Possible Involvement of Multidrug-Resistant Hepatitis B Virus sW172* Truncation Variant in the ER Stress Signaling Pathway during Hepatocarcinogenesis

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SUMMARY: We investigated the biological effect of hepatitis B virus (HBV) rtA181T/sW172* point mutation on HBsAg secretion and the potential mechanisms involved in hepatocarcinogenesis. Full-length HBV wild type (wt) and HBV rtA181T/sW172* expression plasmids were transfected into HepG2 cell lines or were injected into C57BL/6 mice. The extracellular and intracellular expression levels of HBsAg and HBeAg proteins, in mouse serum and liver tissues were detected by ELISA. The localization of the truncated protein was characterized in vitro. The mRNA expression of endoplasmic reticulum (ER) stress gene GRP78 was determined. HBsAg levels were significantly higher in both supernatant of cells transfected with HBV wt and serum of mice injected with HBV wt, compared with that of HBV rtA181T/sW172* mutant. The reversed trend was observed in intracellular cells and intrahepatic liver cells. Wild type S protein alone could rescue this dysfunction. HBV rtA181T/sW172* truncated surface proteins showed a more aggregated cytoplasmic pattern which were also localized to the ER in comparison with HBV wt. Furthermore, GRP78 mRNA expression was increased 72 h post-transfection in HBV rtA181T/sW172*cells relative to HBV wt cells (P = 0.0154). The HBV sW172* truncation variant has a defect on HBsAg secretion which can lead to surface protein retention in the ER, where it may contribute to hepatocarcinogenesis through activating the ER stress signaling pathway.

INTRODUCTION

Chronic infection with hepatitis B virus (HBV) is common, affecting more than 350 million people globally, where it frequently leads serious complications, such as cirrhosis and hepatocellular carcinoma (HCC) (1,2). Long-term treatment of chronic hepatitis B (CHB) carriers with long-term nucleos(t)ide analogues (NAs), including lamivudine (LMV), adeovir (ADV), telbivudine (ETV), and clevudine (LdT) has significantly increased the rate of anti-HBe seroconversion; thus, it reduced the impact of CHB on liver disease. However, therapies using NAs have been confronted with viral resistances which are often associated with worsening of liver disease (2).

HBV is a partially double-stranded DNA virus containing 3.2 kb genome, which has 4 open reading frames encoding viral polymerase, the core and e antigen (HBeAg), and the HBx protein. The pre-S/S region encodes the 3 surface antigens (i.e., the large [pre-S1 with HBV polymerase gene that generate a stop codon in the surface reading frame (sW172stop), leading to the truncation of surface proteins. This point mutation was recently reported in CHB patients with viral breakthrough after LMV or ADV treatment (5–9). Recent studies have indicated that the rtA181T/sW172* mutant has a dominant negative secretion effect as well as an increased oncogenic potential through its transactivation activity (4,10–13). It can also be speculated that is these truncated proteins cause endoplasmic reticulum (ER) stress and cell damage by intracellular retention (12,14). Accumulation of unfolded or misfolded proteins in the ER (i.e., ER stress) induces the unfolded protein response (UPR) (15,16), which may lead to HCC in hepatocytes (17,18). However, this speculation has been inconclusive so far.

In this study, we developed a mouse model of HBV replication with the hydrodynamic injection method and attempted to investigate the effect of HBV rtA181T/sW172* point mutation on HBV surface protein expression, secretion, and hepatocarcinogenesis in vitro and in vivo. We confirmed that the in vitro and vivo results showed the HBV rtA181T/sW172* expressed truncated proteins had a weak capacity for surface protein expression and secretion. This can lead to surface protein accumulation in the ER lumen of hepatocellular carcinoma cell lines, which causes the constitutive overexpression of glucose-regulated protein 78 (GRP78) gene, an ER stress signaling pathway-relat-
ed gene that can be induced by physiological and environmental stress conditions (16,19). Our results provided a novel potential mechanism which implied ER stress may contribute toward the oncogenic potential of HBV rtA181T/sw172* truncation variant related hepatocarcinogenesis.

MATERIALS AND METHODS

HBV expression vector construction: The pHBV-wt plasmid was constructed based on HBV genotype C (GenBank: AB644286). It was cleaved with Pst I, then blunt-ended and digested with EcoRI, the resulting product was a 1.2-genome-length HBV DNA. The pZac2.1 vector (a kind gift from Dr. Gao, University of Pennsylvania, USA) was cleaved with Bgl II, then blunt-ended and digested with EcoRI. The 1.2-genome-length HBV DNA was cloned into pZac 2.1 vector to generate pZac-HBV-wt.

