Original Paper

Intervirology

Intervirology 2008;51:26–32
DOI: 10.1159/000118793

Received: August 8, 2007
Accepted after revision: January 3, 2008
Published online: February 29, 2008

Effects of Hepatitis B Virus X Protein (HBx) on Cell-Growth Inhibition in a CCL13-HBx Stable Cell Line

Chan-Yen Kuo[a] Jing-Chyi Wang[a] Cheng-Chung Wu[b] Shih-Lan Hsu[c]
Guang-Yuh Hwang[a, d]

[a]Department of Life Science, Tunghai University, Departments of [b]Surgery and [c]Education and Research, Taichung Veteran General Hospital, and [d]Life Science Research Center, Tunghai University, Taichung, Taiwan

Key Words
Hepatitis B virus X protein · Proliferation · Apoptosis · Wnt-3/β-catenin

Abstract
Objective: The known function of hepatitis B virus X protein (HBx) is to determine the fate of cells by modulating various signaling pathways. In our previous study, we demonstrated that HBx inhibits tumor formation in nude mice injected with CCL13-HBx stable cell lines; however, the mechanism underlying this inhibition is unclear. ***Methods:*** To investigate the possible mechanisms underlying HBx involvement in CCL13-HBx cells, gene profiles were initially analyzed by DNA microarray technology and subsequently confirmed by performing semiquantitative RT-PCR and Western blotting. Furthermore, the phenomenon of cell death via apoptosis was detected via DNA fragmentation, TUNEL staining, caspase-3 activity assay, and propidium iodide (PI) staining. ***Results:*** The results indicated that HBx induction downregulated Wnt-3 and β-catenin that are involved in cell proliferation. Moreover, HBx induction repressed cell growth and downregulated the expressions of cyclin D1, CDK4, cyclin E, CDK2, and cyclin B1. Furthermore, HBx induction triggered cell death via apoptosis, as determined by DNA fragmentation, TUNEL staining, caspase-3 activity assay, and PI staining. ***Conclusion:*** Most importantly, our results indicated that HBx induction in the CCL13-HBx stable cell line downregulated Wnt-3/β-catenin expression and suppressed cell growth by repressing cell proliferation or triggering cell apoptosis.

Introduction

The hepatitis B virus (HBV) is a member of the Hepadnaviridae family and a well-known risk factor in the development of human hepatocellular carcinoma (HCC) [22]. The HBV-encoded X protein (HBx) has been demonstrated to play a critical role in mediating the pathological effects of this virus [11, 21, 22]. HBx mediates cell growth, which includes cell death and cell survival, via proliferation and apoptosis [3], and it is associated with signal transduction cascades [4]. Currently, the signal pathways involving HBx include the ras/raf/MAPK [8], PKB/Akt [16], and JAK/STAT pathways [33]. Furthermore, the transcriptional factors involving HBx include NF-κB, AP-1, CREB/ATF [34], RNA polymerase subunit RBP5 [7], and TBP [1, 11, 24]. Although the contribution of HBx to the development of HCC via transcriptional activation and signal alteration has been demonstrated, the critical role played by HBx in hepatocarcinogenesis remains unclear. Recently, β-catenin mutation and accu-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0300–5526/08/0511–0026$24.50/0

Accessible online at:
www.karger.com/int

Guang-Yuh Hwang
Department of Life Science, Tunghai University
No. 181, Sec. 3, Chungkang Road, Taichung (Taiwan)
Tel. +886 4 2359 0121, ext. 2464, Fax +886 4 2359 0296
E-Mail gyhwang@thu.edu.tw

mulation have been documented among the clinical and pathological features of HCC patients. In addition, tumors exhibiting β-catenin accumulation are associated with a prognosis of poorly differentiated morphology, high proliferative activity, and vascular invasion [4, 5, 15]. Recently, Ding et al. [10] reported a significant correlation between HBx expression and β-catenin accumulation in HCC tumor samples. These findings raise the possibility that HBx may mediate HCC development via the Wnt/β-catenin pathway.

Wnt proteins are a large family of cysteine-rich glycoproteins, comprising 19 members that play critical roles in embryogenesis, prostate cancer, bone metastases, colon cancer, and tumorigenesis [2, 12, 25]. In the absence of Wnt, the downstream molecule GSK-3β is activated in the Wnt-3/β-catenin pathway; the resulting inactivation of transcriptional transactivation inhibits cell proliferation and alters the fate of cells [14, 20]. Although Cha et al. [6] suggested that HBx is essential for the activation of Wnt-1/β-catenin signaling in hepatoma cells, their report lacked information regarding HBx involvement in the Wnt-3/β-catenin pathway.

