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Polymorphism Analyses of Hepatitis B Virus X Gene in Hepatocellular Carcinoma Patients from Southern China

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Abstract Chronic hepatitis B virus (HBV) infection is one of the major causes of hepatocellular carcinoma (HCC), and the HBV X (HBx) gene plays a critical role in the molecular pathogenesis of HBV-related HCC. We have investigated whether there are particular HBx gene mutations associated with HCC in patients from southern China. The HBx gene was examined in 51 paraffin-embedded tumor tissue samples from patients with HCC and 25 serum samples from the HBV carrier by nested polymerase chain reaction (PCR), single-stranded conformational polymorphism and heteroduplex analysis. The HBx genes with potentially important mutations from tumor tissue samples were cloned, sequenced and aligned with the published HBx gene sequence. HBV genotypes in tumor tissue samples were analyzed by nested PCR. Analyses of HBx gene polymorphism showed that 31.3% of HBx gene fragments in tumor tissue samples had a special pattern. A common deletion at nt 382–400 of the HBx gene accompanied by 29 point mutations was detected in four randomly selected tumor tissue samples with this pattern which caused a frame-shift in the HBx open reading frame with a new stop codon at nt 1818, resulting in an HBx polypeptide chain truncated at the C end in these cases. Among the four randomly selected samples, three were HBV genotype B, and one was not detected by our present assay. In another tumor tissue sample, amplification of the full-length HBx gene yielded a shorter fragment. Sequencing of this fragment revealed a 264 bp deletion between nt 1577 and 1840 of the HBV gene. These results suggest that HBx gene mutation occurs frequently in HCC samples, and the deletion at nt 382–400 of the HBx gene might play a role in carcinogenesis of HCC in southern China.

Key words hepatocellular carcinoma; hepatitis B virus; HBx; polymorphism; mutation

Hepatocellular carcinoma (HCC) is the second most common fatal cancer in Southeast Asia and China [1]. Epidemiological studies have convincingly shown that HCC is closely associated with chronic hepatitis B virus (HBV) infection [2]. The incidence of HCC in chronically HBV infected individuals is approximately 100-fold higher than in the uninfected population and the lifetime HCC risk of males infected with HBV at birth is estimated to be approximately 40%. Most cases of HCC occur after many years of chronic hepatitis which can provide the mitogenic and mutagenic environment to precipitate random genetic alterations and lead to the development of HCC. A study from Taiwan showed a decline in the incidence of HCC in children after implementation of a universal hepatitis B vaccination program [3].

HBV is a partial double-stranded DNA virus with a 3.2 kb genome containing four known open reading frames, namely, S, C, P, and X. The HBV X gene (HBx) is the smallest one, with a length of 465 bp, encoding a 154 amino acid activator protein with a molecular weight of 17 kDa [4]. Of these four genes, HBx is most frequently integrated into the host genome, and the HBx protein can function as a transactivator of various cellular genes associated with growth control and is often considered as an important factor in HBV-related hepatocarcinogenesis [5–7]. The expression of HBx in the majority of HCC

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samples strongly suggests that it might play a critical role in regulating the proto-oncoproteins necessary for tumorigenicity and the anti-apoptotic ability of HCC [8].

Furthermore, during the natural history of HBV infection, mutations tend to accumulate in the HBV genome. Some types of HBx changes have been detected preferentially in patients with advanced liver disease. First, some point mutations in HBx, in particular the double substitution K130M and V131I, are more frequent in the sera of patients with cirrhosis and/or HCC than in patients with mild liver disease [9–12]. Second, an insert mutation at position 204 (insert AGGCCC) always accompanied with point 260 (G→A) and 264 (G/C/T→A) was found to be the most frequent mutant pattern in either tissue (40.0%) or serum (70.7%) samples from HCC patients [13]. Finally, disruptions in the HBx leading to the synthesis of C-terminally truncated proteins have been more frequently detected in tumor than in non-tumor liver [14–17]. These C-terminally truncated HBx proteins had lost their antiproliferative effect and cooperated with *ras* and *myc* oncogenes in cell transformation [17,18].

