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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Hepatitis B Virus Activates Signal Transducer and Activator of Transcription 3 Supporting Hepatocyte Survival and Virus Replication

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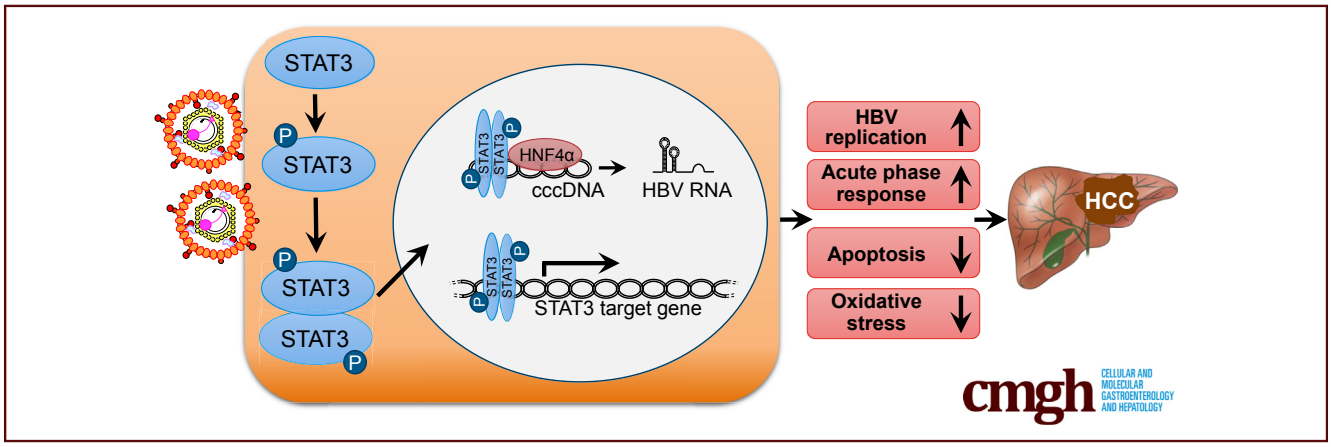

SUMMARY

We show that hepatitis B virus infection activates STAT3 signaling that supports virus replication and prevents apoptosis of infected hepatocytes potentially supporting malignant transformation. Our findings provide new insides into hepatitis B virus–host interaction and open a new avenue to the development of drugs that control the infection and may help to prevent carcinoma development.

BACKGROUND & AIMS: The human hepatitis B virus (HBV) is a major cause of chronic hepatitis and hepatocellular carcinoma, but molecular mechanisms driving liver disease and carcinogenesis are largely unknown. We therefore studied cellular pathways altered by HBV infection.

METHODS: We performed gene expression profiling of primary human hepatocytes infected with HBV and proved the results in HBV-replicating cell lines and human liver tissue using real-time polymerase chain reaction and Western blotting. Activation of signal transducer and activator of transcription (STAT3) was examined in HBV-replicating human hepatocytes, HBV-replicating mice, and liver tissue from HBV-infected individuals using Western blotting, STAT3-luciferase reporter assay, and immunohistochemistry. The consequences of STAT3 activation on HBV infection and cell survival were studied by chemical inhibition of STAT3 phosphorylation and small interfering RNA–mediated knockdown of STAT3.

RESULTS: Gene expression profiling of HBV-infected primary human hepatocytes detected no interferon response, while genes encoding for acute phase and antiapoptotic proteins were up-regulated. This gene regulation was confirmed in liver tissue samples of patients with chronic HBV infection and in HBV-related hepatocellular carcinoma. Pathway analysis revealed activation of STAT3 to be the major regulator. Interleukin-6–dependent and –independent activation of STAT3 was detected in HBV-replicating hepatocytes in cell

culture and in vivo. Prevention of STAT3 activation by inhibition of Janus tyrosine kinases as well as small interfering RNA–mediated knockdown of STAT3-induced apoptosis and reduced HBV replication and gene expression.

CONCLUSIONS: HBV activates STAT3 signaling in hepatocytes to foster its own replication but also to prevent apoptosis of infected cells. This very likely supports HBV-related carcinogenesis. *(Cell Mol Gastroenterol Hepatol 2017;4:339–363; http://dx.doi.org/10.1016/j.jcmgh.2017.07.003)*

Keywords: Hepatitis B Virus Infection; STAT3 Signaling; Hepatocellular Carcinoma; Apoptosis.

