Hepatitis B Virus X Protein Blunts Senescence-Like Growth Arrest of Human Hepatocellular Carcinoma by Reducing Notch1 Cleavage

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One of the serious sequelae of chronic hepatitis B virus (HBV) infection is hepatocellular carcinoma (HCC). Among all the proteins encoded by the HBV genome, hepatitis B virus X protein (HBx) is highly associated with the development of HCC. Although Notch1 signaling has been found to exert a tumor-suppressive function during HCC development, the mechanism of interaction between HBx expression and Notch1 signaling needs to be explored. In this study, we report that HBx expression in hepatic and hepatoma cells resulted in decreased endogenous protein levels of Notch1 intracellular domain (ICN1) and messenger RNA levels of its downstream target genes. These effects were due to a reduction of Notch1 cleavage by HBx through the suppression of presenilin1 (Psen1) transcription rather than inhibition of Notch1 transcription or its ligands' expression. Through transient HBx expression, decreased ICN1 resulted in enhanced cell proliferation, induced G1-S cell cycle progression, and blunted cellular senescence in vitro. Furthermore, the effect of blunted senescence-like growth arrest by stable HBx expression through suppression of ICN1 was shown in a nude mouse xenograft transplantation model. The correlation of inhibited Psen1-dependent Notch1 signaling and blunted senescence-like growth arrest was also observed in HBV-associated HCC patient tumor samples. Conclusion: Our results reveal a novel function of HBx in blunting senescence-like growth arrest by decreasing Notch1 signaling, which could be a putative molecular mechanism mediating HBV-associated hepatocarcinogenesis. (HEPATOLOGY 2010;52:142-154)

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm and the third leading cause of cancer-related death in humans, with nearly 600,000 deaths annually worldwide.1,2 Chronic hepatitis B virus (HBV) infection has been identified as a major risk factor for the development of HCC, especially in southeastern Asia and sub-Saharan Africa.3-5 Several processes are involved in the development of HBV-associated hepatocellular carcinoma, including integration of HBV genes into host cell genome, sustained cycles of necrosis-inflammation-regeneration, activation of oncogenic pathways, and inact-
vation of tumor-suppressive pathways by various viral proteins.5,7

The viral genome of HBV is a partially double-stranded circular DNA that contains four overlapping open reading frames, including S for the surface or envelope gene, C for the core gene, X for the X gene, and P for the polymerase gene.8 Hepatitis B virus X protein (HBx), a 17.5-kDa nonstructural protein encoded by the X gene, is a key multifunctional regulatory protein that may participate in viral pathogenesis and carcinogenesis.9 Investigations on tumor samples from HCC patients, cell culture studies in vitro, and transgenic animal model experiments collectively support the oncogenic role of HBx in HBV-associated hepatocarcinogenesis.10-14

Notch signaling defines an evolutionary conserved local cell interaction mechanism that participates in a variety of cellular processes that involve cell fate determination, differentiation, proliferation, apoptosis, adhesion, epithelial-mesenchymal transition, migration, and angiogenesis.15 In mammals, four Notch molecules (Notch1, Notch2, Notch3, and Notch4) are single-pass, heterodimeric transmembrane proteins that serve as receptors for the five ligands (Jagged-1, Jagged-2, DLL-1, DLL-3, and DLL-4) expressed on the neighboring cells.16 Ligand binding renders the Notch receptor susceptible to two consecutive proteolytic cleavages mediated by tumor necrosis factor-α-converting enzyme (TACE) and γ-secretase, respectively, which in turn results in the release of truncated constitutively Notch intracellular domain, the active form of the Notch receptor, from the plasma membrane to translocate into the nucleus, leading to transcription of its downstream target genes such as Hes and Herp.17,18 Experimental data suggesting a tumor suppressive function of Notch1 signaling in hepatocarcinogenesis are increasing.19-23

Although the respective oncogenic role of HBx and tumor suppressive function of Notch1 in hepatocarcinogenesis have been studied, the interaction between them remains poorly understood. In this study, we tested the hypothesis of whether HBx could promote HBV-associated hepatocarcinogenesis through inhibition of Notch1 signaling activity. We show for the first time that HBx expression suppressed Notch1 signaling by decreasing Notch1 cleavage, which contributed to overcoming senescence-like growth arrest of HCC cells in vitro and in vivo. This may serve as an important molecular mechanism for HBV-associated hepatocarcinogenesis.