Although it is known that HCC develops many years after HBV infection, the exact molecular mechanisms of HBV in HCC have not yet been well understood. Therefore, we analyzed the HBx polymorphism and investigated whether there were characteristic HBx mutations associated with HCC patients from southern China using nested polymerase chain reaction (PCR), single-stranded conformational polymorphism (SSCP), heteroduplex analysis (HA) and sequencing. We also tried to analyze the relationship between the HBx mutation and HBV genotype.

Materials and Methods

Specimens

Primary hepatoma samples of 63 patients (53 males and 10 females; mean age 44 years, range 26–70 years) were collected from the Affiliated Xiangya Hospital of Central South University (Changsha, China) in 2004 and 2005. These patients were natives and permanent residents of different places of non-HCC-prevalent area in southern China such as Hunan, Hubei and Jiangxi provinces, which were diagnosed by liver biopsy or the combination of increased α fetoprotein (>25 ng/ml) and typical features on angiography, sonography or computed tomography. None of these patients had previously received HBV vaccine. The control group comprised 25 hepatitis B surface antigen (HBsAg) carriers without HCC who were frequency-matched to the case patients by sex and year of birth within 10-year categories. All the HBV carrier controls are being followed up in various ways, including a clinical evaluation with ultrasonography measurement and conventional liver function tests every 6–12 months. None of the controls were related to the patients involved in this study. All serum samples were tested for HBsAg, hepatitis B surface antibody (anti-HBs), hepatitis B e antigen (HBeAg), hepatitis B e antibody (anti-HBe) and hepatitis B core antibody (anti-HBc) using commercially available kits (Abbott Laboratories, North Chicago, USA). Serum α fetoprotein levels were measured by radioimmunoassay. All patients were tested negative for antibody to hepatitis C virus and positive for HBV surface antigen. Tumor tissues were collected after surgical resection, fixed in formalin and preserved in paraffin sections. The sera of HBV carriers were collected separately and stored at –80 ºC until used.

Preparation of DNA for PCR analysis

Genomic DNAs from the above-mentioned 63 patients were isolated from the paraffin-embedded HCC tissues as previously described [19], with some modifications. Briefly, approximately 100 mg of HCC tissues was dewaxed with xylene and cleaned with anhydrous ethanol. The samples were homogenized in 1.5 ml lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA) and incubated overnight with 0.4 mg/ml proteinase K and 1% sodium dodecyl sulfate at 37 ºC. After extraction with phenol:chloroform (1:1; *V*/*V*), the pooled aqueous phase was transferred to a fresh centrifuge tube and followed by an addition of 0.2 volume of 3 M sodium acetate (pH 7.0) and 2 volumes of ethanol and precipitated overnight at –20 ºC. The DNA pellet was washed with 70% cold ethanol and resuspended in 50 μl of 10 mM Tris-HCl, pH 7.4, and 1 mM EDTA. Serum DNAs were extracted using an HBV DNA PCR-fluorescence quantitative diagnostic kit (SKHB, Shanghai, China). The concentration of DNA was determined by spectrophotometry at 260 nm. The purity was determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. The purified DNA was diluted to a final concentration of 500 ng/μl.

Nested PCR

The HBV genomic fragment containing HBx was amplified by a two-step nested PCR with four primers: P1, 5′-ATCGTTTCCATGGCTGCTAGGCT-3′ (nt 1365–1387, outer sense); P2, 5′-CACAGCTTGGAGGCTTGAACA-3′ (nt 1881–1861, outer antisense); P3, 5′-GTTCACGGTGG-TCTCCAT-3′ (nt 1625–1608, inner antisense); and P4, 5′-CTCTGCACGTCGCATGGAGAC-3′ (nt 1595–1615, inner

