Abstract

Hepatitis B virus (HBV) infection is one of the major causes of chronic and cancerous liver disease. Elimination of HBV from chronically infected patients by recombinant interferon α (IFNα) monotherapy shows that the mechanisms of the innate immunity play an important role in suppressing viral infection. However, the mechanisms of recognition of the HBV genome and its escape from the mechanisms of natural immunity are still little known.

One of the principal factors enabling the virus to escape from cellular restriction mechanisms is the HBx viral protein. HBx is a 154 amino acid pleiotropic multifunctional protein affecting transcription, signal transduction, cell cycle, protein degradation, apoptosis, and chromosomal stability in the host cell.

Previous results from our laboratory have shown that activation of the MEK1/2-ERK signaling pathway in plasmacytoid dendritic cells leads to inhibition of IFNα production. The aim of my work was to determine whether HBx activates the MEK1/2-ERK pathway and thus inhibits IFN type I production also in hepatocytes. For this purpose, I monitored HBx production in the Huh7 hepatoma cell line by transfecting the bicistronic plasmid pHBx-IRES-EGFP and Western blotting. Using the same method, I monitored activation of the MEK1/2-ERK signaling pathway by ERK kinase phosphorylation. My results show that maximum expression of HBx in Huh7 cells was reached 1 day after transfection of pHBx-IRES-EGFP. Western blot densitometric analysis showed that ERK phosphorylation reached approximately 5-fold higher level after transfection with the pHBx-IRES-EGFP plasmid carrying the wild-type HBx allele than after transfection with the plasmid carrying the mutant HBx^{R96E} allele or the empty vector. Transfection of pHBx-IRES-EGFP results in activation of the MEK1/2 kinase cascade, which could be blocked by the MEK1/2 kinase inhibitor PD0325901. Type I IFN production and inhibition was followed by induction of IFNβ expression by polyI:C reaction by RT-PCR. Quantitative PCR showed that transfection of pHBx-IRES-EGFP inhibited IFNβ expression approximately 5 times. The obtained results confirm the stimulation of IFNβ expression by poly I:C as well as the inhibitory effect of HBx on this stimulation in the stable human hepatocyte line Huh7.

Key word: HBV; suppression of virus replication; restriction factors; virus escape; Toll-like receptors (TLR); regulatory immunoreceptors; cell signaling; interferon (IFN); proteinkinases and their inhibitors