Biochemical and Biophysical Research Communications 450 (2014) 1492–1497

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

HBx affects CUL4–DDB1 function in both positive and negative manners

Liandi Guo [a,1], Xiaobo Wang [b,1], Laifeng Ren [c,1], Ming Zeng [b], Si Wang [b], Yuding Weng [b], Zizhi Tang [b], Xin Wang [b], Yaxiong Tang [d], Huaidong Hu [e], Mingyuan Li [a,*], Chongjie Zhang [a,*], Cong Liu [b,*]

[a] Department of Immunology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu 610041, PR China
[b] Developmental and Stem Cell Institute, Department of Pediatrics, West China Second University Hospital, Chengdu 610041, PR China
[c] Department of Medical Microbiology & Immunology, Fenyang College of Shanxi Medical University, Fenyang 032200, PR China
[d] Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, PR China
[e] Key Laboratory of Molecular Biology for Infectious Disease, Ministry of Education, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, PR China

ARTICLE INFO

Article history:
Received 20 June 2014
Available online 11 July 2014

Keywords:
Hepatitis B virus
HBx
DDB1
cccDNA
PRMT1

ABSTRACT

Hepatitis B virus (HBV) infection is a major public health problem by affecting 350 million people worldwide. The mechanisms that regulate HBV gene expression and viral replication remain poorly understood. HBx is known as the central regulator for HBV replication and is associated with the CUL4–DDB1 ubiquitin ligase through H-box motif. Here, we show that blocking the activity of DDB1 by RNA interfering inhibited viral production and gene expression of HBV, and direct association of HBx with DDB1 promoted viral activities, indicating that DDB1 function is required for viral production. On the other hand, HBx interfered with DDB1-dependent polyubiquitination of PRMT1, arginine methyltransferase 1, suggesting that HBx can also block the function of a subset of CUL4–DDB1 E3 ligases. Thus, we conclude that HBx regulates the function of DDB1 in both positive and negative manners in the context of distinct CUL4–DDB1 complexes and plays different roles in HBV replication cycle.

1. Introduction

HBV infection affects 350 million people worldwide and is regarded as a major public health problem. Chronic infection of HBV causes wide-range of liver diseases including cirrhosis, hepatocellular carcinoma (HCC) that is refractory to medical treatments. HBV is the prototype member of the Hepadnaviridae family with partially double stranded genome of 3.2 kb in length [1]. It encodes seven major viral proteins: three surface antigens, two core proteins, a polymerase and a transcriptional activator (HBx). HBx, a 17 KDa protein, is essential for establishing natural viral infection and can potently activate transcription of episomal viral DNA genome [2,3].

Upon HBV infection, its double stranded DNA genome is converted into a covalently close circular DNA (cccDNA). The establishment and maintenance of viral cccDNA are critical for HBV infection. cccDNA exists as episomes in the nucleus with an estimated copy number of 5–50 molecules per infected cell [4–6] and serves as the sole transcriptional template for the synthesis of viral RNAs. Transcription of cccDNA mainly relies on HBx and is regulated by multiple epigenetic mechanisms including methylation and acetylation [7,8]. PRMT1, a methyltransferase, has recently been identified as a novel binding partner of HBx and represses HBV transcription through its direct recruitment to the HBV cccDNA [9].

HBx carries out many-faceted functions by interacting with a number of cellular proteins in the cytoplasm and nucleus of host cells [10]. Notably, binding of HBx to damage-specific DNA binding protein 1 (DDB1) is essential for HBx to activate HBV transcription [11]. DDB1 forms ubiquitin E3 ligase with CUL4 and associates with adaptor subunits of DCAFs (DDB1–CUL4-associated factors) to target substrates for ubiquitination, which plays important roles in DNA repair, replication and transcription [12–16].

To dissect the role of CUL4–DDB1 in HBV viral production, we analyzed gene expression profile and cccDNA production by interfering the DDB1 function. We demonstrate that the function of viral protein HBx is linked to the multifaceted activities of DDB1. The overall function of DDB1 must be retained to maintain viral production, and direct association of HBx with DDB1 can promote viral gene expression instead of inhibiting it. On the contrary, HBx also suppresses DDB1-mediated polyubiquitination of PRMT1. Thus, the role of HBx in terms of targeting

* Corresponding authors. Address: Department of Microbiology, West China School of Pre-clinical and Forensic Medicine, Sichuan University, Chengdu, Sichuan 610041, PR China (M. Li). Fax: +86 28 85503990 (C. Zhang). Address: Department of Pediatrics, West China Second University Hospital, Sichuan University, Chengdu 610041, PR China. Fax: +86 28 85501727 (C. Liu).
E-mail addresses: lmingyuan16@yahoo.com (M. Li), cjzhang49@hotmail.com (C. Zhang), congliu@scu.edu.cn (C. Liu).
[1] These authors equally contributed to this paper.

http://dx.doi.org/10.1016/j.bbrc.2014.07.019
0006-291X/

CUL4–DDB1 complexes is double-sided: coordination of HBx and CUL4–DDB1 complex with putative novel functions, or blocking the polyubiquitination of a subset of cellular substrates like PRMT1.

