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HBx-elevated SIRT2 promotes HBV replication and hepatocarcinogenesis

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ABSTRACT

Sirtuin 2 (SIRT2) is a class III histone deacetylase that has been implicated to promote HCC development. However, the functional role of SIRT2 in HBV is still unclear. In this study, we found that HBV could upregulate SIRT2 expression. Additionally, HBx could activate SIRT2 promoter to upregulate the mRNA and protein level of SIRT2. Furthermore, we found that SIRT2 could facilitate HBV transcription and replication. Finally, we demonstrated that upregulation of SIRT2 by HBx promoted hepatocarcinogenesis. In summary, our findings revealed a novel function of SIRT2 in HBV and HBV-mediated HCC. First, SIRT2 could promote HBV replication. And then HBx-elevated SIRT2 could enhance the transformation of HBV-mediated HCC. Those findings highlight the potential role of SIRT2 in HBV and HBV-mediated HCC by interaction with HBx.

1. Introduction

Silent information regulator 2 (SIR2) are the highly conserved NAD^+ −dependent histone deacetylases which has been known to promote life span and mediate gene silencing in yeast [1]. In human, there are seven members in this family (SIRT1−SIRT7) which belong to class III histone deacetylases (HDACs) [2]. As one of this family, SIRT2 participated in a wide range of biological processes including genome maintenance [3], cell proliferation [4] and transformation [5]. Although SIRT2 is primarily a cytoplasmic protein [6], the shuttling of SIRT2 from the cytosol to the nucleus has been reported [7]. Growing evidences have supported the nuclear functions of SIRT2. For instance, nuclear SIRT2 is responsible for deacetylation of H4K16 [7] and methylation of H4K20 [8] during cell cycle. The above studies indicated the potential role of SIRT2 in nucleus.

In recent years, the role of SIR2 in virus has received much attentions. Zhang C et al. have found that SIRT1 can mediate hepatic steatosis during hepatitis C virus (HCV) infection [9]. As for hepatitis B virus (HBV), we have reported that SIRT1 facilitate HBV replication by targeting HBV core promoter through regulating transcription factor AP-1 [10] and the SIRT1 inhibitor, nicotinamide, exhibited an inhibitory effect on HBV promoters [11]. It has reported that the failure of virological remission during HBV infection is a significant risk factor for HCC [12]. In previous study, we have showed that SIRT2 can promote the epithelial to mesenchymal transition (EMT) in HCC [13]. However, the role of SIRT2 and the molecular mechanism in HBV and HBV-induced HCC are largely unknown.

In this study, we aim to evaluate the biological function of SIRT2 in HBV and HBV-induced HCC. We revealed that HBV or HBx could upregulate the expression of SIRT2 by targeting its promoter. Meanwhile, the positive functional role of SIRT2 in HBV replication and HBV-induced HCC has been elucidated in this study. Those findings highlight the potential role of SIRT2 in HBV and HBV-induced HCC and provide a new prosperity of HBV treatment.

2. Materials and methods

2.1. Antibodies, plasmids

Rabbit anti-SIRT2 monoclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA); Mouse anti-GAPDH monoclonal antibody was obtained from Zhongshan Golden Brige Biotechnology (ZSGB-Bio, China).

SIRT2 expression vector was purchased from Addgene (Cambridge, MA). SIRT2 short hairpin RNAs (shSIRT2-1 and shSIRT2-2) were kindly provided by Dr. D.Y. Jin (The University of Hong

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Kong, Hong Kong, China). HBx-Flag expression plasmid was stored in our laboratory. HBV expression plasmid pCH9/3091 was kindly provided by Prof. Lin Lan (The Third Military Medical University, Chongqing, China). As previous described [14], HBV expression plasmid with HBx mutation (HBx MUT) was constructed based on pCH9/3091 (as the wild-type HBV, HBV WT) by inserting a stop codon at the beginning of the HBx gene.

2.2. Cell culture

Huh-7 cell line was purchased from the HSRRB (Osaka, Japan) and HepG2 cell line was acquired from ATCC (USA). Huh-7 and HepG2 cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Corning, New York, USA) with 10% fetal bovine serum (FBS). HepAD38 cell line was purchased from the Shanghai Second Military Medical University and cultured in DMEM with 10% FBS, and 400 μg/ml of G418 (Merck, Germany). All the cells were cultured in a humidified incubator at 37 °C with 5% CO2.

2.3. Western blot

The cells were lysed by RIPA lysis buffer which contains protease inhibitor (Roche, Mannheim, Germany). The protein concentration was determined by BCA (Roche, Mannheim, Germany) and the lysates containing 30 μg of total protein was separated by SDS-PAGE. Then the protein was transferred to nitrocellulose membrane (GE Healthcare, Buckinghamshire, UK). After blocked in 5% nonfat milk, the membrane was incubated with primary antibody (Anti-SIRT2 protein 1:3000; Anti-GAPDH 1:10,000) overnight at 4 °C. The corresponding HRP-conjugated secondary antibody was incubated at room temperature for 2 h. The signals were visualized by ECL Western blot reagents (Millipore, Massachusetts, USA). GAPDH was used as a loading control.