The plasmid pZac-HBV-172* was generated to express mutant HBV truncated surface proteins. The mutagenesis was carried out through PCR-mediated site-directed mutagenesis using pZac-HBV-wt as template. The primers that contained the target mutation site were F1(HBV12F) and R1(HBV688R) (Table 1). The PCR product was inserted into EZ-T vector (GenStar Biosolutions, Beijing, China) and designated pHBV-St-T. The PCR product was cloned into the EcoRI and AgeI sites of digested pZac-HBV-wt. The final plasmid, which was named pZac-HBV-172* carried the rtA181T/sw172* mutation.

To express wild-type S proteins only, pcDNA3.1-S-wt was also performed. PCR was also performed using pZac-HBV-wt as template. PCR was performed with PCR primers F2/R2 and F2/R3 (Table 1) to incorporate BamHI and AgeI restriction sites. Then the fragment was cloned into the BamHI and AgeI sites of digested pcDNA3.1/V5-His-TOPO (Invitrogen, Carlsbad, CA, USA) to generate the final plasmids pcDNA3.1-S-wt. All constructs were confirmed by nucleotide sequencing.

Cell culture and transfection: HepG2 cells and HuH-7 cells were grown in Dulbecco’s modified Eagle medium (HyClone Laboratories, S. Logan, UT, USA) supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY, USA) at 37°C in 5% CO2. Cells were seeded at 70% confluence in 6-well cell culture plates. Transient transfection of the plasmids into HepG2 cells and HuH-7 cells were performed using Neofectin DNA Transfection of the plasmids into HepG2 cells supplemented with 10% (HyClone Laboratories, S. Logan, UT, USA) supplemented media. Co-transfection with green fluorescent protein reporter plasmid was performed to normalize transfection efficiency. All experiments were performed in triplicate.

Hydrodynamic injection mouse model of HBV wt and HBV rtA181T/sw172*: In order to establish the mouse model for HBV replication, 16 μg pZac-HBV-wt or pZac-HBV-172* expression plasmid was injected individually into the tail vein of 6 week-old C57BL/6 male mice in a volume of saline equivalent to 9% body mass (e.g., 1.8 mL for a 20-g mouse). The total volume was delivered within 5-8 s. Each injection group contained 5 mice. Mouse peripheral blood samples were collected before injection, and 3 days and 7 days after injection. In addition, mouse liver tissue samples were also separated for the subsequent protein detection. All mice for this experiment were purchased from the Department of Laboratory Animal Science at Peking University Health Science Center. All mice received humane care under the Institutional Review Board in accordance with the Animal Protection Act of Peking University.

ELISA for HBsAg and HBeAg detection: For intra-cellular proteins, cells were lysed in 50 mmol/L Tris-HCl (pH 8.0), 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 1% NP-40, and Phenylmethylsulfonyl fluoride (PMSF) protease inhibitor (AMRESCO, Cleveland, OH, USA). Mouse liver tissues were pestled and lysed with reagents mentioned above to isolate hepatic proteins. For extracellular proteins, cell culture supernatants and mouse serum were used directly. HBsAg and HBeAg were quantified by commercially available microparticle enzyme immunoassay kits (AxSYM; Abbott, Abbott Park, IL, USA).

Immunofluorescence assay: Immunostaining was performed to detect the subcellular localization of HBV surface proteins in pZac-HBV-wt and pZac-HBV-172* transfected HuH-7 cells. At 48 h after transfection, cells were incubated with prewarmed 1 μM ER-tracker Red DPX (Invitrogen) for approximately 15 min at 37°C, then fixed with 4% formaldehyde for 2 min at 37°C. After fixation, cells were washed twice and blocked in 3% BSA/PBS for 2 h at 37°C. Cells were first incubated for 1 h at 37°C with goat polyclonal anti-HBs antibody (1:250 dilution; Keyuezhongkai Biotech, Beijing, China), and then for 1 h with fluorescein-4-isothiocyanate (FITC)-conjugated rabbit anti-goat antibody (1:100 dilution; Invitrogen). Cells were stained with 200 ng/mL DAPI to visualize the nuclei. Confocal microscopy was performed using Leica TCS SP2 Laser Scanning Spectral Confocal System (Leica, Wetzlar, Germany). Cells were identified as “aggregated cells” if they showed an overlap of HBV surface proteins with ER staining. The percentage of the aggregated cells were calculated from 100 cells.

