Hepatitis B virus X protein activates human hepatic stellate cells through upregulating TGFβ1


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ABSTRACT. We investigated the effects of the hepatitis B virus X gene (HBV X) on the activation of human hepatic stellate cells (HSCs) and the possible mechanisms underlying the pathway. Recombinant plasmid pHBV-X-IRES2-EGFP was constructed and transfected into HL-7702 cells using a lipid-mediated method. Transfected cells were screened by G418, which detected stable expression of the X gene by reverse transcription (RT)-PCR and Western blot analysis, and named L02/x. Cells not subjected to G418-selection were analyzed to confirm the transient expression of the X gene and named L02/48x. Subsequently, L02/x and L02/48x, together with non-HBx-expressing cells, were co-cultured with HSCs in a non-contact transwell system. After 36 h of co-culture, the proliferation and migration of HSCs was detected using different cell counting methods. Finally, the mRNA and protein levels of α-SMA, Col I, and TGFβ1 in HSCs were detected by real-time PCR and western blot analysis. RT-PCR and Western blot analysis showed that L02/x and L02/48x cells can express HBV X gene mRNA and protein. Additionally, HSCs co-cultured with L02/x or L02/48x cells showed significantly higher proliferation and migration levels than
control groups. Real-time PCR and Western blot analysis showed that the mRNA and protein expressions of α-SMA, Col I, and TGFβ1 in HSCs co-cultured with HBx-expressing liver cells were higher than those in control groups. HBx protein activated HSCs in vitro, leading to increased proliferation and migration of HSCs and upregulation of α-SMA and Col I. The TGFβ1 gene may be involved in this pathway.

**Keywords:** Hepatitis B virus X (HBV X) gene; Gene activation; TGFβ1; Hepatic stellate cells (HSC); Co-culture system