sense). The first- and second-round PCR amplifications were carried out using *Pfu* DNA polymerase (Tiangen, Beijing, China). The first-round PCR was amplified with primers P1 and P2 and carried out in 25 μl volume containing 2×MasterMix (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.25 mM of each dNTP) (Tiangen), 0.5 μM of primers P1 and P2, 0.1 U/μl *Pfu* DNA polymerase and 2 μl template DNA. Thermocycler conditions were set as 5 min at 94 ºC, 35 cycles of 30 s at 94 ºC, 30 s at 57 ºC, 60 s at 72 ºC, and a final extension at 72 ºC for 5 min. The first-round PCR product was used as a template DNA for second-round amplification. The second-round PCR was amplified with primers P1 and P3 or P2 and P4, in a procedure of 5 min at 94 ºC, 35 cycles of 30 s at 94 ºC, 30 s at 58 ºC, 60 s at 72 ºC and a final extension of 72 ºC for 5 min. One DNA fragment of 517 bp was amplified in the first-round PCR; in the second-round PCR, two DNA fragments of 261 bp and 267 bp were amplified.

Control samples were done in the first and second rounds of PCR. Negative control samples were the DNA specimens extracted from liver tissues of healthy donors, PCR buffer, or water only. DNA samples containing the full-length HBV gene extracted from HepG2.2.15 were used as the positive control.

Single-stranded conformational polymorphism and heteroduplex analysis

One microlitre of second-round PCR product was mixed with 5 μl SSCP loading dye (95% formamide, 20 mM EDTA pH 8.0, 0.05% bromophenol blue and 0.05% xylene cyanol). Prior to running on the gel, the samples were heated to 98 ºC for 10 min, then placed on wet ice. The mixture of 1 μl product of the second-round PCR and 5 μl HA loading dye (40% sucrose, 0.25% bromophenol blue and 0.25% xylene cyanol) was directly used to carry out HA. A portion of 4 μl diluted PCR product was loaded onto 8% non-denaturing polyacrylamide gel and electrophoresed with 1×Tris-borate/EDTA (TBE) buffer at 10 mA and 20 ºC for 5 h. Two PCR product mixtures from the same patient were run on the same gel. Bands on the gel were detected by silver staining. The polyacrylamide gel was stored in 10% ethanol with glycerol. For drying, the gel was placed between gel drying sheets (Promega, Madison, USA) and left to dry overnight.

Cloning of the PCR products and DNA sequencing

According to migration pattern of bands, PCR products from 51 tumor tissue samples were divided into four groups to investigate HBx with potentially important mutations. Fourteen HCC patients were selected at random from the four groups and designated from HC1 to HC14. The PCR products were purified by 1.5% agarose gel electrophoresis. The target band was excised and extracted using an Agarose gel DNA extraction kit (Tiangen). These DNA fragments were cloned into pGM-T (Tiangen). The cloning experiments were carried out following the manufacturer's instructions. Briefly, HBx gene fragment was ligated into the pGM-T vector and transformed into competent cells (*Escherichia coli* TOP10) by electroporation, followed by the addition of 0.4 ml SOC medium and incubation at 37 ºC for 45 min with agitation. Serial dilutions were plated on to the Luria Bertani plates with ampicillin/IPTG/X-gal and incubated overnight at 37 ºC. The white colonies in each plate were picked out and cultured using Luria Bertani broth with ampicillin overnight at 37 ºC with agitation. The recombinant plasmid DNA of the clone cells were then extracted using Plasmid purification mini kits (Tiangen) according to the instructions of the manufacturer and used for HBx sequence analysis. Sequencing was carried out on an ABI PRISM 3730 DNA sequencer (Applied Biosystems, Foster City, USA). The sequences of the cloned variants were aligned and analyzed by BLASTN (ftp://ncbi.nlm.gov/Blast/executables/Blastz.exe) and MULTALIN (http://prodes.toulouse.inra.fr/multalin/multalin.html) software from GenBank

HBV genotype analysis

To study the relationship between HBx mutation and HBV genotype, HBV genotypes A to F were determined by type-specific primer PCR according to Naito *et al.* [20]. Mix A allows for the specific detection of PCR products for types A, B, and C, and mix B allows for detection of types D, E, and F. The two different second-round PCR products from one sample were separately electrophoresed on a 3% agarose gel, stained with ethidium bromide, and evaluated under ultraviolet light. The sizes of PCR products were estimated according to the migration pattern of D2000 marker (Tiangen).