RNA extraction and real time reverse transcription PCR (RT-qPCR): Total RNA was extracted from HepG2 cells transfected with pZac-HBV-wt, pZac-HBV-172*, or pZac-Basic (mock control), respectively for 48 h with TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. cDNA was then reverse transcribed from 3.0 μg of total RNA with random hexamer primers, using Maxima First Strand cDNA Synthesis Kit (Fermentas, Hanover, MD, USA) as recommended by the supplier. RT-qPCR was performed on the LightCycler 480II real-time PCR system (Roche, Mannheim, Germany) using the QuantiTect

Table 1. Oligonucleotide primers used in the study

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<th>Primer</th>
<th>Sequence (5’-3’)</th>
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SYBR Green PCR kit (Qiagen, Hilden, Germany). The 20 μL reaction mix contained 200 nM of each primer, 100 μL LightCycler 480 SYBR green I master mix (Roche), and 1 μL template cDNA. The primers for the GRP78 gene were F (5'-GCCTGTATTTCTAGACCT GCC-3') and R (5'-TTCTATCTGCCCAGCCAGTG-3'), and those for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene were F (5'-AGAAGGCTGG GCCTCATTTG-3') and R (5'-AGGGGCCATCCACA GTCTTC-3'). The PCR conditions for GRP78 and GAPDH consisted of 5 min at 95°C followed by 40 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 60°C, and elongation for 30 s at 72°C. All PCR runs were performed in triplicate using the same batch of synthesized cDNA and starting amount of total RNA. Negative controls containing no cDNA template were included for each gene during each PCR run. Amplification specificity for each gene was confirmed with a single distinct melting curve. Amplified PCR products were separated by 1.5% agarose gel electrophoresis to confirm the presence of a single band at the expected size.

Data analysis: All statistical analysis was performed using GraphPad Prism Ver. 5.0 software (GraphPad Software, San Diego, CA, USA). Data were expressed as mean ± SD. Comparisons between groups were performed using Mann–Whitney U-test. P values <0.05 were considered statistically significant.

RESULTS

The effects of HBV rtA181T/sW172* mutant on HBsAg expression and secretion: Culture supernatant and cell lysate from 24 h, 36 h, 48 h, and 72 h post-transfected HepG2 cells were collected to detect the expression levels of extracellular and intracellular HBsAg. The location of HBsAg target region in the N terminal of the surface gene ensured that the assay was not impacted by sW172* drug-resistant mutants. At each time point, cells transfected with HBV wt showed dramatically high expression levels of HBsAg compared with HBV rtA181T/sW172* mutants. The HBsAg expression level of co-transfectant with 2 plasmids in equal amounts was between those with HBV wt and HBV rtA181T/sW172* transfected individually (Fig. 1A; 24 h, P < 0.0001; 36 h, P = 0.0004; 48 h, P < 0.0001; 72 h, P = 0.0001). In contrast, the intracellular HBsAg levels of HBV rtA181T/sW172* were higher than HBV wt transfected cells (Fig. 1B; 24 h, P = 0.0132; 36 h, P = 0.004; 48 h, P = 0.0003; 72 h, P < 0.0001). In addition, we combined the intracellular and extracellular HBsAg to evaluate total HBsAg expression levels among different
tranfection groups; we found that HBV wt expressed dramatically high levels of total HBsAg compared with HBV rtA181T/sW172* mutants (Fig. 1C; 24 h, $P = 0.0002$; 36 h, $P = 0.0009$; 48 h, $P < 0.0001$; 72 h, $P = 0.0002$). Finally, we analyzed the distribution profiles of extracellular and intracellular HBsAg between HBV wt and HBV rtA181T/sW172*. In the HBV wt group, extracellular HBsAg made up the majority (~75% to 95%) of total HBsAg, whereas, in the HBV rtA181T/sW172* mutant, intracellular HBsAg made up the most (~80% to 94%) of total HBsAg (Fig. 1D). Taken together, the HBV rtA181T/sW172* mutant had a defect in HBsAg expression and secretion, which could have led to intracellular accumulation of HBV surface protein. Additionally, this defect was progressively noticeable as time increased (Fig. 1A–D).

