Reduced growth ability and increased nuclear abnormality in hepatitis B virus genotype C-infected human hepatocytes of humanized chimeric mouse liver

Yuji Ishida,[1,2] Yasumi Yoshizane,[1] Chihiro Yamasaki,[1] Ami Yanagi,[1] Yoko Ogawa,[1] Kazuaki Chayama, [2,3] Chise Tateno[1,2]

[1]PhoenixBio Co., Ltd., [2]Liver Research Project Center, Hiroshima University, [3]Department of Medical and Molecular Science, Hiroshima University.

BACKGROUND & AIMS: Several studies performed in established cell lines and transgenic mice have reported that expression of hepatitis B virus (HBV) proteins, such as surface and X proteins, affect hepatocyte proliferation and induce growth of hepatocellular carcinoma. However, it has not been elucidated how HBV infection affects phenotypes of normal human hepatocytes (HHs), which are the natural host for HBV. In the present study, we examined the effects of HBV infection on the morphology and proliferation of HHs by using chimeric mice with humanized livers.

METHODS: Commercially available cryopreserved HHs were transplanted into urokinase-type plasminogen cDNA activator-transgenic/severely combined immunodeficient (cDNA-uPA/SCID) mice at 3 weeks old. Seven-week-old chimeric mice were inoculated with HBV genotype (Gt.) A and C (10^6 copies/animal). At 12 and 20 weeks after infection, HHs were collected by collagenase perfusion and examined by microscopic observation and flow cytometric analysis to evaluate the effect of HBV-infection on the cell size, ploidy and ratio of binuclear HH. Pegylated interferon (PEG-IFN) alpha-2a, was administered (30 μg/kg, twice/week) to chimeric mice infected with HBV Gt. C for 8 weeks (19-27 weeks old) and HHs were isolated from the mice to confirm the effect of antiviral treatment on the morphological changes induced by HBV infection. To examine the effects of HBV infection on HHs proliferation, HHs were isolated from naïve and HBV Gt. C-infected chimeric mice and transplanted into 3-week-old cDNA-uPA/SCID mice.

RESULTS: Compared with naïve HHs, HBV Gt. C infection induced robust HH hypertrophy, and increased the number of binuclear HHs at 12 weeks after infection. On the other hand, Gt. A infection induced slight HH hypertrophy only. At 20 weeks after infection of HBV Gt. C, the HHs were more enlarged and many atypical nuclei were observed. Flow cytometric analysis and microscopic observation revealed that ploidy increased depending on the duration of infection. PEG-IFN alpha-2a treatment for 8 weeks reduced cell size and inhibited the ploidy increase caused by HBV infection. At 16 weeks after transplantation of naïve HHs, blood human albumin levels reached 9 mg/mL (replacement index: more than 70%). On the other hand, maximum human albumin level was approximately 1 mg/mL (replacement index: less than 5%) in chimeric mice transplanted with HBV Gt. C-infected HHs.

CONCLUSIONS: These results suggest that HBV Gt. C infection not only induced hepatocyte hypertrophy and ploidy increase but also inhibited hepatocyte proliferation *in vivo*.