Materials and Methods

Cell Lines and Human HCC Samples. One immortalized liver cell line (Chang) and three hepatoma cell lines (Huh7, Hep3B, and HepG2) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum in a 5% CO2 incubator at 37°C. Twenty pairs of frozen fresh tumor liver tissues and their peripheral nontumor tissues after surgical resection were collected from HCC patients in the Nantong Cancer Hospital (Nantong University, Jiangsu, China) with informed consent and Institutional Review Board approval. Chronic infection with HBV in these patients was confirmed by way of clinical laboratory examination.

Construction of Plasmids. The full-length amplified HBx gene was inserted into the pcDNA3.1/myc and pEGFP-N3 vectors to generate pcDNA3.1/myc-HBx and pEGFP-N3-HBx plasmids. The amplified presenilin1 (Psen1) promoter was cloned into pGL3-Basic vector to generate pGL3-Psen1-Pro plasmid. pcDNA3–Notch1 intracellular domain (ICN1) plasmid was kindly provided by Dr. Jon C. Aster (Brigham and Women’s Hospital, Boston, MA). The full-length amplified Psen1 was cloned into pcDNA3.1/myc vector to generate pcDNA3.1/myc-Psen1 plasmid. The polymerase chain reaction primer sets used in this study are shown in Supporting Table 1. All plasmid constructs were confirmed by DNA sequencing.

Transient Transfection and Generation of Stable Cell Lines. Chang, Huh7, and Hep3B cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and HepG2 cells were transfected using FuGENE HD Transfection Reagent (Roche Applied Science, Mannheim, Germany) with plasmids according to each manufacturer’s protocol. For stable transfection, transfected Huh7 cells were maintained in Dulbecco’s modified Eagle’s medium containing 800 μg/mL G418 after 48 hours posttransfection. After 4 weeks of selection, individual colonies were isolated and expanded. The overexpression of HBx in these clones was confirmed by way of western blot analysis.

Western Blot Analysis. Protein extraction from cultured cells or tumor tissues and western blotting analysis were performed as described.24 Antibodies used in this study are listed in Supporting Table 2.

Quantitative Real-Time Polymerase Chain Reaction. Total RNA extraction from cultured cells and quantitative real-time polymerase chain reaction (qRT-PCR) were performed as described.25 The PCR primer sets used here are shown in Supporting Table 3.

Immunofluorescence and Flow Cytometry Analysis. Immunofluorescence and flow cytometry procedures are described in detail in the Supporting Information.

Luciferase Reporter Assay. The pGL3-Psen1-Pro plasmid and pGL3-Basic control vector were used for
assessing the effect of HBx expression on Psen1 transcriptional activity. Luciferase reporter assay was performed as in our previous study.26

5-Bromo-2'-deoxyuridine Incorporation Assay. 5-Bromo-2'-deoxyuridine (BrdU) incorporation assay was used to analyze DNA synthesis of transfected Huh7 cells. The procedures are described in detail in the Supporting Information.

Cell Proliferation Assay. Cell proliferation was measured using the Cell Counting Kit-8 (Dojindo, Kamimashiki-gun Kumamoto, Japan) according to the manufacturer’s instructions. At 24 hours posttransfection, Huh7 cells were incubated with CCK-8 for 1 hour. Cell proliferation rate was assessed by measuring the absorbance at 450 nm with the Universal Microplate Reader (BIO-TEK Instruments, Minneapolis, MN).

Colony Formation Assay. Anchorage-independent growth ability was measured using soft agar colony formation assay. The procedures are described in detail in the Supporting Information.

Cell Cycle Analysis. Flow cytometry was performed to analyze the cell cycle status of transfected Huh7 cells. The procedures are described in detail in Supplementary Methods.

Senescence-Associated β-Galactosidase Staining Assay. Senescence-associated β-galactosidase (SA-β-gal) activity was detected using the Cellular Senescence Assay Kit (Millipore, Billerica, MA) according to the manufacturer’s instructions. At day 4 posttransfection, Huh7 cells were fixed and stained at pH 6.0 with X-gal. Clear blue cytoplasmic staining cells were regarded as positive. For quantification purposes, the percentage of SA-β-gal–positive cells relative to total cells was determined by counting 100 cells in three randomly chosen fields per dish using Nikon ECLIPSE TE300 (Nikon, Tokyo, Japan).

Tumor Xenograft Experiments. All animal procedures were performed according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23, revised 1985). The procedures are described in detail in the Supporting Information.

