Inactivation of tumor suppressor TAp63 by hepatitis B virus X protein in hepatocellular carcinoma

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Abstract

Background: The hepatitis B virus X (HBx) protein plays a critical role in the initiation and progression of hepatitis B virus (HBV)-associated hepatocellular carcinoma (HCC). In the early stage of the disease, HBx facilitates tumor onset by inactivating the tumor suppressor p53. The p53-encoding gene, however, is frequently mutated or deleted as the cancer progresses to the late stage and, under such circumstance, the p53 homolog TAp63 can harness HCC growth by transactivating several important p53-target genes.

Methods: To determine whether HBx regulates TAp63, we performed co-immunoprecipitation assay, real-time quantitative polymerase chain reaction, immunoblotting, and flow cytometry analysis in p53-null cancer cell lines, Hep3B and H1299.

Results: HBx interacts with the transactivation domain of TAp63, as HBx was co-immunoprecipitated with TAp63 but not with ΔNp63. The interaction between HBx and TAp63 abolished transcriptional activity of TAp63, as evidenced by the reduction of the levels of its target genes *p21* and *PUMA*, consequently leading to restricted apoptosis and augmented proliferation of HCC cells.

Conclusion: HBV induces progression of HCC that harbors defective p53 by inhibiting the tumor suppressor TAp63.

Keywords: TAp63; hepatitis B virus X; Apoptosis; Proliferation; Liver cancer

Introduction

The deregulation of tumor suppressor genes contributes to tumorigenesis and cancer progression. Transcription factor p53 maintains genomic stability and prevents cancer development by activating a myriad of genes involved in cell cycle arrest, DNA repair, and apoptosis.[1,2] Various mechanisms have evolved to inactivate p53 in cancer cells due to its detrimental cytotoxic effects. In around 50% of human cancers, the *p53*-encoding gene, *TP53*, is mutated by cancer cells. Moreover, p53 mutations abrogate or impair the tumor-suppressive activity of wild-type p53, and some of the p53 mutants are endowed with a "gain of function" to promote cancer cell growth and metastasis.[3,4] In other cancers, p53 is under close surveillance by oncogenic proteins. The master inhibitor for p53 is the E3 ubiquitin ligase MDM2, encoded by a p53-inducible gene amplified or overexpressed in multiple cancers.[5,6] MDM2 binds to the amino- and carboxyl-termini of p53 and inactivates the latter by directly concealing its transactivation domain (TAD)[7] or inducing its ubiquitination and proteolytic degradation,[8,9] thus constituting a negative feedback loop.[10-13] Two oncoproteins, NGFR and PHLDB3, can repress p53 activity by enhancing MDM2-mediated p53 degradation or blocking its DNA-binding domain.[14,15] Thus, the inactivation of p53 by MDM2 and other oncogenic proteins is required for cancer cell growth and proliferation.

The p53 homolog p63 is also regarded as a tumor suppressor by transcriptionally modulating gene expression also regulated by p53.[16,17] The p63-encoding gene generates multiple isoforms from different transcriptional start sites or through the alternative splicing mechanism.[16] The p63 isoforms can be categorized into two groups, the TAp63 isoforms harboring intact TAD and the ΔNp63 isoforms with deleted TAD. TAp63 can transactivate some of the most important p53 target genes, such as *p21* (also known as *CDKN1A*) and *PUMA* (*p53* up-regulated modulator of apoptosis, also known as *BBC3*), leading to cancer cell cycle arrest and apoptosis,[18,19] whereas ΔNp63

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is inactive in transcriptional regulation and endorses tumor growth.[20] TAp63 affects lipid metabolism and liver steatosis, which may progress to hepatocellular carcinoma (HCC); it regulates SIRT1 and AMPKα2, as demonstrated by an aging TAp63$^{-/-}$ mouse model.[21] In addition, TAp63 controls liver *de novo* lipogenesis through the transcriptional induction of *CCDC3* that encodes a secreted protein critical for the increase of long-chain polyunsaturated fatty acids in liver cells and inhibition of hepatic steatosis formation in transgenic *CCDC3* mice.[22] TAp63 suppresses liver cancer cell proliferation, particularly in the p53-mutated or -null context, by activating p53-target gene expression in response genotoxic stress.[23,24] Thus, a thorough understanding of the mechanism underlying TAp63 regulation is crucial for improving therapeutic approaches to treat HCC.