Statistical analysis

Statistical analysis was carried out using Student's *t*-test (two-tailed). The criterion for statistical significance was taken at $P<0.05$.

Results

The genotypes of HBV genomic DNA of 35 HCC tissue samples were successfully determined by PCR with type-specific primers. Among 35 typed HBV genomes from

HCC tissue samples, 12 samples (34.3%) were genotype B, 17 samples (48.6%) were genotype C, 4 samples (11.4%) were genotype B+C and 2 samples (5.7%) were genotype B+D. In 14 sequenced samples, HC1, HC5, HC7 and HC13 were genotype C. HC2, HC3, HC4, HC8, HC10 and HC14 were genotype B. HC6, HC9, HC11 and HC12 had not been amplified and typed. In addition, genotype C was more prevalent in HCC patients who were older than 50 years, whereas genotype B was more common in those with HCC who were aged 50 years or younger. This predominance of genotype B was more substantial in younger patients with HCC, amounting to 90% in those aged 40 years or younger, most of whom did not have cirrhosis.

Of 63 genomic DNA samples from tumor tissues, 51 (81.0%) were successfully amplified by nested PCR with primers P1 and P2, P1 and P3 or P2 and P4. The PCR products were analyzed by agar gel electrophoresis (AGE). Based on the results of AGE analyses, these tumor tissue samples were divided into four groups (**Table 1**). In the first-round PCR, a gene segment of 250 bp was found in sample HC7 (**Fig. 1**), and the amplification yielded a fragment shorter than expected. Further sequencing of this fragment revealed a 264 bp deletion at nt 1577–1840 of the HBV gene (AF282917).

After the second-round PCR products were analyzed by AGE, SSCP and HA, more changes were found in HCC tumor tissues than in sera of HBV carriers (**Table 1** and **Fig. 2**). The detection rate of the HBx mutant in the C group was significantly higher than in the control group ($P<0.05$), and a particular migration band pattern was found in the C group. PCR products of these samples had a common characteristic in that the PCR product amplified by primers P2 and P4 was positive and showed multiple single-strained DNA bands, but the PCR product amplified

Table 1 Hepatitis B virus serological markers and results of agar gel electrophoresis (AGE), single-stranded conformational polymorphism (SSCP) and heteroduplex analysis (HA) in four groups of patients with hepatocellular carcinoma and control group

Group	*n*	HBsAg/HBeAg/HBcAb in serum (*m*)	P1+P2	P1+P3			P2+P4		
			AGE	AGE	SSCP [a]	HA [a]	AGE	SSCP [a]	HA [a]
Control	25	+/+/+(8),+/ –/+(17)	+(7), –(18)	+	4.08±1.61	3.68±1.70	+	3.36±1.32	2.76±0.93
A	6	+/–/+(4),+/+/+(2)	+	+	5.83±1.72*	4.50±1.22	+	4.33±1.75	2.67±0.82
B	27	+/–/+	–	+	4.56±2.87	3.52±2.12	+	3.59±1.69	3.07±1.49
C	16	+/–/+	–	–	0.56±0.73*	0.44±0.81*	+	6.38±0.89*	2.88±0.96
D	2	–/–/+	–	+	5.00±2.83	3.00±1.41	–	3.00±1.41	0.50±0.71

P1+P2, P1+P3 and P2+P4 represent PCR-amplified products using primers P1 and P2, P1 and P3, and P2 and P4, respectively. Mean±SD indicates average and standard deviation of DNA bands of SSCP or HA analysis. There is no statistical analysis for the difference between group D and the control group because the number of samples in group D was too small. +, positive result; –, negative result; HBcAb, hepatitis B core antibody; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen. *$P<0.05$ vs. control group. [a] data were represented as mean±SD.

