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Hepatitis B virus X protein reduces starvation-induced cell death through activation of autophagy and inhibition of mitochondrial apoptotic pathway

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ABSTRACT

The hepatitis B virus X protein (HBx) has been implicated in the development of hepatocellular carcinoma (HCC) associated with chronic infection. As a multifunctional protein, HBx regulates numerous cellular pathways, including autophagy. Although autophagy has been shown to participate in viral DNA replication and envelopment, it remains unclear whether HBx-activated autophagy affects host cell death, which is relevant to both viral pathogenicity and the development of HCC. Here, we showed that enforced expression of HBx can inhibit starvation-induced cell death in hepatic (L02 and Chang) or hepatoma (HepG2 and BEL-7404) cell lines. Starvation-induced cell death was greatly increased in HBX-expressing cell lines treated either with the autophagy inhibitor 3-methyladenine (3-MA) or with an siRNA directed against an autophagy gene, *beclin 1*. In contrast, treatment of cells with the apoptosis inhibitor Z-Vad-fmk significantly reduced cell death. Our results demonstrate that HBx-mediated cell survival during starvation is dependent on autophagy. We then further investigated the mechanisms of cell death inhibition by HBx. We found that HBx inhibited the activation of caspase-3, an execution caspase, blocked the release of mitochondrial apoptogenic factors, such as cytochrome *c* and apoptosis-inducing factor (AIF), and inhibited the activation of caspase-9 during starvation. These results demonstrate that HBx reduces cell death through inhibition of mitochondrial apoptotic pathways. Moreover, increased cell viability was also observed in HepG2.2.15 cells that replicate HBV and in cells transfected with HBV genomic DNA. Our findings demonstrate that HBx promotes cell survival during nutrient deprivation through inhibition of apoptosis and activation of autophagy. This highlights an important potential role of autophagy in HBV-infected hepatocytes growing under nutrient-deficient conditions.

1. Introduction

Hepatitis B virus (HBV) is one of the most significant human pathogens, with an estimated 350 million chronically infected people worldwide. Many chronically infected individuals will eventually acquire severe liver disease that may progress to hepatocellular carcinoma (HCC), which is the fifth most common cancer and the third leading cause of cancer death worldwide [1]. Although chronic infection of HBV has been linked epidemiologically to the development of HCC for more than 40 years [2], the mechanisms by which HBV infection results in HCC are still unclear. The hepatitis B virus X protein (HBx) has generally been viewed as an oncoprotein in viral carcinogenesis. It is involved in liver cell transformation because of its pleiotropic effects on cell cycle regulation, activation of signaling pathways, inhibition of DNA repair, and modulation of apoptotic pathways [3].

Macroautophagy (hereafter referred to as autophagy) is a fundamental catabolic process in which cellular components can be sequestered within double membrane-bound vesicles and targeted to lysosomes for degradation and use as an energy source [4]. Autophagy occurs in most eukaryotes and contributes to the clearance of damaged organelles, misfolded proteins, and pathogens. Alterations in autophagy have been proposed to contribute to human pathologies including cancer [5]. There is accumulating evidence that autophagy plays seemingly contradictory roles in cancer. An anticancer role for autophagy is implied by the inactivation of pro-autophagy genes (e.g. *beclin 1*, *UVRAG*, and *TSC1*) and activation of anti-autophagy genes (e.g. *PI3KCI*, *Akt*, and *Ras*) in human cancers. On the other hand, the tumor microenvironment is commonly deprived of nutrients and oxygen. Cancer cells growing under such conditions may exploit autophagy for survival; such improved autophagic capabilities will benefit the cancer cells [6].

Abbreviations: HBV, hepatitis B virus; HBx, HBV X protein; HCC, hepatocellular carcinoma; 3-MA, 3-methyladenine; siRNA, small interfering RNA; LC3, microtubule-associated proteins light chain 3; AIF, apoptosis-inducing factor.

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We have previously demonstrated that HBx sensitizes cells to starvation-induced autophagy via upregulation of the expression of autophagy gene *beclin 1* [7]. Results from another group also indicated that HBx induces autophagy in liver cell lines and the livers of transgenic mice; such activation of autophagy is required for viral DNA replication [8]. However, it remains unknown whether the regulation of autophagy by HBx has any relationship to host cell viability, which is potentially relevant for both viral pathogenicity in HBV infection as well as the late development of HCC.

In this study, we investigated the effect of HBx on cell viability of hepatic or hepatoma cell lines. Our results showed that HBx increases cell survival rather than cell death during nutrient depletion. HBx-promoted cell survival depends on the activation of autophagy. HBx inhibited caspase-3 activity and blocked the mitochondrial apoptotic pathway induced by starvation. Suppression of autophagy impaired the anti-apoptosis activity of HBx. We suggest that HBx reduces starvation-induced cell death through autophagy.