2. Materials and methods

2.1. Cell culture, DNA and siRNA transfections

7702, T43, HepG2.2.15 (2215) and 293T (for lentiviral packaging) cells were maintained in DMEM (Hyclone) supplemented with 10% fetal bovine serum (FBS). siRNAs were purchased from Ribobio (Guangzhou, China). The targeting sequence of human DDB1 siRNA was 5′-CCTGTTGATTGCCAAAAAC-3′. Transfection was carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction.

2.2. DNA constructs

HBx, DDB1 and HBx-DDB1 were cloned into pLVX-IRES-ZsGreen1 vector through PCR cloning. pcDNA3.1-Myc-His-PRMT1 was kindly provided by Dr. Christine Neuveut.

2.3. Establishment of stable cell line

Human hepatocyte line 7702 was obtained from IBCB CAS. 7702 cells were transfected with pcDNA3.1-HBV containing the 1.3-fold HBV genome. The medium was replaced with complete medium supplemented with 200 μg/ml of G418 after 18 h. After 2 weeks of selection, colonies were screened for HBsAg and HBeAg by ELISA.

2.4. Lentivirus packaging

For packaging of lentivirus encoding HBx, DDB1 or HBx-DDB1, 5×10^6 of 293T cells were co-transfected with Lentivirus package system (pLVX-IRES-ZsGreen1, pMD.2G and psPAX2) and X-treme GENE HP DNA Transfection Reagent (Roche, 06366244001). Supernatant of culture medium containing secreted virus was collected 72 h after transfection and stored at −80 °C freezer.

2.5. Semi-quantitative RT-PCR

Total RNA was extracted from transfected or infected T43 cells using the total RNA extraction kit (OMEGA, R6934-01). One microgram of RNA was reverse-transcribed using Oligo-dT primers and MMLV reverse transcriptase (Promega, K1005S). Semi-quantitative RT-PCR was performed under the following amplification conditions: initial denaturation at 95 °C for 3 min followed by 28 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 50 s and extension at 72 °C for 5 min. The PCR products were captured by electrophoresis with 1.5% agarose gel. In parallel, GAPDH was selected as internal control. Primers used for PCR amplification are listed below: HBe: 5′-ATGCAACTTTTTCACCTCTG-3′(forward) and 5′-CTAACATTGAGATTC CCGAG-3′(reverse); HBs: 5′-ATGGAGAACATCACATCAGGA-3′(forward) and 5′-TTAAATGTATACCCAAAGAC-3′(reverse); HBx: 5′-ATG GCTGCTCGGTTGTGCTG-3′(forward) and 5′-TTAGGCAGAGGTGAAAAAGT-3′(reverse); DDB1: 5′-GCTCTTCATGCTGCTTTTGG-3′(forward) and 5′-CCAAAAGCAGCATGAAGAGC-3′(reverse); PRMT1: 5′-AGGCCGCGAACTGCATCATG-3′(forward) and 5′-GGCCTTGGCAGCAAACATGC-3′(reverse) and GAPDH: 5′-GAAGGTGAAGGTCGGAG TC-3′(forward) and 5′-GAAGATGGTGATGGGATTTC-3′(reverse).

2.6. ELISA and cccDNA assays

To evaluate gene expression and viral replication of HBV in T43 cells, HBsAg and HBeAg levels in culture media from transfected cells were measured using ELISA kits (Kehua Biotechnology Company, Shanghai, China); cccDNA extraction and Real-time PCR assays were carried out according to the manufacturer's instructions (Suoao Biomedtech Company, Beijing, China). All experiments were performed in triplicate.

2.7. Western blotting and chromatin extraction

Whole-cell lysates were prepared by lysing cells in SDS sample buffer (50 mM Tris–HCl pH 6.8, 1% SDS, 10% glycerol, 5% -mercaptoethanol, 0.01% bromophenol blue). Chromatin fraction was prepared using Mendez and Stillman's method [17]. Proteins were probed with the following antibodies: DDB1 (Cell Signaling, 5428S), PRMT1(Millipore, 07-404), H3 (Millipore, 05-1341), HA (Roche, 11583816001), α-tubulin (Sigma, T6074), followed by visualization with horseradish peroxidase-conjugated secondary antibodies (DAKO) and Chemiluminescent exposure detected with ChemiDoc XRS system (Bio-Rad, 170-8265).

