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TOPIC HIGHLIGHT

2016 Hepatitis B virus: Global view

X region mutations of hepatitis B virus related to clinical severity

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Abstract

Chronic hepatitis B virus (HBV) infection remains a major health problem, with more than 240 million people chronically infected worldwide and potentially 650000 deaths per year due to advanced liver diseases including liver cirrhosis and hepatocellular carcinoma (HCC). HBV-X protein (HBx) contributes to the biology and pathogenesis of HBV *via* stimulating virus replication or altering host gene expression related to HCC. The HBV X region contains only 465 bp encoding the 16.5 kDa HBx protein, which also contains several critical cis-elements such as enhancer Ⅱ, the core promoter and the microRNA-binding region. Thus, mutations in this region may affect not only the HBx open reading frame but also the overlapped cis-elements. Recently, several types of HBx mutations significantly associated with clinical severity have been described, although the functional mechanism in most of these cases remains unsolved. This review article will mainly focus on the HBx mutations proven to be significantly related to clinical severity *via* epidemiological studies.

Key words: Hepatitis B virus infection; Hepatitis B virus-X protein mutation; Hepatocellular carcinoma; Clinical severity

Core tip: Of hepatitis B virus (HBV)-X protein (HBx) mutations related to clinical severity, the A1762T/G1764A BCP mutation is one of the most frequently

encountered HBx mutations and plays a significant role in liver disease progression in chronic patients with HBV infections. It also further contributes to disease progression by inducing mutations of other HBx mutations related to clinical severity, such as G1386A/C (V5M/L), C1653T (H94Y), T1753V (I127V) and HBx C-terminal deletion or insertion. Moreover, T1753V (I127L,T,N,S) and C1653T (H94Y) mutations in the enhancer Ⅱ/BCP region and A1383C, G1386A/C (V5M/L) and C1485T (P38S) in the negative regulation domain are significantly related to severe types of liver diseases, including hepatocellular carcinoma. Furthermore, deletions or insertions affecting the C-terminal region of HBx may also contribute to the severity of the clinical outcome in chronic patients. In addition, our recent study indicated that a novel mutation type (X8Del) composed of an 8-bp deletion in the C-terminal region of the HBx could contribute to occult HBV infection in vaccinated Korean individuals *via* a reduced secretion of HBsAg and virions. Therefore, several distinct types of HBx mutations may contribute to HBV pathogenesis by regulating HBV replication or host genes related to cell homeostasis.

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains a major health problem with more than 240 million people chronically infected worldwide, which potentially causes 650000 deaths per year due to advanced liver diseases including liver cirrhosis and hepatocellular carcinoma (HCC), particularly in endemic areas such as China and South Korea[1,2]. It is generally accepted that HBV infection accounts for approximately 50% of the HCC cases worldwide and even 80%-90% in highly endemic areas[1].

HBV is an enveloped Hepadnavirus belonging to the *Hepadnaviridae* family, with an incomplete double-stranded DNA genome of approximately 3.2 kb in length with four overlapping open reading frames (ORFs) encoding the polymerase (P), core (C), surface antigen (S), and X protein[3]. The S gene encodes a family of surface antigen polypeptides embedded within the viral envelope, which is a major target for diagnosis and protective vaccines. The C gene encodes the core antigen, which forms the nucleocapsid, within which reverse transcription of pre-genomic RNA occurs. The P gene encodes the virus reverse transcriptase, which also has RNase H and DNA polymerase activities[4-6]. Transcription of HBV proteins is controlled under four promoters (preS1, preS2, core and X) and two enhancers (EnhI and EnhII) in the viral genome, which overlap with those ORFs. Because it contains a polymerase without proofreading activity and uses an RNA intermediate (pgRNA) during its replication, the HBV genome has a higher mutation ratio than other DNA viruses[7-11]. Moreover, host immune pressures and interventions such as antiviral drugs and vaccines make the viral mutations more complicated[12-18].

Based on an intergroup divergence of > 8% in its complete genome sequence, the HBV strains are classified into eight genotypes, which are designated A-H, with a distinct ethnic and geographical distribution[1,19-21]. Different genotypes have distinct geographical distributions and usually induce various clinical outcomes. For instance, genotype C, the most prevalent genotype in Asia, is more prone to mutations and is associated with more severe liver diseases and lower antiviral responses compared with genotype B[3,22,23]. In particular, genotype C2 is reportedly responsible for the most chronic infections in South Korea. Indeed, several types of HBV mutations that are never or rarely encountered in other areas have been found in South Korea and have been proven through molecular epidemiologic or functional studies to be related to disease progression in chronic patients[24-44].

