Name of journal: *World Journal of Hepatology*

ESPS Manuscript NO: 15543

Columns: MINIREVIEWS

**Key role of hepatitis B virus mutation in chronic hepatitis B development to hepatocellular carcinoma**

Zhang X *et al*. HBV mutation and hepatocellular carcinoma

Xin Zhang, Hui-Guo Ding

**Xin Zhang, Hui-Guo Ding,** Department of Gastroenterology and Hepatology, Beijing You’an Hospital affiliated with Capital Medical University, Beijing 100069, China

**Author contributions:** Zhang X was contributed to the data collection and preparing the manuscript; Ding HG was responsible for this project and the final manuscript.

**Supported by** the Capital Science and Technology Development Foundation, Major Projects on Infectious Disease, No. 2012ZX1002-008-05, and High-Level Talent Academic Leader Training Program.

**Conflict-of-interest:** The authors have no other financial interests or conflict with the subject or materials and results in the manuscript apart from those disclosed.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to: Hui-Guo Ding, MD, PhD,** Director of Gastroenterology and Hepatology, Department of Gastroenterology and Hepatology, Beijing You’an Hospital affiliated with Capital Medical University, Fengtai District, Beijing 100069, China.dinghuiguo@medmail.com.cn

**Telephone:** +86-10-83997155

**Fax:** +86-10-63295525

**Received:** November 28, 2014

**Peer-review started:** November 28, 2014

**First decision:** January 8, 2015

**Revised:** January 19, 2015

**Accepted:** March 16, 2015

**Article in press:**

**Published online:**

**Abstract**

Chronic hepatitis B virus (HBV) infection is a major risk factor of hepatocellular carcinoma (HCC). The HBV mutation which included point mutation, deletion, insertion, truncation mutation of *HBV* gene in 4 open reading frame (S, C, P, X) are closely associated with HCC pathogenesis. Some mutations accumulated during chronic HBV infection and could be regarded as a biomarker to predict occurrence of HCC. The detection of the mutations in clinical practice could be more helpful for defining better preventive and therapeutic strategies, moreover predicting the progression of liver disease.

**Key words:** Chronic hepatitis B; Hepatitis B virus; Mutations; Hepatocellular carcinoma; Carcinogenesis

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This mini-review is more fully described the relationship between hepatitis B virus (HBV) genome mutations in different regions with liver disease progression and development to hepatocellular carcinoma (HCC) according to the recent data of mutations in chronic hepatitis B patients. Accordingly, the HBV mutations, either in the preS or PreC or/and core promoter region, are significantly associated with HCC, could be regarded as a biomarker to predict occurrence of HCC.

Zhang X, Ding HG. Key role of hepatitis B virus mutation in chronic hepatitis B development to hepatocellular carcinoma. *World J Hepatol* 2015; In press

**INTRODUCTION**

Chronic HBV infection is a medical problem and the main reason of chronic hepatitis, cirrhosis as well as hepatocellular carcinoma (HCC) in the world. The major risk factor of HCC carcinogenesis is chronic HBV infection, especially in China. Other factors including products of HBV, HBV integration and mutation, host susceptibility contribute to HBV-related HCC. At present, the carcinogenesis in HBV infection has not been well understanded[1]. The main contribution of HBV infection development to HCC is progressive stages of liver fibrosis and nonresolving inflammation[2,3]. The *HBV* genome contains four partially overlapping open reading frames (ORFs) (pre-S/S, pre-C/C, P and X), which have diverse kinds of patterns of mutations. The mutations were included point mutation, deletion,insertion, truncation in 4 ORFs are closely associated with HCC pathogenesis and liver disease progression[4,5]. This mini-review provides an overview according to the recent data of HBV mutation in chronic hepatitis B patients development to HCC.

***S* REGION GENE MUTATION**

The S region of ORFs are composed of three AUG codons coding the expression of three kinds of proteins:namely large (L), middle (M) and small (S). Pre-S1 region is unique for L protein. Pre-S2 region is the shared sequence with the M protein and the S region is seen in all three kinds of proteins. The L and S proteins are fundamental for virion formation and the M could enhance the virion secretion efficiency[6,7]. The S and M proteins are detected as HBsAg. The dominant epitopes of HBsAg are the targets of neutralizing B cell responses, which located in the “a” determinant (aa 124–147) in the major hydrophilic region (MHR)[8].

