HTLV-1 basic leucine zipper factor downregulates cyclin D1 expression via interactions with NF-κB

YUNYUN MA[1], BO ZHANG[1], DONG WANG[1], LILI QIAN[1], XIANMEI SONG[1], XUEYIN WANG[1], CHAOKUAN YANG[1] and GUOQIANG ZHAO[2]

[1]Henan Medical College, Zhengzhou, Henan 451191; [2]School of Basic Medical Sciences, Zhengzhou University, Zhengzhou, Henan 450001, P.R. China

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Abstract. Human T cell leukemia virus type 1 (HTLV-1) is an oncogenic retrovirus. It can cause adult T cell leukemia (ATL) and other diseases. The HTLV-1 basic leucine zipper (bZIP) factor (HBZ), which is encoded by the minus-strand of the provirus, is expressed in all cases of ATL and involved in T cell proliferation. However, the exact mechanism underlying its growth-promoting activity is poorly understood. Herein, we demonstrated that HBZ suppressed cyclin D1 expression by inhibiting the nuclear factor (NF)-κB signaling pathway. Among the potential mechanisms of cyclin D1 inhibition mediated by HBZ, we found that HBZ suppressed cyclin D1 promoter activity. Luciferase assay analysis revealed that HBZ repressed cyclin D1 promoter activity by suppressing NF-κB-driven transcription mediated by the p65 subunit. Using an immunoprecipitation assay, we found that HBZ could bind to p65, but not p50. Finally, we showed that HBZ selectively interacted with p65 via its AD+bZIP domains. By suppressing cyclin D1 expression, HBZ can alter cell cycle progression of HTLV-1-infected cells, which may be critical for oncogenesis.

Introduction

Nuclear factor (NF)-κB is a transcription factor member of the Rel family. The Rel family includes five members, RelA/p65, RelB, c-Rel, p50 and p52 (1-3), which have sequence similarity to over ~300 amino acids in the amino-terminal half of these proteins. These Rel family members can form heterodimers or homodimers with a range of DNA-binding and activation potentials. Even though all family members contain a Rel homology domain that is required for DNA binding, dimerization, and nuclear localization, only RelA/p65 (hereafter referred to as p65), RelB and c-Rel, have a specific carboxy terminal transactivation domain that can regulate NF-κB-dependent gene expression (3,4). The most widely studied active form of NF-κB is a p65-p50 heterodimer, which is a main activator of gene transcription (5,6).

Although this transcription factor was first known as a regulator of inflammation and immune responses, recent studies show that NF-κB also regulates cell proliferation (7,8). The mechanism of proliferation regulation involves the stimulation of cyclin E/CDK2 activity and c-Myc expression (9-11), as well as other proliferation-associated genes, such as colony stimulating factor, platelet-derived growth factor, and cyclin D1 (4,12-14).

Cyclin D1 is a member of the D-type cyclins. It regulates the G1/S phase transition of the cell cycle. Cyclin D1 is induced by mitogens and can dimerize with CDK4/CDK6 to phosphorylate retinoblastoma (Rb) protein, which then derepresses E2F transcriptional activity to allow entry into the S-phase (15,16). Analysis of the cyclin D1 promoter revealed the presence of two κB sites located at positions -840 and -33, which have been deemed to be κB1 and κB2 (12), respectively. Mutational analysis demonstrated that binding of the p65/p50 complex to the -33 κB2 site is important for *cyclin D1* transcriptional regulation during the proliferative response induced by growth factors or serum or by stimulation via the Ras/Rac pathway (12,13,17).

Human T cell leukemia virus type 1 (HTLV-1) is a complex retrovirus that can cause adult T cell leukemia (ATL) (18,19). HTLV-1 encodes several unique proteins that can participate in viral infectivity, replication, persistence and transformation (20). Among these proteins, HTLV-1 basic leucine zipper (bZIP) factor (HBZ) expression is conserved in all ATL cells and is thought to be essential for leukemogenesis (21). Additionally, the HBZ gene can promote ATL cell proliferation (21). Recent studies have shown that HBZ can inhibit Tax-mediated NF-κB activation and suppress the transcription of various NF-κB target genes (22). These findings suggest that HBZ is involved in the NF-κB signaling pathway, and also contributes to the regulation of *cyclin D1*, an NF-κB target gene.