The hepatitis B virus (HBV) is a small, enveloped DNA virus characterized by a pronounced liver tropism and replication via reverse transcription (RT) of an RNA pregenome. Despite an effective prophylactic vaccine, HBV infection still is a major health problem, with more than 240 million chronically infected individuals, who are at high risk to develop liver cirrhosis, end-stage liver disease and hepatocellular carcinoma (HCC). The virus escapes efficient immune elimination by a very limited activation of innate and adaptive immune responses in the liver.[1] With the introduction of antivirals, treatment options for chronic hepatitis B improved over the last years, but a curative treatment is still lacking.[2] Although rates of HBV-related HCC are slowly decreasing,[3] HCC still rates number 6 among the most frequent cancers and is the number 3 cause of cancer-related death with about half of all HCC related to HBV infection.[4] Advanced liver disease with liver cirrhosis due to ongoing hepatocellular activation and inflammation are major risk factors.[5,6] Persistent viral replication, male sex and a positive family history increase the risk for HBV-related HCC.[6]

A strong correlation between HBV viral load and the risk of HCC development has been established in large clinical trials.[5] In the absence of a dominant oncogene encoded by the HBV genome, the role of HBV in carcinogenesis is complex[7] and still incompletely understood. Direct as well as indirect roles of HBV have been proposed.[8] Integration of HBV DNA into the host genome occurs at early steps of clonal tumor expansion. This may activate cellular cancer-related genes and very likely induces the host chromosomal instability. Hereby, prolonged expression of the viral regulatory HBV X protein may contribute to deregulating cellular transcription and influences protein degradation, cell proliferation and apoptotic signaling pathways (summarized in Neuveut et al,[8] Ringelhan et al,[9] and Tan[10]).

In a number of clinical settings and disease entities chronic liver inflammation seems to be sufficient to induce HCC development.[11] Pioneering work by Nakamoto et al[12] provided first experimental evidence that HBV-related HCC may develop in the absence of viral transactivation, insertional mutagenesis, and genotoxic chemicals[12] solely triggered by the immune response to HBV and resulting chronic inflammation. Key signaling pathways contributing to HCC development, however, have only partially been identified.

Lymphotoxins (LTs) and their receptor are up-regulated in viral hepatitis and related HCC, and sustained triggering of the LT-beta receptor resulting in canonical and noncanonical NF-kb signaling leads to HCC development.[13] We have previously demonstrated that nonparenchymal liver cells, particularly liver macrophages, recognize HBV particles resulting in an activation of NF-kb signaling and production of proinflammatory cytokines (eg, tumor necrosis factor-α, interleukin 6 [IL-6]).[14] IL-6 induces signal transducer and activator of transcription (STAT3) signaling in hepatocytes, and NF-kB and STAT3 have been described to be key players in liver inflammation and cancer (reviewed in He and Karin[15]).

In this study, we aimed at identifying major signaling pathways activated by HBV infection in hepatocytes. As signaling cascades may be largely affected in immortalized cell lines as well as by overexpression of viral proteins, we studied the influence of HBV replication on cellular gene expression in infected primary human liver cell cultures prepared from different donors and in human liver tissues derived from patients with chronic hepatitis B and HBV-related HCC. Finally, we corroborated our findings in transgenic mice expressing the complete genome of HBV in hepatocytes[16] and in mice challenged intravenously with adenoviral vectors encoding a replication competent HBV genome (Ad HBV).[17–19]

Methods

Chemicals

The pharmacological inhibitor AG-490 was purchased from Calbiochem (San Diego, CA). Dimethyl sulfoxide (DMSO) and N-acetyl-L-cysteine (NAC) were obtained from Sigma (St. Louis, MO).

Ethics Statement

The study followed the ethical guidelines of the Declaration of Helsinki and use of human material was approved by the local ethics committees of the University Hospital

***Marianna Hösel and Maria Quasdorff contributed equally to this work.**

Abbreviations used in this paper: **APR, acute phase response; cDNA, complementary DNA; cccDNA, covalently closed circular DNA; cRNA, complementary RNA; CRP, C-reactive protein; DMSO, dimethyl sulfoxide; FCS, fetal calf serum; HBeAg, hepatitis B early antigen; HBV, Hepatitis B virus; HBV pg RNA, hepatitis B pregenomic RNA; HBVtg, hepatitis B transgenic; HCC, hepatocellular carcinoma; HNF, hepatocyte nuclear factor; IFN, interferon; IL-6, interleukin 6; IRF3, interferon regulatory factor 3; mRNA, messenger RNA; NAC, N-acetyl-L-cysteine; p.i., postinfection; PCR, polymerase chain reaction; pgRNA, pregenomic RNA; PHH, primary human hepatocyte; pSTAT3, phosphorylated signal transducer and activator of transcription 3; ROS, reactive oxygen species; RT, reverse transcription; siRNA, small interfering RNA; STAT3, signal transducer and activator of transcription 3.**

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2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2017.07.003