The relationship between *S* gene mutation and HCC were studied focused on pre-S region. HBV *pre-S* mutations are closely related with an increased risk of HCC. Both the single and combined mutations with the other region gene mutations may be predictive for hepatocarcinogenesis.

Qu *et al*[9] in a study found T53C mutation, pre-S2 start codon mutations and pre-S deletions were related with HCC significantly. The longitudinal study showed that the pre-S deletion mutations occurred during the long course of liver diseases, but not at the beginning of HBV infection.

Wang *et al*[10] found that pre-S mutant large HBV surface antigens (LHBs) can initiate ER stress to induce oxidative DNA damage and genomic instability. *Pre-S* mutant LHBs can upregulate cyclooxygenase-2 and cyclin A to induce cell cycle progression and proliferation of hepatocytes. Dysplasia of hepatocytes could be induced by *pre-S* mutants in transgenic mice. In a nested control study, the presence of *pre-S* mutants were closely associated with development to HCC in HBV carriers.

In an interesting recent study, Su *et al*[11] observed that in transgenic mice model *pre-S2* mutants induced ER stress-dependent and-independent pathways, leading to oxidative DNA damage, genomic instability, and transforming capabilities. In their study, the combined expression of HBx and *pre-S2* mutant showed enhanced oncogenic effects in HCC development, however, the concrete role of *HBx* and *pre-S2* mutant protein in HCC carcinogenesis remains to be clarified.

 T53C, preS1 deletion, preS2 start codon mutation, C7A, A2962G, C2964A, and C3116T in the preS region are significantly related with increased risk of HCC[5]. Furthermore, the effects of other HBV *preS*/*S* mutations in hepatocarcinogenesis is still limited.

***PRE C /C* REGION GENE MUTATION**

Mutations in the core promoter and precore regions of HBV lead to down-regulate HBeAg. These mutations are related with chronic hepatitis, cirrhosis and HCC. C ORF of HBV genome encodes core protein and HBeAg[12]. The core shell of HBV is an effective immune stimulator, activating a intense neutralizing immune response to foreign epitopes. Mutations in this region of HBV genome focus in the region of basal core promoter (BCP) and PreC[13].

T1762/A1764 double mutations in BCP is the most convincing association between HBV mutation and the development of HCC. The relation between BCP double mutations and HCC were proved in two large prospective cohort studies[14]. Moreover, V1753, T1766, A1768 in BCP and T1653 mutations in box-α of Enhancer II have been showed to be associated with the development of HCC in several reports.

Park *et al*[15] analysed the 8 key mutations (G1613A, C1653T, T1753V, A1762T, G1764A, A1846T, G1896A and G1899A) in 442 serum samples contained of 310 non-HCC and 132 HCC patients to confirm the combinations with HCC. They reported that the BCP combination mutations of ≥6 mutations that include G1613A + C1653T + A1846T + G1896A and ≤5 mutations with reduced HBeAg production may increase the risk of HCC occurrence than only the number of mutations.

In our study we also found five high frequency mutations (≥ 10%) in BCP and preC region. We observed thirteen types of multimutations in one fragment, among which 3 types were common combinations (≥ 5%). The most three multi-mutations were A1762T/G1764A (36%), A1762T/G1764A/G1896A (11%) and T1753 (A/C)/A1762T/G1764A/G1896A (8%). The multi-mutations in HBV genomes (≥ 3) may carry a high risk of liver cirrhosis or HCC. G1896A mutation had an effect on liver disease progression independent of the patient age. Additionally, in our study the results showed that more viral mutations detected (≥ 3) and G1776A mutation contribute to HBeAg negativity[16].

Finally, accumulation of mutations including V1753 or/and A1768 aside from T1762/A1764 in BCP region were closely associated with HCC among the patients infected with HBV/C1 as shown in a study by Li *et al*[17]. The BCP mutations have effect on the biological functions of HBx, increasing risk of HCC. T1653 mutation in the box α of the core upstream regulatory sequence and V1753 mutation of BCP region in HBV-infected patients has also been reported to increase the risk of HCC[17,18]. However, the mechanism between BCP mutation and hepatocarcinogenesis is mainly unknown.