Despite the wide use of hepatitis B virus (HBV) vaccine and availability of potent antiviral treatments, HBV infection remains the leading etiology of HCC, responsible for 50% to 80% of cases worldwide.[25,26] HBV contributes to the development of HCC through diverse mechanisms. On the one hand, HBV DNA integration into the host genome leads to genomic instability and mutagenesis of many cancer-associated genes, including *TP53*. On the other hand, the prolonged expression of the viral proteins, such as hepatitis B virus X (HBx), has been shown to promote HCC progression by modulating epigenetic modifications and interacting with multiple transcription factors or co-factors.[27] Importantly, HBx can inhibit p53 transcriptional activity on the p53-responsive promoter[28,29] or sequester p53 in the cytoplasm[30,31] by directly interacting with this protein, leading to restricted apoptosis and accelerated liver cancer cell proliferation. Thus, HBx-mediated inactivation of p53 serves a role in the early stages of hepatocellular carcinogenesis. Mutations of p53, however, are found in up to 48% of HCC cases,[32,33] particularly when the cancer progresses to the late stage. Of note, HBV infection is highly associated with the p53-R249S mutation, the only hotspot p53 mutant in HCC.[34,35] In p53-mutated HCC, TAp63 may substitute wild-type p53 for suppressing cell growth and proliferation.[23,24] Therefore, it is worth exploring whether HBx inactivates TAp63, with an additional mechanism underlying the progression of HCC harboring p53 mutation.

In this study, we sought to investigate if HBx promotes HCC progression by regulating TAp63 activity and, if so, what is the underlying mechanism. To this end, we performed co-immunoprecipitation assay, real-time quantitative polymerase chain reaction, immunoblotting, and flow cytometry analysis in p53-defective cancer cell lines as detailed below. These results demonstrate that HBx interacts with and suppresses TAp63, resulting in reduced apoptosis and enhanced cell proliferation.

Methods

Cell culture and transient transfection

Human cancer cell lines H1299 and Hep3B were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. All cells were maintained at 37 °C in a 5% CO_2-humidified atmosphere. Cells were seeded on the plate one day before transfection and then transfected with plasmids, as indicated in the figure legends using Hieff Trans liposomal transfection reagent following the manufacturer's protocol (Yeasen, Shanghai, China). Cells were harvested at 30 to 48 h post-transfection for use in future experiments.

Plasmids and antibodies

The Flag-tagged HBx plasmid was generated by inserting the full-length cDNA amplified by PCR into the 2xFlag-pcDNA3 vector between the *Eco*RI and *Xho*I sites, using the following primers, 5′-CGGAATTCAATGGCTGC TCGGGTGTGC-3′ and 5′-CCGCTCGAGTTAGGCA-GAGGTGAAAAAGTTGC-3′. The plasmids encoding TAp63γ and ΔNp63 were described previously.[22] The antibodies of anti-Myc (Catalog No. 60003-1, Proteintech, Hubei, China), anti-p21 (Catalog No. #2947, Cell Signaling Technology, Danvers, MA, USA), anti-β-actin (Sc-47778, Santa Cruz, CA, USA), anti-p63 (4A4) (Sc-8431, Santa Cruz), anti-Flag (Sigma-Aldrich, F3165-2MG, St louis, MO, USA), and the secondary antibody for mice (Catalog No. ARG65350, Arigo, Shanghai, China) were commercially purchased.

Reverse transcription and quantitative RT-PCR analyses

Total RNA was isolated from cells using RNAiso Plus (Takara, Japan) following the manufacturer's protocol. Total RNAs of 0.5 to 1 μg were used as templates for reverse transcription using PrimeScript RT reagent Kit with gDNA Eraser (Takara). A real-time quantitative polymerase chain reaction (RT-qPCR) was conducted using TB Green Premix according to the manufacturer's protocol (Takara). The primers for *p21*, *PUMA*, and GAPDH cDNA detection[36] were as follows: *p21*, 5′-GGCAGACCAGCATGACAGATT-3′ and 5′-GCGGCC AGGGTATGTACATGA-3′; *PUMA*, 5′-TGACCACTGG CATTCATTTGG-3′ and 5′-CCTCCCTCTTCCGAGA TTTCC-3′; GAPDH, 5′-ACGGATTTGGTCGTATTGGG-3′ and 5′-CCTGGAAGATGGTGATGGGATT-3′.