2. Materials and methods

2.1. Cell lines and culture conditions

Human hepatic cell lines (L02 and Chang) and hepatoma cell lines (BEL-7404 and HepG2) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The HepG2.2.15 cell line was provided by Prof. Yumei Wen. All cells were cultured in the recommended media supplemented with 10% (vol/vol) fetal bovine serum, 100 U/mL penicillin, and streptomycin at 37 °C in 5% CO_2. HBx stable expression cell lines, including *HBX*/7404, *HBX*/Chang, *HBX*/HepG2, and *HBX*/L02, and control cell lines were generated by stable transfection of pcDNA3-Flag-HBx or pcDNA3-Flag plasmids as described in our previous study [7].

2.2. Transfection and RNA interference

The plasmids pcDNA3-Flag-Beclin1, pcDNA3-Flag-HBx, and pEGFP-LC3, were generated as described previously [7]. The plasmid p3.8II contains the wild-type HBV genome, and p3.8IIXm consists of an *HBX*-mutated HBV genome (provided by Prof. Wang Yuan). All transfections were performed using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's protocol. For the RNA interference assay, the cells were transfected with annealed double-stranded Beclin 1 small interfering RNA (*beclin 1* siRNA) or a nonspecific scrambled control siRNA at a final concentration of 40 nM as described previously [7].

2.3. Autophagy assays

For starvation, the cells were incubated in serum-free Earle's balanced salt solution (EBSS; starvation medium; Invitrogen) for indicated number of hours. Autophagy was measured as described previously [7]. Briefly, the cells were transfected with pEGFP-LC3 and were maintained in normal or starvation medium on coverslips. The cellular localization pattern of GFP-LC3 was photographed using a Leica TCS SP2 confocal microscope (Leica, Wetzlar, Germany). The percentage of GFP-LC3-positive cells with GFP-LC3 punctate dots was determined from three independent experiments.

2.4. Cell viability analysis

The cells were incubated in starvation medium for indicated number of hours in the presence or absence of Z-Vad-fmk (25 μM; BD Pharmingen) or 3-methyladenine (3-MA; 10 mM; Sigma) or dimethyl sulfoxide (DMSO; 1 μL; Sigma). Cells were observed under an inverted Leica DMIL microscope (Leica, Wetzlar, Germany). Cell viability was assessed by propidium iodide (PI; 1 μg/ml) staining for 15 min at 37 °C followed by fluorescence activated cell sorter (FACS) analysis using a FACSCalibur flow cytometer (BD Biosciences). All experiments were repeated three times. Data were analyzed by WinMDI29.

2.5. Western blotting analysis

The cells were solubilized in SDS-sample buffer containing 2% SDS, boiled, and used for western blotting with anti-LC3 (Novus Biologicals), anti-cleaved-caspase 3, anti-caspase 9 (Cell signaling), anti-AIF (Santa Cruz Biotechnology), anti-cytochrome *c* (BD Biosciences Pharmingen), and anti-actin (1:3000; Sigma).

2.6. Isolation of cytosolic and mitochondrial pellet fractions

Cytosolic and mitochondrial pellet fractions were prepared as described previously [9]. Briefly, the cells were lysed in lysis buffer (80 mM KCl, 250 mM sucrose, and 50 μg/ml digitonin (Sigma),1 mM DTT, and complete proteinase inhibitors (Roche Diagnostics)), and centrifuged at 10,000*g* for 5 min. The supernatants were designated cytosolic fractions. The pellets were resuspended in lysis buffer, lysed by freeze–thaw, and then centrifuged at 20,000*g* for 10 min. The resulting supernatants, containing mitochondrial proteins, were designated mitochondrial pellet fractions.

2.7. Immunofluorescence analysis

Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100, and then stained with anti-cytochrome *c* (BD Biosciences Pharmingen) or anti-AIF (Santa Cruz Biotechnology) via standard techniques. Images were obtained with a confocal microscope.

2.8. Statistical analysis

The data have been presented as the mean ± standard deviation (x ± SD) or as the mean ± standard error of the mean (x ± SEM). The significance of the differences was determined by Student's t-test. Differences with a P value of <0.05 were considered statistically significant. Statistical analyses were performed in Microsoft Excel.

3. Results

3.1. Stable expression of HBx enhances autophagy and promotes cell survival during starvation

The hepatoma cell lines HepG2 and BEL-7404 were stably transfected with pcDNA3-Flag-HBx or pcDNA3-Flag vector to establish the HBx stable cell lines *HBX*/HepG2 and *HBX*/BEL-7404 and their control cell lines HepG2 Control and BEL-7404 Control. The confocal microscopic images and quantitation of the autophagic cells in the HBx-stable and control cell lines (Fig. 1A) confirmed our previous finding that HBx enhances autophagy following nutrient deprivation [7]. Moreover, we observed that the control cells were shrunken and rapidly lost their attachment to the substrate, whereas most of the HBx stable cells remained attached to the plate after 48 h of starvation (Fig. 1B); the percentages of cell death (PI-positive cells) in HBx stable cells were significantly lower than their control cells (35.0% vs. 64.1% for *HBX*/HepG2 vs. HepG2 Control, $P < 0.01$; 17.7% vs. 47.7% for *HBX*/BEL-7404 vs. BEL-7404 Control, $P < 0.001$, Fig. 1C). Prolonged starvation (72 h) resulted in