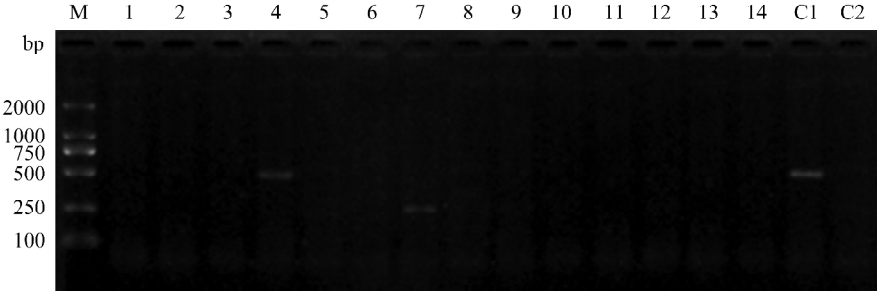

Fig. 1 Amplification of hepatitis B virus (HBV) gene fragment by first-round polymerase chain reaction (PCR) with primers P1 and P2

Amplified HBV gene fragment contains the coding region for the HBV X gene. Lanes 1–14 represent PCR results from genomic DNA of corresponding hepatocellular carcinoma tissue. The PCR amplification generated a DNA fragment of expected size (517 bp). Lane 7 shows the 250 bp DNA band, which is clearly different from the control and other cases. No band is seen in lane 1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13 or 14. M, DNA size marker; C1, positive control for the PCR amplification; C2, negative controls for the PCR amplification.