Several studies have showed that HBV mutations including C1653T, T1674C/G, T1753V, A1762T/G1764A and C1766T/T1768A in the enhancer Ⅱ/BCP regions; G1899A, C2002T, A2159G, A2189C, and G2203A/T in the precore/core region are significantly related with increased risk of HCC[19].

***P* REGION GENE MUTATION**

One of the regions with mutation susceptibility in HBV ORF is P region, which encodes the polymerase protein (reverse transcriptase) or the POL. The envelope (*S*) gene is completely over-lapped by the polymerase gene, so it is logical to assume that changes in virus encoding, related with antiviral resistance in the polymerase, may have impact on the envelope gene, showing a close relationship between mutations in *S* and *P* regions of HBV genome[20]. Mutations in HBV *P* gene are frequently associated with drug resistance. Cross-sectional studies on the mutations of this gene are rare. Mutations in this region have not been assumed to be responsible for HCC, as frequently as other regions spoken above, but anti-viral therapy associated mutations did impact the disease progression on the subject. Several approved antiviral therapeutic agents are available at present, including regular or pegylated interferon and nucleoside/nucleotide analogues (NUCs) such as lamivudine (LAM), adefovir dipivoxial (ADV), entecavir (ETV), telbivudine and tenofovir. The mutation of rtM204V/I with LAM-resistant is the most frequent, located at the catalytic YMDD motif. The rtL180M mutation often occurs at the same time. In LAM resistant patients the rtA181T mutation was reported largely. The rtN236T and rtA181T/V mutations were the most frequent in ADV resistant patients. ETV resistance is rare in naïve patients anti-viral therapy (1.5% by the fifth year). But if the rtI169T, rtT184A/F/G/I/L/S, rtS202G/I, or rtM250V mutation coexists, ETV resistance can occur in the presence of rtM204I/V mutations. The mostly resistant mutant of telbivudine was rtM204I. Tenofovir has low risk of drug resistance.

Yeh *et al*[21] in a study on 123 LAM-resistant chronic hepatitis B patients reported occurrence of the rtA181T/sW172\* mutant in LAM-resistant patients could increase the risk of HCC development in the subsequent courses of antiviral therapy.

In our study, we followed up 131 cases of HBV-infected patients who have been taken anti-viral therapy. The results showed that the anti-viral drug resistance was not significantly associated with the progression of the liver disease. Once happened resistance, between CHB patients with successful and unsuccessful rescue therapy had no difference after 6 years. I126T mutation in S region may be associated with poor prognosis of patients with CHB.I269L mutation in P region and I68T mutation in S region may be associated with poor response of NUCs treatment, and these mutations could be potential new resistant mutations. Whether the mutations mentioned above are related to progression of the liver disease remains to be proved. In our hepatitis B related cirrhosis cohort study, the two-year cumulative incidence of HCC in antiviral resistance (DR) patients(30.6%) was significantly higher than that in both CVR patients (4.3%). Of these DR patients, especially, the extremely high incidence of HCC was 55.6% in the failed rescue therapy. The rtA181T mutation was closely associated with rescue therapy failure[22]. It is suggested that P region gene mutation related DR associated with chronic hepatitis B development to HCC.

***X* GENE MUTATION**

X ORF produces the X protein (HBx) and despite the specific function of HBx is still undefined during HBV replication, many studies show that HBx is essential for viral replication in vivo and in vitro. HBV *X* gene, extremely easy to mutate and integrate into hepatocytes, plays a significant role in HBV infection and HCC development. Mutations in X region can effect viral replication, through the BCP and the enhancer II. Because the BCP region overlaps with the *X* gene in the concomitant reading frame, the *X* gene at xK130M and xV131I had been changed by the A1762T plus G1764A core promoter mutations in the aforementioned study[23].

Wild-type HBx has been proved to activate hypoxia-inducible factor-1α(HIF-1α), which could contribure to HCC development and progression. Liu *et al*[24] in Hong kong sequenced 101 HCC tissues. In their study,the double mutations K130M/V131I increased the function of HBx as they upregulated the HIF-1α expression and transcriptional activity. Wang *et al*[25] reported that during the infection and replication of HBV, HBx mutates to adjust itself to the hepatocyte and increase the carcinogenesis. COOH-terminal truncated HBX may play a stimulative part in HBV-related HCC development as well as hydrophobic/hydrophilic character changes in some specific amino acid sites.

