = 0.001)	[70]
	115	NC	2245	C2245T	C11	Immuno-inactive	HCC (P = 0.007)	[70]
	130	P130T	2288	C2288A	C12	Ⅰ and Ⅱ	HCC (P = 0.022)	[70]
	182	Q182K/ Stop	2444-2445	C2444A/T, A2445G	C13	Immuno-inactive	HCC (n = 4) *vs* Mild (n = 0) (P = 0.039)	[27]

ASC: Asymptomatic S antigen carrier; AVH: Acute viral hepatitis; CHB: Chronic hepatitis B; FHF: Fulminant hepatic failure; HCC: Hepatocellular carcinoma; LC: Liver cirrhosis; MHC: Major histocompatibility Complex; NC: No change.

expression of HBcAg induced a robust IFN response in mice that facilitated control of the viral infection[100]. However, a recent study reported that the majority of mice that received a capsid assembly-deficient HBV mutant with the Y132A mutation in HBcAg failed to elicit the appropriate HBV-specific immune responses for eliminating hepatitis B surface antigenemia, suggesting that nucleocapsid formation is important for triggering a proper antiviral immune response, perhaps *via* the induction of innate immunity against HBV[101]. Thus, the I97F/L mutation, which is responsible for defective nucleocapsid assembly, may partially contribute to the progress of severe liver disease by failing to elicit a proper host immune response against HBV infection. We have previously reported that the lower level of HBV DNA in patients infected with mutated strains in preC/C region than in those with wild strains were found[27], suggesting preC/C mutations could lead to inhibition of HBV replication, generally. But, the identification of mutation types affecting HBV replication should also be done *via* functional study in the future.

Mutations in residue 5 of HBcAg (P5H/L/T or P5R) are reported to be significantly more frequent in HCC patients relative to a reference group, not only in Korean patients infected with sub-genotype C2[27] but also in Indian patients infected with genotypes A or D[72]. Recently, we demonstrated that P5H/L/T mutations in HBV genotype C2 can elicit the ER stress-ROS axis in hepatocytes[75]. This response then leads to inflammatory cytokine production, TGF-β secretion, apoptosis and HBsAg secretion, all of which are related to liver disease progression. The resulting prolonged inflammation, liver damage and increased HBsAg secretion induced by these mutations may contribute to the progression of liver disease in chronic patients.

Xie *et al*[70] recently reported that 5 mutation sites in the preC/C region, namely 1915, 2134, 2221, 2245, and 2288, were identified as statistically significant independent predictors of HCC survival by multivariate survival analysis. Of these, only the C2288A mutation in HBcAg (P130T) results in an amino acid change, while the other 4 mutations are silent. However, further validation in other populations and functional studies will be required to elucidate the mechanism by which these mutations, particularly the 4 silent mutations, affect HCC progression. The preC/C mutations that have been previously reported as associated with liver disease progression in chronic patients and particularly in HCC patients are summarized in Table 3.

CONCLUSION

Mutations in the preC/C region can affect HBeAg serostatus, HBV replication, nucleocapsid formation, and even pgRNA encapsidation[27,45,48,49,74]. Despite disparities in the infecting genotypes and in patient factors such as co-infection with HIV and patient ethnicity and geographical location, several preC/C mutation types that affect disease progression in chronic patients have been identified[61,77,78,87]. In general, mutations such as G1896A, which decrease or cause a loss of HBeAg, may contribute to disease progression *via* persistent

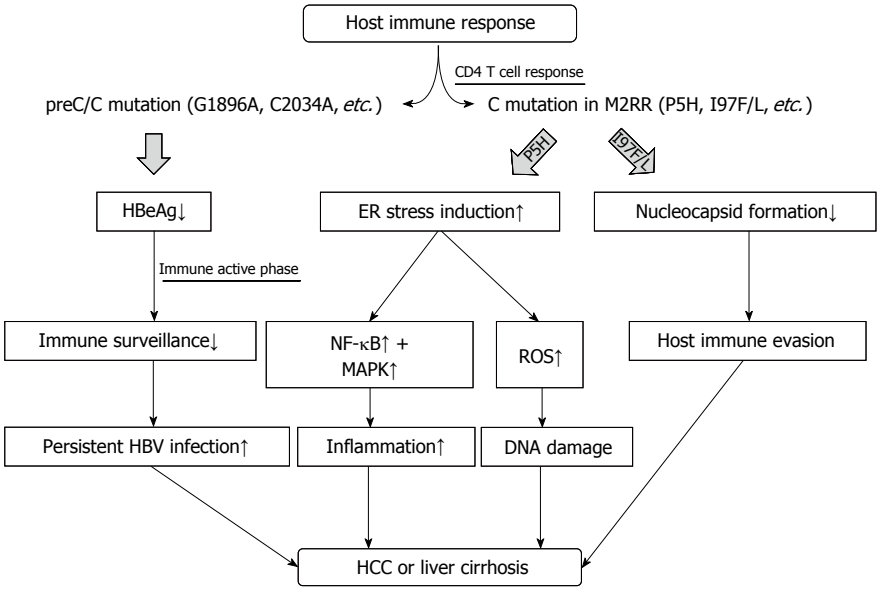

Figure 1 Schematic representation indicating role of mutations in the hepatitis B virus preC/C region in the disease progression of chronic patients. HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; ER: Endoplasmic reticulum; NF-κB: Nuclear factor kappa B.

HBV infection[21,102,103]. Immune-escape mutations in the M2RR of HBcAg, particularly in the hotspot region comprising amino acid residues 81-105, may contribute to persistent HBV infection that is related to disease progression[53,73,79,104]. Mutations such as I97F/L and P135Q, which inhibit core nucleocapsid formation, may also contribute to disease progression by evading host innate immunity[27,73,98,99]. The P5H/L/T mutation was shown to lead to hepatocarcinogenesis by inducing the ER stress-ROS axis (Figure 1). However, to better understand the clinical relevance of preC/C mutations, large, worldwide prospective studies on the clinical relevance of the different types of preC/C mutations are required, as are functional studies that relate the mutations to liver disease progression.

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