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Original Article

Hepatitis B virus PreS1 facilitates hepatocellular carcinoma development by promoting appearance and self-renewal of liver cancer stem cells

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ABSTRACT

Hepatitis B virus (HBV) is a major etiologic agent of hepatocellular carcinoma (HCC). However, the molecular mechanism by which HBV infection contributes to HCC development is not fully understood. Here, we initially showed that HBV stimulates the production of cancer stem cells (CSCs)-related markers (CD133, CD117 and CD90) and CSCs-related genes (Klf4, Sox2, Nanog, c-Myc and Oct4) and facilitates the self-renewal of CSCs in human hepatoma cells. Cellular and clinical studies revealed that HBV facilitates hepatoma cell growth and migration, enhances white blood cell (WBC) production in the sera of patients, stimulates CD133 and CD117 expression in HCC tissues, and promotes the CSCs generation of human hepatoma cells and clinical cancer tissues. Detailed studies revealed that PreS1 protein of HBV is required for HBV-mediated CSCs generation. PreS1 activates CD133, CD117 and CD90 expression in normal hepatocyte derived cell line (L02) and human hepatoma cell line (HepG2 and Huh-7); facilitates L02 cells migration, growth and sphere formation; and finally enhances the abilities of L02 cells and HepG2 cells to induce tumorigeneses in nude mice. Thus, PreS1 acts as a new oncoprotein to play a key role in the appearance and self-renewal of CSCs during HCC development.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer death worldwide with rising incidence in the past decades [1,2]. Hepatitis B virus (HBV) infection is a major risk factor for the development of HCC, with an estimate of 53% cases worldwide were related to HBV [3–5]. Studies showed that hepatitis B surface antigen (HBsAg)-positive patients have a 25–37 times increased risk of developing HCC compared to non-infected people [6,7]. Many factors were proposed for HBV-mediated hepatocarcinogenesis, including the integration of viral DNA within host genome in tumors and tumor-derived cell lines [8], persistent inflammation [9,10], and chromosomal instability and insertional mutagenesis caused by viral integration [11–17]. Expression of certain viral proteins such as hepatitis B X (HBx) and HBsAg may exert effects on cell cycle, cell growth and apoptosis by interfering with cell signaling and gene transcription [18–20]. However, the mechanism by which HBV mediates HCC development is still not fully understood.

Cancer stem cells (CSCs) contribute to tumorigenesis, metastasis, recurrence and chemoresistance. Microarray analyses of human HCC specimens, cancer cell lines and transgenic models established the molecular similarities between CSCs and hepatic

Abbreviations: CSCs, cancer stem cells; c-Myc, cellular myelocytomatosis oncogene; CC, colon carcinoma; ESC, embryonic stem cell; GIST, gastrointestinal stromal tumor; HSC, hepatic stem cell; HBV, hepatitis B virus; HBx, hepatitis B X protein; HCC, hepatocellular carcinoma; hrEGF, human recombinant epidermal growth factor; HBsAg, hepatitis B surface antigen; Nanog, human homeobox protein; hrbFGF, human recombinant basic fibroblast growth factor; Klf-4, Kruppel-like factor 4; NB, neuroblastoma; Oct-4, octamer-binding transcription factor 4; PBMC, peripheral blood mononuclear cell; PDGF, platelet-derived growth factor; SCM, stem cell marker; shRNA, short hairpin RNA; SGC, salivary gland carcinoma; SCM, stem cell marker; TGCT, testicular germ cell tumor; WBC, white blood cell.