Besides, Tuteja *et al*[26] reported 222 cases with HBs Ag positive patients and they found that T36A and G50R mutations in *X* gene were associated with HCC. The integration of the viral genome into the host cellular genome were detected in 80%-90% of these cancers. The viral DNA integration also may cause insertional mutagenesis and result in a 3′-terminal truncation of HBX,that deleted at the C-terminal region by 20-40 amino acids. In the HBX sequence, multiple point mutations may be consequent change with integration. Moreover, it showed that both truncation and point mutations may increase the oncogenic activation processes. It has been found that the C-truncated HBx proteins transform immortalized liver cell lines and interact with the mutant p53 protein p.R249S to change genetic stability and proliferation of non-transformed hepatocytes in experimental models[27]. Lee *et al*[19] found that a specific HBx mutation may contribute to development HCC in chronic hepatitis B patients by activating NF-κB activity. The HBx5 mutation in genotype C2 HBV was showed to increase a risk of the development of HCC.

HBV *X* gene multi-site mutations were found frequently in the clinical HCC tissues. Wang *et al*[28] analyzed the *HBx* gene sequences of 60 cases of HCC tumor tissues and paratumor tissues from China. The results showed that the most frequent mutations were at amino acid 30, 88, 144 from tumor samples and at amino acid 31, 43, 87, 94 from non-tumor samples. It has been found that HBx linked-mutations such as at aa L30F/S144A, was 29.5% positive in the tumor tissues.

Among the HCC-associated mutations, combined rather than single mutation are associated with the risk of HCC significantly. In the preS region, the frequencies of combined mutations (haplotypic carriages) including 2964C-3116T-preS2 start codon wildtype-7A, 2964C-3116T-7A-76C, and 2964A-3116T-7C-76A/T are significantly higher in the patients with HCC than in those without HCC, and yet the haplotypic carriages with single mutation are inversely associated with HCC. In the preS and EnhⅡ/BCP regions, HCC patients have more frequent occurrence of a haplotypic carriage with 105C and 2962G than those without HCC, and the frequency of 2962G-preS2 start codon wild type-105C-1762T/1764A is 47.9% in HCC and 4.3% in those without HCC.

Accordingly, the HBV mutations, either in the preS or in the core promoter region, are significantly associated with HCC, whereas the wild-type nucleotides in these regions are mostly associated with liver cirrhosis. HBV mutations can be used as indicators for the prediction of end-stage liver diseases including HCC. Although these mutations and the combinations are specific for HCC to some extent, it will be more practicable if they can predict the malignancy in the HBV-infected subjects before the occurrence of HCC.

**IN THE FUTURE**

Many factors have effect on the development of HBV associated HCC, including products of HBV, HBV integration and mutation, and host susceptibility. HBV sequences from these individuals demonstrate numerous mutations/deletions and alterations that can result in decreased immune recognition of the virus, thereby affecting the expression and functions of specific genes and contributing to liver disorders. However, the aforementioned studies mostly lacked a series of observation and detection. Additionally, sequencing HBV genome for finding the HCC-related HBV mutations have conflicting results suggesting that the pathogenesis of development to HCC is a combination. The hepatocarcinogenesis of chronic inflammation, host immunity, and environment in chronic hepatitis B patients with different patterns of mutation should be further studied.

**REFERENCES**

1 **Chemin I**, Zoulim F. Hepatitis B virus induced hepatocellular carcinoma. *Cancer Lett* 2009; **286**: 52-59 [PMID: 19147276 DOI: 10.1016/j.canlet.2008.12.003]

2 **Brechot C**, Kremsdorf D, Soussan P, Pineau P, Dejean A, Paterlini-Brechot P, Tiollais P. Hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC): molecular mechanisms and novel paradigms. *Pathol Biol* (Paris) 2010; **58**: 278-287 [PMID: 20667665 DOI: 10.1016/j.patbio.2010.05.001]

3 **Neuveut C**, Wei Y, Buendia MA. Mechanisms of HBV-related hepatocarcinogenesis. *J Hepatol* 2010; **52**: 594-604 [PMID: 20185200 DOI: 10.1016/j.jhep.2009.10.033]

4 **Xu C**, Zhou W, Wang Y, Qiao L. Hepatitis B virus-induced hepatocellular carcinoma. *Cancer Lett* 2014; **345**: 216-222 [PMID: 23981576 DOI: 10.1016/j.canlet.2013.08.035]

