Hepatitis B Virus X Protein Confers Resistance of Hepatoma Cells to Anoikis by Up-regulating and Activating p21-Activated Kinase 1

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BACKGROUND & AIMS: Patients with chronic hepatitis B virus (HBV) infection are at risk for metastatic hepatocellular carcinoma (HCC). Metastatic cancer cells develop resistance to anoikis. The serine/threonine p21-activated kinase (PAK) 1 regulates cytoskeletal dynamics and protects cells from anoikis; it also promotes virus replication. We investigated the effects of PAK1 on anoikis resistance in human hepatoma cells and in mice. **METHODS:** We transfected human hepatoma cells with pHBV1.3 (to mimic HBV replication) or plasmids encoding different HBV proteins; we performed colony formation and anoikis assays. We knocked down levels of PAK1 and Bcl2, or inhibited their activity, in hepatoma cells and quantified anoikis and growth of tumor xenografts in nude mice; we also measured anoikis of tumor cells isolated from ascites of the mice. We performed immunohistochemical analysis of PAK1 levels in HCC samples from patients. **RESULTS:** Human hepatoma cells transfected with pHBV1.3 expressing hepatitis B virus X protein (HBx) underwent anchorage-independent proliferation, were resistant to anoikis, and had higher levels of Bcl2 than nontransfected cells. Expression of HBx increased mitochondrial levels of Bcl2 and PAK1, which interacted physically. Anoikis resistance of Huh7 and SK-Hep1 cells required PAK1 activity and Bcl2. Expression of HBx promoted growth of Huh7 xenograft tumors in mice; PAK1 knockdown reduced growth of these tumors in mice and anoikis of cells isolated from these tumors. In human HCC samples, increased levels of PAK1 correlated with poor prognosis, HBV infection, and portal vein tumor thrombosis. **CONCLUSIONS: The HBV protein HBx up-regulates PAK1, allows hepatoma cells to become resistant to anoikis, and promotes growth of aggressive xenograft tumors in mice. HBx induction of PAK1 might promote progression of HCC in patients with chronic HBV infection.**

Keywords: Liver Cancer; Mouse Model; Metastasis; Apoptosis Signal Transduction.

Although the prevalence of hepatitis B virus (HBV) infection has fallen remarkably due to the availability of efficacious hepatitis B vaccine and multiple antiviral agents, chronic hepatitis B remains a challenging global health problem, with approximately 350 million chronic carriers at risk of cirrhosis, liver failure, and hepatocellular carcinoma (HCC).[1,2] Epidemiological studies have provided overwhelming evidence for endemic HBV infection and aflatoxin-B1–contaminated food constituting 2 major etiological risk factors for the development of HCC in China, but the molecular mechanisms underlying HBV-associated hepatocarcinogenesis remain poorly understood.[3–5] Despite the absence of a dominant oncogene encoded by HBV genome, hepatitis B virus X protein (HBx), a key regulatory multifunctional protein of the virus, has been reported to exert a direct hepatocarcinogenic effect in the development of HCC.[6]

The p21-activated kinase (PAK) family of serine/threonine kinases defines an evolutionally conserved crucial convergent signaling nodule that participates in pleiotropic physiological processes, including cytoskeleton dynamics and cell motility, survival, mitosis, transcription, and translation.[7,8] On the basis of structural and functional similarities, the 6 members of PAK family are classified into 2 groups, with 3 members in each group.[7] In contrast to the constitutively active group II PAKs (PAK4–6), extracellular signals activate group I PAKs (PAK1–3) through GTPase-dependent and -independent mechanisms.[8] Specific upstream signals, including Rho GTPase family members cell division cycle 42 and Rac1, dictate the degree of activation and subcellular localization of PAKs and, in turn, PAKs mediate downstream signaling events that bring about the physiological effects of extracellular signaling via activating additional kinases and other effectors by phosphorylating them at specific serine or threonine residues or through protein–protein interactions.[9]

As a major confluent nodule in growth factor and cytoskeleton signaling downstream of various oncogenic pathways, up-regulated and hyperactivated PAK1 occurs frequently in a variety of human cancers, such as breast, ovary, colorectal, thyroid, pancreatic, and liver.[8,10,11] In addition, a plethora of evidence indicates that PAK1 also

Abbreviations used in this paper: HBV, hepatitis B virus; HBx, hepatitis B virus X protein; HCC, hepatocellular carcinoma; Mcl1, myeloid cell leukemia sequence 1; PAK, p21-activated kinase; PI, propidium iodide; PVTT, portal vein tumor thrombosis; RT-PCR, reverse transcription polymerase chain reaction; siRNA, small interfering RNA; TUNEL, terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling.