2.4. Real-time PCR

The total RNA was extracted by TRNzol (TIANGEN, Beijing, China) methods. IScript™ cDNA Synthesis Kit was acquired from Bio-Rad (Bio-Rad, California, USA). Relative expression level of SIRT2, HBV total RNA and pregenome RNA (pgRNA) were detected by Fast Start Universal SYBR Green Master. β-actin mRNA was used as an endogenous control. The fold changes of various genes were calculated by using the $2^{-\triangle\triangle CT}$ method. The sequences of primers are as follows: SIRT2: forward, CTCTCTAGCCGTTCCCACAT, reverse, GGACTACGAGTCCCAGAAGG. HBV total RNA: forward, 5′-ACCGACCTTGAGGCATACTT-3′, reverse, 5′- GCCTA-CAGCCTCCTAGTACA-3′. HBV pgRNA: forward, 5′- GCCTTA-GAGTCTCCTGAGCA-3′, reverse, 5′- GAGGGAGTTCTTCTTCTAGG-3'. β-actin: forward, 5′-CTCTTCCAGCCTTCCTTCCT-3′, reverse, 5′-AGCACTGTGTTGGCGTACAG-3′.

HBV replicative intermediates were prepared as described previously [10]. The absolute quantification of the HBV replicative intermediates was determined by using Fast Start Universal SYBR Green Master (Roche, Mannheim, Germany). The sequences of HBV replicative intermediates primer: forward, 5′-CCTAGTAGTCAG TTATGTCAAC-3′, reverse, 5′-TCTATAAGCTGGAGGAGTGCGA-3'.

2.5. Luciferase reporter assay

The luciferase report vectors (pGL3-Basic SIRT2 promoter) were cotransfected with HBx-Flag or vector into HepG2 or Huh-7 cells and the relative luciferase activity was measured by dual luciferase reporter assay (Promega, USA). The transfection efficiency was normalized by cotransfecting with pRL-TK.

2.6. Southern blot

The HBV DNA replicative intermediates were separated by agarose gel and then denatured by alkali solution for 30 min. After transferred to the nylon membrane, the DNA was fixed by UV cross-linking. The membrane containing interest DNA was hybridized with digoxigenin-labeled DNA probe at 42 °C overnight. And then, the membrane was washed by SSC/0.1% SDS solution. Following blocked at 37 °C for 30 min in blocking solution, the membrane incubated with anti-digoxin secondary antibody at 37 °C for 30 min. The signal was collected by X-ray film.

2.7. Transwell migration and invasion assays

Cell metastasis ability was assessed by the transwell migration and invasion assay. 80,000 cells and 100,000 cells were seeded for the migration and invasion assay, respectively. After fixed by methanol, the cells migrated to the underside of the membrane were stained with 0.1% crystal violet and were enumerated for 10 microscope fields.

2.8. Enzyme-linked immunosorbent assay (ELISA)

The secretion level of HBsAg and HBeAg in supernatant were determined by ELISA assay (KHB, China) according to the manufacturer's instructions.

2.9. Statistical analysis

Results are expressed as mean ± SD. The data between two groups were compared by the Student's t-test. A difference was considered significant when $P < 0.05$. All statistical analysis was performed by the SPSS 19.0 software.

3. Results

3.1. HBV upregulated SIRT2 expression

We have previously reported that SIRT2 has been in implicated in epithelial-mesenchymal transition of hepatocellular carcinoma [13]. However, the relation between SIRT2 and HBV replication has not been investigated. The mRNA and protein levels of SIRT2 was examined in HepAD38 cell lines which is an HBV stably transfected cell line constitutively producing HBV under the control of tetracycline (Fig. 1A and B) and human hepatoma Huh-7 cells transiently transfected with HBV expressing plasmid pCH9/3091 (containing a 1.1-unit length HBV genome driven by a cytomegalovirus promoter) (Fig. 1C and D). Both mRNA and protein levels of SIRT2 were upregulated in HBV-expressing cells relative to control cells.

3.2. HBx enhanced SIRT2 expression in HCC cell lines

To determine whether viral protein HBx is responsible for SIRT2 upregulation, HepG2 or Huh-7 cells were transfected with HBV 1.1-mer replicons in which the expression of X gene was abrogated: HBV-HBx mutant. As expected, HBx mutation abolished the SIRT2 upregulation induced by wild type HBV replicons expression in both two cell lines (Fig. 2A and B), which suggesting the potential role of HBx in SIRT2 expression.

To further confirm the relationship between HBx and SIRT2, HBx-Flag or vector plasmid were transfected into HepG2 or Huh-7 cells and the expression level of SIRT2 were determined by Real-time PCR and Western blot. Both mRNA and protein levels of SIRT2 in HepG2 or Huh-7 were significantly elevated when transfected with HBx expression plasmid (Fig. 2C and D). HBx is closely related

