RESEARCH ARTICLE

Tax is Involved in Up-regulation of HMGB1 Expression Levels by Interaction with C/EBP

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Abstract

The high mobility group box 1 (HMGB1) protein is a multifunctional cytokine-like molecule that plays an important role in the pathogenesis of tumors. In this study, real-time polymerase chain reactions and Western blot assays indicated that HMGB1 transcriptional activity and protein level are increased in Tax\textsuperscript{+}-T cells (TaxP). To clarify the mechanisms, a series of HMGB1 deletion reporter plasmids (pHLuc1 to pHLuc6) were transfected into Tax\textsuperscript{-}-T cells (TaxN, Jurkat) and Tax\textsuperscript{-}-T cells (TaxP). We found that promoter activity in Tax\textsuperscript{-}-T cells to be higher than that in Tax\textsuperscript{-}-T cells, indicating a significant increase in pHLuc6. Bay11-7082 (NF-κB inhibitor) treatment did not block the enhancing effect. Chromatin immunoprecipitation assays revealed that Tax was retained on a HMGB1 promoter fragment encompassing -1163 to -975. Bioinformatics analysis showed six characteristic cis-elements for CdxA, AP-1, AML-1a, USF, v-Myb, and C/EBP in the fragment in question. Mutation of cis-elements for C/EBP reduced significant HMGB1 promoter activity induced by Tax. These findings indicate that Tax enhances the expression of HMGB1 gene at the transcriptional level, possibly by interacting with C/EBP.

Keywords: Human HMGB1 promoter - Tax - transcriptional regulation - C/EBP

Introduction

Adult T cell leukemia (ATL), an aggressive T-cell malignancy without an effective cure (Yoshida et al., 1982), is found to be caused by human T-cell leukemia virus type I (HTLV-I) infection and occurs in HTLV-I-endemic areas, such as southwestern Japan, Central and South America, the Caribbean islands, Middle East, Aboriginal regions in Australia, and Intertropical Africa (Uchiyama et al., 1977; Vidal et al., 1994). The prognosis is poor, with less than 1 year survival for patients (Goncalves et al., 2010). However, investigators have also shown that low prevalence of HTLV-I infection in Iranian gastric cancer patients in comparison to controls (Tahaei et al., 2011). HTLV-I encodes Tax protein that participates in viral replication, viral infectivity, persistence and transformation (Kannian and Green, 2010). Oncoprotein Tax exhibits diverse functions in host cells, resulting in persistent activation of NF-κB and deregulation of its responsive gene expressions for T cell survival (Patrick et al., 2001; Easley et al., 2010; Alfonso et al., 2012). Tax protein predominantly localizes to the nucleus. However, Tax alone does not bind DNA; moreover, it functions as a transcriptional activator through interactions between the Tax and a large array of transcriptional regulators to regulate cellular processes (Boxus et al., 2008). Notably, the viral-transforming protein Tax is an important part of the oncogenic mechanism of HTLV-I in enhancing transcription by interacting with transcriptional factors. High mobility group box 1 protein (HMGB1), a chromatin-binding nuclear protein and damage-associated molecular pattern molecule, is a multifunctional cytokine-like molecule that has a critical role in the regulation of transcription. Various studies have shown that HMGB1 has pleiotropic effects outside and inside cells. In the nucleus, HMGB1 functions as a non-histone nucleosomal protein that binds DNA, contributes to stabilization of nucleosomes, and promotes DNA repair and replication (Bustin, 1999). In the extracellular milieu, HMGB1 is a pro-inflammatory cytokine that acts as an alarmin via passive release from damaged or necrotic cells (Beyer et al., 2012; He et al., 2012; Yi et al., 2013) or by active secretion from innate immune system cells in response to LPS, TNF-α or IL-1β stimulation to induce T-cell activation, cytokine production, and inflammatory responses by the transduction of cellular signals through its receptors (Akirav et al., 2012; Kang et al., 2013; Mohammad et al., 2013). Aside from these pro-inflammatory functions, HMGB1 protein also promotes regeneration processes and accelerates cell cycle progression. This paradoxical