In our previous study, we demonstrated that HBx inhibits tumorigenicity in the Chang liver cell line [32]. The CCL13-HBx stable cell line was established as an HBx-inducible expression that is regulated by a tetracycline-dependent expression system (tet-off system). In this tet-off system, HBx expression was detected in the CCL13-HBx stable cell line in the absence of tetracycline (Tet–); further, inhibition of tumorigenicity was observed in the CCL13-HBx stable cell line in nude mice [32]. In this study, we used the CCL13-HBx stable cell line as a model to study the effects of HBx, which mediates cell growth via proliferation and apoptosis. To elucidate the possible mechanism underlying these effects of HBx, we used the microarray approach for analyzing gene expression in the CCL13-HBx stable cell line. The CCL13-HBx cell growth rates in the absence (Tet–) and presence (Tet+) of tetracycline were studied and compared. Moreover, the phenomenon of cell death via apoptosis was detected via DNA fragmentation, TUNEL staining, caspase-3 activity assay, and propidium iodide (PI) staining.

Methods

Cell Lines and Cell Culture

The CCL13-HBx stable cell line was established in our laboratory by using the culture conditions described by Wang et al. [32].

RNA Isolation, cDNA Microarray Analysis, and Semiquantitative RT-PCR

Total RNA was isolated from the CCL13-HBx stable cell line using TRIzol reagent (Invitrogen). The cDNA microarray experiment was commissioned to Welgene Biotech. Co. Ltd (Taipei, Taiwan). The primer sequences and procedure used for the RT-PCR analysis of HBx have been described previously [32]. The cDNAs for the *Wnt-3*, *GSK-3β*, and *β-catenin* genes were synthesized using primer sets with the following sequences: 5′-AGT GGA CTT TGT TCC AAC-3′ and 5′-CCC CGG AAC TGA TGC TGG-3′ for Wnt-3, 5′-TCC TTT GGA ATC TGC CAT CGG-3′ and 5′-GTC GGA AGA CCT TAG TCC AAG-3′ for GSK-3β, 5′-ATG GCA TCA GAA CTG GCA-3′ and 5′-ATG GCA TCA GAA CTG GCA-3′ for β-catenin.

Western Blotting

Proteins extracted from the CCL13-HBx stable cell line were subjected to Western blotting. The membranes were immunodetected using anti-HBx [32], anti-Wnt-3, anti-β-catenin, anti-CDK4, anti-CDK2, anti-proliferating-cell nuclear antigen (PCNA), anti-actin (Santa Cruz), anti-cyclin E, anti-cyclin A, anti-cyclin B1, anti-p53 (Novocastra), anti-GSK-3β (Cell signaling), or anti-cyclin D1 (Zymed).

Cell Viability

Cells were seeded in a 6-well culture dish at a concentration of 0.5×10^5 cells per well and were grown in the presence of 1.5 μg/ml tetracycline in a DMEM culture medium containing 10% FBS. The culture medium was replaced once in 2 days. Further, the culture medium lacking 1.5 μg/ml tetracycline was replaced every alternate day, and the cells were counted until they attained approximately 60–70% confluence. After the cells were counted, they were stained with trypan blue.

Immunofluorescence Staining

The CCL13-HBx stable cell line was fixed in 3% formaldehyde, blocked with PBS containing 3% FBS, and incubated with anti-HBx [32]. Subsequently, the cells were incubated with an FITC-labeled secondary antibody (Jackson Immunoresearch Laboratories). The nuclei were stained with diamidino-2-phenylindole (DAPI, Molecular Probes), which is a DNA groove-binding dye, and the cells were examined under a Nikon E400 microscope.

PI Staining

The cells were trypsinized, collected, and washed with PBS. They were then fixed with 70% ethanol in PBS, and the lysate was incubated with 40 μg/ml PI (Molecular Probes) and 100 μg/ml DNase-free RNase A. The samples were examined on the CyFlow system (Partec GmbH, Münster, Germany).

DNA Fragmentation Analysis

The cells were collected and washed with PBS. The analysis was performed using the Tissue & Cell Genomic kit (GeneMark, Taiwan), according to the manufacturer's instructions. DNA was extracted using phenol/chloroform and subjected to 1.8% agarose gel electrophoresis.

TUNEL Staining and Caspase-3 Activity Assay

Cells were seeded in a 24-well culture dish at a concentration of 1×10^4 cells per well and were grown in the presence of 1.5