HBV X PROTEIN STRUCTURE AND FUNCTION

The HBV X protein (HBx) is a multifunctional non-structural protein that contributes to HBV biology and pathogenesis by stimulating virus replication or altering host gene expression related to HCC. HBx contains only 465 bp encoding the 16.5 kDa protein, which also contains several critical cis-elements such as EnhII, the core promoter and the microRNA-binding region[45-47] (Figure 1).

HBx plays a significant role in sustained HBV replication, which is a major risk factor for HCC development *via* proteasome inhibition[48,49], transactivation of HBV enhancer or promoters[50], autophagy induction[51,52], or polymerase activation by Ca^{2+}-dependent signaling[53-55]. HBx can also regulate HBV replication through epigenetic modifications, by being recruited onto the viral minichromosome in the nuclei of infected hepatocytes along with cellular histone acetyltransferases such as CREB-binding protein (CBP)/p300[56,57] and histone deacetylases such as HDAC1 and hSirt1[58]. HBx can help establish and maintain chronic infection by altering the patterns of host innate immunity, which causes the development and progression of chronic liver diseases in the absence of virus elimination[59,60]. HBx blocks apoptotic signaling and activates signaling pathways (such as NF-κB and PI3K) that override apoptotic signals from extrinsic ligands such as Fas or TNF-alpha[61,62]. HBx also plays an important role in hepatocarcinogenesis by inactivating the tumor suppressor p53[63], promoting Rb inactivation by

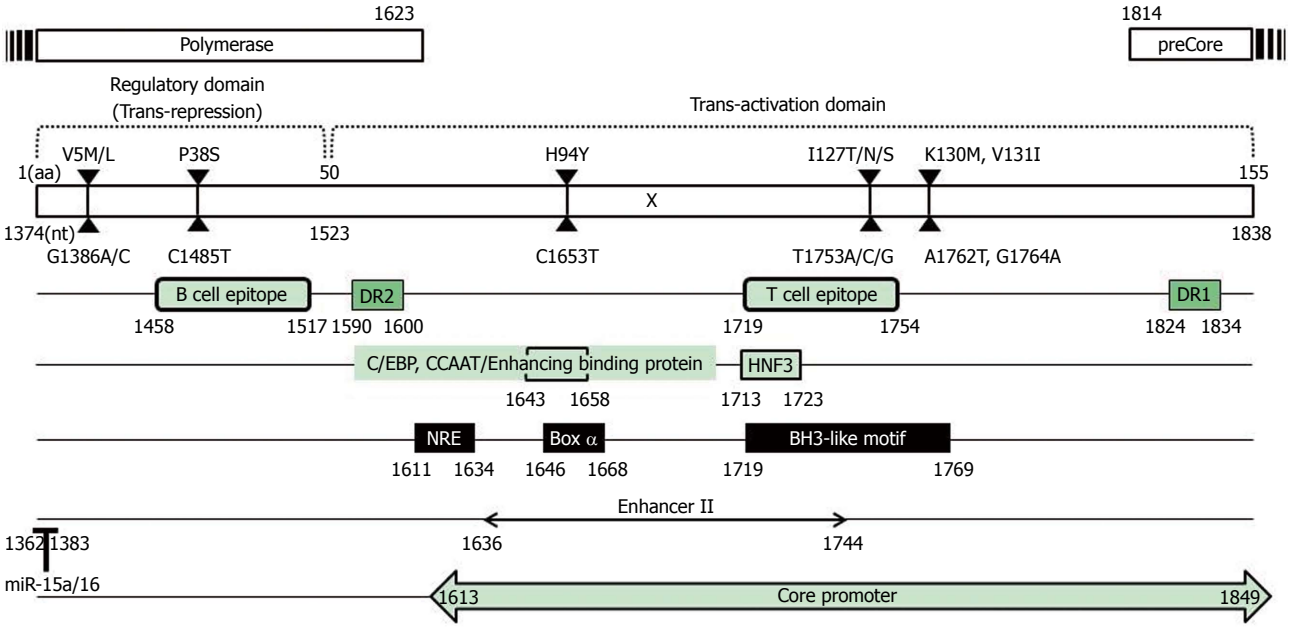

Figure 1 Hepatitis B virus-X protein genome structure. The HBV X region contains 465 bp (nt 1374 to 1838) encoding the 16.5 kDa HBx protein composed of 154 aa, which also contains several critical cis-elements such as EnhII (nt 1636 to 1744), the core promoter (nt 1613 to 1849) and the microRNA-binding region (nt 1362 to 1383). Thus, HBx mutations affected not only the HBx open reading frame but also the overlapped cis-elements. HBx: Hepatitis B virus-X protein.