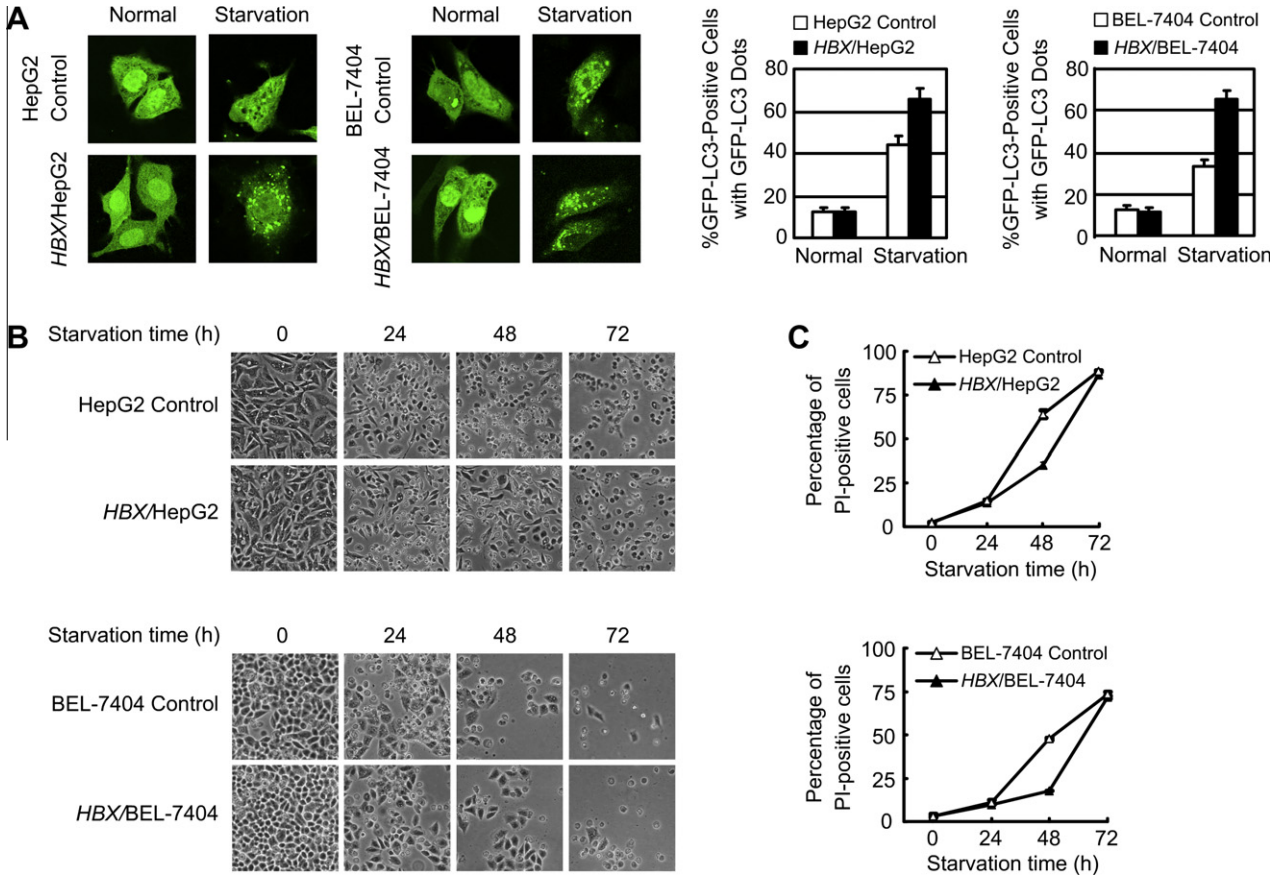

Fig. 1. HBx increases autophagy and cell viability during starvation. (A) Representative confocal images of GFP-LC3 staining, and quantitation of the autophagic cells in *HBX*/HepG2, *HBX*/BEL-7404 and their control cell lines under 24 h of starvation. (B) Representative phase-contrast microscopy images, and (C) Quantitation of cell viability of the above cell lines under starvation conditions over a 72-h time course. Cell viability was assessed by PI staining and FACS analysis at 0, 24, 48 and 72 h. Data are shown as mean ± SEM for three independent experiments.

massive cell death in both HBx stable cells and control cells. Results from the 72-h time course assay showed that cell death was inhibited most significantly at 48 h of starvation in HBx stable cells relative to control cells. We therefore chose the 48-h starvation condition for the following studies. Our results demonstrate that enforced expression of HBx enhances autophagy and resistance to starvation-induced cell death.