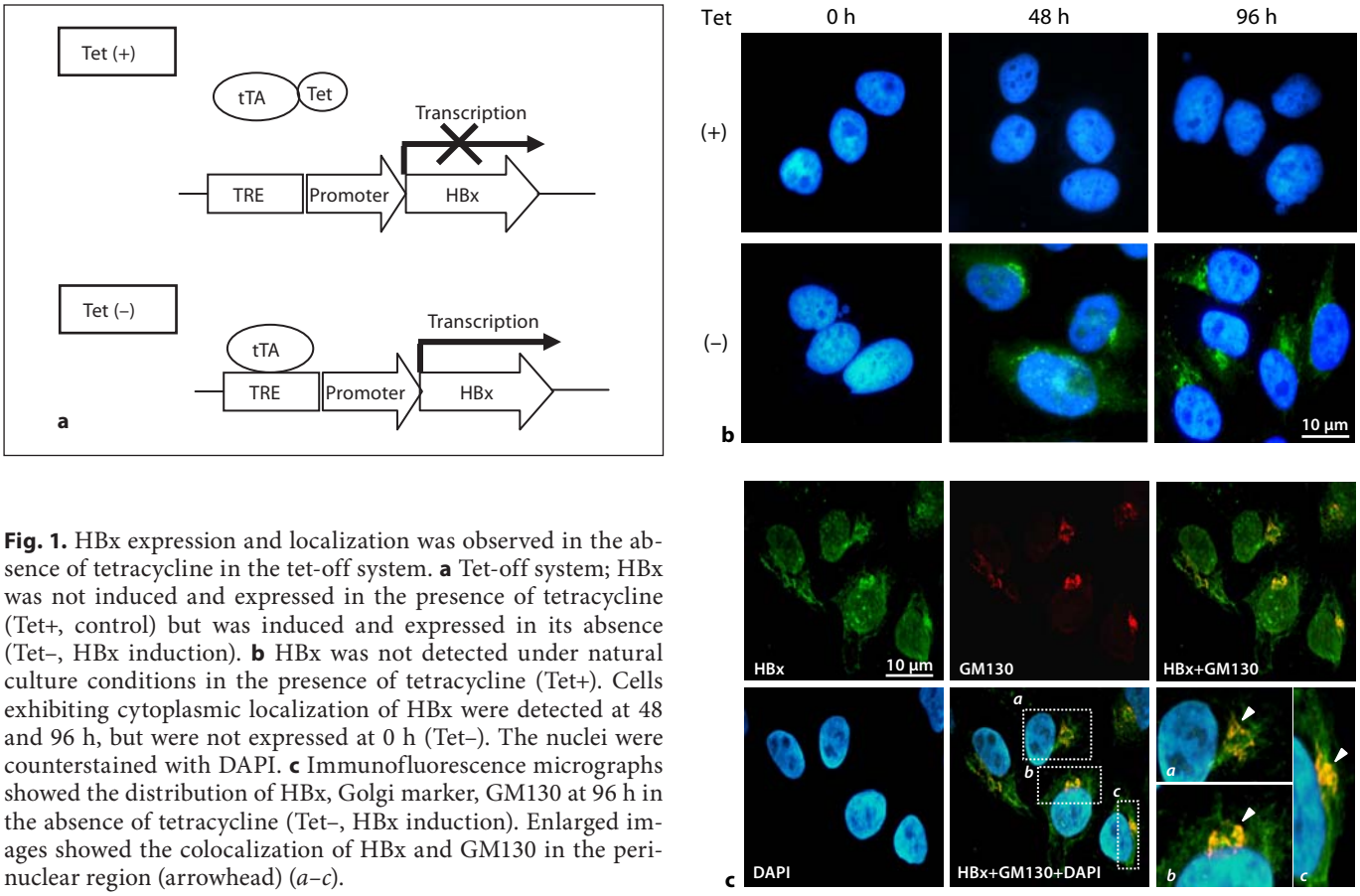

Fig. 1. HBx expression and localization was observed in the absence of tetracycline in the tet-off system. **a** Tet-off system; HBx was not induced and expressed in the presence of tetracycline (Tet+, control) but was induced and expressed in its absence (Tet–, HBx induction). **b** HBx was not detected under natural culture conditions in the presence of tetracycline (Tet+). Cells exhibiting cytoplasmic localization of HBx were detected at 48 and 96 h, but were not expressed at 0 h (Tet–). The nuclei were counterstained with DAPI. **c** Immunofluorescence micrographs showed the distribution of HBx, Golgi marker, GM130 at 96 h in the absence of tetracycline (Tet–, HBx induction). Enlarged images showed the colocalization of HBx and GM130 in the perinuclear region (arrowhead) (*a–c*).

μg/ml tetracycline in DMEM culture medium containing 10% FBS. The analysis was performed using the TUNEL apoptosis detection kit (Upstate) and the caspase-3 activity detection kit (Upstate) according to the manufacturer's instructions. Statistical significance for all the experiments was determined by performing the t test. Error bars are used to indicate the standard errors of the means and p values of <0.01 were considered significant.

Results

Characteristics of HBx in the Chang-HBx Stable Cell Line

In the tet-off system, *HBx* gene expression is triggered on removing tetracycline from the culture medium (fig. 1a, Tet–). In our system (tet-off system), *HBx* was induced in the CCL13-HBx stable cell line in the absence of tetracycline (Tet–). HBx expression was induced and detected at 48 h, and it progressively increased with time following tetracycline deprivation (fig. 1b, 2c, Tet–). However, immunofluorescence staining and Western blotting revealed no HBx expression at 0 h following tetracycline deprivation (fig. 1b, 2c, Tet–). Cells exhibiting cytoplasmic localization of HBx were detected at 48 and 96 h but were not expressed at 0 h (fig. 1b, lower panel). In addition, similar to 0 h following tetracycline deprivation (fig. 1b, Tet–), the HBx signal was not detected under natural culture conditions in the presence of tetracycline (fig. 1b, 2c, Tet+). Thus, HBx expression was detected and analyzed in the absence of tetracycline in the CCL13-HBx stable cell line (Tet–) in a time-dependent manner. Cells grown in the presence of tetracycline (Tet+) were used as a control.