phosphorylation[64], and compromising DNA repair mechanisms[65]. Consequently, mutations in the HBx ORF region may affect not only the HBx ORF and the overlapped cis-elements but also its binding capacity for host proteins. Recently, several types of HBx mutations significantly associated with clinical severity have been described mainly from chronic patients infected with genotype C[28,31,39,66-83], although the functional mechanism in most of these cases remains unsolved. This review article will mainly focus on the HBx mutations that have been proven to be significantly related to clinical severity *via* epidemiological studies (Table 1).

HBx MUTATIONS RELATED TO CLINICAL SEVERITY

Mutations in EnhII and (or) the core promoter region (BCP mutation, T1753V, and C1653T)

In general, 3 types of mutations in the EnhII/BCP region [one mutation in EnhII (H94Y: C→T of nt 1653) and two mutations in BCP (I127L,T,N,S: T→V of nt 1753, K130M and V131I: A→T of nt 1762 and G→A of nt 1764)] are mutational "hot spots", namely, the most frequently encountered among naturally occurring HBx mutations related to clinical severance from chronic hepatitis B patients, irrespective of genotype or geographical distributions (Table 1). The A1762T/G1764A BCP mutation leading to two overlapped HBx amino acid changes, K130M and V131I, is the most frequent HBV DNA mutation identified in many studies as being associated with HCC risk and outcomes[72,74,84-87]. The exact mechanism underlying the role of this mutation in hepatocarcinogenesis is still unknown. However, some underlying mechanisms have been recently elucidated. The mutation can cause a substantial decrease in HBeAg expression and enhancement of viral genome replication, which contribute to the liver disease progression *via* increased inflammation and viral invasion[88,89]. The mutation also leads to a truncated HBx protein, which not only promotes hepatocellular proliferation but also enhances HCC cell invasion and metastasis[90,91]. In particular, in chronic patients infected with genotype C2, this mutation is reported to be related to HBV genome deletion[26,31] or to be positively correlated with HBx M5V/L or H94Y mutations[28,37]. In addition, it may also contribute to hepatocarcinogenesis *via* reduced p21 expression, leading to rapid and uncontrolled cell proliferation[92]. A recent meta-analysis by Yang *et al*[82] revealed that the BCP mutation is present at significantly higher frequencies in HCC patients than in non-HCC controls, including patients with liver cirrhosis, chronic hepatitis and asymptomatic carriers. Our previous data using a Korean cohort with genotype C2-infected chronic patients also showed that the BCP mutation was the most frequently encountered mutation related to clinical severity (66.1%, 123/184 strains) and was significantly related to HCC [HCC (86.7%) *vs* chronic hepatitis (61%), $P = 0.017$; HCC (86.7%) *vs* asymptomatic carrier (24.4%), $P < 0.001$][28]. Our data also showed that during the natural course of HBV chronic infection, the most significant rise in the rate of the BCP mutation was found during the progression from asymptomatic carrier to chronic hepatitis (24.4%-61.0%), suggesting that the BCP mutation may play a major role in liver

Table 1 Mutations in the hepatitis B virus X region as related to clinical severity