2.8. His tag pulldown assay

His tag pulldown assay was performed as previously described [18]. Cells transfected with plasmids were incubated with 20 μM MG132 for 4 h before lysed in HUBA (His–ubiquitin buffer A: 6 M guanidine–HCl, 0.1 M Na_2HPO_4/NaH_2PO_4, 10 mM imidazole, pH8.0). His-tagged proteins in cell lysates were absorbed by Ni-NTA resin at room temperature for 3 h, followed by washing in HUBA, HUBC (1 volume HUBA, 3 volumes HUBB) and HUBB (25 mM Tris–HCl, 20 mM imidazole, pH6.8), respectively. Purified proteins were solubilized in SDS sample buffer and separated by SDS–PAGE gel.

3. Results

3.1. Knockdown of DDB1 decreases viral transcription and replication of HBV

HepG2.2.15 cell line is the laboratory cell line for studies of HBV gene expression and replication. However, this cell line is refractory for DNA or RNA transfection and thus put an obstacle for genetic study on cellular factors such as DDB1. To circumvent this problem and to characterize the function of DDB1 in HBV replication, we first established a cell line that is capable of supporting HBV replication and liable to transfection of nucleic acid including small interfering RNA or plasmids. A pcDNA3.1 plasmid containing the 1.3-fold length of HBV genome was introduced into HBV-negative 7702 cell line and screened for G418-resistant and HBV-replicating colonies. A clone with stably integrated HBV genome was selected and expanded, which is referred to as T43 in the following studies.

Subsequently, T43 was characterized for the viral production activities. Secreted viral antigens (HBsAg and HBeAg) in the culture media were detected by enzyme-linked immunosorbent assay (ELISA). T43 indeed secreted these antigens but at a relatively lower level in comparison with HBV positive line HepG2.2.15(2215) (Fig. 1A).

To evaluate the role of cellular DDB1 in HBV gene expression, we introduced DDB1-specific siRNA into T43 cells to decrease gene expression and protein level of DDB1 (Fig. 1B). Viral transcription was then analyzed by semi-quantitative reverse transcription PCR. Notably, decreased DDB1 expression in T43 cells resulted in dramatic reduction of RNA levels of HBe, HBs and HBx genes compared to mock transfection (Fig. 1C). These changes in gene expression were consolidated by antigen secretion (HBsAg and HBeAg) as well as cccDNA levels (Fig. 1D and E), which all rely on the activity

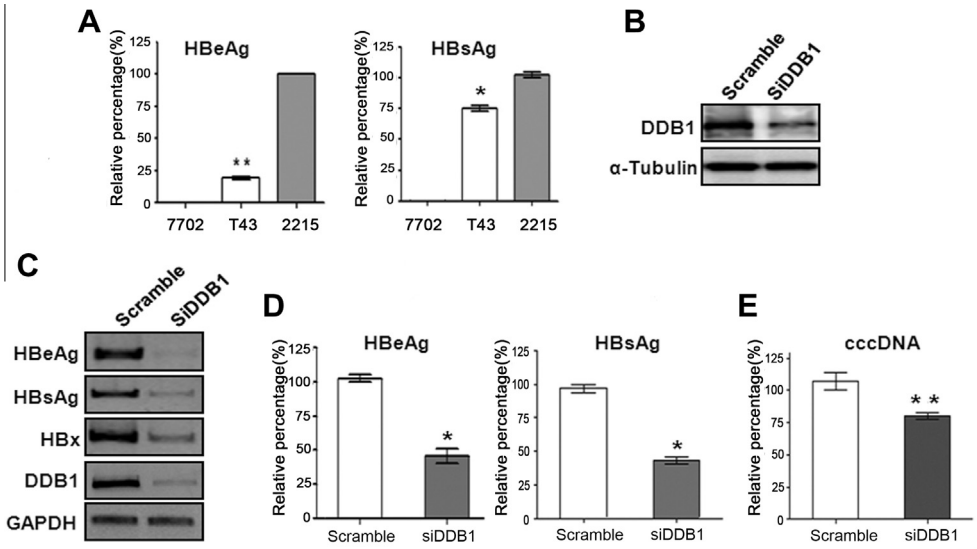

Fig. 1. DDB1 is required for replication and gene expression of HBV. (A) A stably HBV-integrated cell line (T43) with active viral replication was selected and measured for the secretion of HBV antigens (HBsAg and HBeAg) by ELISA. HepG2.2.15(2215) was used as positive control for HBV antigen production. (B) T43 cells were transfected with DDB1 siRNA, whole cell lysates were prepared and resolved by SDS–PAGE, followed by Western blotting with anti-DDB1 antibody. (C) Cells transfected with DDB1 siRNA were collected to evaluate relative expression levels of indicated genes by semi-quantitative RT-PCR. (D) Secreted viral antigens (HBeAg and HBsAg) in the media of siRNA-transfected cell culture were measured by ELISA. (E) DDB1 knockdown cells (as described above) were harvested and quantified for the level of nuclear cccDNA by Real-time PCR. Values are the mean standard error of three independent experiments for all of the related experiments. Error bar, s.e.m. ($n = 3$). Student's two-tailed t-test:$^{*}P < 0.05$; $^{**}P < 0.01$ vs. control.

of HBx [7,8]. Based on these results, in accordance with previous studies by Leupin and colleagues, we conclude that the overall activity of DDB1 plays a positive role in viral replication of HBV.