To speculate the detailed intracellular localization of HBx in the CCL13-HBx stable cell line, we used immunofluorescence microscopy as an approach to analyze. Results indicated that the HBx was intimately colocalized with the Golgi marker, GM130 (abcam) in the perinuclear region (fig. 1c).

Repression of Cell Growth in the Chang-HBx Cell Line

HBx is a multifunctional protein and may mediate cell growth [21, 22]. To elucidate the involvement of HBx in

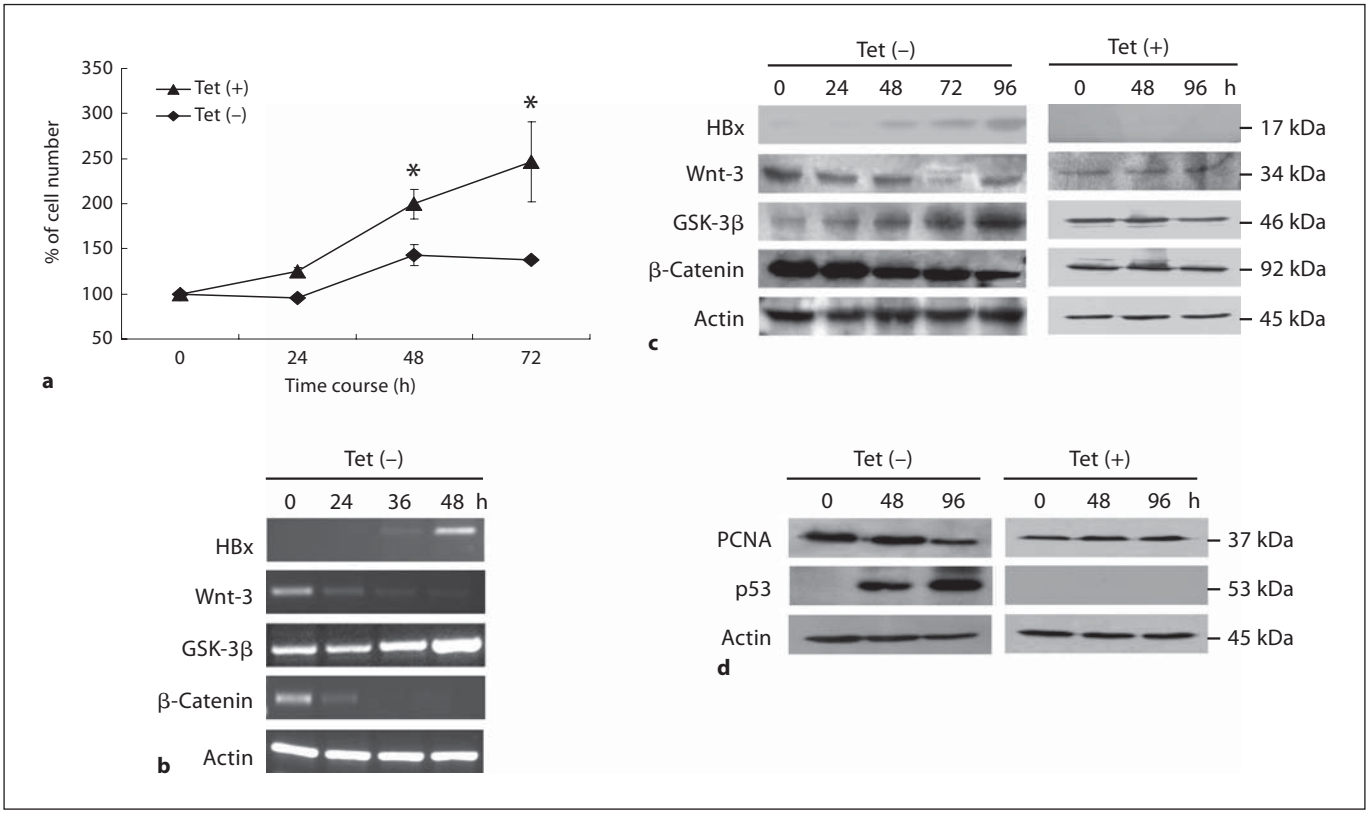

Fig. 2. HBx repressed cell growth and downregulated Wnt-3/β-catenin. **a** Change in the effects of HBx on cell growth with time in the absence (Tet–, ◆) and presence (Tet+, ▲) of tetracycline. The cells were counted as the mean of triplicates. * $p < 0.05$. **b** Transcription of HBx, Wnt-3, GSK-3β, β-catenin, and actin (internal control) was measured by performing semiquantitative RT-PCR at various time points following tetracycline deprivation (Tet–). **c** Changes in the expressions of HBx, Wnt-3, GSK-3β, β-catenin, and actin (internal control) with time were observed by Western blotting following HBx induction (Tet–) or in the absence of induction (Tet+). **d** HBx mediated cell growth via apoptosis or proliferation. The expression of p53 was upregulated, while that of PCNA was downregulated on HBx induction (Tet–), but these expressions were not detected or remained unaltered in the absence of HBx induction (Tet+). Actin was used as an internal control.