In a previous study, we showed that HBZ downregulated cyclin D1 expression via interactions with cAMP-response

Correspondence to: Dr Guoqiang Zhao, School of Basic Medical Sciences, Zhengzhou University, 100 Kexue Road, Zhengzhou, Henan 450001, P.R. China
E-mail: zhaogq@zzu.edu.cn

Professor Chaokuan Yang, Henan Medical College, 8 Shuang Hu Road, Xinzheng, Zhengzhou, Henan 451191, P.R. China
E-mail: yckuan0930@sina.com

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element binding protein CREB (23). To characterize other mechanisms of cyclin D1 suppression via HBZ, we utilized 293T and Jurkat cells in which HBZ was stably expressed and studied the effects of HBZ on cyclin D1 expression. We found that HBZ mediated the suppression of cyclin D1 by interacting with p65.

Materials and methods

Cell culture. Jurkat cells (purchased from the China Center for Type Culture Collection, Wuhan, China) were cultured in RPMI-1640 medium (HyClone, Logan, UT, USA). 293T cells (purchased from the China Center for Type Culture Collection) were cultured in Dulbecco's modified Eagle's medium (DMEM) (HyClone). Media were supplemented with 10% fetal bovine serum (FBS; HyClone), 2 mM glutamine, 50 mg/ml streptomycin, and 100 U/ml penicillin. Cells were synchronized by serum starvation for 18 h, followed by stimulation in medium containing 10% FBS for 12 h.

Lentiviral vector construction and transfection with the recombinant lentivirus. Full-length sHBZ (amino acids 1-209) and deletion mutants (sHBZ-AD, amino acids 1-73; sHBZ-basic leucine zipper domain (bZIP), amino acids 155-209; sHBZ-ΔAD, amino acids 74-209; sHBZ-ΔbZIP, amino acids 1-154; and sHBZ-AD+bZIP, amino acids (1-73)+(155-209) were generated by PCR from pET-29a-HBZ (provided by Dr H.J. Zhi; Uniformed Services University of the Health Sciences, Bethesda, MD, USA) with an HA epitope tagged at the C- or N-terminus (Fig. 1). These fragments were subcloned into a lentiviral vector (LV5) and were transfected into 293T cells using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA) as reported previously (23). Infection or transduction with lentiviral vectors was also performed as described previously (23).

Synthesis of cDNA and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the stable lentiviral-infected 293T cells, Jurkat cells and uninfected control cells using Total RNA kit I (R6834-01; Omega, Norcross, GA, USA) according to the manufacturer's instructions. For cDNA synthesis, 2.5 μg total RNA was reverse transcribed using the RevertAid™ First-Strand cDNA Synthesis kit (K1621; Fermentas, Carlsbad, CA, USA). For RT-PCR, cDNA products were quantified with Power SYBR-Green PCR Master Mix and an ABI 7500 Fast Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA transcript levels were quantified to normalize the amount of cDNA that was loaded. The customized primers used were: *cyclin D1* forward, 5'-GACCATCCCCCTGACGGCCGAG-3' and reverse, 5'-CGCACGTCGGTGGGTGTGC-3'; and *GAPDH* forward, 5'-AGAGGCAGGGATGATGTTCTG-3' and reverse, 5'-GACTCATGACCACAGTCCATGC-3'. All PCR experiments were repeated at least 3 times. A melting curve analysis was performed to ensure the specificity of the products after amplification. The median in each triplicate dataset was used to calculate the relative *cyclin D1* mRNA concentration (ΔCt = Ct median mRNA - Ct median *GAPDH*), which was then converted to x-fold-changes ($2^{-\Delta\Delta Ct}$) (24).