3.2. Direct association of HBx with DDB1 does not suppress HBV replication and gene expression

To further demonstrate that DDB1 is a positive regulator for HBV replication, we constructed lentiviral plasmids coding for DDB1 and expressed in T43 cell line (Fig. 2A). Changes of HBV replication under the condition of elevated level of DDB1 expression were analyzed. Obviously, overexpression of DDB1 enhanced the expression of HBe, HBs and HBx, reversely corresponding to the RNAi experiments (Fig. 2B). Again alteration of gene expression pattern was mirrored by elevation of secretion of HBV antigens as well as more robustly production of cccDNA (Fig. 2C and D). HBx interacts through a C-terminal motif (H-box) with DDB1 and selectively replaces a subset of DCAF proteins [20]. This leads to the prediction that binding of HBx to DDB1 would disrupt the function of CUL4–DDB1 E3 ligase and thus suppress the HBV replication. To test this prediction, the C-terminal of HBx was put in proximity of DDB1 by fusing the two proteins together. Surprisingly, as shown in Fig. 2A–D, although the expression of HBx-DDB1 fusion gene was less rigorous than DDB1 alone, its induction of viral gene expression and replication was even more pronounced than the effects of the DDB1 construct. It suggests that HBx-DDB1 fusion protein retains the stimulatory instead of inhibitory effect on HBV transcription and replication activities, implicating that HBx coordinates with DDB1 in replication of HBV, possibly by ubiquitinating specific targets of CUL4–DDB1 or by diverging the physiological function of this E3 ligase.

3.3. DDB1 down-regulates PRMT1 protein level to promote HBV gene expression

The above results demonstrate that CUL4–DDB1 is a positive regulator for HBV replication, though these can neither corroborate nor rule out the hypothesis that HBx disrupts the assembly and function of CUL4–DDB1–DCAF complexes. Previous study shown that PRMT1 is a negative regulator of HBV transcription from cccDNA and it is associated with DDB1 and HBx [9]. Therefore, PRMT1 could be a target of CUL4–DDB1 and loss of DDB1 function may lead to the accumulation of PRMT1. To investigate the role of PRMT1 in viral replication and how it is regulated by HBx and DDB1, we overexpressed the Myc-His-tagged PRMT1 (MH-PRMT1) in T43 cells to monitor its effect on viral gene expression (Fig. 3A). Consistent to previous report, we found that there was a significant reduction of the expression level for HBe and HBs genes, as shown by semi-quantitative RT-PCR and ELISA (Fig. 3B and C).

Since PRMT1 is associated with DDB1 and HBx [9], we were intrigued in the biological implication of this association. Upon knockdown of DDB1, there was an elevation of total protein level of PRMT1 (Fig. 3D). It suggests that DDB1 negatively regulates PRMT1 protein function that potentially stimulates the gene expression of HBV.

3.4. Polyubiquitination of PRMT1 requires DDB1

PRMT1 was distributed in both soluble and insoluble fractions of nuclear extracts, indicating it is associated with chromatin compartments (Fig. 3E). As PRMT1 is speculated to function at the transcription or replication level, we focused on characterizing the chromatin fraction of PRMT1. Clearly, knockdown of DDB1 displayed lower level of slow migration forms of PRMT1 in the chromatin fraction of 7702 cells in comparison with mock transfected cells, which are likely to be polyubiquitinated forms of PRMT1 (Fig. 3F). Upon knocking down of DDB1 in 7702 cells, these high molecular weight species were dramatically reduced, indicating that this modification is likely DDB1-dependent polyubiquitin species in the consideration that CUL4–DDB1 is a RING-finger type of ubiquitin ligase [19]. Loss of these putative polyubiquitination is consistent with the elevated level of PRMT1 upon loss of DDB1. More convincingly, when 7702 cells pre-treated with MG132 were lysed in guanidine buffer and subject to