Because the clinical presentation of HBV in drug resistant CHB patients exhibited as a mixture of HBV wt and HBV rtA181T/sW172*, we also detected the expression level of HBsAg in cell populations with mixed HBV wt and HBV rtA181T/sW172*. HepG2 cells were co-transfected with pZac-HBV-wt and pZac-HBV-172* at different ratios, though intracellular HBeAg was far less than supernatant HBeAg (Fig. 2B). This result also verified that the significant differences of HBsAg expression and secretion were not due to the tranfection efficiency differences between pZac-HBV-wt and pZac-HBV-172*, thus, our results accurately reflected the different HBsAg secretion capabilities between HBV wt and HBV rtA181T/sW172*.

**A**

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<tr>
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<tr>
<td></td>
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<tr>
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**B**

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<tr>
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</tr>
<tr>
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Fig. 2. Dominant negative effect on the HBsAg expression of HBV wt by HBV rtA181T/sW172* variant. HepG2 cells were co-transfected with pZac-HBV-wt and pZac-HBV-172* in different ratio. The HBsAg and HBeAg expression levels in culture supernatants and cell lysates were estimated by ELISA. (A) HBsAg expression levels in culture supernatants and cell lysates. (B) HBeAg expression levels in culture supernatants and cell lysates. Results are shown as the means ± SD of 3 independent experiments. S/CO, signal to cutoff ratio.

We also detected HBeAg expression levels in the supernatant and cell lysate of HepG2 cells co-transfected with pZac-HBV-wt and pZac-HBV-172* at different ratios. Both intracellular and extracellular HBeAg showed similar expression levels between different transfection ratios, though intracellular HBeAg was far less than supernatant HBeAg (Fig. 2B). This result also verified that the significant differences of HBsAg expression and secretion were not due to the tranfection efficiency differences between pZac-HBV-wt and pZac-HBV-172*, thus, our results accurately reflected the different HBsAg secretion capabilities between HBV wt and HBV rtA181T/sW172*.

**Activation of ER Stress Pathway by HBV sW172***

Because the clinical presentation of HBV in drug resistant CHB patients exhibited as a mixture of HBV wt and HBV rtA181T/sW172*, we also detected the expression level of HBsAg in cell populations with mixed HBV wt and HBV rtA181T/sW172*. HepG2 cells were co-transfected with pZac-HBV-wt and pZac-HBV-172* at different ratios but equal total amounts (Fig. 2). Cells transfected with only pZac-HBV-wt harvested maximal HBsAg secretion in the supernatant, whereas cells transfected with only pZac-HBV-172* secreted minimal HBsAg. As the amount of HBV rtA181T/sW172* increased and the amounts of HBV wt decreased, the expression level of HBsAg remarkably decreased. When the 2 plasmids were in equal amounts, HBsAg expression level was lower than the simple sum of HBV wt only and HBV rtA181T/sW172* only transfected cell HBsAg expression level (Fig. 2A). Therefore, results indicated that the HBV rtA181T/sW172* mutant had a dominant negative secretion effect. This showed a coincidence with previous reports (4, 10). The opposite trend was observed in intracellular cell lysates (Fig. 2A), which also proved that the HBV rtA181T/sW172* mutant had defects in HBsAg secretion and subsequently intracellular accumulation.

We also detected HBeAg expression levels in the supernatant and cell lysate of HepG2 cells co-transfected with pZac-HBV-wt and pZac-HBV-172* at different ratios. Both intracellular and extracellular HBeAg showed similar expression levels between different transfection ratios, though intracellular HBeAg was far less than supernatant HBeAg (Fig. 2B). This result also verified that the significant differences of HBsAg expression and secretion were not due to the tranfection efficiency differences between pZac-HBV-wt and pZac-HBV-172*, thus, our results accurately reflected the different HBsAg secretion capabilities between HBV wt and HBV rtA181T/sW172*.
Fig. 3. HBsAg and HBeAg secretion levels in mouse serum and hepatocytes. The HBsAg and HBeAg expression levels in peripheral blood and liver tissue from C57BL/6 mice on 0 day, 3 days, and 7 days after hydrodynamic injection of HBV wt and HBV sW172*, were estimated by ELISA. (A) The HBsAg expression levels in mouse serum. (B) The HBsAg expression levels in hepatocytes. (C) The HBeAg expression levels in mouse serum. (D) The HBeAg expression levels in hepatocytes. Results are shown as the means ± SD of 3 independent experiments (*, \( P < 0.05 \)).