cell growth, the growth rate of the CCL13-HBx cell line was measured (fig. 2a). The cells were counted every alternate day, and the results revealed that the cells cultured in the absence of tetracycline (fig. 2a, Tet–, ◆) grew slower than those cultured in its presence (fig. 2a, Tet+, ▲). This data indicated that cell growth was repressed on HBx induction (Tet–). To elucidate the effects of HBx on cell growth and the possible mechanisms involving HBx, we used the microarray approach to analyze multiple gene expression in the Chang-HBx stable cell line. The results indicated that the *Wnt-3*, *v-maf*, and *Endothelin-2* candidate genes, which are largely involved in cell proliferation and angiogenesis, were significantly downregulated on HBx induction (data not shown).

Influence of HBx Induction on Wnt-3/β-Catenin and Cell Cycle Regulation

To conclusively illustrate the functions of HBx, we analyzed the expressions of *HBx*, *Wnt-3*, *GSK-3β*, and *β-catenin* by performing semiquantitative RT-PCR. The data revealed that *Wnt-3* and *β-catenin* were downregulated, but *GSK-3β* was upregulated on HBx induction (fig. 2b); Western blotting analysis confirmed this observation and revealed that the expression of these genes was unaltered in the absence of HBx induction (fig. 2c, Tet+). In the absence of Wnt, the downstream molecule GSK-3β is reportedly activated in the Wnt-3/β-catenin pathway [14, 20]. Interestingly, we observed that GSK-3β expression increased on HBx induction (fig. 2c, Tet–) but was unaltered in the absence of HBx induction (fig. 2c, Tet+).

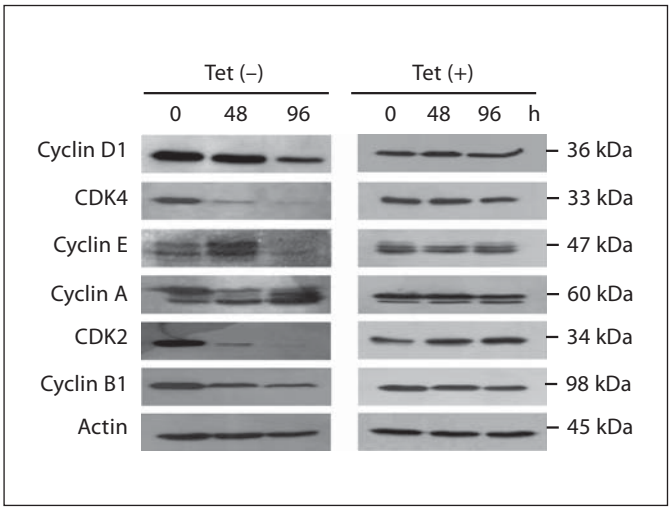

Fig. 3. HBx repressed cell proliferation and arrested cell growth at the G1 phase. Changes in the expressions of cyclin D1, CDK4, cyclin E, CDK2, and cyclin B1 with time were detected by performing Western blotting following HBx induction (Tet–) or in the absence of (Tet+) induction. Actin was used as an internal control.

Thus, the data revealed that on HBx induction, the expression of Wnt-3 was downregulated, while that of GSK-3β was upregulated. The Wnt-3 expression increased at 96 h after a steady decline from 0 to 72 h was due to cells' rescue after the cell-growth inhibition at 72 h upon HBx induction. HBx is reported to mediate cell growth, including cell death and cell survival, via proliferation and apoptosis [3]. In this regard, we also investigated the expression patterns of PCNA and p53. It is well known that p53 activation is associated either with cell cycle arrest and DNA repair or with apoptosis [17]. We observed that p53 expression was upregulated on HBx induction (fig. 2d, Tet–), while its expression was not detected in the absence of HBx induction (fig. 2d, Tet+). PCNA is considered to be a marker of proliferation during the late G1 phase, exhibiting maximal levels during the S phase [18]. Based on our data, PCNA expression was downregulated on HBx induction (fig. 2d, Tet–) but was unaltered in the absence of HBx induction (fig. 2d, Tet+). Thus, the results indicated that HBx either represses cell growth or triggers cell death. Further, the expressions of cyclin D1, CDK4, cyclin E, CDK2, and cyclin B1 were downregulated on HBx induction (fig. 3, Tet–) but were unaltered in the absence of HBx induction (fig. 3, Tet+). Thus, our data suggest that HBx induction may arrest cell growth at the G1 phase and may repress cell proliferation.

