Identification of novel oncogenic hepatitis B virus X gene mutations in Entecavir-treated patients

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Background

Being one of the major leading causes of liver diseases, chronic hepatitis B virus (HBV) infection and its therapeutic strategies have puzzled clinician for dozen of years. As one of the anti-HBV drugs, Entecavir (ETV) has been approved and widely used in Taiwan for treating chronic HBV infection. However, recent studies have discovered that a sustained a proportion of patients developed hepatocellular carcinoma (HCC) when receiving ETV treatment for an unknown reason.

Aims

Hepatitis B virus X gene has been well-known for its role in HBV-mediated carcinogenesis. In this study, we aimed to investigate whether sequence variations in HBx are responsible for hepatocarcinogenesis under ETV therapy. Accordingly, in vitro experiments were performed to search for its possible underlying mechanism(s).

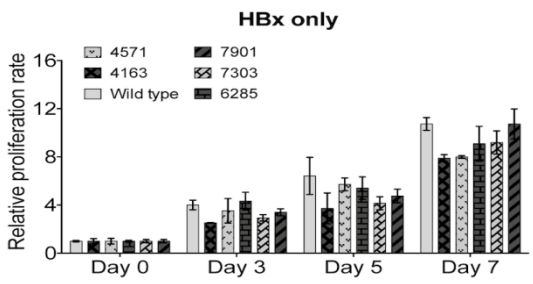

Figure 1.

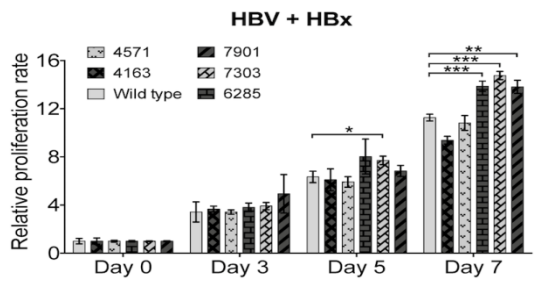

Figure 2.

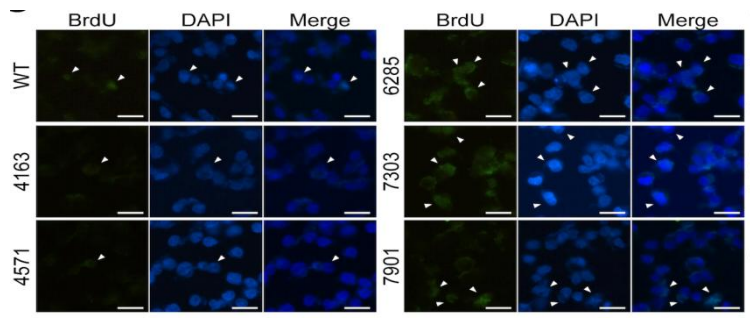

Figure 3.

Methods

A total of 5 patients diagnosed as HCC during ETV antiviral therapy and receiving surgical resection were enrolled. HBV DNA was extracted to sequence analysis. The cell-based experiments including assays about cell proliferative and apoptotic abilities were performed to investigate the hepatocarcinogenesis of HBx mutations.

Results

After HBx mutations plasmid construction, interestingly, we found no increase of cell proliferation rate when the scarcity of full-length HBV genome (figure 1). However, in contrast, three of them significantly enhanced cell proliferative capability when co-existence of HBV, implyingt hat their oncogenic role was HBV-dependent (figure 2). To more confirm this finding, the BrdU incorporation assay was also conducted (Figure 3). Further quantification of the cells with GFP-positive in nucleus also supported this notion (Figure 4). Additionally, the **t**ransferased **U**TP nick end labeling (TUNEL) assay was conducted (figure 5)and showed that two of these three variants markedly impede cell apoptotic capability, suggesting that they promote HBx-mediated oncogenesis by suppressing programmed cell death.

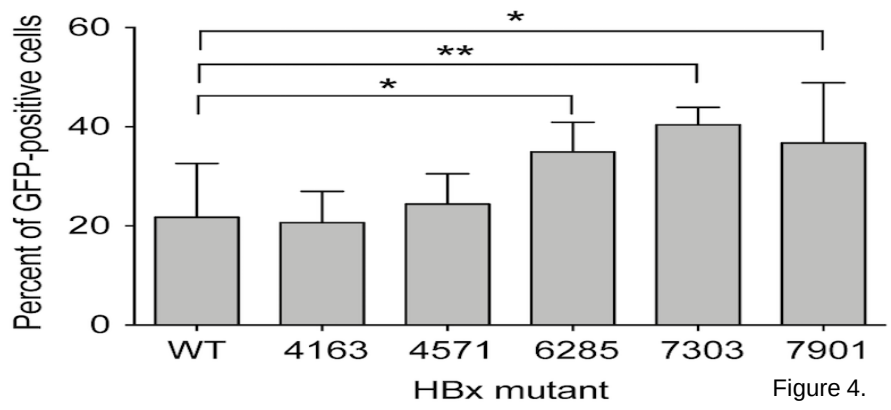

Figure 4.

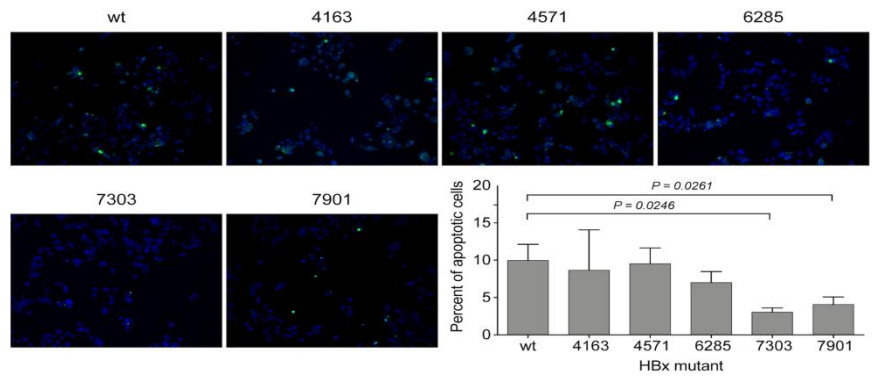

Figure 5.

Conclusions

We identified three oncogenicity-enhancing HBx variants from ETV-treated patients. Of them, two could assess it by reducing programmed cell death.