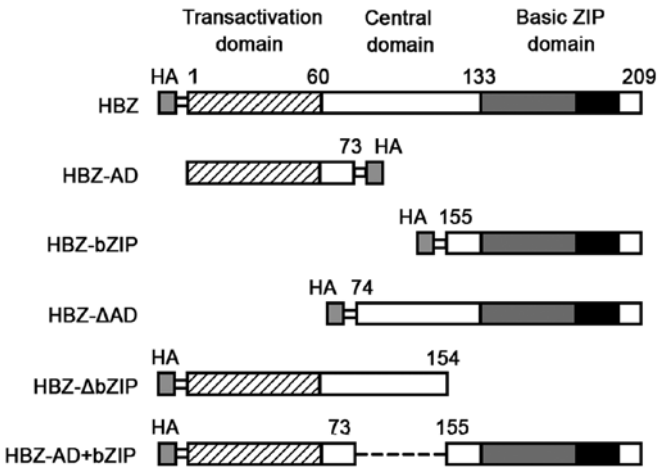

Figure 1. A schematic diagram of HBZ and its mutants used in this study. Characteristic domains of HBZ are indicated as follows: transactivation domain (AD), central domain (CD), and basic leucine zipper domain (bZIP). An HA epitope tag was introduced at the N- or C-terminus of HBZ and its mutants.

Construction of cyclin D1 promoter deletion mutants and luciferase assays. The deletion mutants of human *cyclin D1* promoter that lacked AP1-, STAT-/SP1-, CREB- and NF-κB-binding sites (CD1 to CD5) were generated by PCR and then cloned into the vector pGL3-enhancer (Promega, Madison, WI, USA), as previously reported (23). Luciferase assays were performed using 293T and Jurkat cells, as described previously (23). All reporter assays were performed in triplicate and repeated in three independent experiments. Firefly luciferase values were normalized by *Renilla* luciferase values from pRL-TK (Promega).

Immunoprecipitation and immunoblotting. To examine the protein-protein interactions in the 293T cells, subconfluent cells were transfected with various combinations of expression vectors. Cells were lysed 48 h later in RIPA buffer containing 50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM $MgCl_2$, 1% Triton X-100, 0.5% Nonidet P-40 and protease inhibitors. Lysates were precleared by incubation with 20 μl suspension of 50% protein G-agarose (GE Healthcare Life Sciences, Uppsala, Sweden) for 1 h at 4˚C. Precleared lysates were incubated with anti-HA (Y-11) or anti-NF-κB p65 (C-20) (both from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) antibodies for 1 h at 4˚C, and immune complexes were collected by incubation with protein G-agarose at 4˚C for 1 h. After extensive washing, immunoprecipitated proteins were resolved by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by western blotting with anti-p65 (SC-109), anti-p50 (H-119), or anti-HA (F-7) (all from Santa Cruz Biotechnology, Inc.) antibodies. Membranes were developed with ECL (GE Healthcare Life Sciences). Other antibodies were as follows: anti-mouse IgG and anti-rabbit IgG (GE Healthcare Life Sciences).

Statistical analysis. All data are presented as the means ± standard deviation (SD) from at least 3 independent experiments. Statistical analyses were performed with SPSS (version 19.0; SPSS Inc., Chicago, IL, USA). Statistical significance