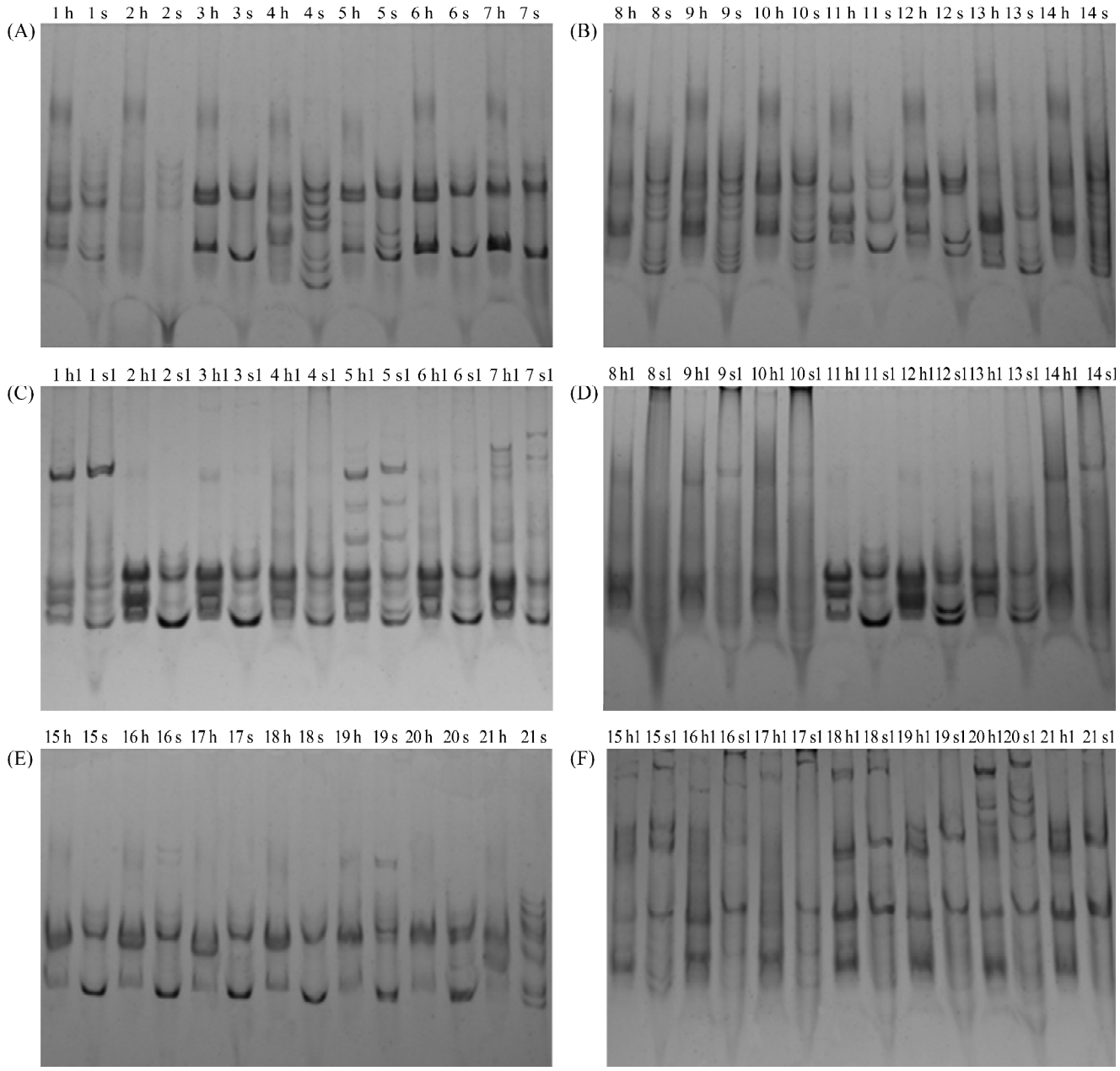

Fig. 2 Single-stranded conformational polymorphism (SSCP) and heteroduplex analysis (HA) of hepatitis B virus X gene

(A, B and E) The PCR amplified products using primers P2 and P4. (C, D and F) The PCR amplified products using primers P1 and P3. (A–D) Templates from hepatocellular carcinoma tissues. (E, F) Templates from serum samples of hepatitis B virus carrier. s and h, polymerase chain reaction (PCR)-SSCP and HA map of the PCR amplified products using primers P2 and P4. s1 and h1, PCR-SSCP and HA map of the PCR amplified products using primers P1 and P3. Number 4, 6 and 7 represent A group SSCP/HA analyses map; number 1, 3, 5, 11, 12 and 13 represent B group SSCP/HA analyses map; number 8, 9, 10 and 14 represent C group SSCP/HA analyses map; number 2 represents D group SSCP/HA analyses map. Lanes 8s, 9s, 10s and 14s show multiple single-strained DNA bands. Lanes 8s1, 9s1, 10s1 and 14s1 show single strain deletions, which are clearly different from the control and other cases.

by primers P1 and P3 was negative and showed single strain deletion (**Fig. 2**). Four PCR products using HBx-specific internal primers and template with the first-round PCR product from these particular pattern samples were randomly selected, purified, cloned and sequenced. HBx sequences obtained from these samples were compared with HBV genotype B (AF282917) (**Table 2**), and the identity was only 87.31%–87.96%. The results also indicated that a total of 30 different types of mutations were identified in HBx of these samples, and 96.7% HBx

Table 2 Distribution of hepatitis B virus X (HBx) gene mutations in four selected HBx genes from tumor tissue with a particular pattern