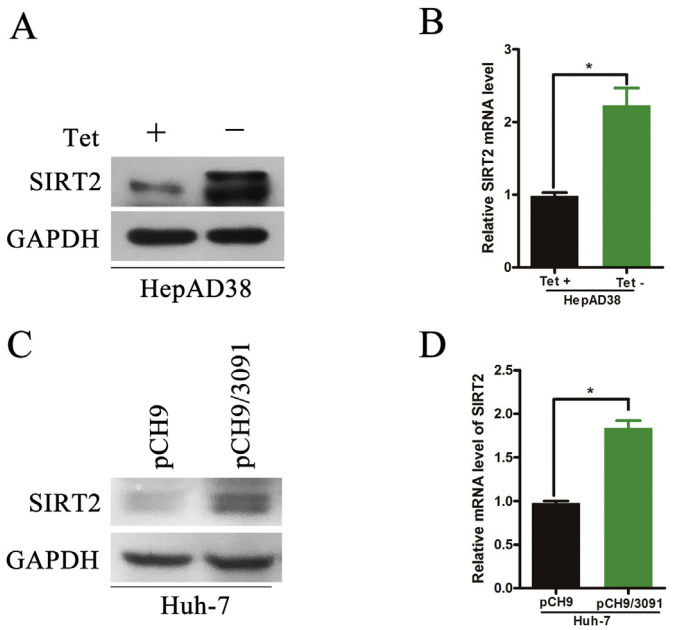

Fig. 1. SIRT2 is upregulated in HBV expression cells. (A, B) The mRNA and protein levels of SIRT2 in HepAD38 cells with or without tetracycline were detected by real-time PCR and Western blot. β-actin was used as an internal control in real-time PCR and GAPDH was used as the loading control in Western blot, $^{*}p < 0.05$. (C, D) The HBV expressing plasmid pCH9/3091 was transfected into Huh-7 cells. Both mRNA and protein levels of SIRT2 were detected by real-time PCR and Western blot.

to the transcription activity of host genes via interaction with the promoter of corresponding genes [15] and has a significant role in viral pathogenesis and carcinogenesis [16]. Based on above data, we hypothesized that HBx may regulate SIRT2 gene transcription.

3.3. HBx activates the transcription of SIRT2 by targeting its promoter

To further investigate that whether the HBx would activate the transcription activity of SIRT2 through regulating its promoter, we cloned the promoter region of SIRT2 into the luciferase reporter vector pGL3-Basic. Dual-luciferase reporter assay showed that promoter activity of SIRT2 was significantly upregulated in HepG2 or Huh-7 cells expressing HBx (Fig. 2E and F). The above data revealed that HBx upregulated the expression of SIRT2 by targeting its promoter.

3.4. SIRT2 promotes HBV replication in HBV expression cells

To further investigate the functional role of SIRT2 on HBV replication, studies were conducted in HepAD38 cells. The overexpression efficiency of SIRT2 in HepAD38 was first confirmed by Western blotting (Fig. 3A). SIRT2 overexpression resulted in increased levels of HBV DNA replicative intermediates, as evidenced by both Southern blot and Real-time PCR analysis (Fig. 3B and C). Consistently, SIRT2 overexpression also enhanced HBV total RNA and pgRNA level (Fig. 3D and E). Furthermore, the secretion level of HBsAg and HBeAg in supernatant were also elevated in SIRT2 overexpressed cells compared with control cells (Fig. S1A,B).

In contrast, the effect of SIRT2 silencing on HBV replication was further investigated. HepAD38 cells was transfected with shRNA targeting SIRT2 (shSIRT2-1 and shSIRT2-2) or nontargeting shRNA (shCont). The silencing efficiency were first examined by Western blot (Fig. 3F). The HBV DNA level was significantly decreased in cells expressing shSIRT2-1 or shSIRT2-2 compared with shCont (Fig. 3G and H). Consistently, the HBV total RNA and pgRNA level were decreased in SIRT2-depleted cells compared with the shCont group (Fig. 3I and J). Also, the decreased secretion level of HBsAg and HBeAg in supernatant were observed in SIRT2 depletion cells compared with shControl cells (Fig. S1C,D). In general, those data supported that SIRT2 facilitated HBV transcription and replication.

3.5. HBx facilitates HCC cell proliferation through SIRT2

To explore the role of SIRT2 on HBV-expressing cell proliferation, the SIRT2 overexpression plasmid or shRNA were transfected into HepAD38 cells and the cell numbers at indicated time points were recorded. Comparison with control cells, overexpressed SIRT2 promotes HBV-expressing cells proliferation (Fig. 4A) and silencing of SIRT2 could decrease the proliferation rate in HBV-expressing cells (Fig. 4B) which suggested that SIRT2 could facilitate HBV-expressing cell proliferation.

It has reported that HBx plays important role in HBV-mediated hepatocellular carcinoma [17]. In this study, we evaluated the significance of SIRT2 in hepatocarcinogenesis mediated by HBV. To explore the mechanism of HBx on HCC cell proliferation, the SIRT2 overexpression plasmid or shRNA were cotransfected with HBx into the HCC cells and the cell numbers at indicated time points were recorded. As shown in Fig. 4CF, HBx facilitates HepG2 and Huh-7 cells proliferation depended on SIRT2. The proliferation rate was obviously elevated in SIRT2 overexpression cells compared with control cells (Fig. 3C and D). However, the loss of SIRT2 by scramble RNA significantly decreased the proliferation rate of HCC cell lines (Fig. 3E and F).

3.6. HBx enhances HCC cell migration and invasion by a SIRT2-dependent way

To evaluate whether the HBx-elevated SIRT2 plays a role in the motility of HCC cells, transwell migration and invasion assays were carried out. The overexpression plasmid of SIRT2 was cotransfected with HBx into the Huh-7 cells. Significant increased migration ability was observed in cells expressing SIRT2 compared to the cells expressing vector (Fig. 4G, upper panel). Concordantly, overexpression of SIRT2 also upregulated the cell invasion ability (Fig. 4G, lower panel).

shRNA targeting SIRT2 was cotransfected with HBx into the Huh-7 cells. Notable reduction of cell migration was detected in shSIRT2 group compared with shCont group (Fig. 4H, upper panel). Meanwhile, depletion of SIRT2 could impair the cell invasion ability compared with shCont group (Fig. 4H, lower panel).