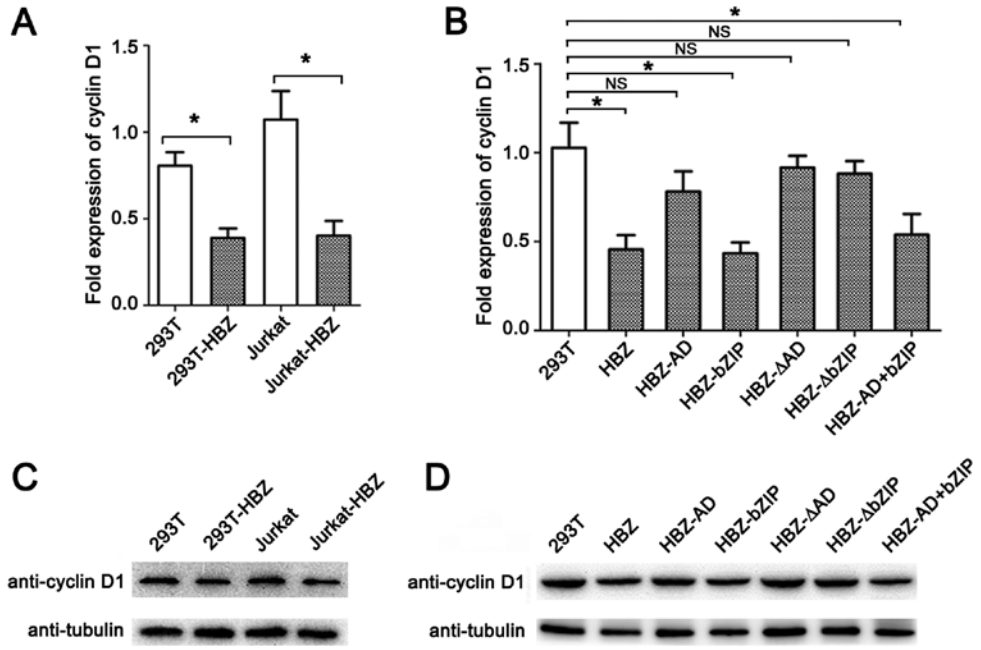

Figure 2. Repression of *cyclin D1* gene expression by HBZ. Cells that stably expressed HBZ or its mutants were generated by lentiviral-mediated transfection of the HBZ genes (HBZ, HBZ-AD, HBZ-bZIP, HBZ-ΔAD, HBZ-ΔbZIP or HBZ-AD+bZP). 293T cells were transduced with either LV-HBZ, LV-HBZ-AD, LV-HBZ-bZIP, HBZ-ΔAD, HBZ-ΔbZIP or HBZ-AD+bZP at an MOI of 5. Jurkat cells were transduced with LV-HBZ at an MOI of 30. Cells were selected in liquid media containing 1 mg/ml puromycin. (A) Levels of *cyclin D1* mRNA transcripts were reduced in the HBZ-expressing cells. Real-time PCR was performed to analyze the expression of *cyclin D1* mRNA in untransduced (293T or Jurkat) cells and transduced (293T-HBZ or Jurkat-HBZ) cells; error bars represent standard deviation (SD). (B) *Cyclin D1* mRNA transcript levels were reduced by the bZIP and bZIP+AD domains of HBZ. Stable expression of HBZ and its mutants in 293T cells and empty 293T cells was confirmed by real-time PCR, as in (A); error bars represent SD. (C) Cyclin D1 expression was reduced by HBZ. The blot shows that 50 μg total cell protein from each HBZ stably transfected cell line was resolved using 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted using cyclin D1, HA and β-tubulin antibodies. (D) Cyclin D1 expression was reduced by HBZ via its bZIP and bZIP+AD domains. Cell lysates were prepared from 293T cells expressing stable HBZ and its mutants and empty-vector transduced 293T cells; immunoblots were carried out as in (C). *P<0.01; NS, no significant difference.

was assessed by one-way analysis of variance (ANOVA) or the Student's t-test. Differences were considered statistically significant when P-values were <0.05.

Results

HBZ inhibits the expression of cyclin D1. To analyze the effect of HBZ on cyclin D1 expression, we first transduced 293T cells with a lentiviral vector carrying the HBZ gene and evaluated cyclin D1 levels by RT-qPCR and western blot analysis. The expression of HBZ resulted in reduced expression levels of cyclin D1 mRNA and protein (Fig. 2A and C). To further characterize this effect in T cells, we transduced HBZ into Jurkat cells using a lentiviral vector. As in 293T cells, HBZ expression resulted in reduced cyclin D1 mRNA and protein levels in the Jurkat cells as detected by RT-qPCR and western blot assays.

HBZ has three domains, and each has one of the following distinct functions (Fig. 1): an activation domain (AD), a central domain (CD), and a bZIP domain (25-27). To identify the portion of HBZ that is necessary to inhibit the expression of cyclin D1, we constructed deletion mutants (Fig. 1). 293T cells were transduced with the lentiviral vectors carrying each of the aforementioned HBZ mutants, followed by RT-qPCR and western blot assays. Two mutants (HBZ-bZIP and HBZ-AD+bZIP) repressed cyclin D1 mRNA and protein levels (Fig. 2B and D). By contrast, three other mutants (HBZ-AD, HBZ-ΔAD and HBZ-ΔbZIP) caused no significant changes in cyclin D1 mRNA and protein levels. These findings indicate that the bZIP domain, with or without an AD domain, is needed to suppress levels of cyclin D1 mRNA and protein.