Type of mutation	Mutations				Genotype	Clinical Significance			Region	Description	Ref.
	Amino acid		Nucleotide			HCC (%)	Non-HCC (%)	*P* value			
	aa	mutations	nt	mutations							
AS	4	NC	1383	A1383C	B/C	HCC (postoperative survival in patients with HBV-HCC) (P = 0.028)				Independent predictors of HCC survival	[65]
						52.8	25.8	0.003		miRNA binding site	[66]
AS	5	V5M/L	1386	G1386A/C	C	50.0	4.9	< 0.001			[27]
						47.8	0.0	< 0.001			[38]
						37.7	13.3	0.002			[67]
AS	30	NC	1461	G1461A/T/C	B/C	HCC (postoperative survival in patients with HBV-HCC) (P = 0.005)			B cell epitope	Independent predictors of HCC survival	[65]
AS	36	T36P/S/A	1479	A/G1479C/T/G	A/C/D	49.3	22.7	0.034	B cell epitope	B cell epitope	[66]
						15.3	7.8	< 0.001		B cell epitope	[66]
						80.0	17.1	< 0.010			[68]
AS	38	P38S	1485	C1485T	B/C	21.7	4.9	0.023	B cell epitope		[27]
						HCC (postoperative survival in patients with HBV-HCC) (P = 0.006)				Independent predictors of HCC survival	[65]
						48.7	13.9	0.001		Independent risk factor for the HCC	[69]
						29.9	16.8	0.001		B cell epitope	[66]
						30.4	15.0	0.038			[67]
AS	44	A44V	1504, 1505	C1504G, C1505G	A/D	35.9	6.9	0.012	B cell epitope		[70]
AS	50	G50R	1521	G1521A/C	A/D	60.0	4.3	< 0.010			[68]
AS	57	NC	1544	T1544A/C	B/C	HCC (postoperative survival in patients with HBV-HCC) (P = 0.039)				Independent predictors of HCC survival	[65]
AS	80	NC	1613	G1613A	B/C/C2	HCC (postoperative survival in patients with HBV-HCC) (P = 0.006)			Core promoter	Independent predictors of HCC survival	[65]
						54.7	28.3	0.001		Significance of association with HCC	[71]
						38.0	10.0	< 0.050			[72]
AS	81	NC	1613	G1613A	B/C	50.0	8.6	0.001	Core promoter		[73]
AS	86	NC	1631	C1631T	C	8.3	1.8	0.010	Core promoter	CP, NRE	[66]
AS	94	H94Y	1653	C1653T	B/C/C2	40.0	4.9	< 0.001	Box α, C/EBP,CCAAT/enhancing binding protein, Core promoter, EnhII		[27]
						HCC (postoperative survival in patients with HBV-HCC) (P = 0.015)				Independent predictors of HCC survival	[65]
						61.3	25.3	< 0.001		Significance of association with HCC	[71]
						56.0	30.0	0.0013			[74]
						45.0	19.0	< 0.050			[72]
						31.6	19.1	0.016		Box α	[75]
						35.4	18.6	< 0.001		Box α, CP, C/EBP, EnII	[66]
						41.2	13.3	< 0.001			[67]
						55.5	2.9	< 0.001			[73]
						8.9	2.2	0.017			[76]
AS	101	S101Stop	1675	C1675A	B/C	35.3	5.3	0.001	Core promoter, EnhII	EnhII	[77]
AS	106	S106T	1689	T1689A	C2	19.3	4.4	< 0.001	Core promoter, EnhII		[76]

AS	116	L/V116V/L	1719	T/G1719G/T	B/C	HCC (postoperative survival in patients with HBV-HCC) (P = 0.020)			BH3-like motif, Core promoter, EnhII, NRE	Independent predictors of HCC survival	[65]
						82.6	57.6	0.010		BH3-like motif, CP, EnhII, HNF3, T cell epitope	[66]
AS	117	NC	1724	T1724C	B/C	41.1	2.6	0.000	BH3-like motif, Core promoter, EnhII, NRE	EnhII	[77]
AS	118	NC	1727	A1727G	D1	35.0	5.0	0.001	BH3-like motif, Core promoter, EnhII, NRE		[78]
AS	123	L123S	1741	T1741C	D1	30.0	7.5	0.006	BH3-like motif, Core promoter, EnhII, NRE		[78]
AS, DM	127	I127L/T/N/S	1753	T1753C/A	C	36.7	12.2	0.007	BH3-like motif, Core promoter, NRE		[27]
			1753	T1753C	B/C	HCC (postoperative survival in patients with HBV-HCC) (P = 0.047)				Independent predictors of HCC survival	[65]
			1752, 1753	A1752C + T1753A/C/G	D	52.2	20.0	0.033			[79]
			1753	T1753A/C/G	C2	50.7	16.0	< 0.001		Significance of association with HCC	[71]
			1753	T1753A/C/G	C2	50.0	24.0	0.001			[74]
			1753	T1753C/G	A/D	43.6	17.2	0.041			[70]
			1753	T1753A/C/G	B/C	30.9	17.6	0.006		EnhII/BCP	[75]
			1753	T1753A/C/G	C	29.0	5.0	< 0.001			[67]
AS	130	K130M	1762	A1762T	C2	94.7	74.7	0.001	BH3-like motif, Core promoter	Significance of association with HCC	[71]
					B/C	80.0	25.7	< 0.001			[73]
AS	131	V131I	1764	G1764A	C2	98.7	78.7	< 0.001	BH3-like motif, Core promoter	Significance of association with HCC	[71]
					B/C	95.0	31.4	< 0.001			[73]
AS, DM	134	NC	1773	C1773T	D1	95.0	52.5	0.000	Core promoter		[78]
			1773, 1775	C1773T + A1775G	D1	17.5	0.0	0.010			[78]
AS	143	C143R	1800	T1800C	C	3.5	0.3	0.008	Core promoter	CP	[66]
AS, DM	100, 102	NC	1673, 1679	C1673T + A1679G	D1	17.5	0.0	0.010	Core promoter		[78]
AS, TM	128, 131	NC + V131L	1757, 1764, 1766	G1757A, G1764C + C1766G	D1	37.5	12.5	0.010	Core promoter		[78]
AS, DM	130, 131	K130M + V131I	1762, 1764	A1762T + G1764A	C	86.7	24.4	< 0.001	Core promoter		[27]
					D	HCC (HBV-DNA ⩾ 5 log copies/mL) *vs* CLD (HBV-DNA < 5 log copies/mL) (P < 0.05)					[79]
					C2	91.0	73.0	0.0035			[74]
					A/D	62.5	14.3	0.034			[80]
					A/D	64.1	20.7	0.000			[70]
					B/C	71.1	55.7	0.009		EnhII/BCP	[75]
					B/C	64.0	50.8	< 0.00001			[81]
					A/D	44.9	20.7	< 0.001			[82]
					C	91.5	53.3	< 0.001			[67]
					C2	60.7	22.2	< 0.001			[76]
Del, Ins	129-154, 120-148, 115-149, 135-154, 137-151	Deletion	93-94 (4aa), 79-80 (2aa), 93-94 (4aa), 151-152 (3aa)	Insertion	C2	HCC + LC (7.6%) *vs* CH + C (1.5%) (P = 0.017)					[30]