5 **Liu S**, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101**: 1066-1082 [PMID: 19574418 DOI: 10.1093/jnci/djp180]

6 **Yuan Q**, Ou SH, Chen CR, Ge SX, Pei B, Chen QR, Yan Q, Lin YC, Ni HY, Huang CH, Yeo AE, Shih JW, Zhang J, Xia NS. Molecular characteristics of occult hepatitis B virus from blood donors in southeast China. *J Clin Microbiol* 2010; **48**: 357-362 [PMID: 19940057 DOI: 10.1128/JCM.01781-09]

7 **Hsu CW**, Yeh CT. Emergence of hepatitis B virus S gene mutants in patients experiencing hepatitis B surface antigen seroconversion after peginterferon therapy. *Hepatology* 2011; **54**: 101-108 [PMID: 21503942 DOI: 10.1002/hep.24363]

8 **Huang CH**, Yuan Q, Chen PJ, Zhang YL, Chen CR, Zheng QB, Yeh SH, Yu H, Xue Y, Chen YX, Liu PG, Ge SX, Zhang J, Xia NS. Influence of mutations in hepatitis B virus surface protein on viral antigenicity and phenotype in occult HBV strains from blood donors. *J Hepatol* 2012; **57**: 720-729 [PMID: 22634131 DOI: 10.1016/j.jhep.2012.05.009]

9 **Qu LS**, Liu JX, Liu TT, Shen XZ, Chen TY, Ni ZP, Lu CH. Association of hepatitis B virus pre-S deletions with the development of hepatocellular carcinoma in Qidong, China. *PLoS One* 2014; **9**: e98257 [PMID: 24849936 DOI: 10.1371/journal.pone.0098257]

10 **Wang HC**, Huang W, Lai MD, Su IJ. Hepatitis B virus pre-S mutants, endoplasmic reticulum stress and hepatocarcinogenesis. *Cancer Sci* 2006; **97**: 683-688 [PMID: 16863502 DOI: 10.1111/j.1349-7006.2006.00235.x]

11 **Su IJ**, Wang LH, Hsieh WC, Wu HC, Teng CF, Tsai HW, Huang W. The emerging role of hepatitis B virus pre-S2 deletion mutant proteins in HBV tumorigenesis. *J Biomed Sci* 2014; **21**: 98 [PMID: 25316153 DOI: 10.1186/s12929-014-0098-7]

12 **Nguyen DH**, Ludgate L, Hu J. Hepatitis B virus-cell interactions and pathogenesis. *J Cell Physiol* 2008; **216**: 289-294 [PMID: 18302164 DOI: 10.1002/jcp.21416]

13 **Roseman AM**, Borschukova O, Berriman JA, Wynne SA, Pumpens P, Crowther RA. Structures of hepatitis B virus cores presenting a model epitope and their complexes with antibodies. *J Mol Biol* 2012; **423**: 63-78 [PMID: 22750730 DOI: 10.1016/j.jmb.2012.06.032]

14 **Lin CL**, Kao JH. The clinical implications of hepatitis B virus genotype: Recent advances. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 123-130 [PMID: 21199523 DOI: 10.1111/j.1440-1746.2010.06541.x]

15 **Park YM**, Jang JW, Yoo SH, Kim SH, Oh IM, Park SJ, Jang YS, Lee SJ. Combinations of eight key mutations in the X/preC region and genomic activity of hepatitis B virus are associated with hepatocellular carcinoma. *J Viral Hepat* 2014; **21**: 171-177 [PMID: 24344773 DOI: 10.1111/jvh.12134]

16 **Zhang D**, Ma S, Zhang X, Zhao H, Ding H, Zeng C. Prevalent HBV point mutations and mutation combinations at BCP/preC region and their association with liver disease progression. *BMC Infect Dis* 2010; **10**: 271 [PMID: 20846420 DOI: 10.1186/1471-2334-10-271]

17 **Li W**, Chen G, Yu X, Shi Y, Peng M, Wei J. Accumulation of the mutations in basal core promoter of hepatitis B virus subgenotype C1 increase the risk of hepatocellular carcinoma in Southern China. *Int J Clin Exp Pathol* 2013; **6**: 1076-1085 [PMID: 23696925]