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function of HMGB1 protein has been revealed in the growth and spread of many types of tumors such as hepatocellular carcinoma, colon cancer, breast cancer, and leukemia (Kostova et al., 2010; Ohmori et al., 2011; Jube et al., 2012; Lee et al., 2012; Liu et al., 2012; Xing et al., 2012; Yu et al., 2012; Stoetzer et al., 2013). Thus, HMGB1 has become the focus of recent cancer research and is currently a relevant target for cancer treatment (Ohmori et al., 2011; Yu et al., 2012).

Some studies have demonstrated that HMGB1 protein binds virus nucleoprotein, modulates viral replication, and promotes retrovirus HIV dissemination and latency, which indicate that the alarmin HMGB1 contributes to the activation of the immune system (Barqasho et al., 2010; Marius et al., 2011; Moisy et al., 2012; Pirot et al., 2012). However, the pathogenic mechanisms for persistent immune activation remain unknown. HTLV-I, similar to HIV retrovirus, is the etiological agent of an aggressive malignancy of the CD4+ T-cells. Its virally encoded Tax oncoprotein is a key factor in HTLV-I pathogenicity by up-regulation of its responsive gene expressions. To date, the role of HMGB1 in the pathogenesis of HTLV-I infection has not been clearly defined. In this work, we demonstrated that HTLV-I virally encoded Tax protein enhanced HMGB1 transcription levels, and that Bay11-7082 (NF-κB inhibitor) did not repress Tax-mediated transcription activation of the HMGB1 gene. Tax protein was recruited at the -1103 HMGB1 site. In order to elucidate further the association of Tax and HMGB1, and the involvement of putative transcription factor-binding motifs targeted by Tax in the regulation of the HMGB1 promoter activity, site-directed mutation was introduced into these putative ciselements within the pHLuc6 (−1163/+83). Mutation in the C/EBP-motif reduced the Luc activity to 52% of the pHLuc6 in TaxP cells. Taken together, our results revealed that Tax protein, a transcription regulator, subtly regulated HMGB1 transcription by interacting with transcription factor C/EBP.

Materials and Methods

Reagents

Plasmid pGL3-neo-luc was donated kindly by Prof. Guoqiang Zhao of Zhengzhou University. pCMV-Tax and pCMV-Neo were from Dr. Sho ji Yamaoaka of Kyoto University. Tax M22 and Tax M47 were given by Edward W. Harhaj of Miami University, and HMGB1 and pHLuc6 mutants were from Dr. Sho ji Yamaoka of Kyoto University, Tax M22 and Tax M47 were given by Edward W. Harhaj of Miami University, and HMGB1 and pHLuc6 mutants were from Dr. Sho ji Yamaoka of Kyoto University. Transfection and luciferase (Luc) assays

Jurkat cells were purchased from ACTT (USA), pCMV-Tax and pCMV-Neo were stably transduced into Jurkat cells and were selected with 600 μg/ml G418 to obtain TaxP and TaxN cells, respectively. TaxP and TaxN cells were preserved in the immunity research center of Xinxiang Medical University. All cells were cultured in RPMI 1640 medium supplemented with FBS (10%), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (10 mM), antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin), and L-glutamine (2 mM) in a humidified atmosphere incubator with 5% CO2. For Luc assays, Jurkat, TaxP, and TaxN cells were each plated at 5×104 cells per well on 24-well plates and transfected with 0.2 μg reporter plasmids (pHLuc1 to pHLuc6); Jurkat cells were cotransfected with 0.2 μg reporter plasmids (pHLuc1 to pHLuc6) together with 0.3 μg expression vectors (pCMV-Tax or its mutants) or with increasing doses of pCMV-Neo vectors. For Western blot assay, Jurkat cells were plated at 2×106 cells per well on 6-well plates and transfected with 1.2 μg expression vectors (pCMV-Tax or its mutants) or cotransfected with 0.8 μg reporter plasmid pHLuc6 together with increasing doses of pCMV-Neo vectors. For reverse transcription PCR assay, Jurkat cells were transfected with each 0.3 μg expression vectors (pCMV-Tax and its mutants) and 0.3 μg empty vector (pCMV-Neo). The amount of transcribed DNA was equalized by the addition of empty vector (pCMV-Neo or pGL3-neo-luc). After 24 hours, cells were harvested and lysed with reporter lysis buffer, and Luc activity in the resulting supernatants was measured using a 20/20n luminometer (Turner BioSystems, USA) according to the manufacturer’s instructions. All cells were cotransfected with pSV-β-gal as a control for transfection efficiency, and galactosidase activity was measured using a β-galactosidase enzyme assay system.