3.2. HBx-promoted cell survival during starvation is autophagy-dependent

The HBx stable cell lines (*HBX*/L02, *HBX*/Chang, *HBX*/HepG2 and *HBX*/BEL-7404), and their respective controls were cultured in normal or starvation medium containing 3-MA, a pharmacological inhibitor of autophagy, or Z-Vad-fmk, a broad-spectrum caspase inhibitor, for 48 h followed by PI staining and FACS analysis. As shown in Fig. 2A, starvation-induced cell death was significantly reduced in HBx stable cell lines compared to controls, confirming the above findings that HBx increases cell viability during starvation. Cell death was greatly increased in HBx stable cells treated with 3-MA (3-MA treatment vs. DMSO control, $P < 0.05$; Fig. 2A), indicating that inhibition of autophagy blocked HBx's effects on cell survival. In contrast, cell death was suppressed in both HBx stable cells and control cells treated with Z-Vad-fmk (Z-Vad-fmk treatment vs. DMSO control, $P < 0.05$, Fig. 2A). Moreover, when autophagy and apoptosis were simultaneously inhibited with 3-MA and Z-Vad-fmk, we did not observe the increased cell death associated with suppression of autophagy (Z-Vad-fmk plus 3-MA treatment vs. 3-MA only or vs. DMSO control, $P < 0.05$; Fig. 2A). Similar results were obtained from all four cell lines tested, indicating that the promotion of cell survival from starvation by HBx is dependent on autophagy. When cells were cultured in the normal medium, no significant increased or decreased cell death was induced by HBx ($P > 0.05$, Supplementary Fig. S1).

Our previous work revealed that the expression of *beclin 1*, an essential gene for the initiation of autophagy, was upregulated by HBx [7]. Here we examined the effect of *beclin 1* on HBx-promoted cell survival. Overexpression of *beclin 1* enhances cell survival during starvation (Supplementary Fig. S2A). We used siRNA to knockdown *beclin 1* in HBx stable cell lines (*HBX*/HepG2 and *HBX*/Chang) and control cell lines. As shown in Fig. 2B, after *beclin 1* siRNA transfection, HBx did not inhibit cell death, and the percentages of cell death in *HBX*/HepG2 and *HBX*/Chang were identical to those in their Control cells during starvation ($P > 0.05$). After transfection with a control siRNA, the *HBX*/cells still exhibited reduced cell death compared to control cells during starvation ($P < 0.05$ for *HBX*/HepG2 vs. HepG2; $P < 0.01$ for *HBX*/Chang vs. Chang, Fig. 2B). These results demonstrate that HBx-mediated suppression of cell death is blocked by *beclin 1* siRNA, suggesting that Beclin 1 is required for HBx-enhanced cell viability during starvation. Under normal conditions, there was no difference in cell viability between *HBX*/cells and their control cells ($P > 0.05$) or those treated with *beclin 1* siRNA ($P > 0.05$, Supplementary Fig. S2B).

To determine whether the effect of autophagy on HBx-inhibited apoptosis reflects the events in HBV-infected cells, we performed additional cell viability assays comparing non-transfected HepG2 cells with HepG2.2.15 cells that constitutively replicate HBV and

