Abstract
Within the hepatic system, chronic hepatitis caused by hepatitis B and C virus predisposes into hepatocellular carcinoma. Hepatocellular carcinoma (HCC) is one of the major causes of cancer death in South-East Asian countries and frequently caused by Hepatitis B virus (HBV) infection. Critical 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, IKKβ, Erk, GSK-3β and β-catenin, which is critical for hepatocytes transformation by HBV infection. TSC1, a well-known tumor suppressor responsible for the development of tuberous sclerosis complex (TSC), negatively regulates the mammalian target of rapamycin (mTOR) pathway. Dysregulation of the TSC/mTOR signaling pathway has been implicated in the development of cancers. To elucidate the molecular pathways of Hepatitis B virus-X protein induced liver tumorigenesis, the signaling pathway involving IκB kinases β (IKKβ), tuberous sclerosis complex 1 (TSC1), and mammalian target of rapamycin (mTOR) downstream effector S6 kinase (S6K1) was confirmed to be activated in HBV X gene stable expression hepatoma cancer Hep 3B and Hep G2 cells. Phosphorylation of TSC1 and S6K1 was induced in Hepatitis B virus-X protein expression cells. Moreover, overexpression of HBV X protein stimulates activation of IKKβ and mTOR and increases secretion of VEGF in the culture supernatants of Hep3B and HepG2 cells. Treatment of these cells with the mTOR inhibitor rapamycin or the IKKβ inhibitor Bay 11-7082 suppressed X protein induced cell proliferation and VEGF production. We next evaluated immunostaining of clinical hepatitis B associated hepatoma tissue specimens which from 95 patients who received curative surgery. Of interest, we found that pIKKβ expression was strongly correlated with pTSC1 (P<0.01) and pS6K1 (P<0.01) level. Furthermore, the over expression of pIKKβ (P<0.001), pTSC1 (P=0.04) and pS6K1 (P<0.001) in hepatitis B associated hepatoma patients revealed very poor prognosis survival. Together, these results show that HBV X protein can deregulate TSC1/mTOR through IKKβ signaling, which may play a critical role in hepatoma progression. In addition, Bay 11-7082 and rapamycin may potentially be novel targets to develop effective therapies for hepatitis B associated hepatoma.
Introduction
Within the hepatic system, chronic hepatitis caused by hepatitis B and C virus predisposes into hepatocellular carcinoma. Hepatocellular carcinoma (HCC) is one of the major causes of cancer death in South-East Asian countries and frequently caused by Hepatitis B virus (HBV) infection. Critical 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, IKKβ, Erk, GSK-3β and β-catenin, which is critical for hepatocytes transformation by HBV infection. TSC1, a well-known tumor suppressor responsible for the development of tuberous sclerosis complex (TSC), negatively regulates the mammalian target of rapamycin (mTOR) pathway. Dysregulation of the TSC/mTOR signaling pathway has been implicated in the development of cancers. The outcomes of this proposal will elucidate signaling pathways that are critical for hepatitis B x antigen associated HCC development. Moreover, they will help us to better understand molecular pathogenesis of the HCC tumor angiogenesis and identify novel targets to develop effective therapies for HCC.

Material and Methods

Cell line
We used human hepatoma cell lines Hep 3B and HepG2 cells which were derived from hepatoma patients and these two cell lines were transfected with the hepatitis B x gene plasmid to stable expression the hepatitis x protein that we named as Hep 3Bx and Hep G2x cell lines. All these cell lines were maintained at 37°C in a 5% CO₂ incubator with Dulbecco’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 S6 kinase (T389), anti-S6 kinase, anti-phosphorylated IKKβ (S181), anti-phosphorylated AKT (S473), anti-AKT, anti-actin, and anti-IKKβ. Antibodies against the phosphorylation sites of TSC1 S511 were produced using the synthetic phosphorylated peptides SPFYRDpSLPGSQ as antigens and purified on a phosphopeptide column at Bethyl Laboratories, Inc. BAY 11-7082 was purchased from Calbiochem. Recombinant human TNFα was obtained from Roche US. Cells were treated with BAY 11-7082 (40 μM, 45 minutes pretreatment) or TNFα (20 nM, 1 hour pretreatment). Cell proliferation/viability was measured by MTT assay and the cell culture medium was measured VEGF production by ELISA
method. Western blotting was performed to determine the expression of members of the IKKβ/TSC1/mTOR pathway (e.g., IKKβ, TSC1, and S6K1)

Patients and Tumor samples
Immunohistochemical staining for pIKKβ, pTSC1 and pS6K1 protein expression was performed on adjacent 4-μm formalin-fixed paraffin-embedded tissue sections. Ninety-Five hepatitis B associated hepatoma patients’ samples that received curative surgery, assembled in a tissue micro-array, were examined.

Result
To elucidate the molecular pathways of Hepatitis B virus-X protein induced liver tumorigenesis, the signaling pathway involving IκB kinases β (IKKβ), tuberous sclerosis complex 1 (TSC1), and mammalian target of rapamycin (mTOR) downstream effector S6 kinase (S6K1) was confirmed to be activated in HBV X gene stable expression hepatoma cancer Hep 3B and Hep G2 cells. Phosphorylation of TSC1 and S6K1 was induced in Hepatitis B virus-X protein expression cells. Moreover, overexpression of HBV X protein stimulates activation of IKKβ and mTOR and increases secretion of VEGF in the culture supernatants of Hep3B and HepG2 cells. Treatment of these cells with the mTOR inhibitor rapamycin or the IKKβ inhibitor Bay 11-7082 suppressed X protein induced cell proliferation and VEGF production. We next evaluated immunostaining of clinical hepatitis B associated hepatoma tissue specimens which from 95 patients who received curative surgery. Of interest, we found that pIKKβ expression was strongly correlated with pTSC1 (P<0.01) and pS6K1 (P<0.01) level. Furthermore, the over expression of pIKKβ (P<0.001), pTSC1 (P=0.04) and pS6K1 (P<0.001) in hepatitis B associated hepatoma patients revealed very poor prognosis survival.

Conclusion
Together, these results show that HBV X protein can deregulate TSC1/mTOR through IKKβ signaling, which may play a critical role in hepatoma progression. In addition, Bay 11-7082 and rapamycin may potentially be novel targets to develop effective therapies for hepatitis B associated hepatoma.

References