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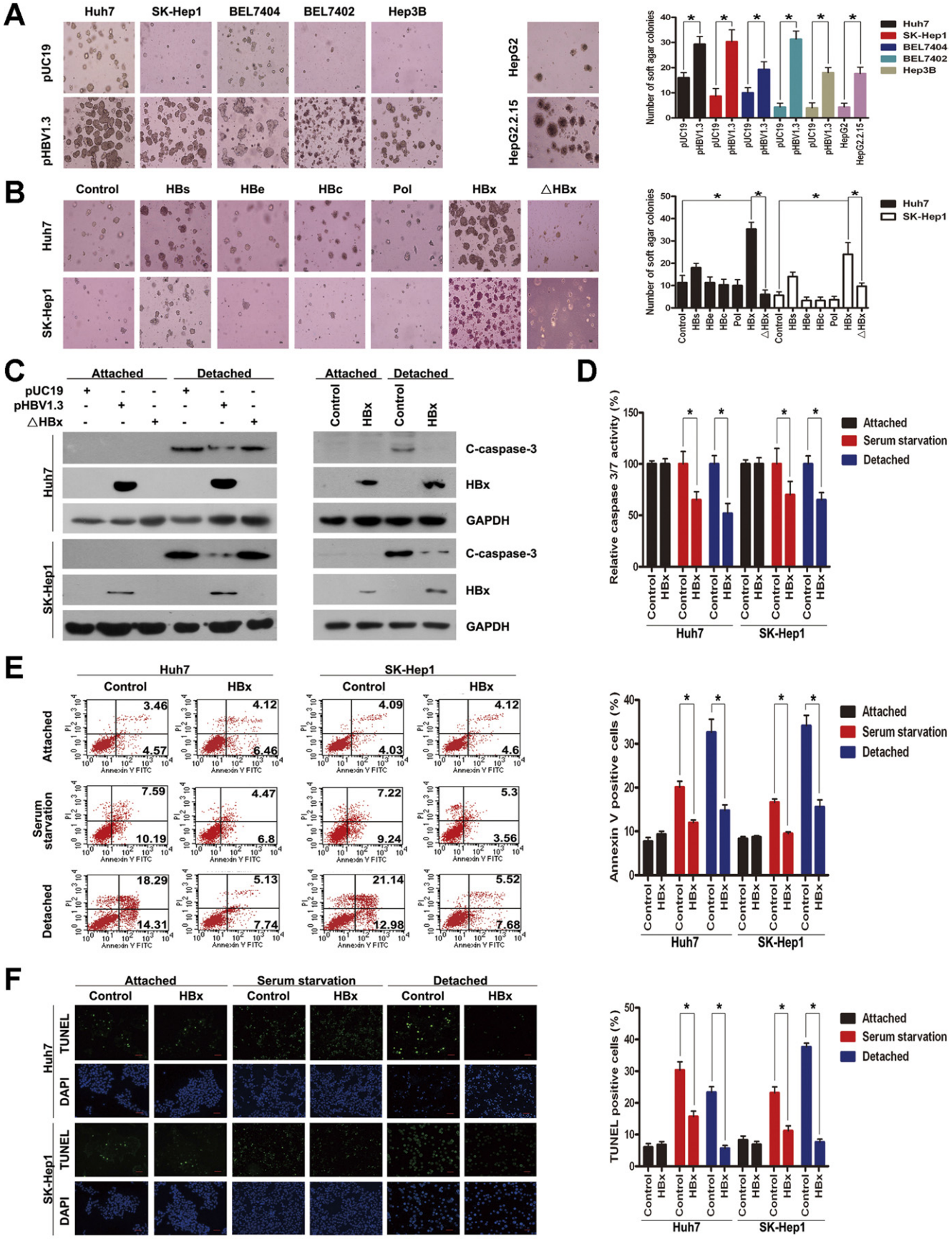
A
Huh7
SK-Hep1
BEL7404
BEL7402
Hep3B
pUC19
pHBV1.3
HepG2
HepG2.2.15
Number of soft agar colonies
B
Control
HBs
HBe
HBc
Pol
HBx
△HBx
C
Attached
Detached
C-caspase-3
HBx
GAPDH
D
Relative caspase 3/7 activity (%)
Serum starvation
E
Annexin V positive cells (%)
F
TUNEL
DAPI
TUNEL positive cells (%)