Hepatitis B virus-X protein upregulates TSC1/m-TOR pathway and enhance angiogenesis in hepatocellular carcinoma

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Introduction

Within the hepatocytic system, chronic hepatitis caused by hepatitis B and C virus precursors into hepatocellular carcinoma. Hepatocellular carcinoma (HCC) is one of the major causes of cancer deaths in South-East Asian countries and is frequently caused by Hepatitis B virus (HBV) infection. Genetic factors involved in the development of HCC by HBV infection are yet to be identified. Recent reports suggest that the Hepatitis B virus-X protein may induce a signaling pathway involving AKT, ERK, GSK3β and mTOR, which is critical for hepatocellular transformation by HBV infection. TSC1, a well-known tumor suppressor, is responsible for the development of hepatocellular carcinoma (HCC). TSC2, negatively regulates the mammalian target of rapamycin (mTOR) pathway. Dysregulation of the TSC1/mTOR signaling pathway has been implicated in the development of cancer. The outcome of these previous studies indicate signaling pathways that are critical for hepatocellular carcinoma development. Moreover, they will help us to better understand molecular pathogenesis of the HCC tumor angiogenesis and find novel targets to develop effective therapies for HCC.

Material and Methods

Cell line

We used human hepatoma cell lines Hep3B and HepG2 cells which were derived from hepatoma patients and these two cell lines were transfected with the hepatitis B X gene plasmid to stably express the hepatitis X protein that we injected a Hep3B and HepG2 cells on long term. All these cell lines were maintained at 37°C in 5% CO2 incubator with Dhalo’s modified Eagle’s/F12 medium plus 10% fetal bovine serum.

Antibody and Reagent

The antibodies used in this study were anti-TSC1, anti-phosphorylated 56 kinase (MAP3K1), anti-AKT, anti-actin, and anti-β-actin. Antibodies against the phosphorylation sites of TSC1/251 were produced using the synthetic phosphorylated peptides S215/219/223 P-mTOR or S409/413 P-mTOR conjugated on a solid support. The TSC1 and TSC2 siRNAs were annealed in Dulbecco’s modified Eagle’s/F12 medium plus 10% fetal bovine serum.

Patients and Tumor samples

Immuno-histochemical staining for pAkt, pTSC1 and pS6K is protein expression was performed on adjacent 4-μm formalin-fixed paraffin-embedded tissue sections. Ninety-five hepatitis B-associated hepatocellular carcinoma patients’ samples that received curative surgery, resected in a tissue micro-array, were examined.

Result

To disclose the molecular pathways of Hepatitis B virus-X protein induced liver cancer, the signaling pathway involving AKT kinase (mTOR) and mammalian target of rapamycin (mTOR) downstream effector 56 kinase (S6K1) was confirmed to be activated (Akt phosphorylation) in Hep3B and HepG2 cells. Phosphorylation of TSC1 and S6K was induced in Hepatitis B virus-X protein expression cells. Moreover, overexpression of HBV X protein stimulates activation of TSC2 and mTOR and increases secretion of VEGF in the culture supernatant of Hep3B and HepG2 cells. Treatment of these cells with the mTOR inhibitor siRNA resulted in a decrease in cell proliferation and VEGF production. We next evaluated immunostaining of clinic hepatitis B associated hepatocellular carcinoma specimens which from 95 patients who received curative surgery. Of interest, we found that pS6K expression was strongly correlated with pTSC1 (P < 0.01) and pS6K1 (P < 0.01) and these factors were associated with poor survival.

Conclusion

Together, these results show that Hepatitis B virus-X protein can deregulate TSC1/mTOR through AKT, signaling, which may play a critical role in hepatoma progression. In addition, we observed TSC1/mTOR inhibition may potentially be novel targets to develop effective therapies for hepatitis B associated hepatoma.

References