HBZ suppresses cyclin D1 promoter activity through the CRE and NF-κB sites. Based on the ability of HBZ to down-regulate *cyclin D1* gene transcription, we assessed whether *cyclin D1* promoter activity was under the control of HBZ. We constructed a *cyclin D1* promoter reporter plasmid and co-transfected it into HBZ stably expressing Jurkat cells with a control plasmid, pRL-TK. Our findings revealed that HBZ reduced *cyclin D1* promoter activity in Jurkat T cells (data not shown).

The *cyclin D1* promoter has multiple transcription factor-binding sites, such as AP-1, E2F, NF-κB and Oct-1 (28). Extracellular signals act via signal transduction pathways to converge at binding sites to activate or inhibit promoter activity and regulate cell cycle progression. To identify *cis*-element(s) in the *cyclin D1* promoter region that are responsive to HBZ, we transfected luciferase report constructs that contained *cyclin D1* promoters of various lengths into empty 293T cells and stable HBZ-expressing 293T cells. The promoter activity of full-length pGL3-CD1/-1021 in 293T-HBZ cells was reduced by ~30% compared with the activity noted in the empty 293T cells (Fig. 3). These findings are in accord with the aforementioned studies carried out in the Jurkat human

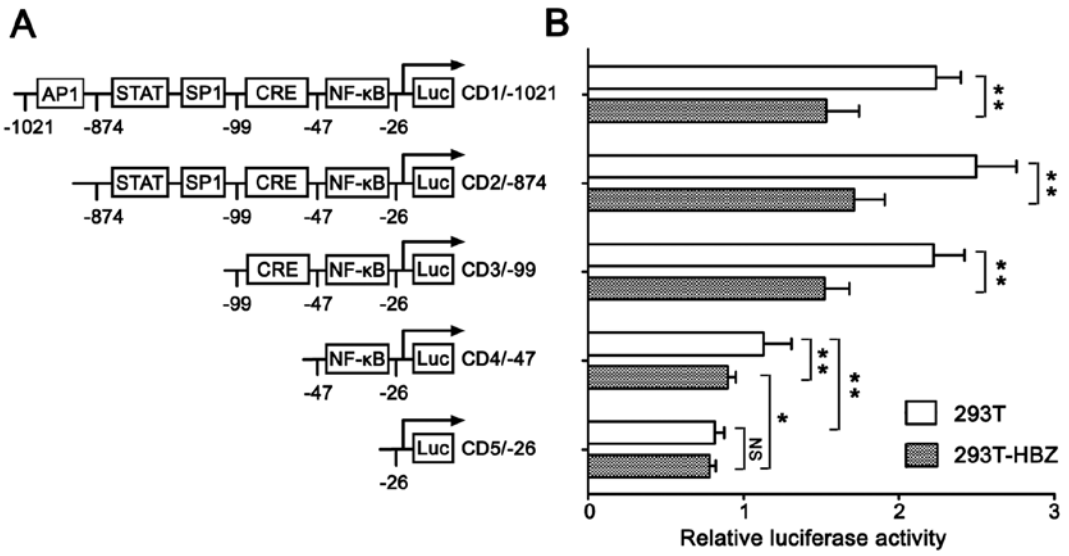

Figure 3. Mutational analysis of *cyclin D1* promoter activity. (A) A schematic representation of luciferase reporter constructs that contained *cyclin D1* promoters of various lengths. Diagrams depict locations of the potential transcription factor binding sites in the *cyclin D1* promoter. (B) The differential effects of HBZ on the activity of *cyclin D1* promoter mutants. A total of 2.5 μg luciferase reporter plasmids (CD1-CD5) and 0.25 μg control *Renilla* luciferase reporter plasmid, pRL-TK, were transfected into empty 293T and 293T-HBZ cells. Luciferase activity was measured 24 h after transfection and the fold-induction is presented; plots represent the average of three independent experiments; error bars represent SD. *P<0.05; **P<0.01; NS, no significant difference.