Statistical Analysis. Experimental data are presented as the mean ± standard deviation (SD). Statistical significance of the differences in the experimental data was determined using the Student t test. Differences were considered significant at values of P < 0.05.

Results

HBx Expression Inhibits Notch1 Signaling Activity. To study the effect of HBx expression on Notch1 signaling, endogenous protein levels of ICN1 from one immortalized liver cell line (Chang) and three hepatoma cell lines (Huh7, Hep3B, and HepG2) were assayed after being transiently transfected with the HBx gene. HBx expression decreased ICN1 protein levels in all four cell lines (Fig. 1A) and was shown in a dose-dependent manner in Huh7 cells (Fig. 1B). Furthermore, qRT-PCR analysis of messenger RNA (mRNA) levels of ICN1 target genes such as Hes1, Hes5, and Herp1 in Huh7 cells transfected with increasing amounts of HBx showed that the mRNA levels of these three target genes were down-regulated by HBx expression in a dose-dependent manner (Fig. 1C). Immunofluorescence analysis on Huh7 cells transfected with HBx verified that HBx expression suppressed Notch1 signaling (Fig. 1D). To investigate whether other proteins encoded by the HBV genome, mutated HBx gene incompetent to express HBx protein (ΔHBx), or HBx expression in the presence of the entire panel of HBV proteins under the control of endogenously driven HBV replication affected Notch1 signaling, western blotting analysis of ICN1 on Huh7 cells after being transfected with HBs, HBe, or ΔHBx transfection had no significant effect on ICN1 protein levels, but HBx expression during endogenously driven HBV replication decreased ICN1 protein levels in Huh7 cells (Fig. 1E). Taken together, these results indicate that only HBx expression inhibits Notch1 signaling activity and its downstream target gene expression.

HBx Expression Decreases ICN1 Protein Level Through Reduction of Notch1 Cleavage. To further elucidate the mechanism for inhibiting Notch1 signaling activity by HBx expression, we first performed qRT-PCR to investigate the effect of HBx expression on the transcriptional level of Notch1. We were not able to show differences of Notch1 mRNA levels between control and HBx-transfected Huh7 cells using qRT-PCR (Fig. 2A). qRT-PCR and flow cytometry analyses were used to determine the effect of HBx on the expression levels of the five ligands of Notch1. Neither mRNA nor protein levels of Jagged-1, Jagged-2, DLL-1, DLL-3, and DLL-4 were affected by HBx expression (Fig. 2B,C). It was postulated that expression of HBx might suppress Notch1 signaling through the inhibition of Notch1 cleavage. Western blotting of
the protein levels of full-length Notch1 (Notch1-FL) and extracellular truncated Notch1 (NEXT1) revealed that in contrast to decreased ICN1 protein level, NEXT1 protein level was increased, whereas Notch1-FL protein level was unaffected by HBx transfection in Huh7 cells (Fig. 2D). Because Notch1 signaling activation requires two consecutive proteolytic cleavages mediated by TACE and γ-secretase, respectively, which means TACE-mediated extramembranous cleavage releases NEXT1 from Notch1-FL, and the consecutive γ-secretase–mediated intramembranous cleavage releases ICN1 from NEXT1, decreased ICN1 protein level and increased NEXT1 protein level in the presence of unaffected Notch1-FL protein level might be due to reduced ICN1 releasing from NEXT1 through inhibited γ-secretase–mediated proteolytic cleavage by HBx expression. These results suggest that HBx decreases ICN1 protein level through reduction of Notch1 cleavage rather than affecting Notch1 transcription or its ligand expression.
HBx Expression Reduces Notch1 Cleavage by Suppressing Psen1 Transcription. Two consecutive Notch1 cleavages after ligand binding are mediated by TACE and γ-secretase.\textsuperscript{18} γ-Secretase is a large protease complex composed of catalytic subunits (Psen1 and Psen2) and accessory subunits (PEN2, APH1, and Nct).\textsuperscript{27} To further characterize whether TACE or γ-secretase, and which component of γ-secretase was involved in the process of reduced Notch1 cleavage by HBx, qRT-PCR for TACE, Psen1, Psen2, PEN2, APH1, and Nct were performed on HBx-transfected Huh7 cells. Compared with control cells, HBx-overexpressing cells displayed no significant differences of TACE, Psen2, PEN2, APH1, or Nct mRNA levels, but reduction of Psen1 mRNA levels was observed (Fig. 3A). Consistent with the reduction of Psen1 mRNA levels, western blotting revealed that Psen1 protein level was also decreased after HBx transfection in Huh7 cells (Fig. 3B). To examine the effect of HBx expression on Psen1 promoter activity, luciferase
reporter assay with pGL3-Psen1-Pro plasmid and pGL3-Basic control vector were employed in transfected Huh7 cells. The relative luciferase activity of Psen1 promoter was reduced in the HBx-overexpressing cells compared with that of control cells (Fig. 3C). To assess the effect of Psen1 transcriptional suppression on decreased ICN1 protein level by HBx expression, western blotting analysis of ICN1, Psen1, HBx, and GAPDH protein in Huh7 cells transiently transfected with pcDNA3.1/myc control vector or pcDNA3.1/myc-HBx plasmid, respectively. GAPDH protein level was used as the internal control. These results demonstrate that HBx expression reduces Notch1 cleavage through suppression of Psen1 transcription.