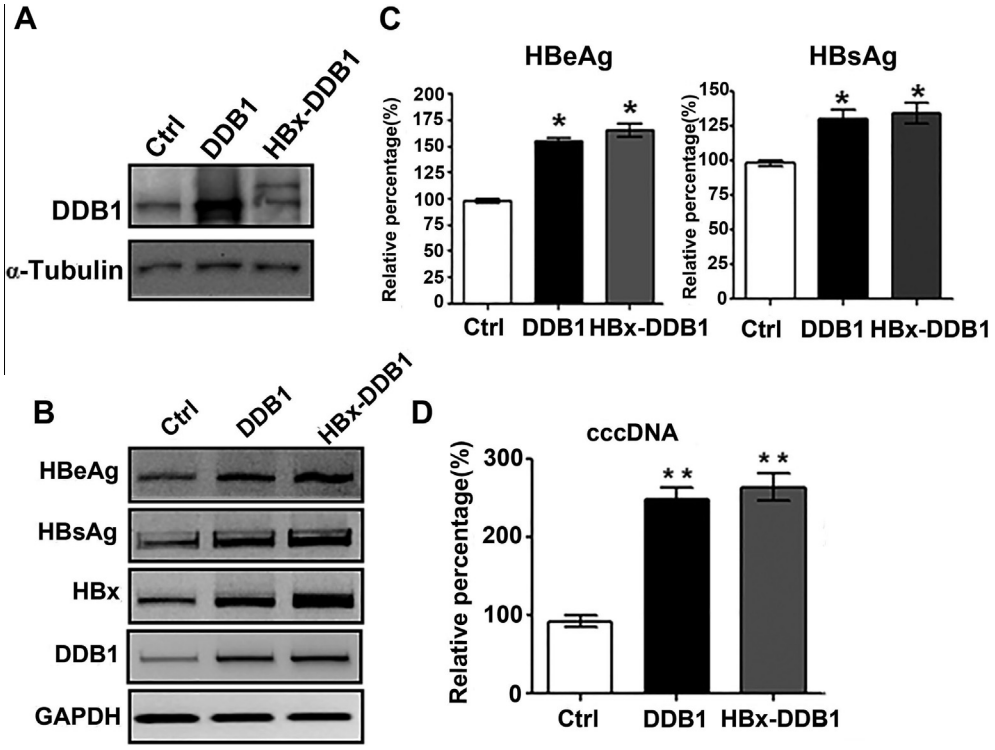

Fig. 2. Excessive amount of DDB1 promotes HBV replication and gene expression. T43 cell line was infected with lentivirus expressing GFP alone, DDB1 or DDB1-HBx fusion proteins. Related DDB1 protein was detected by Western blotting using anti-DDB1 antibody. (B) Semi-quantitative RT-PCR was performed to determine the relative RNA levels of indicated genes after viral infection. (C) ELISA analysis for HBsAg and HBeAg in the supernatant of lentiviral-infected cultures. (D) Infected cells were collected for detection of cccDNA by Real-time PCR. Error bar, s.e.m. ($n = 3$). Student's two-tailed t-test: $^{**}P < 0.01$ vs. control.