T cell line. When the AP1 site was deleted (pGL3-CD2/-874), the promoter activity was not reduced. Furthermore, deletion of the STAT and SP1 (pGL3-CD3/-99) sites also did not reduce promoter activity, and pGL3-CD3/-99, which contained CRE and NF-κB sites, had promoter activity that was similar to pGL3-CD1/-1021, which contained a full-length *cyclin D1* promoter. However, when the CRE site was deleted (pGL3-CD4/-47), promoter activity was decreased to 50% of the pGL3-CD3/-99 activity in the empty 293T cells, while in the 293T-HBZ cells it decreased to 40%. The promoter activity of pGL3-CD4/-47 in the 293T-HBZ cells was reduced 20% compared with that noted in the empty 293T cells. Further deletion of the NF-κB site (pGL3-CD5/-26) resulted in reduction of the promoter activity to ~30% of pGL3-CD4/-47 in the empty 293T cells. By contrast, there was no significant difference in pGL3-CD5/-26 promoter activity between the 293T and 293T-HBZ cells. Although we previously reported that the CRE sites were critical for HBZ-mediated suppression of *cyclin D1* promoter activity (23), these findings indicate that the NF-κB site is also responsible for HBZ-mediated suppression of *cyclin D1* promoter activity.

Domains of HBZ that are responsible for NF-κB p65 inactivation. Previous studies suggest that NF-κB can activate *cyclin D1* promoter activity primarily by the proximal binding site interacting with the classical p50/p65 complex (12,13,29). We aimed to ascertain whether HBZ contributes to the regulation of this signaling pathway. Accordingly, we first evaluated the effect of HBZ on the NF-κB signaling pathway using a luciferase assay. We transfected 293T and 293T-HBZ cells with pGL3-CD4/-47 luciferase reporter plasmids with or without p65-expressing vectors. HBZ expression suppressed p65-mediated pGL3-CD4/-47 (NF-κB) activation (Fig. 4A). Next, we sought to identify the region of HBZ responsible for repressing NF-κB activation. To this end, we transfected pGL3-CD4/-47 luciferase reporter plasmids with p65 vectors into HBZ-deletion mutants that were stably expressed in 293T cells and then performed a luciferase assy. Wild-type HBZ markedly downregulated p65-mediated pGL3-CD4/-47 (NF-κB) activation (Fig. 4B). Compared with other mutants, only the HBZ-AD+bZIP mutant exhibited significant suppressive activity.

HBZ represses NF-κB activity by binding to p65. The data described above suggest that HBZ influences *cyclin D1* promoter activity via the NF-κB p65 signaling pathway. To further investigate whether HBZ could physically interact with NF-κB p65, and to identify whether the HBZ protein was necessary for binding with the NF-κB p65 protein, we performed a co-immunoprecipitation assay. We found that three mutants (HBZ-ΔAD, HBZ-ΔbZIP and HBZ-AD+bZIP) could bind to p65 (Fig. 5A), demonstrating that at least two of the three domains in HBZ were required for binding between HBZ and p65. However, only HBZ-AD+bZIP suppressed NF-κB activation in the luciferase assay (Fig. 4B), indicating the significance of the AD and bZIP domains for p65 binding. In normal cells, p65 and p50 form heterodimers and bind to the κB site in the *cyclin D1* promoter. We next studied the binding affinity of HBZ and p50 by immunoprecipitation. As shown in Fig. 5B, HBZ did not bind to p50, nor did it affect binding between p65 and p50.

Taken together, these observations demonstrate that the HBZ-AD and bZIP domains directly physically associate with NF-κB p65, and this interaction acts to inhibit *cyclin D1* expression.

Discussion

Accumulating evidence indicates that the cell cycle is regulated by oncogenes and tumor-suppressor genes, and that cell cycle alterations can occur in response to various carcinogens (30,31),