18 **Yuen MF**, Tanaka Y, Shinkai N, Poon RT, But DY, Fong DY, Fung J, Wong DK, Yuen JC, Mizokami M, Lai CL. Risk for hepatocellular carcinoma with respect to hepatitis B virus genotypes B/C, specific mutations of enhancer II/core promoter/precore regions and HBV DNA levels. *Gut* 2008; **57**: 98-102 [PMID: 17483190 DOI: 10.1136/gut.2007.119859]

19 **Lee JH**, Han KH, Lee JM, Park JH, Kim HS. Impact of hepatitis B virus (HBV) x gene mutations on hepatocellular carcinoma development in chronic HBV infection. *Clin Vaccine Immunol* 2011; **18**: 914-921 [PMID: 21490166 DOI: 10.1128/CVI.00474-10]

20 **Sheldon J**, Soriano V. Hepatitis B virus escape mutants induced by antiviral therapy. *J Antimicrob Chemother* 2008; **61**: 766-768 [PMID: 18218641 DOI: 10.1093/jac/dkn014]

21 **Yeh CT**, Chen T, Hsu CW, Chen YC, Lai MW, Liang KH, Chen TC. Emergence of the rtA181T/sW172\* mutant increased the risk of hepatoma occurrence in patients with lamivudine-resistant chronic hepatitis B. *BMC Cancer* 2011; **11**: 398 [PMID: 21933446 DOI: 10.1186/1471-2407-11-398]

22 **Li L**, Liu W, Chen YH, Fan CL, Dong PL, Wei FL, Li B, Chen DX, Ding HG. Antiviral drug resistance increases hepatocellular carcinoma: a prospective decompensated cirrhosis cohort study. *World J Gastroenterol* 2013; **19**: 8373-8381 [PMID: 24363530 DOI: 10.3748/wjg.v19.i45.8373]

23 **Datta S**, Ghosh A, Dasgupta D, Ghosh A, Roychoudhury S, Roy G, Das S, Das K, Gupta S, Basu K, Basu A, Datta S, Chowdhury A, Banerjee S. Novel point and combo-mutations in the genome of hepatitis B virus-genotype D: characterization and impact on liver disease progression to hepatocellular carcinoma. *PLoS One* 2014; **9**: e110012 [PMID: 25333524 DOI: 10.1371/journal.pone.0110012]

24 **Liu LP**, Hu BG, Ye C, Ho RL, Chen GG, Lai PB. HBx mutants differentially affect the activation of hypoxia-inducible factor-1α in hepatocellular carcinoma. *Br J Cancer* 2014; **110**: 1066-1073 [PMID: 24346287 DOI: 10.1038/bjc.2013.787]

25 **Wang D**, Cai H, Yu WB, Yu L. Identification of hepatitis B virus X gene variants between hepatocellular carcinoma tissues and pericarcinoma liver tissues in Eastern China. *Int J Clin Exp Pathol* 2014; **7**: 5988-5996 [PMID: 25337243]

26 **Tuteja A**, Siddiqui AB, Madan K, Goyal R, Shalimar V, Kaur N, Panda SK, Narayanasamy K, Subodh S, Acharya SK. Mutation profiling of the hepatitis B virus strains circulating in North Indian population. *PLoS One* 2014; **9**: e91150 [PMID: 24637457 DOI: 10.1371/journal.pone.0091150]

27 **Gouas DA**, Villar S, Ortiz-Cuaran S, Legros P, Ferro G, Kirk GD, Lesi OA, Mendy M, Bah E, Friesen MD, Groopman J, Chemin I, Hainaut P. TP53 R249S mutation, genetic variations in HBX and risk of hepatocellular carcinoma in The Gambia. *Carcinogenesis* 2012; **33**: 1219-1224 [PMID: 22759751 DOI: 10.1093/carcin/bgs068]

28 **Wang Q**, Zhang T, Ye L, Wang W, Zhang X. Analysis of hepatitis B virus X gene (HBx) mutants in tissues of patients suffered from hepatocellular carcinoma in China. *Cancer Epidemiol* 2012; **36**: 369-374 [PMID: 22178505 DOI: 10.1016/j.canep.2011.11.006]

**P-Reviewer:** Di Costanzo GG, Morales-Gonzalez JA, Xu R

**S-Editor:** Tian YL **L-Editor: E-Editor:**