Immunoblotting (IB)

Cells were harvested and lysed in a lysis buffer consisting of 50 mmol/L Tris/HCl (pH 7.5), 0.5% Nonidet P-40 (NP-40), 1 mmol/L ethylenediaminetetraacetic acid, 150 mol/L NaCl, 1 mmol/L dithiothreitol, 0.2 mmol/L phenylmethylsulfonyl fluoride, 10 mmol/L pepstatin A, and 1 mmol/L leupeptin. Equal amounts of clear cell lysates (20–80 μg) were used for IB analyses as previously described.[37]

Immunoprecipitation

Immunoprecipitation (IP) assays were conducted using antibodies as indicated in the figure legends. In brief, ~500 to 1000 μg of proteins were incubated with the indicated antibody at 4 °C for 4 h or overnight. Protein A or G beads (Santa Cruz Biotechnology) were then added, and the mixture was incubated at 4 °C for an additional 1 to 2 h.

Beads were washed at least three times with a lysis buffer. Bound proteins and 10% inputs were detected by IB with antibodies as indicated in the figure legends.

Flow cytometry analysis

Cells were fixed and stained in 500 mL of propidium iodide (PI, Sigma-Aldrich, St louis, MO, USA) stain buffer (50 mg/mL PI, 200 mg/mL RNase A, 0.1% Triton X-100 in phosphate-buffered saline) at 37 °C for 30 min. The cells were then analyzed for DNA content and sub-G1 distribution using an FC500 MPL flow cytometer (Beckham coulter, Indianapolis, IN, USA).

Statistical analysis

All data are presented. The Student's *t*-test or one-way analysis of variance (ANOVA) was performed to evaluate the differences between two groups or more than two groups using SPSS software (SPSS Inc., USA). Additionally, $P < 0.05$ was considered statistically significant. All data are presented as mean ± standard deviation (SD).

Results

HBx interacts with TAp63 in cancer cells

HBx physically binds to and suppresses the transcriptional activity of p53 in HCC. However, this remains elusive if HBx also interacts with the p53 homolog, TAp63. To evaluate this mechanism, we introduced plasmids encoding Flag-HBx and Myc-TAp63γ, a TA isoform that has the strongest transcriptional activity among all the splice variants,[22,24] into the H1299 lung cancer cell line that is a widely used model system with p53 deletion and high transfection efficiency, followed by co-IP-IB analyses. Myc-TAp63γ was co-immunoprecipitated with Flag-HBx using the anti-Flag antibody [Figure 1A]. Conversely, Flag-HBx could be co-immunoprecipitated with Myc-TAp63γ using the anti-Myc antibody [Figure 1B]. These results demonstrate a clear interaction between HBx and TAp63γ in cancer cells.

HBx binds to the TAD of TAp63

There are various p63 variants generated using different promoters or through the C-terminal alternative splicing [Figure 2A]. The TA isoforms of p63 possess transcriptional activity and can transactivate a subset of p53 target genes, while the ΔN isoforms of p63 that lack the TAD are deprived of transcriptional activity.[16] Thus, we considered whether HBx might bind to ΔNp63. By introducing plasmids encoding Flag-HBx and ΔNp63 into cancer cells, we performed co-IP-IB analyses and found that HBx does not interact with ΔNp63 [Figure 2B]. This finding also suggests that HBx may regulate TAp63, but not ΔNp63, transcriptional activity in cancer cells, which is addressed below.

HBx represses TAp63 transcriptional activity

As HBx attenuates p53 transcriptional activity by binding to the latter, we determined if HBx inactivates TAp63 in p53-null cancer cells. H1299 cells were transfected with plasmids encoding Flag-HBx and Myc-TAp63γ individually or in combination. RT-qPCR analysis was then performed to assess the expression of p21, a potent cyclin-dependent kinase inhibitor that can be induced by the p53 family proteins.[38,39] Ectopic expression of Flag-HBx in H1299 cells does not influence the level of p21 but does significantly suppress Myc-TAp63γ-induced p21 expression [Figure 3A], indicating that HBx may regulate TAp63-dependent transcription in p53-null cancer cells. In addition, we tested this idea in p53-null liver cancer Hep3B cells with a high level of endogenous TAp63.[23] Indeed, we demonstrated that ectopic expression of Flag-HBx significantly downregulates the mRNA expression of *p21* and *PUMA*, a BH3-only pro-apoptotic gene,[40,41] in Hep3B cells [Figure 3B,C]. Consistently, the protein level of p21 was also dampened by ectopic HBx [Figure 3D]. These results collectively demonstrate that HBx can