AA: Amino acid; AS: Amino acid substitution; BCP: Basal core promoter; C: Carrier; CH: Chronic hepatitis; CLD: Chronic liver disease; CP: Core protein; Del: Deletion; DM: Double mutation; EnhII: Enhancer II; HCC: Hepatocellular carcinoma; HNF3: Hepatocyte nuclear factor 3; Ins: Insertion; LC: Liver cirrhosis; miRNA: MicroRNA; NC: No change; NRE: Negative regulatory element; TM: Triple mutation.

disease progression, especially in the progression from asymptomatic carrier to chronic hepatitis in chronic patients infected with genotype C2[28]. This finding has also been confirmed by a recent meta-analysis[82]. Yang *et al*[82] also demonstrated that HBV-infected patients with genotype C, including HCC patients, have a significantly higher risk of BCP mutation compared with those with genotype B, suggesting that the BCP mutation can increase the risk of HBV-related hepatocellular carcinoma, particularly in an HBV genotype C population.

An HBV genome transfection-based experiment indicated that the BCP mutation can reduce the synthesis of HBeAg and enhance viral replication. However, a meta-analysis and our previous report also showed that there is no significant difference in BCP mutation prevalence between HBeAg-positive and HBeAg-negative chronic HBV-infected patients[28,82], suggesting that BCP mutation may occur in the HBeAg-positive immune tolerance phase earlier than in the HBeAg-negative immune clearance phase, at least in chronic patients infected with genotype C2.

The other HCC-associated T1753V mutation (I127L,T,N,S: T→V of nt 1753) was also shown to affect HCC survival[93,94]. The mutations in the HBx protein, which include an I127L,T,N,S amino acid substitution, can change the HBx binding affinity to BCL2, thereby affecting HBx-induced apoptosis[95]. Our previous data using Korean HBV-infected patients with genotype C2 showed that the prevalence of this mutation was also significantly higher in chronic patients with severe liver disease, HCC or liver cirrhosis than in patients who had milder types of diseases, were carriers or had chronic hepatitis [HCC and LC (34.3%) *vs* chronic hepatitis and carrier (13.4%), $P < 0.001$][28]. The other study using chronic patients from India who had genotype A or D revealed that this mutation is also usually associated with advanced forms of liver disease and had an increased risk of HCC[69], suggesting that the T1753V mutation may play a significant role in liver disease progression. Our previous report showed that the T1753V mutation is significantly related to the BCP double mutation [patients with the BCP mutation (31.7%) *vs* patients without the BCP mutation (11.5%), $P = 0.003$], but not to HBeAg serostatus[28]. A recent multivariate survival analysis by Xie *et al*[66] showed that the T1753V mutation is an independent predictor of HCC survival.