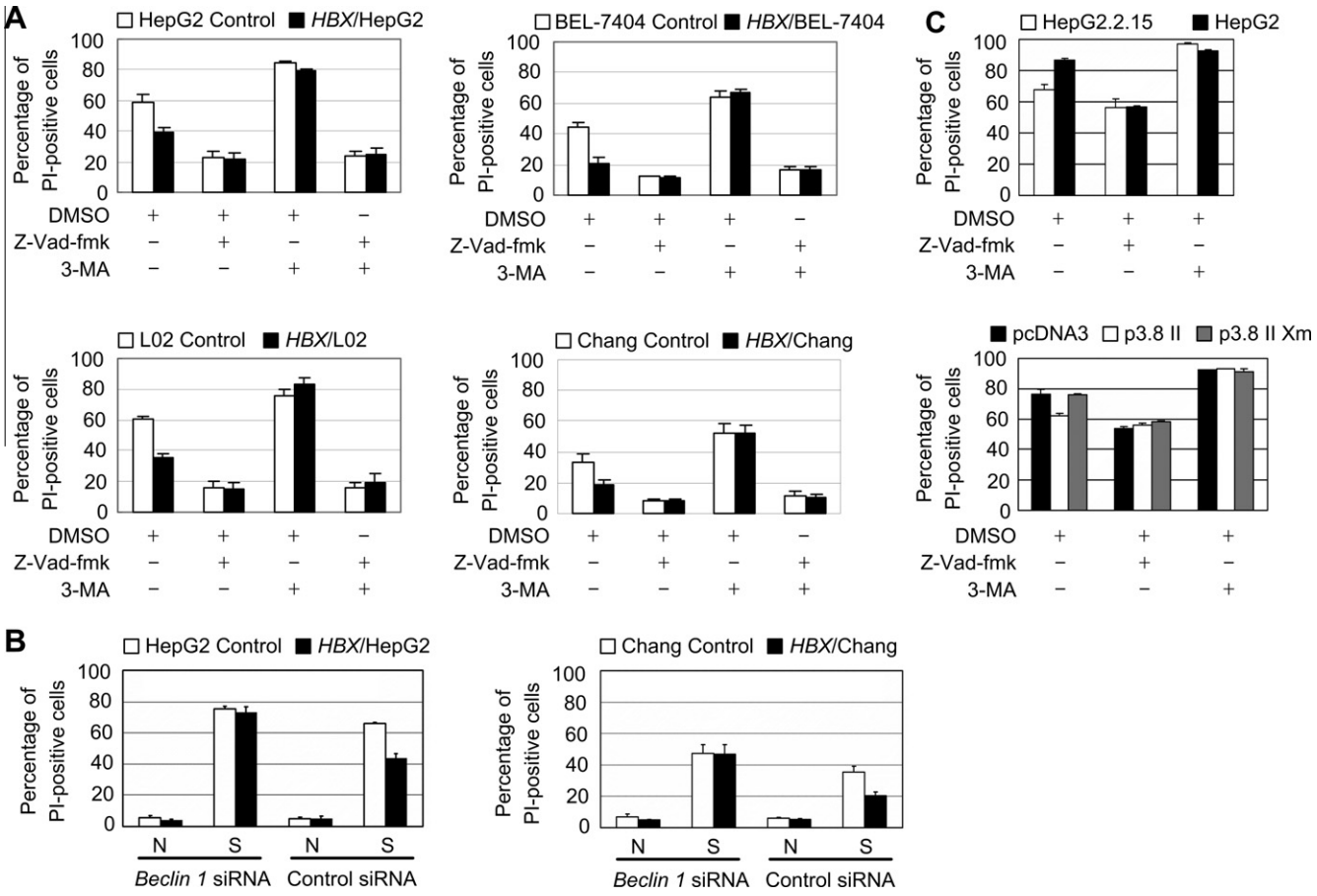

Fig. 2. HBx reduces cell death through autophagy. (A) Quantitation of cell viability in the HBx stable cell lines (*HBX*/HepG2, *HBX*/BEL-7404, *HBX*/L02, and *HBX*/Chang) and their control cell lines. The cells were cultured in a starvation medium for 48 h with 3-MA, Z-Vad-fmk, or DMSO as indicated. (B) Quantitation of cell viability in the HBx stable cell lines and their control cell lines. The cells were transfected with *beclin 1* siRNA or a scrambled control siRNA as indicated. After 72 h posttransfection, the cells were cultured in a normal or starvation medium for 48 h. (C) Quantitation of cell viability in HepG2 and HepG2.2.15 cells (upper panel), and in the HepG2 cells transiently transfected with p3.8II, p3.8IIxm, or pcDNA3 control plasmid as indicated (lower panel). After 36 h posttransfection, the cells were cultured in a starvation medium for 48 h. Cell viability was assessed by PI staining and FACS analysis. Data are shown as mean ± SEM for three independent experiments.

with cells transiently transfected with the p3.8II plasmid containing the wild-type HBV genome or with p3.8IIxm, an HBx-mutated HBV genome. We found that cell death under starvation conditions was reduced in HepG2.2.15 cells and p3.8II-transfected cells, but not in p3.8IIxm-transfected cells, as compared with their respective controls (Fig. 2C). These increases of cell viability also could be blocked by 3-MA. As expected, Z-Vad-fmk inhibited cell death in all tested cells. These results demonstrate that HBV infection also reduces cell death during starvation in an autophagy dependent manner. Taking together, we concluded that HBx-promoted cell survival during starvation is autophagy-dependent.

3.3. HBx reduces starvation-induced caspase-3 activation

Starvation is a typical stimulus for apoptotic cell death. We therefore sought to determine whether HBx inhibits apoptosis to reduce cell death during starvation. Caspase-3 is an effector caspase that performs downstream execution steps of apoptosis. Cleaved caspase-3 is considered an indicator of late-stage apoptosis [10]. We examined the effect of HBx on the activation of caspase-3. Western blotting analysis showed that the cleaved form of caspase-3 appeared at 48 h of starvation, and the level of cleaved caspase-3 in the HBx-expressing cell line *HBX*/ HepG2 was lower than in HepG2 control cells (Fig. 3A). Thus, HBx may delay the activation of caspase-3 during starvation. Meanwhile, the autophagic marker LC3 was also analyzed. LC3 is converted from its cytosolic form (LC3-I) to the autophagosome-associated form (LC3-II) during autophagy [11]. Our results showed that the LC3-II appeared at 24 h of starvation, earlier than cleaved caspase-3, and the amount of LC3-II was increased in *HBX*/HepG2 cells comparing with controls. After 48 h of starvation, LC3-II levels were increased in both *HBX*/HepG2 and HepG2 control cells (Fig. 3A). These results indicated that HBx enhanced autophagy and inhibited apoptosis during starvation.