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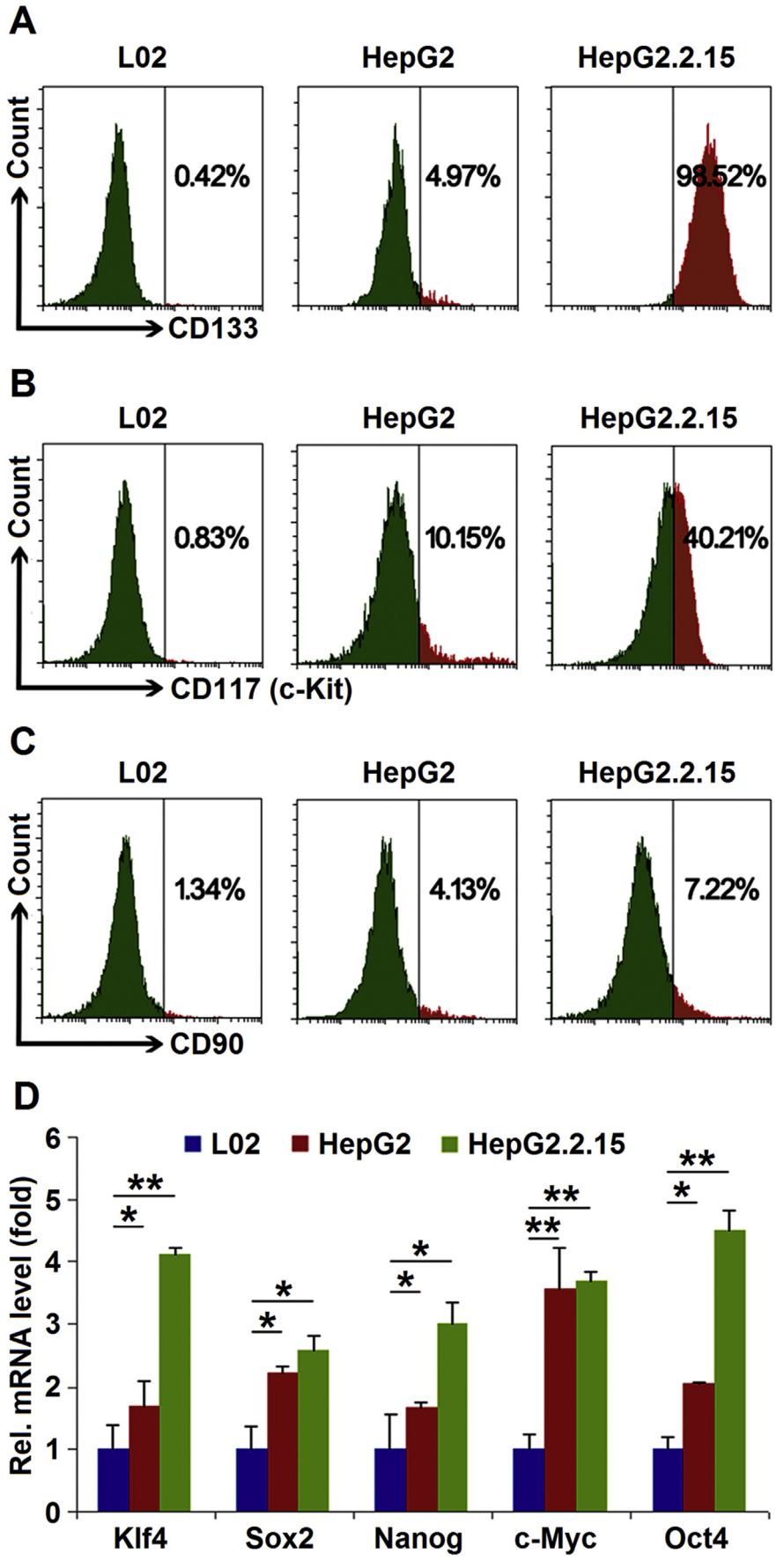
A
L02
HepG2
HepG2.2.15
Count
0.42%
4.97%
98.52%
CD133
B
L02
HepG2
HepG2.2.15
Count
0.83%
10.15%
40.21%
CD117 (c-Kit)
C
L02
HepG2
HepG2.2.15
Count
1.34%
4.13%
7.22%
CD90
D
L02
HepG2
HepG2.2.15
Rel. mRNA level (fold)
0
1
2
3
4
5
6
Klf4
Sox2
Nanog
c-Myc
Oct4