Mutation site (n.p.)	Mutation type	*n* (%)	Mutation site (n.p.)	Mutation type	*n* (%)
52	T→C	4 (100)	275	C→T	4 (100)
88	T→G	4 (100)	279	G→A	4 (100)
91	C→T	4 (100)	283	A→G	1 (25)
100	C→T	4 (100)	300	T→C	4 (100)
107	C→A	4 (100)	303	C→A	4 (100)
115	C→T	4 (100)	308	C→T	4 (100)
118	G→C	4 (100)	322	C→T	1 (25)
124–125	CC→TT	4 (100)	330	A→G	1 (25)
127	C→T	4 (100)	353–354	CT→AA	4 (100)
130–132	CTT→GCC	4 (100)	357	G→C	4 (100)
135	A→T	4 (100)	379	G→A	4 (100)
139	A→G	4 (100)	Deletion 382–400	AGGTTTAAGG-TCTTTGTAC	4 (100)
171	A→T	4 (100)			
239	A→C	4 (100)	426	G→C	4 (100)
241	A→C	4 (100)	430	T→C	1 (25)
257	A→G	4 (100)	435	C→A	4 (100)
259–260	AG→CA	4 (100)	437	T→C	4 (100)
262–265	AACC→GTAT	4 (100)	445	A→G	1 (25)

n.p.,nucleotide position.

mutation was coincident in these samples. We also found a common deletion mutation at position 382–400 (deletion 382 AGGTTTAAGGTCTTTGTAC) in these samples with the particular pattern (**Table 2**). According to the BLASTN results, such a pattern of mutation in HBx has not been recorded. Therefore, we termed this type of mutation a 382 deletion. The mutation created a frame-shift in the HBx open reading frame with a new stop codon at nt 1818. In addition, the deletion mutation was always accompanied by a common point mutation (**Table 2**). The deduced amino acid sequences of these HBV variants were aligned with the genotype B wild-type HBx protein, as shown in **Fig. 3**. The major different regions were located in positions 30–47 and 127–146.

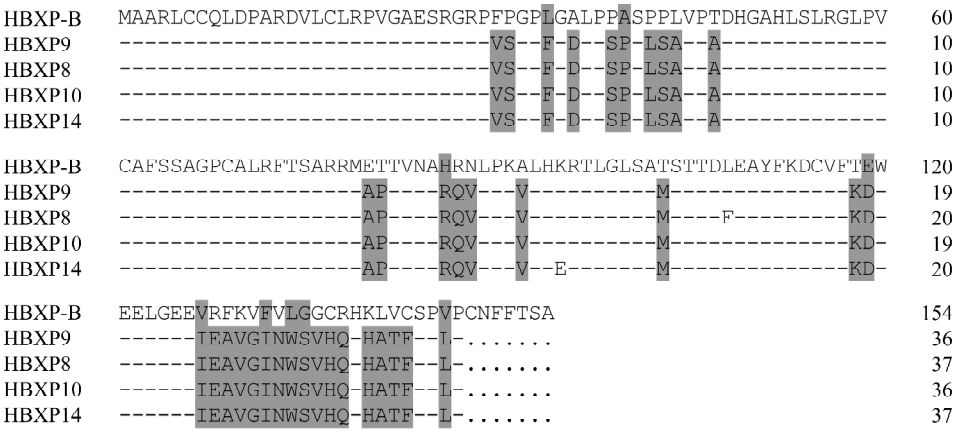

Fig. 3 Comparison of the deduced amino acid sequences among wild-type hepatitis B virus genotype B and naturally occurring hepatitis B virus variants in hepatoma

The major mutated regions were located in amino acid positions 30, 31, 34, 36, 39, 40, 42–44, 47, 80, 81, 86-88, 92, 103, 118, 119, 127–143, 146. The sequences independently obtained from patients 8, 9, 10 and 14 were nearly identical.

Discussion

Chronic HBV infection is a major factor leading to the development of HCC in southern China and HBx mutation plays an important role in hepatocarcinogenesis. Mutational analysis of the HBx gene has been done in other regions [13,16,21]. The hot spots reported by these regions are nt 382–389 (codons 128–130) in HCC samples collected from Qidong, northern China [16], nt 93 (codon 31) in HCC samples from Taiwan [21] and nt 204 and 260–264 (codons 68, 87 and 88) in HCC samples from Hong Kong [13]. However, the study on HBx polymorphism in tumor tissues of HCC patients from southern China has not been reported. Our present study shows that HBx mutation in tumor tissues is very obvious, and a particular migration band pattern has been found in the C group, which is clearly different from the control group and other cases (**Table 1** and **Fig. 2**). We have identified different types of HBx mutations in some tumor tissues and further noted that most of the samples contained more than one type of mutation. The development of multiple types of mutations in a given sample might represent a strategy of HBV to escape immune surveillance and thus contribute to the process of multiple steps in hepatocarcinogenesis. As shown in **Table 2**, the hot spot of mutations in group C of our analyzed samples is different from that in other regions. These studies suggest that HBx might have its own distinct behavior in different geographic regions. However, the biological consequence is likely to be be the same in each region, to stimulate tumor growth.