HBeAg than HBV wt injected mice (Fig. 3D). The HBeAg expression profiles indirectly indicated that there were no differences between expression efficiencies of the pZac-HBV-wt and pZac-HBV-172* plasmids in vivo.

In line with the above in vitro results, the in vivo experiment provided further evidence indicating that the defects of HBsAg secretion existed in the HBV rtA181T/sW172* mutant, but not HBeAg secretion when compared with HBV wt: thus, this resulted in the retention of a large number of truncated surface proteins in hepatocytes.

Wild type S protein alone rescued HBV rtA181T/sW172* mutant defect in HBsAg secretion: Because the overlap of polymerase point mutation and envelope genes in drug-resistant HBV led to the disruption in surface protein secretion, we investigated whether wild-type S protein could rescue this dysfunction. All transfection groups had equal amounts of full-length HBV rtA181T/sW172*, as wild-type S protein level increased, HBsAg secretion level significantly increased (Fig. 4A). When HBV rtA181T/sW172* and wild-type S protein were in equal amounts, HBsAg expression was almost 290-fold higher than that of HBV rtA181T/sW172* alone (Fig. 4A). The rescue effect was not simply due to the increased amounts of pcDNA3.1-S-wt transfected into cells, as the amounts of HBsAg in the HBV rtA181T/sW172* and pcDNA3.1-S-wt cotransfected 1:1 group (17.68 IU) was much higher than HBV rtA181T/sW172* (0.06 IU) or pcDNA3.1-S-wt (4.94 IU) transfected individually (Fig. 4A). The same HBsAg expression trend was also observed in intracellular cell lysates. Thus, we concluded that wild-type S protein alone could also help to increase both intracellular and extracellular HBsAg expression levels (Fig. 4B). As expected, HBeAg levels in both supernatants and cell lysates did not show any significant differences between different transfection ratios. Therefore, wild-type S protein alone could not affect HBV rtA181T/sW172* mutant HBeAg secretion and expression (Fig. 4 C and D).

Intracellular accumulation of HBV surface proteins led to ER stress signal activation: We studied the subcellular localization of HBV wt and truncated surface proteins to observe whether truncated surface proteins accumulated in the ER. HuH-7 cells were transiently transfected with the pZac-HBV wt or pZac-HBV-172* expression plasmid, labeled by ER tracker with HBsAg immunofluorescence staining for confocal microscopy visualization at 48 h post-transfection. Cells transfected with HBV wt showed a diffused cytoplasmic staining pattern for the presence of HBV surface proteins, which were localized to the ER (Fig. 5A). However, cells transfected with the HBV rtA181T/sW172* mutant plasmid showed an aggregated cytoplasmic staining pattern for the presence of HBV surface proteins, which were also localized to the ER (Fig. 5A). We also quantified the aggregated cells in HBV wt or HBV rtA181T/sW172* transfection groups;
HBV rtA181T/sW172* transfected cells showed more aggregation compared with HBV wt transfected cells ($P < 0.0001$; Fig. 5B). The results suggested an accumulation of envelope proteins in the ER of HBV rtA181T/sW172* truncated mutant when compared with HBV wt.

The accumulation of truncated envelope proteins in the ER presumably might have led to ER stress signal activation. In order to evaluate the transcription and translation of ER stress genes in cells transfected with HBV wt or HBV rtA181T/sW172* plasmids, we analyzed the expression of GRP78 gene, a typical marker of ER stress. The analysis by RT-qPCR indicated that GRP78 mRNA expression was found to be induced 1.5-fold ($P = 0.0154$) higher in HBV rtA181T/sW172* cells than in HBV wt cells 72 h post-transfection (Fig. 5C). The above studies might suggest the oncogenic potential of HBV rtA181T/sW172* truncation variant via ER stress and UPR induction.