4. Discussion

Sirtuins are widely related to virus replication and various factors are involved in this pathological process. We have previously reported that SIRT1 can facilitate HBV replication by targeting HBV core promoter through regulating transcription factor AP-1 [10]. However, the role of SIRT2 in HBV replication is still remains unknown.

In this study, HBV replication could induce SIRT2 mRNA and protein levels in HBV-expressing cell models, suggesting SIRT2 might play a role in the HBV replication process. To determine which viral protein is responsible for SIRT2 upregulation, we transfected Huh-7 or HepG2 cells with HBV-HBx mutant plasmid or HBx expression plasmid. The data indicated that SIRT2 upregulation is induced by HBx. HBx is a ubiquitous transactivator, which is essential for HBV replication *in vivo* [18] and plays a significant role in gene expression via activation the promoter of host gene [19]. Consistently, we displayed that HBx could increase the expression

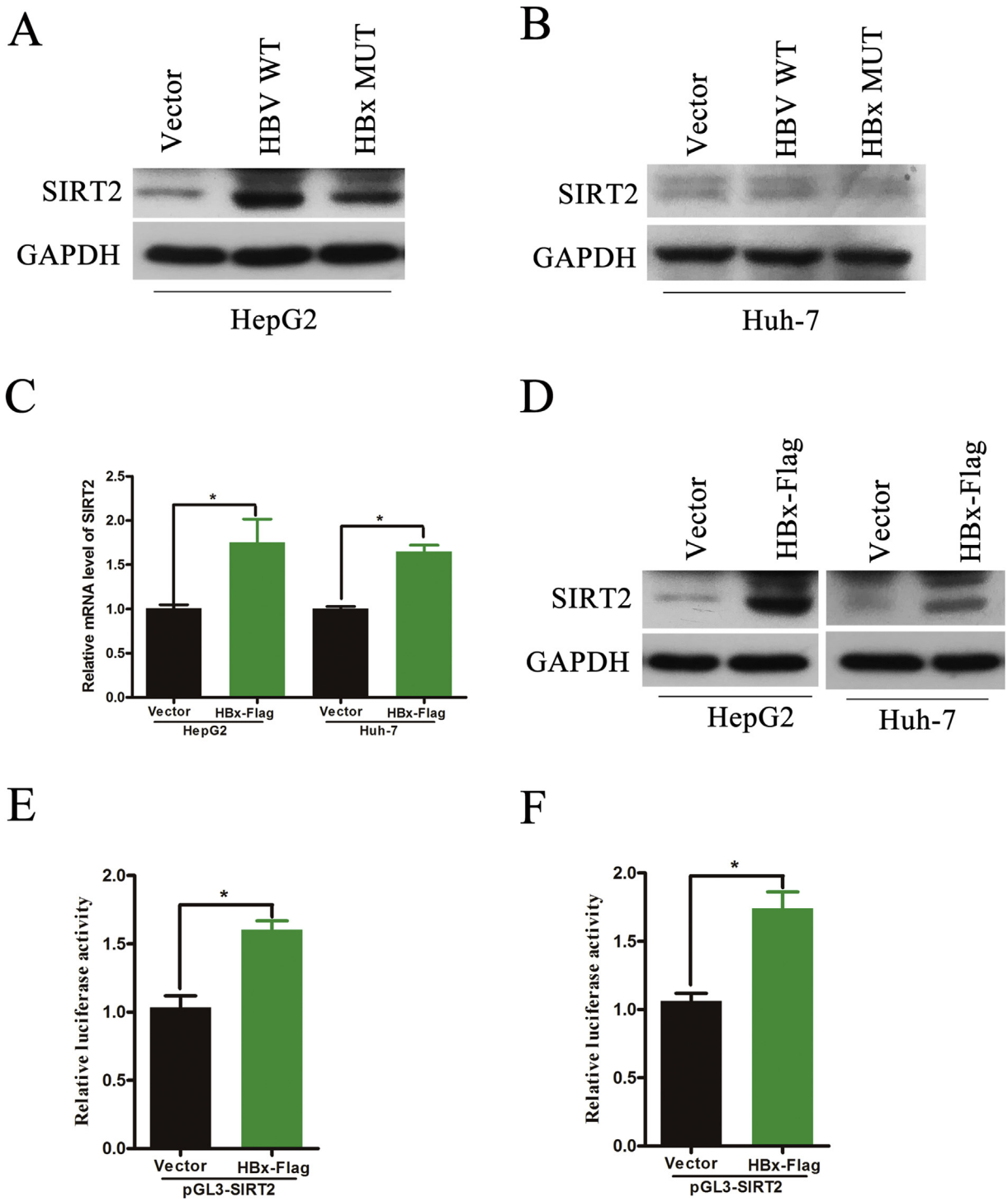

Fig. 2. HBx upregulates SIRT2 expression by targeting its promoter. (A, B) The vector, HBV WT or HBx MUT were transfected into HepG2 or Huh-7 cells. The SIRT2 expression level was detected by Western blot, GAPDH was used as an internal control. (C, D) The vector or HBx-Flag were introduced into HepG2 or Huh-7 cells. The mRNA (C) and protein (D) level of SIRT2 were detected by real time PCR and Western blot, respectively. β-actin and GAPDH were used as the internal control, respectively. $^{*}p < 0.05$ (E, F) The pGL3-Basic SIRT2 promoter plasmid was cotransfected with HBx-Flag or vector into HepG2 or Huh-7 cells and the relative luciferase activity was measured by dual luciferase reporter assay at 36 h posttransfection. $^{*}p < 0.05$.

of SIRT2 by targeting its promoter to facilitate HBV transcription and replication.