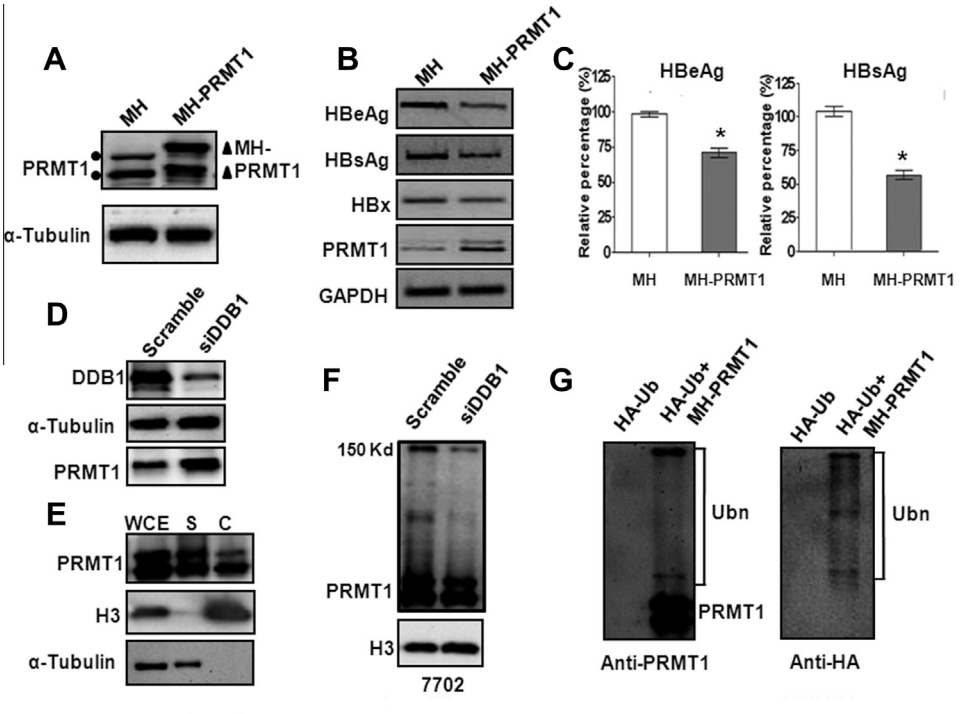

Fig. 3. DDB1 regulates PRMT1 protein level to regulate HBV gene expression. (A) Western blotting of cell extract from T43 transfected with pcDNA3.1-Myc-His(MH) or pcDNA3.1-Myc-His-PRMT1(MH-PRMT1). Endogenous (circle) or MH-PRMT1 protein (triangle) was indicated. (B) For same transfection experiment, total RNA was isolated and semi-quantitative RT-PCR was performed to determine the relative expression levels of indicated genes. (C) Culture media were collected for ELISA analysis for HBsAg and HBeAg. (D) DDB1 was knocked down in 7702 cells and PRMT1 protein level was evaluated by Western Blotting. There are two bands of PRMT1, the upper band possibly represents uncharacterized modified form. (E) Soluble proteins (S) and chromatin proteins (C) were fractionated as described in Section 2. Histone H3 and α-tubulin were used as corresponding markers (F) Chromatin fractions were isolated and resolved for low and high molecular weight species of PRMT1. (G) Plasmids HA-tagged ubiquitin and MH-PRMT1 were co-transfected as indicated. His-tagged proteins were purified and resolved by SDS–PAGE as described above. Polyubiquitinated species were monitored by anti-PRMT1 or anti-HA antibody.

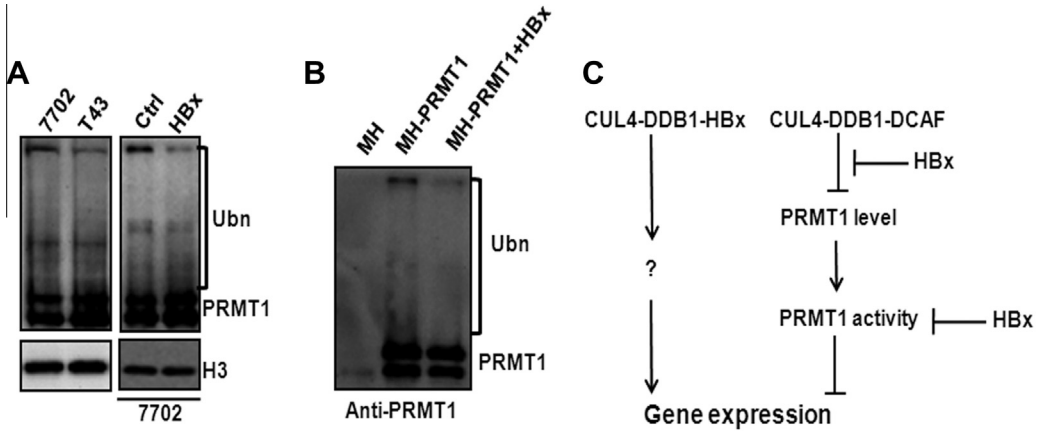

Fig. 4. HBx affects the polyubiquitination and separates functions of PRMT1. (A) Detection of slow migrating forms of PRMT1 in T43 cells and in 7702 cells infected with lentivirus expressing HBx. (B) 7702 cells pre-treated with MG132 were infected with lentivirus expressing HBx, followed by transfection with pcDNA3.1-Myc-His-PRMT1 plasmids. His-tagged species were pulled down and probed with anti-PRMT1 antibody. (C) Postulated model for double-sided roles of DDB1 in HBV replication and its interaction with HBx and PRMT1. DDB1 stimulates HBV transcription and replication through association with HBx. HBx also can displace specific type of DCAF protein and thus perturb the targeting of PRMT1 by DDB1. Increased level of PRMT1 protein inhibits HBV replication activities, but this effect can be counteracted by direct inhibition of PRMT1 methylation activity by HBx.

pulldown assay under denatured condition to purify His-tagged PRMT1, the high molecular weight forms were co-purified with the unmodified form of PRMT1, suggesting that these forms are covalently attached to PRMT1 protein (Fig. 3G). Moreover, these slow migrating forms or putative polyubiquitinated forms of PRMT1 were corresponded to HA-tagged polyubiquitin chains represented by HA signals. These results suggest that PRMT1 is polyubiquitinated and this process is DDB1-dependent.

3.5. HBx affects the polyubiquitination and separates functions of PRMT1

Supposing HBx binds to DDB1 and blocks the assembly of CUL4–DDB1 with PRMT1-specific DCAF [20,21], HBx would also reduce the polyubiquitination forms of PRMT1. Indeed, the high molecular weight forms of PRMT1 in chromatin fraction were significantly reduced in T43 cells in comparison with control cell line (Fig. 4A). This reduction was caused by the presence of HBx, since over-expression of HBx also down-regulated the slow migration forms of PRMT1 (Fig. 4A). His-tagPulldown assay in the denatured condition, combining with Fig. 3G, proved that HBx reduced polyubiquitinated forms of PRMT1 (Fig. 4B). This result consolidates that PRMT1 is indeed polyubiquitinated by DDB1-containing E3 ligase that is antagonized by HBx.

4. Discussion

Although several lines of evidence have pointed out that the physical interaction between HBx and DDB1 is important for HBV gene expression and replication [20,22–24], the underlying mechanism is not fully understood. In this study, we provided evidence that DDB1 and its association with HBx are required for viral replication and transcription of HBV at least partially through PRMT1, one of their binding partners. We propose that DDB1 can negatively regulate protein level of PRMT1 by promoting its polyubiquitination whereas HBx decreases this process by dissembling CUL4–DDB1 E3 ligase.