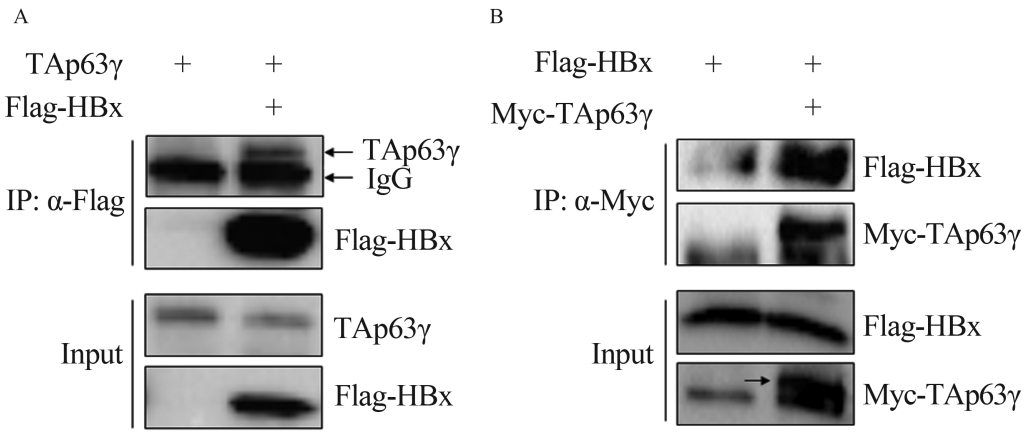

Figure 1: HBx interacts with TAp63. (A) Cells were transfected with plasmids encoding HBx and TAp63γ, followed by co-IP using the anti-Flag antibody. The bound proteins were detected by IB using antibodies as indicated. (B) Cells were transfected with plasmids encoding HBx and TAp63γ, followed by co-IP using the anti-Myc antibody. The bound proteins were detected by IB using antibodies as indicated. co-IP: Co-immunoprecipitation; HBx: Hepatitis B virus X; IB: Immunoblotting.

repress TAp63 transcriptional activity in p53-null HCC cells.

HBx empowers HCC cell growth by negating TAp63 activity

To translate the above findings into biological significance, we conducted flow cytometry analysis to determine if HBx controls apoptosis and cell cycle progression by regulating TAp63. Ectopic expression of HBx slightly reduced apoptosis of Hep3B cells as indicated by the sub-G1 population [Figure 4B]. Remarkably, the overexpression of TAp63γ dramatically boosted apoptosis, while the simultaneous overexpression of HBx completely abrogated TAp63γ-induced apoptosis [Figure 4A,B]. In line with these results, we also showed that the ectopic expression of HBx markedly restored Hep3B cell proliferation by counteracting TAp63γ activity, as illustrated by the S-phase population [Figure 4A,C]. Taken together, these results explicitly demonstrate that HBx impairs apoptosis and promotes the growth and proliferation of liver cancer cells by negatively regulating TAp63 activity.

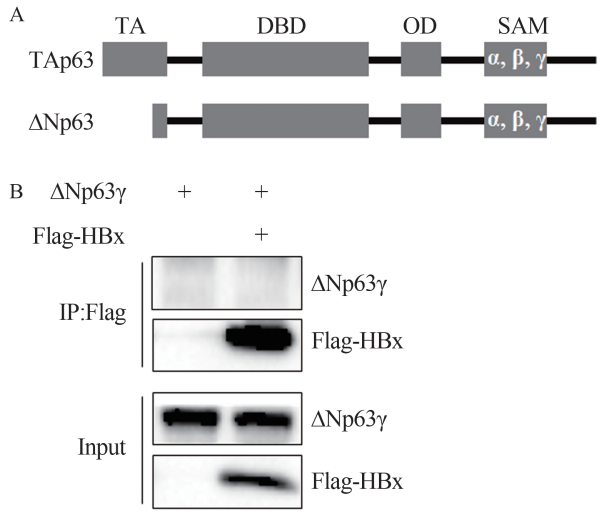

Figure 2: HBx does not bind to ΔNp63. (A) A schematic for the isoforms of p63 proteins. (B) Cells were transfected with plasmids encoding HBx and ΔNp63, followed by co-IP using the anti-Flag antibody. The bound proteins were detected by IB using antibodies as indicated. co-IP: Co-immunoprecipitation; IB: Immunoblotting; HBx: Hepatitis B virus X.

Discussion

HBV infection remains a major contributor to HCC worldwide. The viral regulatory protein HBx is required for HBV DNA transcription and replication, while also being essential for HBV-induced oncogenesis. Here, we reported that HBx can interact with the TAD of TAp63 and represses its transcriptional activity, as evidenced by downregulation of *p21* and *PUMA*, consequently leading to restricted apoptosis and accelerated cell proliferation of p53-null HCC cells. Our study illustrates the interplay between HBx and TAp63, and it demonstrates an important yet unappreciated mechanism for HBV-induced progression of liver cancer with mutation or deletion of *p53*.