HBV and HBx expression presents the host genes with much abnormal regulation. On the one hand, HBx is capable of decreasing the host antiviral protein APOBEC3G [20] and HNF4α [21] to activate HBV replication. Also, many host factors can play a role in HBV replication by interaction with HBx. For instance, DDB1 can stimulate HBV replication via HBx-independent way [22]. Among the numerous HBx-related cellular proteins, many factors are involved in both HBV replication and HCC development. HBx-elevated male-specific lethal 2 (MSL2) modulates HBV cccDNA in hepatoma cells, leading to hepatocarcinogenesis [23]. AKT can be upregulated by

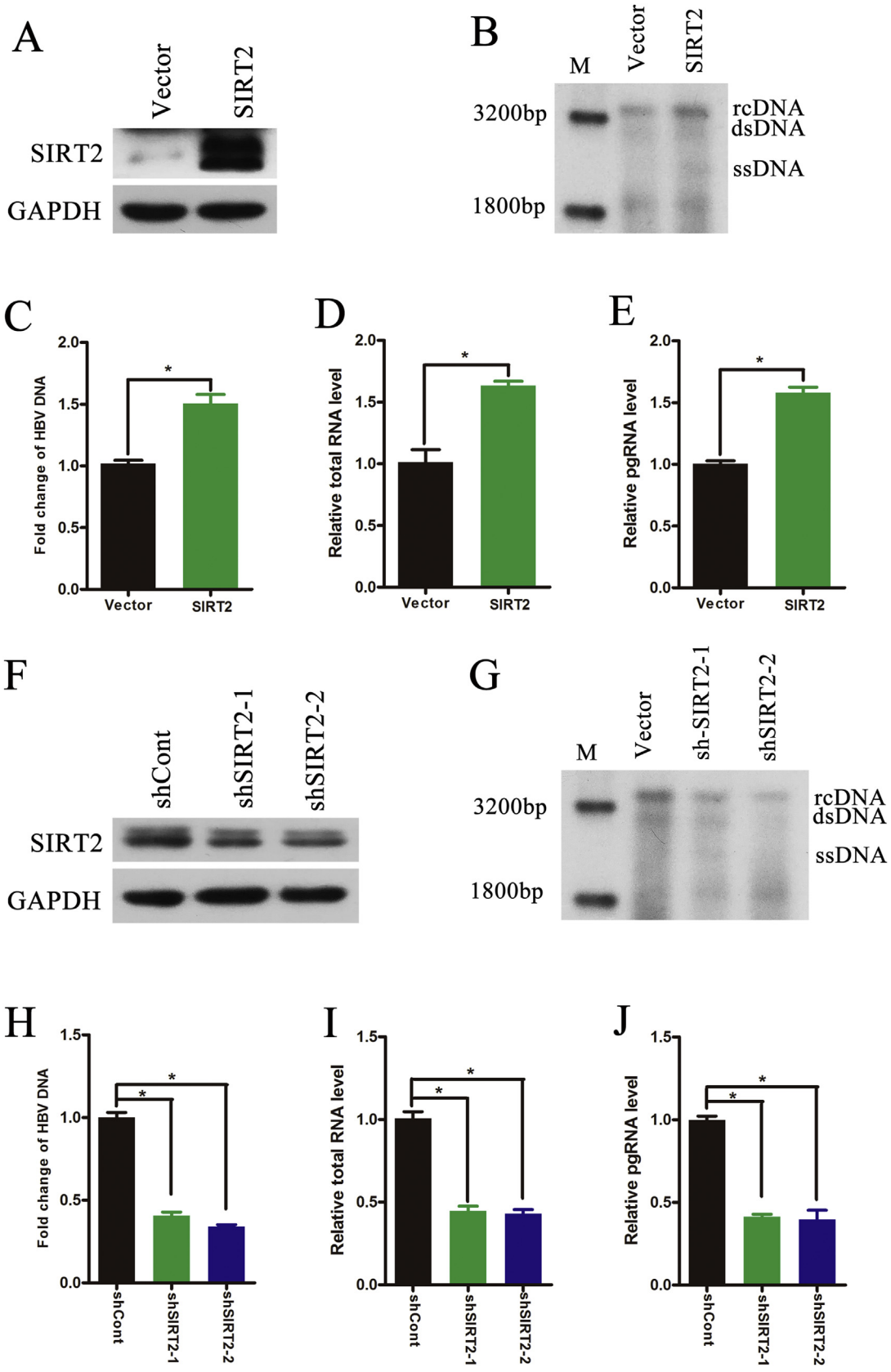

Fig. 3. SIRT2 promotes HBV replication in HBV expressing cells. (A) The overexpression efficiency was first determined by Western blot. (B–E) Overexpression of SIRT2 in HepAD38 cell would increase the level of HBV DNA (B, C), HBV total RNA (D) and pgRNA (E). (F) The depletion efficiency was first determined by Western blot. (G–J) Silencing of SIRT2 in HepAD38 cell would decrease the level of HBV DNA (G, H), HBV total RNA (I) and pgRNA (J).