We further examined whether autophagy affects HBx-mediated inhibition of caspase-3 activation. Under starvation conditions (at 48 h), treatment of *HBX*/HepG2 and control cells with the caspase inhibitor Z-Vad-fmk completely inhibited caspase-3 activation but did not affect LC3 conversion (Fig. 3B). After treatment of cells with the autophagy inhibitor 3-MA, LC3-II levels were dramatically reduced in both *HBX*/HepG2 and control cells. However, cleaved caspase-3 levels in *HBX*/HepG2 cells were increased compared to DMSO-treated *HBX*/HepG2 cells (Fig. 3B). These results indicate that autophagy contributes to HBx's inhibition of caspase-3 activation, implying that autophagy might play a role in suppressing apoptosis.

3.4. HBx inhibits apoptosis through the mitochondrial apoptotic pathway

To identify the anti-apoptotic function of HBx during starvation, we monitored the release of the mitochondrial apoptogenic

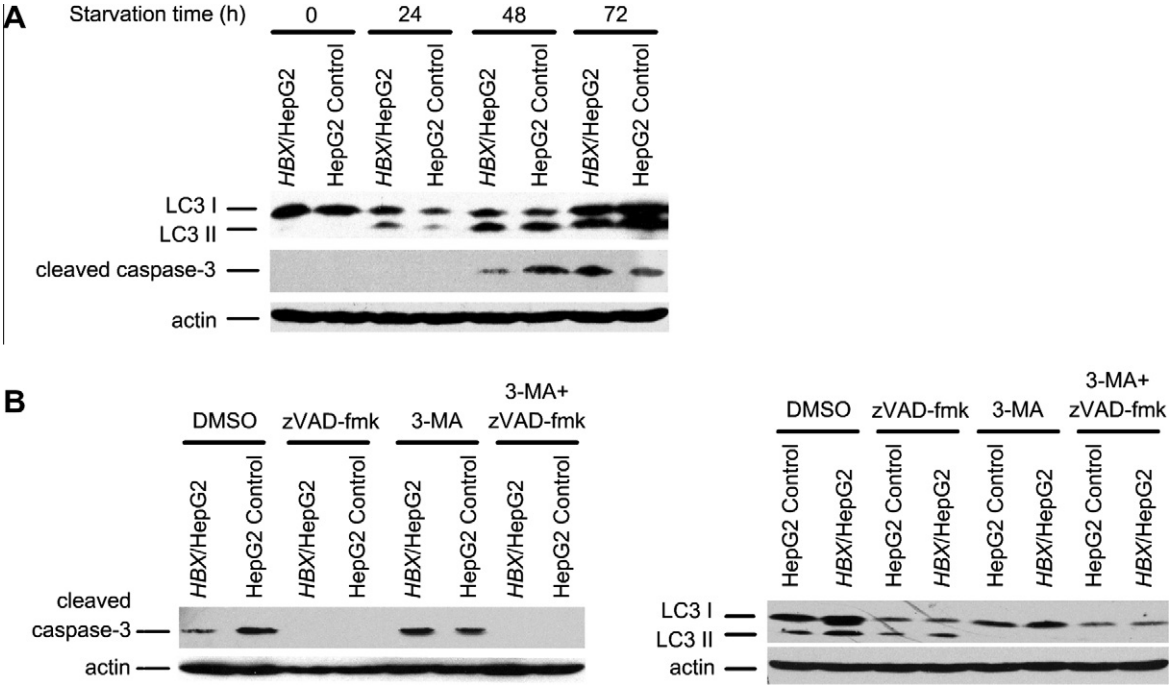

Fig. 3. HBx reduces caspase-3 activity during starvation. Western blot analysis of active caspase-3 (cleaved caspase-3), LC3 inactive form (LC3-I), and LC3 active form (LC3II) in *HBX*/HepG2 and HepG2 Control cell lines. Actin was used as a loading control. (A) Cells were starved at various times as indicated. (B) Cell were starved for 48 h and treated with 3-MA, Z-VAD-fmk, or DMSO as indicated.

factors cytochrome *c* and apoptosis-inducing factor (AIF), as well as AIF nuclear translocation, which is considered the commitment step of apoptosis [12]. Immunofluorescence analysis showed that HepG2 control cells exhibited diffuse cytosolic patterns of cytochrome *c* and AIF staining after 48 h of starvation, indicating that both were released from mitochondria. In contrast, cytochrome *c* and AIF staining in *HBX*/HepG2 cells remained punctate after starvation, indicating that HBx blocked the release of these mitochondrial proteins (Fig. 4A). Moreover, immunofluorescence analysis showed that most AIF in *HBX*/HepG2 cells remained outside the nucleus as compared to control cells, suggesting that HBx also blocked nuclear translocation of AIF during starvation (Fig. 4A).

We next investigated the effect of autophagy on HBx-inhibited release of mitochondrial proteins. Western blotting analysis showed that cytoplasmic levels of cytochrome *c* and AIF were decreased in *HBX*/HepG2 cells compared to control cells after 48 h of starvation, confirming that HBx reduces the release of cytochrome *c* and AIF (Fig. 4B). Treatment of *HBX*/HepG2 cells with 3-MA resulted in accumulation of cytochrome *c* and AIF in the cytosolic fractions, indicating that 3-MA blocks the effect of HBx on the release of mitochondrial proteins (Fig. 4B).