While a number of studies have described the interaction of HBx and p53 that leads to p53 inactivation and liver cancer progression,[28-31] it is unknown whether HBx also regulates p53 family proteins in p53-null liver cancer cells. Our study showed that HBx indeed binds to and dampens transcriptional activity of TAp63. Considering our study, however, the mechanisms behind HBx regulation of p53 and TAp63 may be different. HBx associates with the carboxyl-terminus of p53 to suppresses its transcriptional activity by interfering with the interaction of *p53* with TFIIH complex and *ERCC3*, both of which are involved in nucleotide excision repair.[28,42,43] Furthermore, the HBx-p53 interaction prevents the nuclear entrance of p53 and impairs the activation of apoptotic genes,[30,31] probably because HBx blocks the nuclear localization signal within the carboxyl-terminus of p53. Interestingly, our results showed that HBx binds to the amino-terminal TAD of TAp63, but not to the carboxyl-terminus of p63, as HBx did not interact with ΔNp63. Hence, we hypothesize that the interaction of HBx with TAp63 may expose the transcriptional repression domain of HBx to[29] or directly insulate the TAD from the basal transcriptional machinery.

Lipid metabolic reprograming is crucial to carcinogenesis. Liver steatosis can be a significant cofactor in the establishment of chronic liver disease and even the development of HCC.[44] Prolonged overexpression of HBx promotes lipid accumulation by upregulating FABP1, SREBP1, and PPARγ, consequently inducing liver steatosis;[45,46] this is an essential mechanism for HBV-associated hepatocellular carcinogenesis. Another well-documented finding in lipid metabolism of hepatocytes is that TAp63 can confine lipid biogenesis through

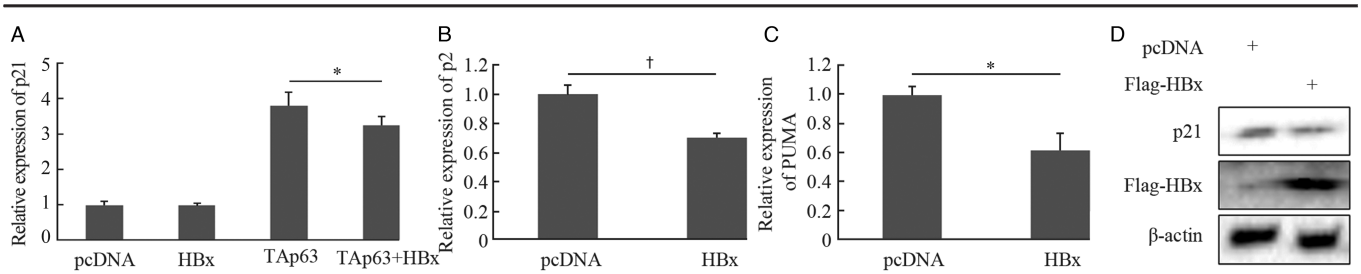

Figure 3: HBx inhibits TAp63 transcriptional activity. (A) H1299 cells were transfected with combinations of plasmids as indicated, followed by RT-qPCR analysis of *p21* expression. (B,C) Hep3B cells were transfected with the plasmid encoding HBx or the empty vector, followed by RT-qPCR analysis of *p21* (B) and *PUMA* (C) expression. (D) Hep3B cells were transfected with the plasmid encoding HBx or the empty vector, followed by IB analysis of *p21* expression. $^{*}P < 0.05$, $^{\dagger}P < 0.01$ by two tailed *t* test. HBx: Hepatitis B virus X; IB: Immunoblotting; RT-qPCR: Real-time quantitative polymerase chain reaction.