Although we have shown that the slow migration forms of PRMT1 are polyubiquitinated species and are subject to regulation of DDB1 and HBx, we cannot exclude the possibility that PRMT1 is ubiquitinated by other E3 ligase without in vitro ubiquitination assay or knowing the identity of the substrate-targeting DCAF. However, in the consideration that PRMT1 is associated with HBx and DDB1 complex, it is likely that polyubiquitination of PRMT1 is promoted directly by CUL4–DDB1 complex and HBx counteracts this activity. Although we detected notable elevation of protein level of PRMT1 in DDB1-knockdown cells, it was difficult to observe accumulation of PRMT1 in T43 and HBx overexpressing cells. We reason it is possible that HBx blocks PRMT1 polyubiquitination in limited region of chromatin or cccDNA, and thus does not influence the total protein level of this protein.

Results from DDB1 RNA interfering assay and HBx or PRMT1 overexpression suggest that binding of HBx to DDB1 could be both positive and negative effect on the function of CUL4–DDB1 E3 ligases: RNAi assay disrupts overall functions of CUL4–DDB1–DCAFs instead of specific subset of substrates, and from this set of assays we conclude that CUL4–DDB1 is indispensable for HBV replication cycle; moreover, HBx could exert positive effect on CUL4–DDB1 as fusion protein of HBx-DDB1 promotes HBV replication and transcription; instead, HBx can also block a subset of CUL4–DDB1–DCAF complexes as ectopic expression of HBx specifically disturbs PRMT1 polyubiquitination. Thus, influence of HBx on DDB1 is multi-faceted, representing complicated function of cellular CUL4–DDB1–DCAF E3 ligases and diverged function of the novel complex CUL4–DDB1 coupled with HBx.

The HBV genome forms cccDNA minichromosomes that serve as the main replicative intermediate and platform for viral RNA transcription in the infected hepatocytes. cccDNA is subject to multiple layers of regulation including epigenetic mechanisms such as methylation and acetylation. Histone H3, H2B as well as non-histone protein including HBcAg, HBx and other regulatory proteins are recruited to cccDNA and exert regulatory roles in viral gene expression and replication. PRMT1 is also deposited to cccDNA and negatively regulates HBV gene expression [9]. Confusingly, as HBx blocks DDB1-dependent polyubiquitination of PRMT1, it is supposed to increases the level or chromatin enrichment of PRMT1, which would inhibit viral transcription mimicking the situation of ectopic expression of PRMT1. And, in HBV-replicating cells, the PRMT1 activity was suppressed by HBx instead of stimulated [9]. One of the explanations that could resolve this contradiction is to suppose the inhibition of PRMT1 polyubiquitination

and its protein enrichment on cccDNA is only a side-effect of HBx. The blockage of PRMT1 degradation and subsequent increase of methyltransferase activity can be counteracted by direct association of HBx with it [9].

All in all, our findings suggest that HBx regulates the CUL4–DDB1 network in both positive and negative ways. The precise mechanisms of how HBx coordinates with DDB1 to promote HBV replicative activities and which subset of cellular targets is interfered remain to be elucidated.

Acknowledgments

We thank Dr. Christine Neuveut for kindly providing constructs pcDNA3.1-Myc-His-PRMT1. This work is supported by the Ministry of Science and Technology of China (2011CB966200 and 2013CB911000), National Natural Science Foundation of China (81071362 and 31171319), Department of Science and Technology of Sichuan Province (2011SZ0002 and 2012JQ0005), and Bureau of Science and Technology of Chengdu (11PPYB072SF).