HBx Triggers Cell Death via Apoptosis

Our previous study revealed that the tumor size was significantly greater in nude mice injected with Chang liver cells than in those injected with Chang-HBx stable cell lines [32]. The HBx protein is known to be a multifunctional protein that not only activates transcriptional transactivation but also mediates cell growth via proliferation and apoptosis [3]. Therefore, to elucidate the role played by HBx in cell death, we investigated the proapoptotic effects of HBx by performing DNA fragmentation, TUNEL staining, caspase-3 activity assay, and PI staining (fig. 4). DNA fragmentation was observed at 72 and 96 h but not at 0, 24, and 48 h following HBx induction (fig. 4a, Tet–), and it was not observed in such a time-dependent manner in the absence of HBx induction (fig. 4a, Tet+). The TUNEL staining results (fig. 4b) revealed the occurrence of apoptotic phenomena such as DNA condensation and fragmentation at 48 h following HBx induction (Tet–); however, these changes were not detected at 0 h and in the absence of HBx induction (data not shown). Interestingly, caspase-3 activity was significantly elevated ($p < 0.01$) at 48 and 72 h as compared to at 0 h following HBx induction (fig. 4c, Tet–). In contrast, caspase-3 activity at 48 h ($p = 0.147364$) and 72 h ($p = 0.165836$) did not differ from that at 0 h in the absence of HBx induction (fig. 4c, Tet+). To further investigate and confirm cell death via apoptosis, cell death was measured by PI staining (fig. 4d). The apoptosis indicator subG1 was detected on HBx induction (fig. 4d, Tet–) but not in the absence of induction (fig. 4d, Tet+) with the same time course. Most importantly, the data suggested that HBx may trigger cell death via apoptosis.

Discussion

The HBx protein is one of the agents involved in hepatocarcinogenesis [3]; however, its critical role in this regard remains unclear. In this study, we investigated the negative regulation of the Wnt-3/β-catenin pathway on HBx induction in the Chang-HBx stable cell line. The Wnt/β-catenin pathway is reported to be involved in embryogenesis, embryo development, and carcinogenesis [2, 12, 25]. However, the involvement of HBx in this pathway has not been investigated in detail.

The HBx protein is known to be a multifunctional protein that not only activates transcriptional transactivation but also mediates cell growth via proliferation and apoptosis. Similar to our results (fig. 3), Sirma et al. [27] reported that HBx expression induces the block or arrest