RNA extraction and cDNA synthesis

Total RNA from TaxP and TaxN cells, and Jurkat cells after transient transfection with pCMV-Neo, pCMV-Tax, and two mutants (M22, M47), was extracted using RNAiso Plus reagent according to the manufacturer’s instructions. The extracted RNA was dissolved in diethylpyrocarbonate-treated water. The quality and concentration of RNA were verified using denaturing gel electrophoresis and by determining the A260/A280 absorbance ratio. Total RNA was then reversely transcribed in a total volume of 10 μL, containing 0.5 μL of 10 mM dNTP, 1 μL of 50 μM Oligo (dT)18 Primers, 0.25 μL of 40 U/μL RNase Inhibitor, 1 μL of 200 U/μL RTase M-MLV (RNase H-), 2 μL of 5×M-MLV buffer, and 1 μg of RNA. The response conditions were as follows: 70 °C for 10 min, ice for 2 min, 42 °C for 60 min, 70 °C for 10 min, and 4 °C for 5 min. The first-strand
cDNA was synthesized and stored at -20 °C.

Reverse transcription PCR (RT-PCR) and quantitative real-time PCR (qRT-PCR)

Primers for the human HMGB1 promoter and GAPDH were synthesized by Invitrogen. The sequences of the primers were as follows: 5’-GCAGCTCTTCACCTCGTACA-3’ (sense), 5’-ACATGGTCTTCCACCTCTTGAC-3’ (anti-sense) for HMGB1 and 5’-TCAACACGCGACACCACCTCC-3’ (sense), 5’-TGAGGTCACCACCCCTTTG-3’ (anti-sense) for GAPDH. RT-PCR parameters were 30 cycles of 94 °C for 1 min, 57 °C for 30 s, and 72 °C for 1 min, after which an additional extension step at 72 °C for 10 min was performed. After the amplification protocol was completed, 10 µL aliquots of the PCR products were subjected to electrophoresis on a 1.2% agarose gel, stained with ethidium bromide, photographed, and then scanned using Band Leader software for gray-scale semi-quantitative analysis. Subsequently, qRT-PCR amplification was performed by using GoTaq®qPCR master mix with cycling conditions of 94 °C for 2 s, 57 °C for 10 s, and 72 °C for 10 s. After the amplification protocol was completed, the PCR product was subjected to melting curve analysis to identify primer dimer formation. Relative gene expression data were calculated using the 2^{-ΔΔCT} method. All samples were measured in triplicate.

Western blot

According to the manufacturer’s instructions, the protein was extracted with ice-cold radioimmunoprecipitation assay buffer supplemented with 1 mM phenylmethylsulfonyl fluoride for 30 min, sonicated with 4 to 5 sets of 1 s pulses on wet ice using a 100 watt-model high-intensity ultrasonic processor/sonicator, and then boiled for 10 min. The lysates were separated by electrophoresis on polyacrylamide gels containing 8% sodium dodecyl sulfate (SDS) and then transferred to the nitrocellulose membranes. The membranes were blocked with blocking buffer and incubated with anti-HMGB1 (1:1000), anti-Tax (1:2000) and mouse anti-beta actin monoclonal antibody (1:2000). This procedure was followed by incubation with anti-mouse IgG (H+L). Membranes were exposed to X-ray film, and each specific band that recorded the targeted proteins was quantified and analyzed by the Band Scan 5.0 system.