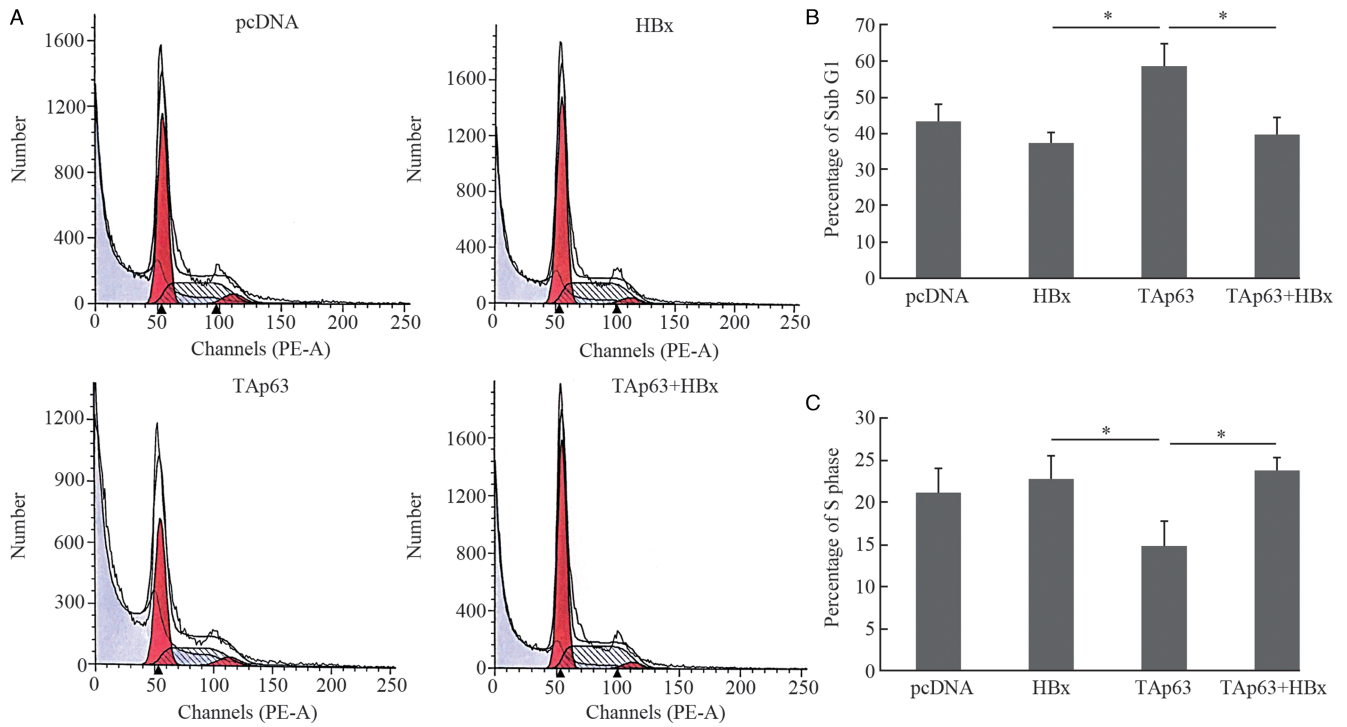

Figure 4: HBx abrogates tumor suppressive activity of TAp63. (A) Hep3B cells transfected with combinations of plasmids as indicated were subjected to flow cytometry analysis. (B) Quantification of the sub-G1 population. (C) Quantification of the S-phase population. $^{*}P < 0.05$ by two tailed *t* test. HBx: Hepatitis B virus X; PE-A: Phycoerythrin area.

diverse mechanisms. The TAp63$^{-/-}$ mice, for example, were found to exhibit obesity, insulin resistance, and glucose intolerance.[21] Mechanistically, depletion of TAp63 selectively reduces the expression levels of Sirt1, AMPKα2, and LKB1, resulting in elevated fatty acid synthesis and declined fatty acid oxidation levels.[21] In addition, a TAp63-specific target gene, CCDC3, has been identified during adipocyte differentiation.[22] The transgenic CCDC3 mice displayed alleviated glucose intolerance, insulin resistance, and steatosis formation upon high-fat diet.[22] Therefore, TAp63 may act more like a metabolic modulator in hepatocytes and HCC compared with p53. Thus, additional studies are necessary to elucidate the interplay of TAp63 and HBx in regulating lipid metabolism, steatosis, and HCC development.

In conclusion, HBV infection is the leading etiology of HCC, and the viral regulatory protein HBx plays a critical role in HBV-induced hepatocellular carcinogenesis by inhibiting p53 activity. However, the *TP53* gene is frequently mutated or deleted due to exacerbated HBV-triggered genomic instability as the cancer progresses to the late stage. In this scenario, TAp63 acts as a tumor suppressor to activate p53-target genes. In this study, we demonstrate that HBx interacts with and represses the transcriptional activity of TAp63, resulting in impaired apoptosis and increased proliferation of HCC cells.

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Availability of data and materials

The datasets used during the present study are available from the corresponding authors upon reasonable request.

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Conflicts of interest

None.

References

1. Levine AJ, Oren M. The first 30 years of p53: growing ever more complex. Nat Rev Cancer 2009;9:749–758. doi: 10.1038/nrc2723.
2. Levine AJ. The many faces of p53: something for everyone. J Mol Cell Biol 2019;11:524–530. doi: 10.1093/jmcb/mjz026.
3. Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. Genes Dev 2012;26:1268–1286. doi: 10.1101/gad.190678.112.
4. Zhou X, Hao Q, Lu H. Mutant p53 in cancer therapy-the barrier or the path. J Mol Cell Biol 2019;11:293–305. doi: 10.1093/jmcb/mjy072.
5. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 1992;69:1237–1245. doi: 10.1016/0092-8674 (92)90644-R.

6. Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B. Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature 1992;358:80–83. doi: 10.1038/358080a0.
7. Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris J, Kinzler KW, Vogelstein B. Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. Nature 1993;362:857–860. doi: 10.1038/362857a0.
8. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. Nature 1997;387:296–299. doi: 10.1038/387296a0.
9. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. Nature 1997;387:299–303. doi: 10.1038/387299a0.
10. Montes de Oca Luna R, Wagner DS, Lozano G. Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. Nature 1995;378:203–206. doi: 10.1038/378203a0.
11. Jones SN, Roe AE, Donehower LA, Bradley A. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. Nature 1995;378:206–208. doi: 10.1038/378206a0.
12. Zhou X, Cao B, Lu H. Negative auto-regulators trap p53 in their web. J Mol Cell Biol 2017;9:62–68. doi: 10.1093/jmcb/mjx001.
13. Liu Y, Tavana O, Gu W. p53 modifications: exquisite decorations of the powerful guardian. J Mol Cell Biol 2019;11:564–577. doi: 10.1093/jmcb/mjz060.
14. Zhou X, Hao Q, Liao P, Luo S, Zhang M, Hu G, *et al*. Nerve growth factor receptor negates the tumor suppressor p53 as a feedback regulator. eLife 2016;5:1–22. doi: 10.7554/eLife.15099.
15. Chao T, Zhou X, Cao B, Liao P, Liu H, Chen Y, *et al*. Pleckstrin homology domain-containing protein PHLDB3 supports cancer growth via a negative feedback loop involving p53. Nat Commun 2016;7:1–12. doi: 10.1038/ncomms13755.
16. Stiewe T. The p53 family in differentiation and tumorigenesis. Nat Rev Cancer 2007;7:165–168. doi: 10.1038/nrc2072.
17. Zhang J, Sun W, Kong X, Zhang Y, Yang HJ, Ren C, *et al*. Mutant p53 antagonizes p63/p73-mediated tumor suppression via Notch1. Proc Natl Acad Sci USA 2019;116:24259–24267. doi: 10.1073/pnas.1913919116.
18. Zeng SX, Dai MS, Keller DM, Lu H. SSRP1 functions as a co-activator of the transcriptional activator p63. EMBO J 2002;21:5487–5497. doi: 10.1093/emboj/cdf540.
19. Liao JM, Zhang Y, Liao W, Zeng SX, Su X, Flores ER, *et al*. IkappaB kinase beta (IKKbeta) inhibits p63 isoform gamma (TAp63gamma) transcriptional activity. J Biol Chem 2013;288:18184–18193. doi: 10.1074/jbc.M113.466540.
20. Rokudai S, Li Y, Otaka Y, Fujieda M, Owens DM, Christiano AM, *et al*. STXBP4 regulates APC/C-mediated p63 turnover and drives squamous cell carcinogenesis. Proc Natl Acad Sci USA 2018;115:E4806–E4814. doi: 10.1073/pnas.1718546115.
21. Su X, Gi YJ, Chakravarti D, Chan IL, Zhang A, Xia X, *et al*. TAp63 is a master transcriptional regulator of lipid and glucose metabolism. Cell Metab 2012;16:511–525. doi: 10.1016/j.cmet.2012.09.006.
22. Liao W, Liu H, Zhang Y, Jung JH, Chen J, Su X, *et al*. Ccdc3: a new P63 target involved in regulation of liver lipid metabolism. Sci Rep 2017;7:1–15. doi: 10.1038/s41598-017-09228-8.
23. Petitjean A, Cavard C, Shi H, Tribollet V, Hainaut P, Caron de Fromentel C. The expression of TA and DeltaNp63 are regulated by different mechanisms in liver cells. Oncogene 2005;24:512–519. doi: 10.1038/sj.onc.1208215.
24. Petitjean A, Ruptier C, Tribollet V, Hautefeuille A, Chardon F, Cavard C, *et al*. Properties of the six isoforms of p63: p53-like regulation in response to genotoxic stress and cross talk with DeltaNp73. Carcinogenesis 2008;29:273–281. doi: 10.1093/carcin/bgm258.
25. Kulik L, El-Serag HB. Epidemiology and management of hepatocellular carcinoma. Gastroenterology 2019;156:477–491.e471. doi: 10.1053/j.gastro.2018.08.065.
26. de Martel C, Maucort-Boulch D, Plummer M, Franceschi S. World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma. Hepatology 2015;62:1190–1200. doi: 10.1002/hep.27969.