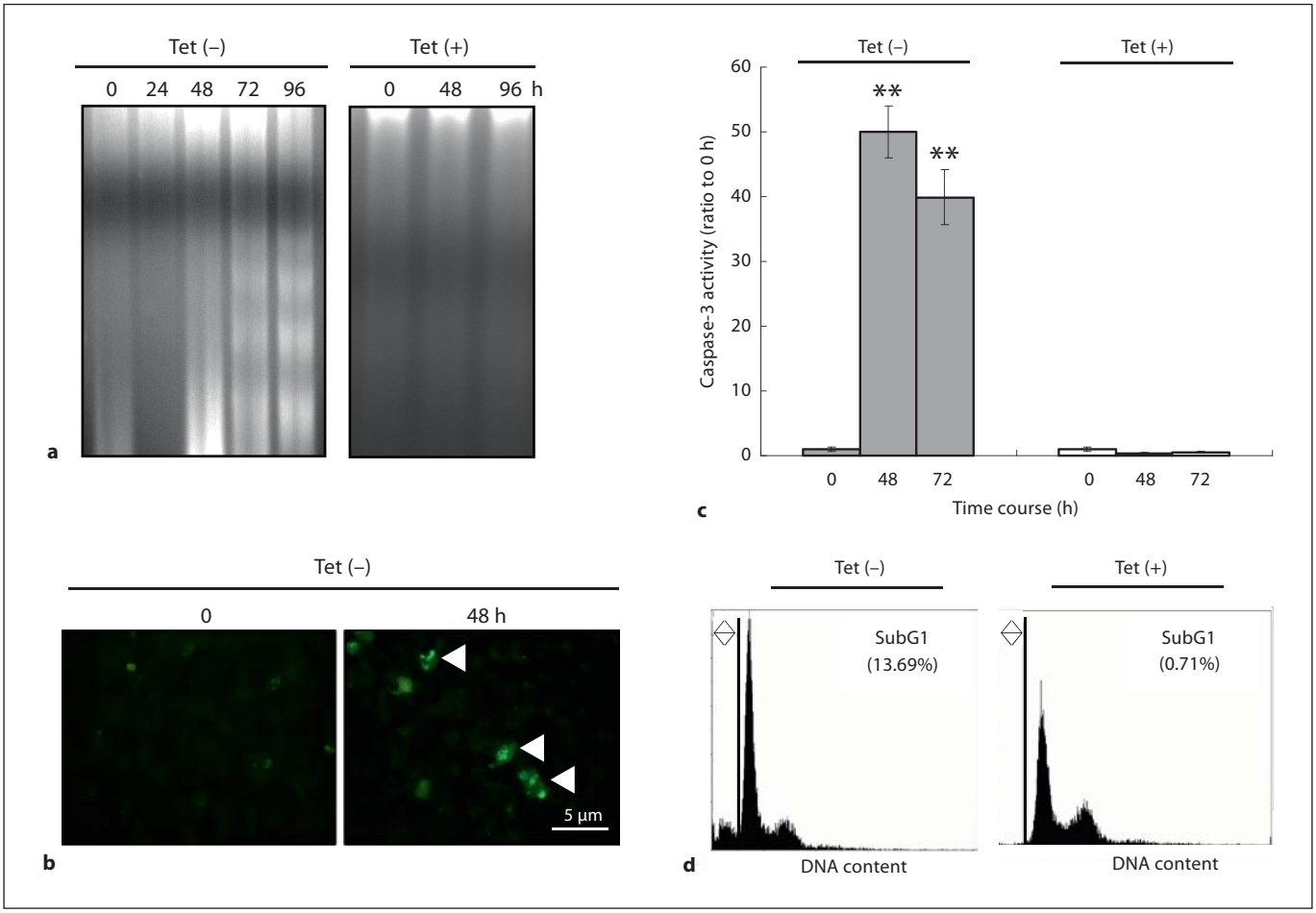

Fig. 4. HBx triggered cell death via apoptosis. **a** DNA fragmentation analysis demonstrated that the HBx-sensitized cells underwent apoptosis at 72 and 96 h but not at 0, 24, and 48 h following HBx induction (Tet–), and apoptosis was not observed in a time-dependent manner in the absence of HBx induction (Tet+). **b** TUNEL staining analysis was performed at 0 and 48 h following HBx induction (Tet–). **c** The variations in caspase-3 activity with time were determined following HBx induction (Tet–) or in the absence of (Tet+) induction. ** $p < 0.01$. **d** Cell death via apoptosis following HBx induction (Tet–) or in its absence (Tet+) at the same time course, as detected by PI staining and FACS analysis.

of the cell cycle at the G1 phase. Moreover, HBx may sensitize CCL13 cells to apoptotic killing via tumor necrosis factor α [29]. Consistent with this, many other studies have demonstrated that HBx mediates or sensitizes cells to apoptosis [9, 13, 19, 23, 26, 28, 30, 31]. In our study, the rate of cell growth was reduced on HBx induction (fig. 2a, Tet–). Further, the expressions of PCNA (fig. 2d, Tet–), cyclin D1, CDK4, cyclin E, CDK2, and cyclin B1 were downregulated on HBx induction (fig. 3, Tet–) but were unaltered in the absence of induction (fig. 3, Tet+). Thus, the data indicates that HBx induction represses cell proliferation.

Moreover, Cha et al. [6] demonstrated that HBx is essential for the activation of the Wnt-1/β-catenin pathway in hepatoma cells. However, the detailed molecular mechanism underlying HBx involvement in the Wnt-3/β-catenin pathway has not been investigated. In this study, we observed that the expressions of Wnt-3 and β-catenin were downregulated, while that of GSK-3β was upregulated on HBx induction (fig. 2b, c, Tet–); this led to repression and the arrest of cell proliferation at the G1 phase (fig. 3, Tet–). HBx coordinates the balance between proliferation and programmed cell death by inducing or inhibiting apoptosis [22]. However, we observed that HBx induction triggered cell death via apoptosis (fig. 2d, 4),

and these results are consistent with those of several other studies [9, 13, 19, 23, 26, 28, 30, 31]. Thus, our results indicated that HBx induction in the CCL13-HBx stable cell line downregulated Wnt-3/β-catenin expression and suppressed cell growth by either repressing cell proliferation or triggering cell apoptosis.

Acknowledgments

We appreciate the support of Dr. V.C. Yang at Tunghai University. This work was supported in part by grants TCVGH-T-957808 and TCVGH-T-967807.