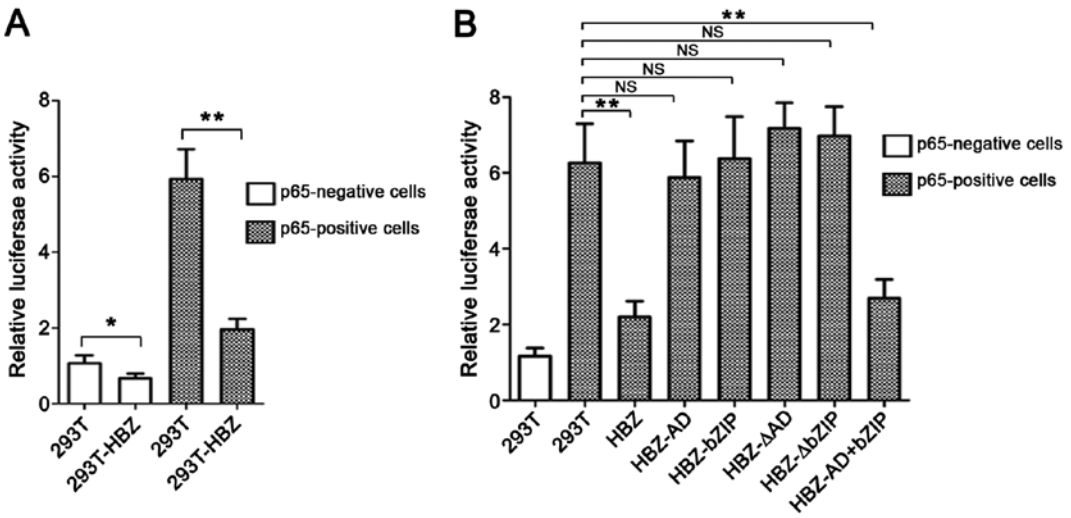

Figure 4. Domains of HBZ responsible for suppressing *cyclin D1* promoter activity at the proximal-κB site. (A) HBZ suppressed p65-mediated activity of the cyclin D1 promoter at the proximal nuclear factor-κB (NF-κB) site. Empty 293T and 293T-HBZ cells were co-transfected with 2.5 μg CD5-Luc reporter plasmid and 0.25 μg pRL-TK plasmid with or without 1 μg expression plasmid that encoded p65. After 24 h, luciferase levels were measured; error bars represent SD. (B) Analysis of HBZ deletion mutants for their effects on p65-mediated NF-κB activation. Empty 293T cells and HBZ mutant-expressing 293T cells were co-transfected with CD5-Luc and pRL-TK, with or without 1 μg p65 expression plasmid. After 24 h, luciferase levels were measured; error bars represent SD. *P<0.05; **P<0.01; NS, no significant difference.

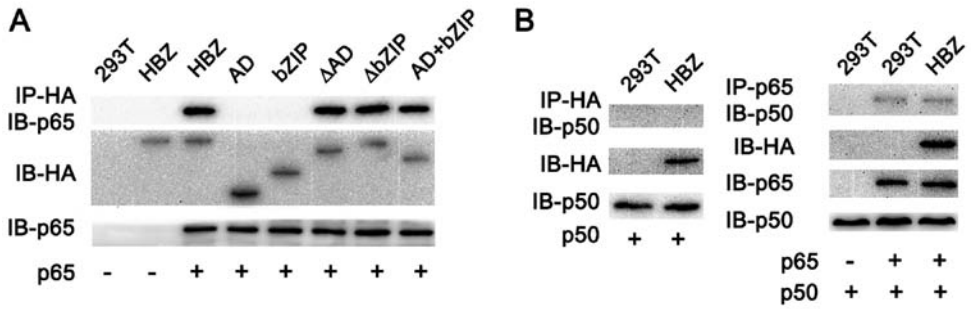

Figure 5. HBZ and its mutants interact with nuclear factor (NF)-κB p65. (A) Identification of the region of HBZ responsible for interacting with p65. Empty 293T cells and cells that stably expressed HBZ and its mutants in 293T cells were transfected with or without 1 μg p65 expression plasmid. Cell lysates were subjected to immunoprecipitation (IP) using anti-HA antibody followed by immunoblotting (IB) using anti-p65 antibody. Expression levels of p65 and HBZ mutants were measured. (B) HBZ did not influence p65/p50 interactions. Empty 293T and 293T-HBZ cells were transfected with p50 expression plasmid with or without p65 expression plasmids. Cell lysates were subjected to IP using anti-HA or anti-p65 antibodies, followed by IB using anti-p50 antibody. Expression levels of p65, p50 and HBZ were evaluated.

implicating cell cycle regulation in carcinogenesis (32). Normal eukaryotic cells have a well-defined cell cycle that consists of four distinct stages: G1, S, G2 and M. G1 and G2 phases represents gaps between the M-S phase and S-M transitions, respectively. These gaps allow for the repair of DNA damage and replication errors. Moreover, G1 represents a period when many pathways can be engaged to influence cell fate (33). Extracellular changes, such as hypoxia, stress and diverse metabolic responses, are integrated and interpreted during this period. Aberrant control of G1, caused by the activation of many oncogenes or the inactivation of tumor-suppressor genes, plays a critical role in tumorigenesis (33). Cyclin D1 is a key regulator of G1-S transition (34). As a sensor that is activated in response to extracellular changes, cyclin D1 can be induced by growth factors and stress in response to the activation of various signaling pathways, including NF-κB (35-37).