ChIP

TaxP cells (1x10^6) were collected and fixed with 1% formaldehyde to crosslink proteins to DNA for 10 min, then quenched with 10 mM glycine for 5 min. After washing with ice-cold phosphate buffered saline, cells were resuspended in SDS lysis buffer. Then, the chromatin was sheared to a manageable size with 5 sets of 10 s pulses on wet ice using a 100 watt-model high-intensity ultrasonic processor/sonicator. ChIPs were performed using the ChIP kit. Protein–DNA complexes were immunoprecipitated with 6 µg anti-Tax antibody overnight followed by protein G-conjugated agarose beads. Then, protein/DNA complexes were reversed by incubating samples at 65 °C overnight for 12 h. Associated DNA was eluted and purified according to the manufacturer’s instructions, and sequences of the HMGB1 target gene and the negative control (HM-mock) were analyzed by PCR and qRT-PCR using specific primers. The primers were as follows: 5’-CCACTACAGCCAGCATTT-3’ (sense), 5’-TAGATGCAAGGAGAGTGC-3’ (anti-sense) for HMGB1 target gene and 5’-TCTGGGAGGATCTTCT-3’ (sense), 5’-GAATCCATCGGTCTTCCA-3’ (anti-sense) for HM-mock. One microliter of precipitated and purified DNA was subjected to standard PCR, and DNA fragment sizes were analyzed by 2% agarose gel electrophoresis. The detection of specific DNA sequences was performed by qRT-PCR analysis of ChIPed samples using the GoTaq® qPCR master mix. The HMGB1 gene site bound by Tax protein was determined by qRT-PCR analysis relative to the Tax signal at the HM-mock site. The 2^{-ΔΔCT} (P value at each site targeted by Tax) was normalized to that at HM-mock, which was set to 1.

Statistical analysis

Data were representative of at least three independent experiments and expressed as mean±SD with one-way ANOVA. An unpaired Student’s t-test was used to evaluate significant differences between groups. A value of p<0.05 was considered statistically significant.

Figure 1. Tax Protein Induces HMGB1 Expression Levels in Different T Cells. (A and C) Results from conventional RT-PCR; (B and D) results from real-time RT-PCR; (E and F) results from Western blot. (A and B) According to the manufacturer’s protocol, 5x10^6 cells (TaxN, TaxP) were directly collected, after which total RNA was extracted. Then, total RNA was reversely transcribed into cDNA to conduct RT-PCR and real-time PCR, respectively. HMGB1 expression level relative to a calibrator was calculated using the formula 2^{-ΔΔCT}. A value of 1 indicates that the expression level is equivalent to calibrator sample. Value was normalized to the expression of GAPDH gene. Data represent mean±SD of three different analyses. Error bars indicate SD. (C and D) Jurkat lymphocytes were transiently transfected with pCMV-Neo (control group), pCMV-Tax, and two mutants (M22, M47). After 24 h, total RNA was reversely transcribed into cDNA to conduct RT-PCR and real-time PCR, respectively. Values of 1 indicates that the expression level is equivalent to calibrator sample. Values were normalized to the expression of GAPDH gene. Data represent mean±SD of three different analyses. Error bars indicate SD. (E and F) To analyze the expression level of HMGB1 protein in TaxN and TaxP cells, and Jurkat cells after transient transfection with pCMV-Neo, pCMV-Tax, and two mutants (M22, M47), Western blot analysis was performed using anti-HMGB1. β-actin was used as a control. Compared with the control group (TaxN or pCMV-Neo), *denotes p<0.05 and **denotes p<0.01.
we found that the regulation trend of HMGB1 gene was increased in Tax