References

1 Anzola M: Hepatocellular carcinoma: role of hepatitis B and hepatitis C viruses proteins in hepatocarcinogenesis. J Viral Hepat 2004; 11:383–393.
2 Behrens J, Lustig B: The Wnt connection to tumorigenesis. Int J Dev Biol 2004;48:477–487.
3 Bouchard MJ, Schneider RJ: The enigmatic X gene of hepatitis B virus. J Virol 2004;78: 12725–12734.
4 Branda M, Wands JR: Signal transduction cascades and hepatitis B and C related hepatocellular carcinoma. Hepatology 2006;43: 891–902.
5 Breuhahn K, Longerich T, Schirmacher P: Dysregulation of growth factor signaling in human hepatocellular carcinoma. Oncogene 2006;25:3787–3800.
6 Cha MY, Kim CM, Park YM, Ryu WS: Hepatitis B virus X protein is essential for the activation of Wnt/β-catenin signaling in hepatoma cells. Hepatology 2004;39:1683–1693.
7 Cheong JH, Yi M, Lin Y, Murakami S: Human RPB5, a subunit shared by eukaryotic nuclear RNA polymerases, binds human hepatitis B virus X protein and may play a role in X transactivation. EMBO J 1995;14: 143–150.
8 Chirillo P, Falco M, Puri PL, Artini M, Balsano C, Levrero M, Natoli G: Hepatitis B virus pX activates NF-κB-dependent transcription through a Raf-independent pathway. J Virol 1996;70:641–646.
9 Chirillo P, Pagano S, Natoli G, Puri PL, Burgio VL, Balsano C, Levrero M: The hepatitis B virus X gene induces p53-mediated programmed cell death. Proc Natl Acad Sci USA 1997;94:8162–8167.
10 Ding Q, Xia W, Liu JC, Yang JY, Lee DF, Xia J, Bartholomeusz G, Li Y, Pan Y, Li Z, Bargou RC, Qin J, Lai CC, Tsai FJ, Tsai CH, Hung MC: Erk associates with and primes GSK-3β for its inactivation resulting in upregulation of β-catenin. Mol Cell 2005;19:159–170.
11 Feitelson MA, Sun B, Satiroglu-Tufan NL, Liu J, Pan J, Lian Z: Genetic mechanisms of hepatocarcinogenesis. Oncogene 2002;21: 2593–2604.
12 Hall CL, Kang S, MacDougald OA, Keller ET: Role of Wnts in prostate cancer bone metastases. J Cell Biochem 2006;97:661–672.
13 Kim H, Lee H, Yun Y: X-gene product of hepatitis B virus induces apoptosis in liver cells. J Biol Chem 1998;273:381–385.
14 Kohn AD, Moon RT: Wnt and calcium signaling: β-catenin-independent pathways. Cell Calcium 2005;38:439–446.
15 Laurent-Puig P, Zucman-Rossi J: Genetics of hepatocellular tumors. Oncogene 2006;25: 3778–3786.
16 Lee YI, Kang-Park S, Do SI, Lee YI: The hepatitis B virus-X protein activates a phosphatidylinositol 3-kinase-dependent survival signaling cascade. J Biol Chem 2001;276: 16969–16977.
17 Levine AL: p53, the cellular gatekeeper for growth and division. Cell 1997;88:323–331.
18 Maga G, Hubscher U: Proliferating cell nuclear antigen (PCNA): a dancer with many partners. J Cell Sci 2003;116:3051–3060.
19 Miao J, Chen GG, Chun SY, Lai PP: Hepatitis B virus X protein induces apoptosis in hepatoma cells through inhibiting Bcl-xL expression. Cancer Lett 2006;236:115–124.
20 Moon RT, Kohn AD, De-Ferrari GV, Kaykas A: WNT and β-catenin signalling: diseases and therapies. Nat Rev Genet 2004;5:691–701.
21 Murakami S: Hepatitis B virus X protein: structure, function, and biology. Intervirology 1999;42:81–99.
22 Murakami S: Hepatitis B virus X protein: a multifunctional viral regulator. J Gastroenterol 2001;36:651–660.
23 Pollicino T, Terradillos O, Lecoeur H, Gougeon ML, Buendia MA: Pro-apoptotic effect of the hepatitis B virus X gene. Biomed Pharmacother 1998;52:363–368.
24 Qadri I, Maguire HF, Siddiqui A: Hepatitis B virus transactivator protein X interacts with the TATA-binding protein. Proc Natl Acad Sci USA 1995;92:1003–1007.
25 Schneikert J, Behrens J: The canonical Wnt signalling pathway and its APC partner in colon cancer development. Gut 2007;56:417–425.
26 Shintani Y, Yotsuyanagi H, Moriya K, Fujie H, Tsutsumi T, Kanegae Y, Kimura S, Saito I, Koike K: Induction of apoptosis after switch-on of the hepatitis B virus X gene mediated by the Cre/loxP recombination system. J Gen Virol 1999;80:3257–3265.
27 Sirma H, Giannini C, Poussin K, Paterlini P, Kremsdorf D, Brechot C: Hepatitis B virus X mutants, present in hepatocellular carcinoma tissue abrogate both the antiproliferative and transactivation effects of HBx. Oncogene 1999;18:4848–4859.
28 Su F, Theodosis CN, Schneider RJ: Role of NF-κB and myc proteins in apoptosis induced by hepatitis B virus HBx protein. J Virol 2001;75:215–225.
29 Su F, Schneider RJ: Hepatitis B virus HBx protein sensitizes cells to apoptotic killing by tumor necrosis factor α. Proc Natl Acad Sci USA 1997;94:8744–8749.
30 Tanaka Y, Kanai F, Kawakami T, Tateishi K, Ijichi H, Kawabe T, Arakawa Y, Kawakami T, Nishimura T, Shirakata Y, Koike K, Omata M: Interaction of the hepatitis B virus X protein (HBx) with heat shock protein 60 enhances HBx-mediated apoptosis. Biochem Biophys Res Commun 2004;318:461–469.
31 Terradillos O, Pollicino T, Lecoeur H, Tripodi M, Gougeon ML, Tiollais P, Buendia MA: p53-independent apoptotic effects of the hepatitis B virus HBx protein in vivo and in vitro. Oncogene 1998;17:2115–2123.
32 Wang JC, Hsu SL, Hwang GY: Inhibition of tumorigenicity of the hepatitis B virus X gene in Chang liver cell line. Virus Res 2004; 102:133–139.
33 Waris G, Huh KW, Siddiqui A: Mitochondrially associated hepatitis B virus X protein constitutively activates transcription factors STAT-3 and NF-κB via oxidative stress. Mol Cell Biol 2001;21:7721–7730.
34 Williams JS, Andrisani OM: The hepatitis B virus X protein targets the basic region-leucine zipper domain of CREB. Proc Natl Acad Sci USA 1995;92:3819–3823.