27. Levrero M, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. J Hepatol 2016;64 (1 Suppl):S84–S101. doi: 10.1016/j.jhep.2016.02.021.
28. Wang XW, Gibson MK, Vermeulen W, Yeh H, Forrester K, Sturzbecher HW, *et al*. Abrogation of p53-induced apoptosis by the hepatitis B virus X gene. Cancer Res 1995;55:6012–6016.
29. Truant R, Antunovic J, Greenblatt J, Prives C, Cromlish JA. Direct interaction of the hepatitis B virus HBx protein with p53 leads to inhibition by HBx of p53 response element-directed transactivation. J Virol 1995;69:1851–1859. doi: 10.1128/jvi.69.3.1851-1859.1995.
30. Elmore LW, Hancock AR, Chang SF, Wang XW, Chang S, Callahan CP, *et al*. Hepatitis B virus X protein and p53 tumor suppressor interactions in the modulation of apoptosis. Proc Natl Acad Sci USA 1997;94:14707–14712. doi: 10.1073/pnas.94.26.14707.
31. Ueda H, Ullrich SJ, Gangemi JD, Kappel CA, Ngo L, Feitelson MA, *et al*. Functional inactivation but not structural mutation of p53 causes liver cancer. Nat Genet 1995;9:41–47. doi: 10.1038/ng0195-41.
32. Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Rebouissou S, *et al*. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet 2015;47:505–511. doi: 10.1038/ng.3252.
33. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, *et al*. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012;44:694–698. doi: 10.1038/ng.2256.
34. Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature 1991;350:427–428. doi: 10.1038/350427a0.
35. Liao P, Zeng SX, Zhou X, Chen T, Zhou F, Cao B, *et al*. Mutant p53 gains its function via c-myc activation upon CDK4 phosphorylation at serine 249 and consequent PIN1 binding. Mol Cell 2017;68:1134–1146. e1136. doi: 10.1016/j.molcel.2017.11.006.
36. Zhou X, Hao Q, Zhang Q, Liao JM, Ke JW, Liao P, *et al*. Ribosomal proteins L11 and L5 activate TAp73 by overcoming MDM2 inhibition. Cell Death Differ 2015;22:755–766. doi: 10.1038/cdd.2014.167.
37. Zhou X, Hao Q, Liao J, Zhang Q, Lu H. Ribosomal protein S14 unties the MDM2-p53 loop upon ribosomal stress. Oncogene 2013;32:388–396. doi: 10.1038/onc.2012.63.
38. el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, *et al*. WAF1, a potential mediator of p53 tumor suppression. Cell 1993;75:817–825. doi: 10.1016/0092-8674(93)90500-p.
39. Shimada A, Kato S, Enjo K, Osada M, Ikawa Y, Kohno K, *et al*. The transcriptional activities of p53 and its homologue p51/p63: similarities and differences. Cancer Res 1999;59:2781–2786.
40. Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. PUMA induces the rapid apoptosis of colorectal cancer cells. Mol Cell 2001;7:673–682. doi: 10.1016/s1097-2765(01)00213-1.
41. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 2001;7:683–694. doi: 10.1016/s1097-2765(01)00214-3.
42. Wang XW, Forrester K, Yeh H, Feitelson MA, Gu JR, Harris CC. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. Proc Natl Acad Sci USA 1994;91:2230–2234. doi: 10.1073/pnas.91.6.2230.
43. Wang XW, Yeh H, Schaeffer L, Roy R, Moncollin V, Egly JM, *et al*. p53 modulation of TFIIH-associated nucleotide excision repair activity. Nat Genet 1995;10:188–195. doi: 10.1038/ng0695-188.
44. Nakagawa H, Hayata Y, Kawamura S, Yamada T, Fujiwara N, Koike K. Lipid metabolic reprogramming in hepatocellular carcinoma. Cancers (Basel) 2018;10:1–14. doi: 10.3390/cancers10110447.
45. Kim KH, Shin HJ, Kim K, Choi HM, Rhee SH, Moon HB, *et al*. Hepatitis B virus X protein induces hepatic steatosis via transcriptional activation of SREBP1 and PPARgamma. Gastroenterology 2007;132:1955–1967. doi: 10.1053/j.gastro.2007.03.039.
46. Wu YL, Peng XE, Zhu YB, Yan XL, Chen WN, Lin X. Hepatitis B virus X protein induces hepatic steatosis by enhancing the expression of liver fatty acid binding protein. J Virol 2016;90:1729–1740. doi: 10.1128/JVI.02604-15.

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