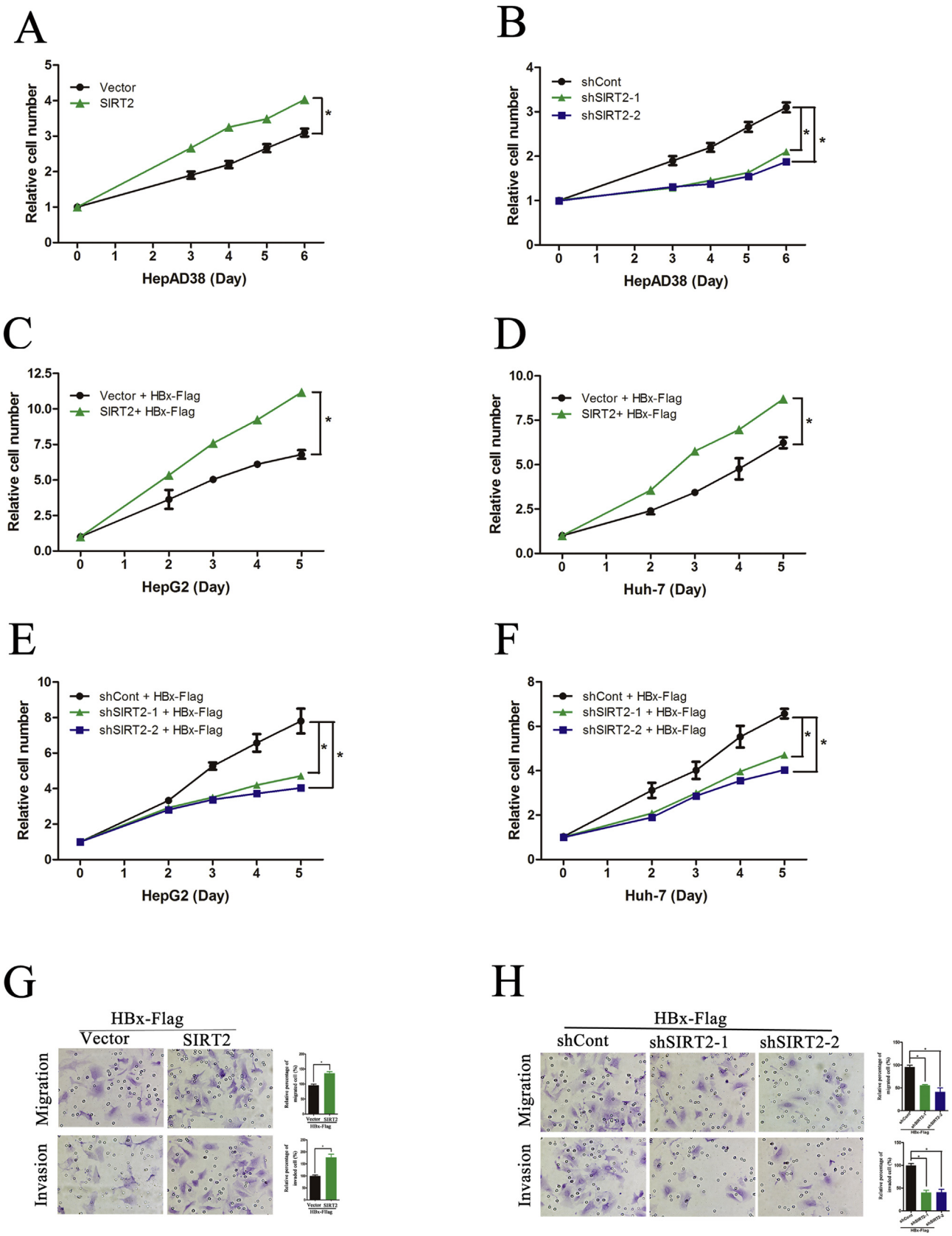

Fig. 4. HBx promotes hepatocarcinogenesis via SIRT2. (A, B) The overexpression or depletion of SIRT2 could promote or inhibit HepAD38 cell growth, *p < 0.05. (C–F) The SIRT2 overexpression plasmid (C, D) or shRNA targeting SIRT2 (E, F) were cotransfected with HBx into the in HepG2 or Huh-7 cells and the cell numbers at indicated time points were recorded, *p < 0.05. (G) The overexpression of SIRT2 could promote HBx mediated cell migration and invasion in Huh-7 cells which determined by transwell migration and invasion assays, *p < 0.05. (H) The deletion of SIRT2 could inhibit HBx mediated cell migration and invasion which were examined by transwell migration and invasion assays, *p < 0.05.