The C1653T mutation, leading to a simultaneous H94Y amino acid change in HBx, is located in box α, which is a strong activation element of the EnhII/core promoter, can enhance the box α binding affinity and EnhII/core promoter activity[96,97]. Because many trans-regulated nuclear factors bind HBV at the 1653 site, this mutation can alter the binding affinity of these nuclear factors. The C1653T mutation has been recently reported to be a predictive factor for HCC in Japan[75,98] and is associated with fulminant hepatitis and the acute exacerbation of HCC[99,100]. A recent multivariate survival analysis by Xie *et al*[66] showed that the C1653T mutation together with the T1753V mutation is also an independent predictor of HCC survival. Furthermore, our previous report showed that the C1653T mutation is significantly related to advanced liver diseases in Korean patients with genotype C2 infections [patients with HCC or LC (36.3%) *vs* patients who have chronic hepatitis or are carriers (12.2%), $P < 0.001$]. It has been reported that the C1653T mutation, together with 1762T/1764A mutations, can reduce the pre-C mRNA level (to approximately 55%) and HBeAg secretion in a transient transfection system using Huh7 cells[101]. Our previous study also demonstrated that this mutation tended to be related to an HBeAg-negative serostatus ($P = 0.087$) and was significantly related to the BCP mutation [patients with the BCP mutation (35.0%) *vs* patients without the BCP mutation (6.6%), $P < 0.001$].

Mutation in the negative regulation domain of HBx (aa 1-50) (A1383C, G1386A/C-V5M/L, C1485T-P38S)

The A1383T synonymous mutation, which does not cause an amino acid change in the HBx protein, is located in the negative regulation domain of HBx (aa 1-50), and this mutation was first found to be associated with HCC in a Korean cohort[28]. In one clinical study using Chinese cohort mostly infected with genotype B and C, this mutation was also associated with a worse prognosis in patients after liver transplantation[66]. Recently, a comprehensive analysis study based on global data by Li *et al*[67] showed that A1383T is one of the HBx mutations identified as independent risk factors for genotype C HBV-related HCC. It has also been reported that tumor suppressor microRNA 15a/16 (miR-15a/16) can directly target the wildtype HBx RNA sequence (nt1362-1383), inducing Bcl-2 expression by acting as a sponge to bind and sequester endogenous miR-15a/16. Consequently, this mutation can lead to a reduced binding capacity of miR-15a/16 to the HBx protein[47], which can prevent the infected cell from apoptosis by altering critical cell signal pathways and thereby contributing to hepatocarcinogenesis.

The G1386A/C mutation leading to a simultaneous V5M/L amino acid change at codon 5 of the HBx protein was first introduced by our previous study using a Korean cohort with genotype C2 infections[28]. Our data showed that this mutation was significantly more frequently found in HCC patients than in patients in other disease groups. Notably, the prevalence of this mutation was abruptly increased in HCC patients rather than in liver cirrhosis patients during disease progression (HCC *vs* liver cirrhosis; 49.2% *vs* 25.6%, $P = 0.024$), strongly suggesting that this mutation is a genuine HCC-specific mutation that possibly plays a pivotal role in the progression from liver cirrhosis to HCC[28]. Recently, the combination of both BCP double

mutations and both types of the V5M mutation, V5M and V5L, has also been reported to increase the HCC risk by 5.34 times compared with the wild type by inducing a higher NF-κB activity in transformed cells[86]. Our previous report showed that this mutation is significantly related to an HBeAg-negative serostatus [HBeAg-negative patients (40%) *vs* HBeAg-positive patients (19.1%), $P = 0.004$], suggesting that it may be generated from the immune clearance phase[28]. This mutation was also significantly related to the BCP mutation [patients with the BCP mutation (36.6%) *vs* patients without the BCP mutation (9.2%), $P < 0.001$]. To date, its clinical relevance has not been introduced except for a Korean cohort with genotype C2 infections. It is tempting to speculate that this mutation may play a pivotal role in hepatocarcinogenesis during the HBeAg-negative immune clearance phase during the natural course of genotype C2 HBV infection.

The C1485T mutation, leading to simultaneous P38S in the HBx protein, were first introduced as an independent risk factor for HCC development in a study by Muroyama *et al*[70] using a Japanese cohort with genotype C infections. Both studies using Korean cohorts with genotype C2 infections[28,68] and a recent investigation based on global data by Li *et al*[67] also revealed that this mutation is significantly related to HCC. A functional study supporting the relationship between the mutations with HCC still remains to be conducted. However, given that its mutation site is located at the B cell epitope region (Figure 1), this mutation may lead to persistent infection by providing a mechanism of evading the humoral immune response of the host.