**HBx Expression Promotes Cell Proliferation by Decreased ICN1.** Because Notch1 signaling was found to exert a tumor-suppressive effect in hepatocarcinogenesis, we predicted that HBx expression might promote hepatocarcinogenesis by decreasing ICN1. To determine whether HBx expression affected cell proliferation through a decrease in ICN1, we first performed western blotting for the proliferative cell marker, namely proliferating cell nuclear antigen expression in transfected Huh7 cells. Our results revealed that enhanced proliferating cell nuclear antigen expression after HBx transfection was reversed by ICN1 cotransfection (Fig. 4A). To further verify this observation, BrdU incorporation assay was used to assess DNA synthesis during cell proliferation by monitoring incorporation of BrdU by way of flow cytometry analysis. Our results confirmed that the increased DNA synthesis of HBx-transfected Huh7 cells was reversed by ICN1 cotransfection (Fig. 4B). In addition, cell proliferation assay using CCK-8 also
confirmed that increased cell proliferation rate of HBx-transfected Huh7 cells was reversed by ICN1 cotransfection (Fig. 4C). Subsequently, colony formation assay was used to verify whether the reduction of ICN1 by HBx expression influenced anchorage-independent growth of cells in soft agar. Consistent with the above results, colony formation was increased by HBx transfection and was mainly inhibited by ICN1 cotransfection (Fig. 4D). Overall, these results indicate that HBx expression promotes cell proliferation through decreased Notch1 signaling.

**HBx Expression Induces G1-S Cell Cycle Progression by Suppressing ICN1.** To identify whether down-regulated ICN1 by HBx expression exerted biological effects on the growth of human HCC cells, flow cytometry analysis of cell cycle was examined among HBx-transfected Huh7 cells. The induced G1-S cell cycle progression after HBx transfection was reversed by ICN1 cotransfection (Fig. 5A). In accordance with the above observation, western blotting of G1-S cell cycle regulatory proteins such as cyclin D1, cyclin D3, CDK2, and CDK4 verified that increased
expression of all four of these proteins by HBx transfection was reversed by ICN1 cotransfection (Fig. 5B). These results strongly suggest that HBx expression induces G1-S cell cycle progression by down-regulation of ICN1.

**HBx Expression Blunts Senescence-Like Growth Arrest by Down-regulating ICN1.** Cellular senescence, also termed senescence-like growth arrest, is an important intrinsic tumor suppression mechanism characterized by an irreversible cell cycle arrest and cell proliferation suppression. The above results indicated that reduction of ICN1 by HBx expression might be involved in the regulation of senescence-like growth arrest. SA-β-gal staining at pH 6.0 is a specific biochemical marker of cellular senescence. The above results indicated that reduction of ICN1 by HBx expression might be involved in the regulation of senescence-like growth arrest. SA-β-gal staining at pH 6.0 is a specific biochemical marker of cellular senescence. To investigate whether HBx affected cellular senescence by decreasing ICN1, we used SA-β-gal staining assay to measure the effect of decreased ICN1 by HBx expression on cellular senescence in transfected Huh7 cells. Results indicated that blunted cellular senescence after HBx transfection was reversed by ICN1 cotransfection (Fig. 6A). Dec1, DcR2, and cell cycle regulatory proteins such as p14ARF, p15INK4b, p16INK4a, p21WAF1/Cip, and p27Kip1 are regarded as characteristic molecular markers for cellular senescence. qRT-PCR analysis revealed that Dec1, DcR2, p14ARF, p15INK4b, p16INK4a, p21WAF1/Cip, and p27Kip1 mRNA were all down-regulated by HBx transfection but up-regulated by ICN1 cotransfection (Fig. 6B). In addition, western blotting analysis confirmed the change of Dec1, p21WAF1/Cip, and p27Kip1 protein levels in transfected Huh7 cells (Fig. 6C). These data indicate that HBx expression blunts cellular senescence by decreasing Notch1 signaling activity.