HBx to balance the HBV replication and cell survival, thus promoting HBV replication and HBV-induced HCC [24,25]. As for HCC, J. Li et al. reported that HBx can function as a oncogene factor via inhibiting the apoptosis of HCC cells by modulating cell endoplasmic reticulum stress response [26]. Bcl-2, which largely involved in cell apoptosis and autophagy, can interact with HBx and participate in cell autophagy [27,28]. Meanwhile, HBx can interrupt the physical interaction between PARP1 and SIRT6 which may be the onset of hepatocarcinogenesis [29]. In line with the above researches that HBx can regulate the host genes to promote the development of HCC, our study found that HBx could promote HCC which rely on the upregulation of SIRT2. Additionally, HBx can promote HCC invasion and metastasis via upregulating thioredoxin interacting protein [30]. *In vivo*, C-terminus of HBx stimulates the expansion and tumourigenesis of hepatic progenitor cells in mice [31]. Coincidence with the above research, we demonstrated that the dysfunction of SIRT2 is closely related to the metastasis ability in HBV-induced HCC which is mediated by HBx.

In general, we described a novel mechanism for HBx-mediated promotion of HBV replication and HCC development by upregulation of SIRT2. And SIRT2 exerts a positive role in HBV replication which indicated the potential role of HBV treatment. Further, SIRT2 is highly associated with HBV-mediated HCC and will be a potential target for HCC treatment. The underlying mechanism of SIRT2 to facilitate HBV replication and HCC development needs further studies.

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Acknowledgements

All the authors declare no potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.01.127.