Deletions or insertions in the C-terminal region of HBx

The C-terminal region of HBx plays a key role in controlling cell proliferation, viability, and transformation[102-105]. Therefore, C-terminally deleted or inserted HBx has reduced transactivation activity and inhibitory effects on cell proliferation and thus may contribute to HCC generation[106]. Moreover, its reduced transacting capacity might reduce HBV replication[107]. The C-terminal deletion or insertion is one of the most frequently reported mutations of HBx and has been frequently detected in tissues and serum samples from HCC patients, irrespective of genotype or geographical distribution[24,108,109]. Our previous report using a Korean cohort with genotype C2 infections showed that the prevalence of deletions or insertions was significantly higher in patients with severe liver disease, HCC, or cirrhosis of the liver (7.2%, 10/132), compared with patients who were carriers or had chronic hepatitis (1.5%, 2/135) ($P = 0.017$)[31]. All deletions in six strains were concentrated at the C-terminal end of HBx, encompassing the 113th to 154th codons. Four types of insertions (PKLL, GM, FFN, and tt) were observed in six patients. Notably, all insertions were accompanied by a BCP double mutation[31] (Figure 2). Furthermore, we first introduced a novel HBxAg vaccine escape mutation, X8Del with an 8-bp deletion in the C-terminal region of the HBx gene from 6 vaccinated Korean subjects[38]. Our *in vitro* and *in vivo* studies showed that this mutation causes a reduced secretion of HBsAg and HBV virions compared with the wild type, suggesting that the X8Del mutation may contribute to occult HBV infection in vaccinated individuals *via* the reduced secretion of HBsAg and virions, possibly by compromising the transacting capacity of HBxAg[38].

Other HBx mutations related to clinical severity

Recently, Xie *et al*[66] have reported 8 HBx mutational sites identified as significant independent risk predictors of HCC survival: 1383, 1461, 1485, 1544, 1613, 1653, 1719, and 1753 from a Chinese cohort mostly infected with genotype B and C. Despite the fact that the G1461V mutation is located at the B cell epitope, it (as a synonymous mutation) did not cause any simultaneous amino acid change in the HBx protein. Its regulatory modification in host cell or virion replication remains to be solved. The T1544V mutation also did not cause an amino acid change in the HBx protein. The G1613A mutation in the core promoter region is also a synonymous mutation, and its relationships with HCC have been reported in other previous studies.

Mutations in the BH-3-like motif of HBx can interfere with its interaction with two other Bcl-2 family members (Bcl-2 and Bcl-xL, which are critical for HBx to increase the intracellular calcium concentration), playing a significant role in viral replication and cell death[110]. Previous studies have reported that several types of mutations in the BH-3-like motif, T/G1719G/T, T1724C, and T1741C, were also significantly related to HCC[66,67,79].

The T1800C mutation leading to a simultaneous C143R amino acid change in the HBx protein is a novel genotype C HCC risk mutation identified by the Li *et al*[67] study, based on global data. To date, the function of this mutation in HCC remains unclear. However, of note, a recent study regarding HBV integration sites in 88 Chinese HCC patients showed that almost 40% of the integrated HBV genomes were cleaved at approximately nt1800, suggesting a potential role of this site in carcinogenesis, given that HBV genome integration has long been considered an important factor in HCC development.

CONCLUSION

In conclusion, HBx mutations may affect not only the HBx ORF but also the overlapped cis-elements. Considering all the HBx mutations related to clinical severity, the A1762T/G1764A BCP mutation is one of the most frequently encountered HBx mutations and plays a significant role in liver disease progression in