**Stable HBx Expression Blunts Cellular Senescence by Decreasing ICN1 in Nude Mice.** To further investigate the effect of decreased ICN1 by HBx on cellular senescence in vivo, tumor xenograft experiments were performed in nude mice with stably HBx-expressing Huh7 cells. Decreased ICN1 through stable HBx expression was confirmed by way of western blotting analysis (Fig. 7A). HBx expression significantly promoted...
overall tumor growth compared with the control group, as assessed by tumor volume (Fig. 7B). Four weeks after tumor xenograft, mice were sacrificed and tumor tissues were examined. Notably, HBx stably transfected Huh7 cells showed enhanced tumor growth compared with control cells (Fig. 7C,D). To determine whether stable expression of HBx blunted cellular senescence and its role in the process of enhanced tumorigenesis in nude mice, western blotting analysis for ICN1 and Dec1 was performed in six tumor tissues from two groups of nude mice. Consistent with the above experimental data in vitro, Dec1 and ICN1 protein levels were both down-regulated by HBx stable expression in vivo (Fig. 7E). Taken together, these data demonstrate that stable expression of HBx in Huh7 cells blunts cellular senescence by decreasing Notch1 signaling in nude mice.

**Decreased Psen1-Dependent Notch1 Signaling Correlates With Blunted Cellular Senescence in Tumor Tissues Obtained from HBV-Associated HCC Patients.** To ascertain whether blunted cellular senescence correlates with decreased Psen1-dependent Notch1 signaling in the presence of HBx expression during the development of HBV-associated HCC, western blotting on 20 paired HBV-associated HCC tissues and adjacent nontumor tissues was analyzed for the expression of Dec1, ICN1, Psen1, and HBx protein levels. As shown in Fig. 8 and Supporting Table 4, although no significant differences of HBx protein levels were found in most of the 20 HCC tissues compared within the relevant adjacent nontumor tissues, 11 of 20 (55%) HCC tissues had lower expression levels of Psen1, ICN1, and Dec1 compared with the relevant adjacent nontumor tissues. The expression levels
of Psen1, ICN1, and Dec1 were unexpectedly higher in three of 20 (15%) HCC tissues compared with the relevant adjacent nontumor tissues. In the other six of 20 (30%) paired patient samples, there was no significant correlation of decreased Psen1-dependent Notch1 signaling with blunted cellular senescence in the HCC tissues compared with the relevant adjacent nontumor tissues. These results show that decreased Psen1-dependent Notch1 signaling correlates with blunted cellular senescence in the majority of human HBV-associated HCC tissues.

Discussion

In the current study, we demonstrate for the first time that the suppressive effect of HBx expression on Notch1 signaling activity contributed to the blunting of senescence-like growth arrest in vitro and in vivo, providing a novel potential mechanism for HBV-associated hepatocarcinogenesis. This finding further supports our previous observation that HBx induced cell cycle progression through the up-regulation of GalTI transcriptionally, which might contribute to HBV-associated HCC development and progression.30

Notch1 signaling was reported to exert an oncogenic or tumor-suppressive function in tumorigenesis depending on the specified cell type and context.31 The roles of Notch1 signaling in the process of HCC development and liver regeneration have been studied previously. It has been reported that Notch1 signaling could inhibit human HCC cells growth by arresting cell cycle and inducing apoptosis in vitro and in vivo.20,23 Recent studies indicated that inducible inactivation of Notch1 caused nodular regenerative hyperplasia due to continuous proliferation of hepatocytes in Notch1 conditional knockout mice.21,22 Our study demonstrates that HBx expression decreased Notch1 signaling activity, thus not only promoting cell...
proliferation and inducing cell cycle progression consistent with previous reports, but also blunting senescence-like growth arrest in vitro and in vivo. In this sense, HBx might exert oncogenic function, partially by decreasing Notch1 signaling in HBV-associated hepatocarcinogenesis. In addition to previous reports in vitro and in mouse model studies, we found that Psen1-dependent Notch1 signaling decreased in 55% (11/20) of HBV-associated HCC tumor tissues compared with the relevant adjacent nontumor tissues, which may play an important role in the promotion of tumor growth in HBV-associated hepatocarcinogenesis. However, we found unexpectedly that Psen1-dependent Notch1 signaling was increased in 15% (3/20) of tumor tissues and showed no significant correlation between protein level changes of Psen1 and ICN1 in 30% (6/20) of tumor tissues compared with the paired nontumor tissues. Because the development of HCC is a multistep process, interaction of Notch1 signaling with other signal pathways in these samples cannot be excluded.