A particular migration band pattern was found in SSCP and HA analysis, occurring in 31.4% of tumor tissue samples tested. HBx sequences obtained for these samples were compared with HBV genotype B (AF282917) (**Table 2**), for which the identity is only 87.31%–87.96%. As shown in **Table 2**, 96.7% HBx gene mutations were coincident in the tumor tissues of HCC with the particular pattern, highlighting that the same types of HBx mutants can occur in different patients. Moreover, a common deletion mutation at position 382–400 has been found in these samples. The change, to the best of our knowledge, has not been described in populations elsewhere, but is found solely in the population of southern China. The identification of deletion mutation at position 382–400 might be of important diagnostic or clinical significance. First, it occurs in a significant number of HCC samples with the particular migration band pattern, up to 31.4% of tumor tissue samples. Second, the 382 deletion leads to a frameshift in the HBx open reading frame with a new stop codon. A previous study has shown *in vitro* that the C-terminally truncated proteins are endowed with biological properties that differ from those of wild-type proteins. As it would no longer be able to inhibit cell proliferation and transformation, but might enhance the transforming capability of *ras* and *myc*, the presence of truncated HBx protein could have important consequences on the infected liver cells [17,22]. Moreover, the HBx deletion mutations (deletion 8 bp or 20 bp at nt 1763–1770 or 1753–1772 of the HBV gene) can cause the reduction of HBsAg, HBcAg and HBeAg, which might enable the mutant viruses to escape immunodetection [23]. Finally, the 382 deletion is always accompanied with some common point mutation (**Table 2**). As shown in **Table 2**, by comparison with the wild-type sequence, these accumulated mutations suggest the existence of the dominant forms of the HBV variants in HCC. The deduced amino acid sequences of these HBV variants were aligned with the genotype B wild-type HBx protein, as shown in **Fig. 3**. The major different regions were located in positions 30–47 and 127–146. It has been shown that there might be three regions of the HBx gene essential for the transactivation function of the HBx protein (at codons 46–52, 61–69 and 132–139) [24]. The mutation in the HBx gene in the four cases reported here (codons 30–47 and 127–146, respectively) are largely located in the regulatory domain of the HBx protein (positions 20–50) [22]. These mutations are within the functional domain of HBx protein, which has been reported to play a central role in transactivation [25].

Our findings also showed that there was an obvious deletion segment of HBx gene in one tumor tissue sample HC7 (**Fig. 1**), and the fragment is shorter than expected. Sequencing further revealed a 264 bp deletion between nt 1577 and 1840 of the HBV gene (AF282917), which has not been reported elsewhere. However, further studies are needed to clarify the significance of the mutations.

We investigated whether there is a link between HBV genotype and 382 deletion mutation found in tumor tissues. In four HCC tumor tissues with the particular pattern, three samples were infected with genotype B, whereas one sample was unsuccessfully tested in HBV genotype analyses. The negative result might be related to HBV S gene disruption/deletion in the HBV genome. The evidence suggests that HBV genotype B might be associated with the development of HCC with 382 deletion mutation in southern China.

In summary, our finding shows that there is a particular HBx gene mutation in patients with HCC from southern China. The 382 deletion mutation could be of diagnostic or clinical significance and serve as a useful molecular

marker for predicting the clinical outcomes of patients with chronic HBV infection. However, the significance of the 382 deletion mutation in the development of hepatocarcinogenesis is still unclear and requires further study.

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