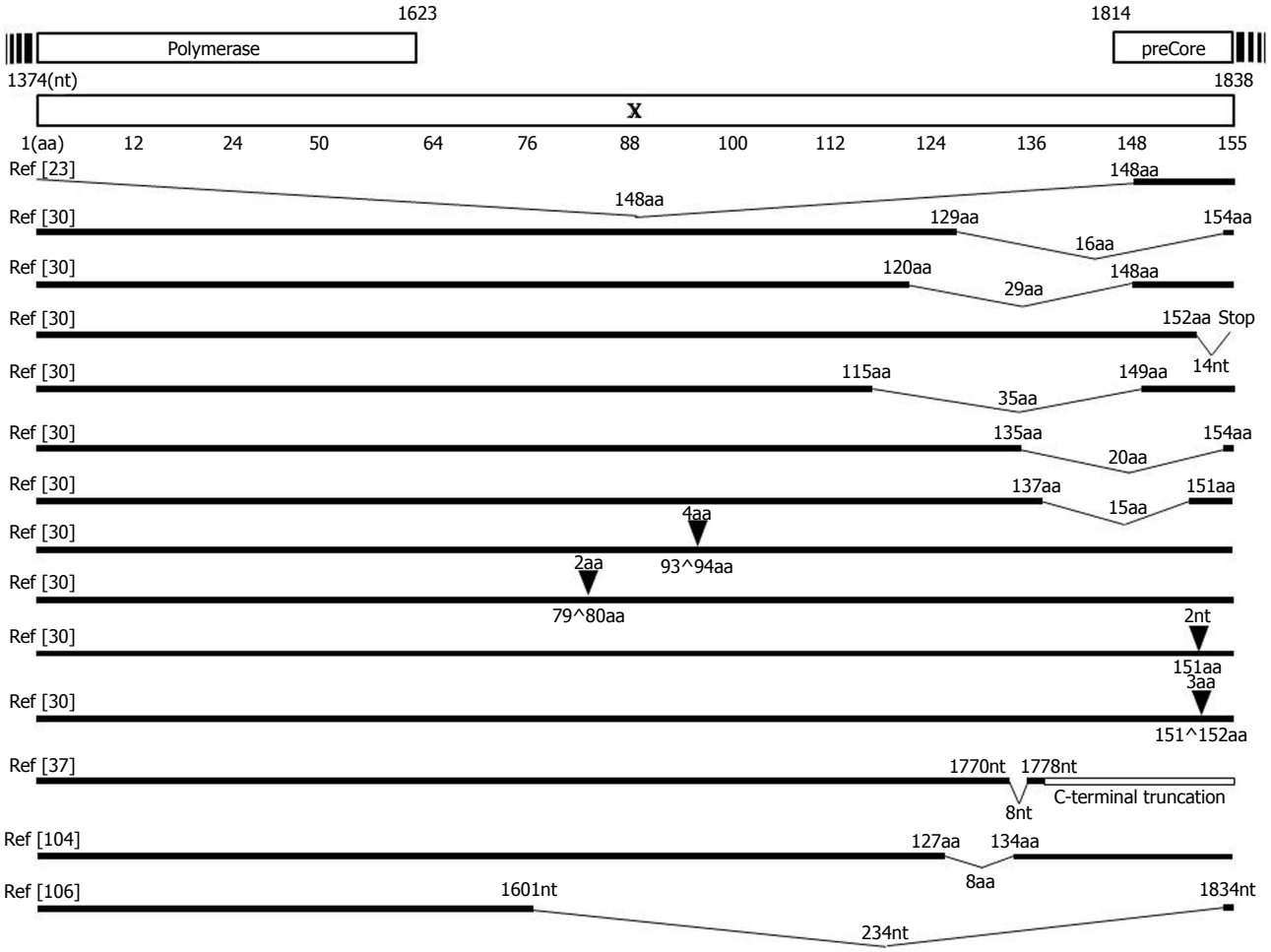

Figure 2 Mapping of deletions or insertions in the hepatitis B virus-X protein region. Deletions or insertions in the HBx region mainly occur in the C-terminal region of HBx, which may also contribute to the clinical outcome severity in chronic patients. HBx: Hepatitis B virus-X protein.

chronic patients with HBV infections. It also further contributes to disease progression by inducing mutations of other HBx mutations related to clinical severity, such as G1386A/C (V5M/L), C1653T (H94Y), T1753V (I127V) and HBx C terminal deletion or insertion. Moreover, T1753V (I127L,T,N,S) and C1653T (H94Y) mutations in the EnhII/BCP region and A1383C, G1386A/C (V5M/L) and C1485T (P38S) in the negative regulation domain were significantly related to severe types of liver diseases, including HCC. Furthermore, deletions or insertions affecting the C-terminal region of HBx can also contribute to the clinical outcome severity in chronic patients. In addition, our recent study indicated that a novel mutation type (X8Del) composed of an 8-bp deletion in the C-terminal region of the HBx contributes to occult HBV infection in vaccinated Korean individuals *via* a reduced secretion of HBsAg and virions. Thus, several distinct types of HBx mutations may contribute to HBV pathogenesis by regulating HBV replication or host genes related to cell homeostasis.

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