A lumbrokinase isozyme targets hepatitis B e-antigen

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Dear Editor,

Hepatitis B Virus (HBV) infection remains a major health problem worldwide despite the availability of a highly effective preventive vaccine. HBV genome codes several distinct proteins, among which the non-particulate e-antigen (HBeAg) is one of the important markers in HBV infection. Sera containing HBeAg are highly infectious but those with anti-HBeAg have little infectivity. The spontaneous or therapy-induced HBeAg seroconversion with HBe antibody development is generally considered a key event in patients with long-lasting HBV-related infection (Fiorino et al., 2017). Serum aminotransferase levels usually decrease, reaching normal and a significant reduction in HBV replication in a large part of the subjects undergoing HBeAb development is generally considered a key event in patients with long-lasting HBV-related infection (Fiorino et al., 2017). Serum aminotransferase levels usually decrease, reaching normal and a significant reduction in HBV replication in a large part of the subjects undergoing HBeAb development.

Among the hepatitis B virus antigens, HBeAg protein appears highly immunogenic and induces important lymphocyte effector functions (Jung et al., 1995). Therefore, decreasing HBeAg levels may be a novel strategy to intervene hepatitis B.

Earthworms (earth-dragon) have been used as a Chinese traditional medicine for a long time (Wang et al., 2018). One of the important efficacies of earthworms is to treat jaundice that is involved in liver disorders (Li and Wang, 1999; Mo et al., 2018). Earthworms such as L. rubellus and E. fetida synthesize a group of isozymes called lumbrokinase with fibrinolytic activity (Nakajima et al., 2003). The group of isozymes has been made into an enteric-coated capsule in the treatment of stroke over 20 years. Here we show that one of the isozymes from E. fetida is able to recognize and degrade HBeAg, suggesting that lumbrokinase could be used as a potential drug to target HBeAg in the treatment of hepatitis B.

HBeAgase markedly decreased HBeAg levels in the culture medium of HepG2.2.15 cells detected by ELISA (Figure 1C). Lamivudine as a control also decreased HBeAg levels. To assess the potential toxicity of HBeAgase to HepG2.2.15 cells, we examined the viability of treated cells using cell-counting kit-8. HBeAgase (33 nmol/L) did not significantly

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affect cell viability, and neither did lamivudine under experimental conditions (Figure S2). As the negative control, neither 10% nor 0% fetal bovine serum medium markedly affected the secretion of HBeAg levels (Figure 1D).

To demonstrate that HBeAgase targets HBeAg, we analyzed the hydrolytic site on HBeAg. HBeAg (∼15 kD) disappeared with time in the presence of HBeAgase followed by appearance of a protein band (∼10 kD) in SDS-PAGE (Figure S3A, B). Marked degradation of HBeAg was not observed in the presence of trypsin under the experimental conditions. The amino acid sequence of HBeAg (14865.70 Da) and ∼10 kD degraded fragment (9553.73 Da) had the same N-terminal sequence detected by mass spectrometry (Table S1, Figure S4). Based on the primary structure in GenBank (CAA01610.1), the cleavage site (R92/D93) was in the linear epitope of HBeAg (Figure 1E).

Some T cells are involved in hepatitis B. For example, NK cells have both pathogenic and protective functions in viral infections (Wu and Tian, 2017). HBeAgase cleaves at the linear epitope of HBeAg that binds to T cells (Jung et al., 1995). Furthermore, HBeAgase degraded HBeAg more actively than trypsin under the same conditions, and HBeAgase suppressed HBeAg secreted by HepG2.2.15 cells compared to lamivudine. It appears that HBeAgase may be developed as a potential drug to inhibit HBV infection through its suppression of HBeAg.