**DISCUSSION**

The prolonged usage of NAs for long-term clinical treatment of CHB patients causes the emergence of drug-resistant HBV mutants, which can lead to treatment failure and progression of liver disease. The selection of rtA181T/sW172* multi-drug resistant mutants by widespread treatment with NAs has raised many concerns due to decreased HBV susceptibility to LMV, LdT, ADV, and tenofovir (8,10,20). Its dominant negative effect on wild-type HBV virion secretion and oncogenic potential through transactivation activities were also reported (10–12). In our study, we used ELISA to quantitatively confirm the secretion defect of this truncated mutant and its dominant negative effect on wild-type surface protein secretion in cell populations mixed with of wild-type and mutant HBV. In addition, wild-type S protein alone was able to rescue this secretion and expression defect. Therefore, we can speculate that wild-type S protein played a major role in rescuing the secretion of HBV rtA181T/sW172* because of the remarkable increase of extracellular HBsAg as shown in Fig. 4A. All information required to produce an HBsAg particle is known to reside within the S protein. The HBV envelope proteins are synthesized at the ER, same as typical membrane proteins. The C-terminal hydrophobic 57 amino acids of S protein are believed to be embedded in the ER membrane. Foreign domains fused to the C terminus of S protein are oriented towards the ER lumen (21). Deletion of the C-terminal hydrophobic...
domain results in a stable, glycosylated, but nonsecreted chain. However, when co-expressed with wild-type S protein this mutant polypeptide can be incorporated into particles and secreted, indicating that the chain is still competent for some of the distal steps in particle assembly (22). The truncation variant with a stop codon in the amino acids 172 of the surface gene can lead loss of functional C-terminal hydrophobic peptide; thus, results in not only the secretion of subviral particles but also the envelopment of mature virions. In natural conditions, subviral particles can be highly over expressed relative to virions and reach a 10,000-fold higher concentration in serum. Subviral particles and virions carry identical surface antigens (HBsAg), even though protein composition is not identical. It is assumed that the massive HBsAg overproduction serves as a mechanism of evading the host immune system, which is advantageous for the virus (23). In a certain sense, this mutant disability of virion particle production assists HBV in surviving and escaping from the immune system.

We also confirmed abrogated HBsAg serum secretion and increased HBsAg retention in liver, but no change of HBeAg profiles in the animal model injected with HBV rtA181T/sW172* mutant strain; these data were comparable with another study (24). Based on results in vivo and in vitro, we further proved that relative to the HBV wt, HBV rtA181T/sW172* mutant had defects in HBsAg secretion but not HBeAg secretion; thus, a large number of viral particles were retained in hepatocytes. The sW172* mutant also showed dysfunction of subviral particle release which led the intracellular retention of large amounts of surface proteins. Because HBV envelope protein synthesis and secretion occurs in the ER, most truncated proteins accumulate in the ER lumen; this can trigger the UPR owing to overloading of the ER with virus-encoded proteins, which could represent one of the ancient evolutionary pressures for linking ER stress to cell suicide in avoiding the replication and spread of viruses (25,26). In our study, HBV sW172* truncated proteins were found to display more aggregated ER lumen distribution in comparison with HBV wild proteins. We also obtained high expression levels of the molecular chaperone GRP78 (also known as BiP) in sW172* cells compared with wt HBV transfected cells 72 h post-transfection. GRP78 plays many roles in ER function, including assisting protein folding, targeting
Activation of ER Stress Pathway by HBV sW172*

misfolded proteins for proteasomal degradation, serving as a sensor for ER stress, and contributing to ER calcium stores (25,26). Taken together, our results demonstrated that intracellular accumulation of truncated proteins may lead to the activation of ER stress signaling pathway. Previous reports have indicated that pre-S mutant HBsAg accumulate in the ER, resulting in strong ER stress, oxidative DNA damage, and genomic instability; these combined effects could potentially lead to hepatocarcinogenesis over the decades of CHB infection. The presence of pre-S mutants in serum sample was reported to carry a high risk of developing HCC (27,28). The oncogenic potential of HBV sW172* was also confirmed through its ability to transactivate the c-Myc and SV40 promoters (12,29). There are still no reports on the oncogenic potential of this mutant in initiating ER stress and oxidative damage. It can be concluded that the HBV sW172* truncation variant not only may influence the envelompement of HBV nucleocapsid and virion secretion, but may also cause cellular stress and damage by intracellular retention. ER stress induces the UPR, which can lead to HCC in hepatocytes.

In conclusion, the prolonged usage of NAs for CHB therapies could significantly suppress the viral genome replication, but could also select for multidrug-resistant mutants that may contribute to the worsening of liver disease, even leading to HCC through activation of ER stress signaling pathways. Therefore, CHB patients should be carefully considered before the treatment with NAs. Furthermore, development of new anti-viral drugs should target other stages of the HBV lifecycle that would not only maintain virologic clearance, but also prevent the emergence of drug resistant mutants with oncogenic potential.

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Conflict of interest None to declare.

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