Furthermore, we measured the activation of caspase-9, the initiator caspase, downstream of cytochrome *c* in the mitochondrial apoptotic pathway [13]. After 24 h of starvation, *HBX*/HepG2 cells contained lower levels of cleaved caspase-9 than did HepG2 control cells (Fig. 4C), indicating that HBx inhibits caspase-9 activation. Treatment of *HBX*/HepG2 cells and control cells with 3-MA increased the amount of cleaved caspase-9 in *HBX*/HepG2 cells after 48 h of starvation, whereas administration of Z-Vad-fmk with or without 3-MA inhibited caspase-9 (Fig. 4C), suggesting a role for autophagy in HBx inhibition of caspase-9 activation. Together, these results demonstrate that HBx inhibits apoptosis during starvation through a mitochondrial pathway.

4. Discussion

HBx behaves as a multifunctional protein that regulates HBV replication, cellular transcription, cell cycle progression, cell growth, and apoptotic cell death. The ability of HBx to modulate cell viability is potentially relevant for both viral pathogenicity in HBV infection as well as the late development of HCC. Numerous studies have linked HBx activity to either promotion of cell survival (anti-apoptotic effect) [14,15] or induction of cell death (pro-apoptotic effect) [16,17] in HBx expressing cells with or without various apoptotic stimuli. Therefore, the fate of infected cells expressing HBx is likely to be determined by signals of viral, cellular and environmental origin, and the basis for HBx function in such processes remains largely elusive.

In this study, we showed that stable expression of HBx promotes cell survival in hepatic (L02 and Chang) and hepatoma (HepG2 and BEL-7404) cell lines grown under nutrient deprivation (Fig. 1A and B, and 2A). The cell death induced by starvation occurs mainly through apoptosis because addition of the general caspase inhibitor Z-Vad-fmk significantly inhibited cell death in all tested cells (Fig. 2A). The pro-survival effect of HBx was confirmed in the HBV-replicative cell line HepG2.2.15 and in HepG2 cells transiently transfected with HBV genomic dimmers (Fig. 2C). The HepG2.2.15 cell line is derived from the hepatoblastoma cell line HepG2 by transfection of tandem copies of the HBV genome [18]. Because HepG2.2.15 cells can constitutively replicate HBV DNA and produce natural HBV particles, it is widely used as an in vitro model for the study of viral infection. This supports the notion that promotion of cell survival by HBx is not an artifact of overexpression of HBx in our system.

The effect of HBx on the apoptotic pathway was also monitored in starved HepG2 cells. We found that HBx blocked the release of cytochrome *c* and AIF from mitochondria (Fig. 4A and B) and inhibited the downstream activity of caspase-9 (Fig. 4C) and caspase-3 (Fig. 4A). Our observations clearly demonstrate an anti-apoptotic