The transcription factor NF-κB belongs to the Rel family. Mature dimeric NF-κB proteins can translocate to the nucleus and activate genes involved in apoptosis, cell proliferation and angiogenesis. It can be activated in response to many viral infections, and is thought to be important in the host protective response to viral pathogens. Thus, many viruses have evolved distinct strategies to regulate the activation of NF-κB. Furthermore, activation of the NF-κB signaling pathway has also been reported in various types of cancer cells and is be thought to play an important role in the development and progression of tumors (38,39).

HTLV-1 was first reported in 1980 (18). After transmission, it increases the viral copy number by driving the clonal proliferation of infected cells, which results in the onset of ATL (40,41). In this strategy, Tax is known to play a critical role in increasing the number of HTLV-1-infected cells by promoting proliferation and inhibiting apoptosis (42,43). Tax can activate NF-κB by both classical and alternative pathways via interactions with IKKγ and p100 (44,45). However, Tax can often be inactivated by genetic and epigenetic modifications

in ATL (40,46). *HBZ*, another viral gene which is encoded by the minus strand of the HTLV-1 genome, is expressed in all HTLV-1-infected and ATL cells, and promotes the proliferation of these cells (21). Similar to other viral proteins of oncogenic viruses, recent studies have demonstrated multiple functions of HBZ. For example, HBZ can deregulate multiple cellular signaling pathways, including the classical NF-κB, AP-1, transforming growth factor-β (TGF-β), and the Wnt pathways, which possibly contribute to viral persistence and the clonal expansion of infected cells (22,47-49). These findings indicate that *HBZ* is a critical viral gene involved in HTLV-1-mediated oncogenesis.

The present study demonstrated a function of the retroviral protein HBZ and provides some insights into the general mechanism of the proliferation of viral-infected cells. Our data indicate that HBZ can inhibit the expression of cyclin D1 via repression of *cyclin D1* promoter activity. Using *cyclin D1* promoter mutant reporter constructs, the luciferase assay showed that HBZ downregulated *cyclin D1* promoter activity through interactions with the CREB and NF-κB binding sites (Fig. 3), suggesting that HBZ likely interacts with these transcription factors. Our previous study showed that HBZ could directly bind to CREB via a bZIP domain and inhibit CREB transcriptional activity at the CRE site, thereby downregulating levels of *cyclin D1* transcription (23). Therefore, in the present study, we further explored the interaction between HBZ and NF-κB. We found that HBZ suppressed activation of the *cyclin D1* promoter, which contained an NF-κB binding site, in a manner induced by p65. This suppression was found to occur through the HBZ-AD and bZIP domains (Fig. 4). This finding suggests that HBZ exerts effects on the classical NF-κB signaling pathway, although not all of the three HBZ domains participate in this process, in accord with a previous study (22). Finally, our co-immunoprecipitation findings confirmed that HBZ could bind to p65 via the HBZ-AD and/or bZIP domains *in vivo* (Fig. 5A). However, HBZ was unable to bind to a single p50 molecule, and binding between HBZ and p65 did not disturb the formation of p65/p50 heterodimers (Fig. 5B).

As HBZ has been found to be an important viral protein, it remains unclear how HTLV-1 induces transformation of T cells into ATL. One viral protein, Tax, induces the expression of many genes and causes the aberrant expression of many cellular genes involved in the growth and survival of T cells (50). However, another viral protein, HBZ, also interacts with many transcriptional factors and exerts an effect that is opposite to Tax on the regulation of cellular signaling pathways. As shown in the present study, HBZ functions as a suppressor of cyclin D1. In accord with previous studies, we identified a mechanism involved in the cell cycle regulation mediated by HBZ. Although Tax has been found to activate cyclin D1 expression and the NF-κB pathway (45,51,52). we believe that HTLV-1 may take advantage of this opposite function of Tax and HBZ in the regulation of signaling pathways to allow for better survival and regulation of the proliferation of HTLV-1-infected cells.

Acknowledgements

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