References

[1] M.C. Haigis, D.A. Sinclair, Mammalian sirtuins: biological insights and disease relevance, Annu. Rev. Pathol. 5 (2010) 253–295.
[2] S. Imai, C.M. Armstrong, M. Kaeberlein, L. Guarente, Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase, Nature 403 (2000) 795–800.
[3] P.E. Head, H. Zhang, A.J. Bastien, A.E. Koyen, A.E. Withers, W.B. Daddacha, X. Cheng, D.S. Yu, Sirtuin 2 mutations in human cancers impair its function in genome maintenance, J. Biol. Chem. 292 (2017) 9919–9931.
[4] Y. Du, J. Wu, H. Zhang, S. Li, H. Sun, Reduced expression of SIRT2 in serous ovarian carcinoma promotes cell proliferation through disinhibition of CDK4 expression, Mol. Med. Rep. 15 (2017) 1638–1646.
[5] P. Nguyen, S. Lee, D. Lorang-Leins, J. Trepel, D.K. Smart, SIRT2 interacts with beta-catenin to inhibit Wnt signaling output in response to radiation-induced stress, Mol. Canc. Res.: MCR 12 (2014) 1244–1253.
[6] E. Michishita, J.Y. Park, J.M. Burneskis, J.C. Barrett, I. Horikawa, Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins, Mol. Biol. Cell 16 (2005) 4623–4635.
[7] A. Vaquero, M.B. Scher, D.H. Lee, A. Sutton, H.L. Cheng, F.W. Alt, L. Serrano, R. Sternglanz, D. Reinberg, SirT2 is a histone deacetylase with preference for histone H4 Lys 16 during mitosis, Genes Dev. 20 (2006) 1256–1261.
[8] L. Serrano, P. Martinez-Redondo, A. Marazuela-Duque, B.N. Vazquez, S.J. Dooley, P. Voigt, D.B. Beck, N. Kane-Goldsmith, Q. Tong, R.M. Rabanal, D. Fondevila, P. Munoz, M. Kruger, J.A. Tischfield, A. Vaquero, The tumor suppressor SirT2 regulates cell cycle progression and genome stability by modulating the mitotic deposition of H4K20 methylation, Genes Dev. 27 (2013) 639–653.
[9] C. Zhang, J. Wang, H. Zhang, S. Liu, H.J. Lee, W. Jin, J. Cheng, Hepatitis C virus core protein induces hepatic steatosis via Sirt1-dependent pathway, Liver Int.: Off. J. Int. Assoc. Study Liver 37 (9) (2017).
[10] J.H. Ren, Y. Tao, Z.Z. Zhang, W.X. Chen, X.F. Cai, K. Chen, B.C. Ko, C.L. Song, L.K. Ran, W.Y. Li, A.L. Huang, J. Chen, Sirtuin 1 regulates hepatitis B virus transcription and replication by targeting transcription factor AP-1, J. Virol. 88 (2014) 2442–2451.
[11] W.Y. Li, J.H. Ren, N.N. Tao, L.K. Ran, X. Chen, H.Z. Zhou, B. Liu, X.S. Li, A.L. Huang, J. Chen, The SIRT1 inhibitor, nicotinamide, inhibits hepatitis B virus replication in vitro and in vivo, Arch. Virol. 161 (2016) 621–630.
[12] W. Zhang, X. Wang, Y. Wang, X. Zhao, W. Duan, Q. Wang, X. Wu, Y. Kong, H. Ma, H. You, X. Ou, J. Jia, Effective viral suppression is necessary to reduce hepatocellular carcinoma development in cirrhotic patients with chronic hepatitis B: results of a 10-year follow up, Medicine 96 (2017), e8454.
[13] J. Chen, A.W. Chan, K.F. To, W. Chen, Z. Zhang, J. Ren, C. Song, Y.S. Cheung, P.B. Lai, S.H. Cheng, M.H. Ng, A. Huang, B.C. Ko, SIRT2 overexpression in hepatocellular carcinoma mediates epithelial to mesenchymal transition by protein kinase B/glycogen synthase kinase-3beta/beta-catenin signaling, Hepatology 57 (2013) 2287–2298.
[14] J.H. Ren, X. Chen, L. Zhou, N.N. Tao, H.Z. Zhou, B. Liu, W.Y. Li, A.L. Huang, J. Chen, Protective role of Sirtuin3 (SIRT3) in oxidative stress mediated by Hepatitis B virus X protein expression, PLoS One 11 (2016), e0150961.
[15] D.Y. Wang, S.H. An, L. Liu, S.S. Bai, K.X. Wu, R. Zhu, Z.J. Wang, Hepatitis B virus X protein influences enrichment profiles of H3K9me3 on promoter regions in human hepatoma cell lines, Oncotarget 7 (2016) 84883–84892.
[16] T. Zhang, N. Xie, W. He, R. Liu, Y. Lei, Y. Chen, H. Tang, B. Liu, C. Huang, Y. Wei, An integrated proteomics and bioinformatics analyses of hepatitis B virus X interacting proteins and identification of a novel interactor apoA-I, J. Proteonomics 84 (2013) 92–105.
[17] T. Shi, Q. Hua, Z. Ma, Q. Lv, Downregulation of miR-200a-3p induced by hepatitis B Virus X (HBx) Protein promotes cell proliferation and invasion in HBV-infection-associated hepatocarcinoma, Pathol. Res. Pract. 213 (2017) 1464–1469.
[18] P. Gong, X. Zhang, J. Zhang, J. Zhang, H. Luo, Z. Wang, Hepatitis B virus X protein in the proliferation of hepatocellular carcinoma cells, Front. Biosci. 18 (2013) 1256–1265.
[19] R. Colgrove, G. Simon, D. Ganem, Transcriptional activation of homologous and heterologous genes by the hepatitis B virus X gene product in cells permissive for viral replication, J. Virol. 63 (1989) 4019–4026.
[20] R. Chen, X. Zhao, Y. Wang, Y. Xie, J. Liu, Hepatitis B virus X protein is capable of down-regulating protein level of host antiviral protein APOBEC3G, Sci. Rep. 7 (2017) 40783.
[21] E. Ktistaki, N.T. Ktistakis, E. Papadogeorgaki, I. Talianidis, Recruitment of hepatocyte nuclear factor 4 into specific intranuclear compartments depends on tyrosine phosphorylation that affects its DNA-binding and transactivation potential, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 9876–9880.
[22] W. Kim, S. Lee, Y. Son, C. Ko, W.S. Ryu, DDB1 stimulates viral transcription of Hepatitis B virus via HBx-independent mechanisms, J. Virol. 90 (2016) 9644–9653.
[23] Y. Gao, J. Feng, G. Yang, S. Zhang, Y. Liu, Y. Bu, M. Sun, M. Zhao, F. Chen, W. Zhang, L. Ye, X. Zhang, Hepatitis B virus X protein-elevated MSL2 modulates hepatitis B virus covalently closed circular DNA by inducing degradation of APOBEC3B to enhance hepatocarcinogenesis, Hepatology 66 (2017) 1413–1429.
[24] S. Rawat, M.J. Bouchard, The hepatitis B virus (HBV) HBx protein activates AKT to simultaneously regulate HBV replication and hepatocyte survival, J. Virol. 89 (2015) 999–1012.
[25] C. Wang, B. Yao, M. Xu, X. Zheng, RIP1 upregulation promoted tumor progression by activating AKT/Bcl-2/BAX signaling and predicted poor post-surgical prognosis in HCC, Tumor Biol.: J. Int. Soc. Oncodevelopmental Biol. Med. 37 (2016) 15305–15313.
[26] J. Li, J. He, Y. Fu, X. Hu, L.Q. Sun, Y. Huang, X. Fan, Hepatitis B virus X protein inhibits apoptosis by modulating endoplasmic reticulum stress response, Oncotarget 8 (2017) 96027–96034.
[27] T. Jiang, M. Liu, J. Wu, Y. Shi, Structural and biochemical analysis of Bcl-2 interaction with the hepatitis B virus protein HBx, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) 2074–2079.
[28] L. Zhong, W. Shu, W. Dai, B. Gao, S. Xiong, Reactive oxygen species-mediated c-Jun NH2-terminal kinase activation contributes to Hepatitis B virus X protein-induced autophagy via regulation of the Beclin-1/Bcl-2 interaction, J. Virol. 91 (2017).
[29] T.Y. Na, N.L. Ka, H. Rhee, D. Kyeong, M.H. Kim, J.K. Seong, Y.N. Park, M.O. Lee, Interaction of hepatitis B virus X protein with PARP1 results in inhibition of DNA repair in hepatocellular carcinoma, Oncogene 35 (2016) 5435–5445.
[30] Z. He, Y. Yu, Y. Nong, L. Du, C. Liu, Y. Cao, L. Bai, H. Tang, Hepatitis B virus X protein promotes hepatocellular carcinoma invasion and metastasis via upregulating thioredoxin interacting protein, Oncol. Lett. 14 (2017) 1323–1332.
[31] L. Yu, S. Chen, N. Luo, S. He, The C-terminus domain of the hepatitis B virus x protein stimulates the proliferation of mouse foetal hepatic progenitor cells, although it is not required for the formation of spheroids, Int. J. Mol. Med. 40 (2017) 400–410.