References

[1] T. Li, E.I. Robert, P.C. van Breugel, M. Strubin, N. Zheng, A promiscuousalpha-helical motif anchors viral hijackers and substrate receptors to the CUL4–DDB1 ubiquitin ligase machinery, Nat. Struct. Mol. Biol. 17 (2010) U105–U131.
[2] J. Lee, P. Zhou, DCAFs, the missing link of the CUL4–DDB1 ubiquitin ligase, Mol. Cell 26 (2007) 775–780.
[3] X. Zhang, J. Hou, M. Lu, Regulation of hepatitis B virus replication by epigenetic mechanisms and microRNAs, Front. Genet. 4 (2013) 202.
[4] H.S. Chen, S. Kaneko, R. Girones, R.W. Anderson, W.E. Hornbuckle, B.C. Tennant, P.J. Cote, J.L. Gerin, R.H. Purcell, R.H. Miller, The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks, J. Virol. 67 (1993) 1218–1226.
[5] F. Zoulim, J. Saputelli, C. Seeger, Woodchuck hepatitis virus X protein is required for viral infection in vivo, J. Virol. 68 (1994) 2026–2030.
[6] T.T. Wu, L. Coates, C.E. Aldrich, J. Summers, W.S. Mason, In hepatocytes infected with duck hepatitis B virus, the template for viral RNA synthesis is amplified by an intracellular pathway, Virology 175 (1990) 255–261.
[7] Y.Y. Zhang, B.H. Zhang, D. Theele, S. Litwin, E. Toll, J. Summers, Single-cell analysis of covalently closed circular DNA copy numbers in a hepadnavirus-infected liver, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 12372–12377.
[8] S. Locarnini, W.S. Mason, Cellular and virological mechanisms of HBV drug resistance, J. Hepatol. 44 (2006) 422–431.
[9] L. Belloni, L. Allweiss, F. Guerrieri, N. Pediconi, T. Volz, T. Pollicino, J. Petersen, G. Raimondo, M. Dandri, M. Levrero, IFN-alpha inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome, J. Clin. Invest. 122 (2012) 529–537.
[10] L. Belloni, T. Pollicino, F. De Nicola, F. Guerrieri, G. Raffa, M. Fanciulli, G. Raimondo, M. Levrero, Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 19975–19979.
[11] S. Benhenda, A. Ducroux, L. Riviere, B. Sobhian, M.D. Ward, S. Dion, O. Hantz, U. Protzer, M.L. Michel, M. Benkirane, O.J. Semmes, M.A. Buendia, C. Neuveut, Methyltransferase PRMT1 is a binding partner of HBx and a negative regulator of hepatitis B virus transcription, J. Virol. 87 (2013) 4360–4371.
[12] M.J. Bouchard, R.J. Schneider, The enigmatic X gene of hepatitis B virus, J. Virol. 78 (2004) 12725–12734.
[13] D. Sitterlin, F. Bergametti, C. Transy, UVDDB p127-binding modulates activities and intracellular distribution of hepatitis B virus X protein, Oncogene 19 (2000) 4417–4426.
[14] S. Angers, T. Li, X. Yi, M.J. MacCoss, R.T. Moon, N. Zheng, Molecular architecture and assembly of the DDB1–CUL4A ubiquitin ligase machinery, Nature 443 (2006) 590–593.
[15] Y.J. He, C.M. McCall, J. Hu, Y. Zeng, Y. Xiong, DDB1 functions as a linker to recruit receptor WD40 proteins to CUL4-ROC1 ubiquitin ligases, Genes Dev. 20 (2006) 2949–2954.
[16] L.A. Higa, M. Wu, T. Ye, R. Kobayashi, H. Sun, H. Zhang, CUL4–DDB1 ubiquitin ligase interacts with multiple WD40-repeat proteins and regulates histone methylation, Nat. Cell Biol. 8 (2006) 1277–1283.
[17] P. Shiyanov, A. Nag, P. Raychaudhuri, Cullin 4A associates with the UV-damaged DNA-binding protein DDB, J. Biol. Chem. 274 (1999) 35309–35312.
[18] B. Iovine, M.L. Iannella, M.A. Bevilacqua, Damage-specific DNA binding protein 1 (DDB1): a protein with a wide range of functions, Int. J. Biochem. Cell Biol. 43 (2011) 1664–1667.
[19] J. Mendez, B. Stillman, Chromatin association of human origin recognition complex, cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis, Mol. Cell Biol. 20 (2000) 8602–8612.
[20] H. Shimizu, D. Saliba, M. Wallace, L. Finlan, P.R. Langridge-Smith, T.R. Hupp, Destabilizing missense mutations in the tumour suppressor protein p53 enhance its ubiquitination in vitro and in vivo, Biochem. J. 397 (2006) 355–367.
[21] E.S. Fischer, A. Scrima, K. Bohm, S. Matsumoto, G.M. Lingaraju, M. Faty, T. Yasuda, S. Cavadini, M. Wakasugi, F. Hanaoka, S. Iwai, H. Gut, K. Sugasawa, N.H. Thoma, The molecular basis of CRL4DDB2/CSA ubiquitin ligase architecture, targeting, and activation, Cell 147 (2011) 1024–1039.
[22] O. Leupin, S. Bontron, C. Schaeffer, M. Strubin, Hepatitis B virus X protein stimulates viral genome replication via a DDB1-dependent pathway distinct from that leading to cell death, J. Virol. 79 (2005) 4238–4245.
[23] K.F. Tang, J. Xie, M. Chen, Q. Liu, X.Y. Zhou, W. Zeng, A.L. Huang, G.Q. Zuo, Y. Wang, R. Xiang, H. Ren, Knockdown of damage-specific DNA binding protein 1 (DDB1) enhances the HBx-siRNA-mediated inhibition of HBV replication, Biologicals 36 (2008) 177–183.
[24] H. Tang, L. Delgermaa, F. Huang, N. Oishi, L. Liu, F. He, L. Zhao, S. Murakami, The transcriptional transactivation function of HBx protein is important for its augmentation role in hepatitis B virus replication, J. Virol. 79 (2005) 5548–5556.