In this study, we further explored the mechanism by which HBx expression decreased Notch1 signaling activity. Psen1-dependent γ-secretase–mediated proteolytic cleavage is necessary for the release of ICN1 from plasma membrane and activation of Notch1 signaling. Interestingly, we found that HBx inhibited γ-secretase–mediated cleavage of Notch1 by suppressing proliferation and inducing cell cycle progression consistent with previous reports, but also blunting senescence-like growth arrest in vitro and in vivo. In this sense, HBx might exert oncogenic function, partially by decreasing Notch1 signaling in HBV-associated hepatocarcinogenesis. In addition to previous reports in vitro and in mouse model studies, we found that Psen1-dependent Notch1 signaling decreased in 55% (11/20) of HBV-associated HCC tumor tissues compared with the relevant adjacent nontumor tissues, which may play an important role in the promotion of tumor growth in HBV-associated hepatocarcinogenesis. However, we found unexpectedly that Psen1-dependent Notch1 signaling was increased in 15% (3/20) of tumor tissues and showed no significant correlation between protein level changes of Psen1 and ICN1 in 30% (6/20) of tumor tissues compared with the paired nontumor tissues. Because the development of HCC is a multistep process, interaction of Notch1 signaling with other signal pathways in these samples cannot be excluded.

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Psen1 transcription, thus down-regulating the protein level of ICN1. Therefore, inhibited Psen1 transcription could serve as an oncogenic function of HBx in HBV-associated hepatocarcinogenesis. It has been reported that Psen1/γ-secretase functions as a tumor suppressor in epithelia by regulating EGFR and Notch pathways. In another report, loss of Psen1 promoted skin tumorigenesis by enhancing Wnt/β-catenin signaling in Psen1 knockout mice. Psen1 was also reported to serve as a scaffold protein that affects β-catenin phosphorylation and stability independently of the Wnt-regulated axin-CK1z complex. HBx has been reported to be essential for the activation of Wnt/β-catenin signaling in hepatoma cells. Together with previous studies, our results suggest that suppressed Psen1 transcription by HBx might link decreased Notch1 signaling with activated Wnt/β-catenin signaling in the complex process of HBV-associated hepatocarcinogenesis.

It has been reported that HBx might contribute to carcinogenesis through binding with p55γ-secretase, which is a protein isolated from senescent human cells and similar to Notch ligand. Recent investigation revealed that significantly diminished p16INK4a, p21WAF1/Cip1, and p27Kip1 cell cycle checkpoint markers; decreased telomere length; increased DNA damage markers; and decreased SA-β-gal activity were found in HBV-associated HCC tumor tissues compared with normal hepatocytes. Our current study reveals that decreased senescence-like growth arrest was found in HBx-transfected hepatoma cells and HBV-associated HCC tumor tissues. Senescence-like growth arrest, which limits the replicative capacity of uncontrolled cells, thus preventing the proliferation of tumor cells, plays an important tumor-suppressor role in cancer development. The blunted senescence-like growth arrest by HBx shown in this study could extend the replicative capacity of transformed cells and result in promoting cell proliferation, thus exerting oncogenic function in HBV-associated hepatocarcinogenesis.

In conclusion, our results presented here reveal a novel association between HBx expression and inhibited Notch1 signaling in the development of HBV-associated HCC. This inhibition was mediated through decreased Notch1 cleavage by suppressing Psen1 transcription. The inhibited Notch1 signaling could enhance tumor growth through blunting senescence-like growth arrest, thereby revealing a putative molecular mechanism for the development and progression of HBV-associated HCC. These results provide clues for future potential clinical application of Notch1 signaling reactivation to prevent hepatocarcinogenesis in the high-risk group of chronic hepatitis B patients.

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