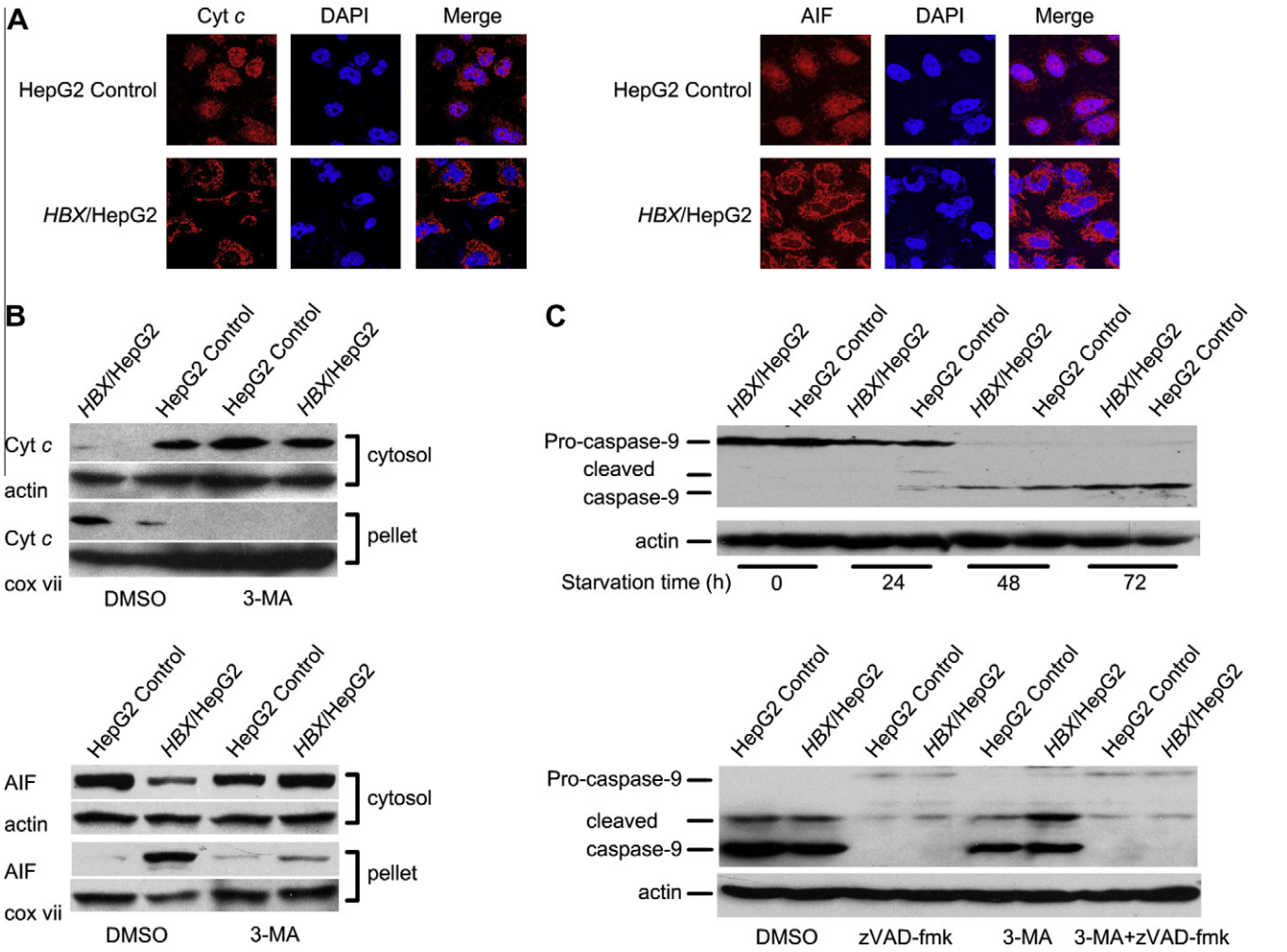

Fig. 4. HBx inhibits the release of mitochondrial apoptogenic factors and reduces caspase-9 activity during starvation. (A) Immunolocalization of ytochrome *c* (left panel) and AIF (right panel) in *HBX*/HepG2 and HepG2 Control cells under 48 h of starvation. Cytochrome *c* and AIF were stained with specific antibodies (red), the nucleus was stained with DAPI (blue), and colocalization was detected by merging the two channels. (B) Western blot analysis of cytochrome *c* (upper panel) and AIF (lower panel) in cytosolic and pellet fractions prepared from the above cells under 48 h of starvation and treated with DMSO or 3-MA as indicated. Actin and cox vii were used as cytosolic and pellet fraction markers respectively. (C) Western blot analysis of pro-caspase-9 and cleaved caspase-9 levels in the above cells at various times after starvation (upper panel), or under 48 h of starvation and treated with 3-MA, Z-VAD-fmk, or DMSO as indicated (lower panel)(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

function of HBx via the mitochondrial pathway. A previous report also showed that HBx protein is a potent caspase 3 inhibitor; cells synthesizing HBx were resistant to various apoptotic stimuli, such as growth factor depletion, tumor necrosis factor α, or anti-Fas antibody administration [19]. The mechanisms for these processes were revealed. For example, HBx protects against anti-Fas-mediated apoptosis by inducing NF-kappa B [14] and SAPK/JNK signaling [15]. However, there was no evidence as to whether HBx pro-survival activity involves cross-talk between apoptosis and autophagy.

Through self-degradation of cellular components, autophagy not only provides nutrients to maintain vital cellular functions during starvation, but also removes superfluous cells or damaged organelles and invasive pathogens [5]. On the other hand, many viruses have developed multi-pronged strategies to avoid autophagic degradation or utilize aspects of host autophagy to their own advantage [20]. Our previous work has demonstrated that HBx enhances starvation-induced autophagy by upregulation of *beclin 1* gene expression [7]. Subsequent studies from other laboratories have confirmed the enhancement of autophagy in HBV infection and pointed out the key roles of autophagy in viral DNA replication and envelopment [8,21]. Here, we have provided evidence that HBx promotion of cell survival was greatly attenuated by the autophagy inhibitor 3-MA and by *beclin 1* siRNA (Fig. 2). The inhibitory effect of HBx on mitochondrial protein release and caspase activation was completely blocked by the autophagy inhibitor 3-MA (Fig. 3 and 4). Our results demonstrate for the first time that the anti-apoptotic function of HBx can be suppressed by autophagy inhibitors, implying cross-talk between apoptosis and autophagy.

In conclusion, our study demonstrates that HBx inhibits apoptosis and increases cell viability in hepatic and hepatoma cell lines during nutrient deprivation. This anti-apoptosis and pro-survival function of HBx is dependent on enhanced autophagy by HBx. Our results propose an important role for autophagy in HBV-infected hepatocytes growing under nutrient-deficient conditions. Further understanding of HBx-regulated cross-talk between autophagy and apoptosis may shed a light on the development of HBV-associated HCC and lead to the development of new therapeutic strategies for the treatment of HBV patients.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.10.013.

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