The regulation of HMGB1 transcription in Tax- and Tax-T cells

As shown in the above results, HMGB1 expression increased in Tax-T cells. To clarify the basal promoter activity of the human HMGB1 gene and observe how Tax affects the regulation of HMGB1 transcription in T lymphocytes, the available HMGB1 regulatory genes were subcloned upstream of the reporter gene pGL3-neo-luc to construct a series of deletion reporter vectors (pHLuc1 to pHLuc6) (Figure 2). Using transient transfection with reporter vectors (pHLuc1 to pHLuc6) and pGL3-neo-luc into Tax-T cells (TaxN) and Tax-T cells (TaxP), we found that the regulation trend of HMGB1 gene was slightly similar, but not identical. We also observed the maximal promoter activity in pHLuc3 containing -504 to +83 fragment. Interestingly, a significant increase in Tax-T cells was observed in the pHLuc6 group (Figure 2). These findings suggested that the up-regulation expression of HMGB1 gene in pHLuc6 was responsible for the interaction of Tax protein and some transcriptional regulators.

Tax mediates HMGB1 transcription via non-NF-κB pathway

The above analysis indicated that HMGB1 transcription activity from reporter vector pHLuc6 in Tax-T cells was higher than that in Tax-T cells. To further confirm the involvement of Tax in HMGB1 promoter activation, we transiently cotransfected pHLuc1 to pHLuc6 individually...
with pCMV-Tax. The results of relative Luc activity (Tax/Neo) showed that HMGB1 transcription activity increased by 2–3-fold in the pHLuc6 group. By contrast, minimal effect was observed in the other pHLuc groups (Figure 3A). To further examine this result, pHLuc6 was cotransfected into Jurkat cells with increasing doses of Tax expression plasmids. As shown in Figs. 3B and 3C, the co-expression of Tax significantly increased HMGB1 promoter activity in a dose-dependent manner.

Previous studies have demonstrated that nuclear factor NF-κB and CREB/ATF activation have a critical function in the oncogenesis of ATL by HTLV-1, and its viral protein Tax is indispensable for maintenance of the malignant phenotype by transactivation through the CREB/ATF or NF-κB pathway (Patrick et al., 2001; Alfonso et al., 2012). Tax mutants (M22, M47) are inactive in the CREB/ATF or NF-κB pathway (Kwon et al., 2005). To determine whether Tax alters the level of HMGB1 via CREB/ATF or NF-κB pathway in T lymphocytes, we transiently transfected pCMV-Neo (empty vector), pCMV-Tax (expression vector), and Tax mutants (M22, M47) into Jurkat cells with pHLuc3 and pHLuc6, containing 587 bp HMGB1 fragment (the strongest HMGB1 promoter activation) and 1246 bp HMGB1 fragment (the longest clone), respectively. Compared with the control group, nearly 2–3-fold increase in HMGB1 transcriptional activity was observed in the pHLuc6 group due to Tax and mutants. This phenomenon did not occur in the pHLuc3 group (Figure 3D). To further validate whether Tax protein promotes HMGB1 transcription through the NF-κB pathway by the mediation of BAY11-7082 (NF-κB inhibitor), we treated Jurkat cells transfected with Tax and mutants (M22, M47) together with pHLuc6 and TaxP transfected with pHLuc6. Surprisingly, no significant inhibition phenomenon was observed in Jurkat cells transfected with Tax and mutants (Figure 3E) and in TaxP cells (Figure 3F). Therefore, BAY11-7082 could not inhibit HMGB1 transcriptional activity up-regulated by Tax protein. Taken together, these results suggest that

the up-regulated effect of Tax on cellular HMGB1 gene is mediated by interactions between the viral Tax protein and some transcriptional regulators in the -1163 to -975 region through the non-NF-κB pathway.

Tax is Enriched in the HMGB1 Gene

The above findings indicate that Tax protein is a potential transcriptional regulator for up-regulation of HMGB1 gene in the -1163 to -975 region. To identify HMGB1 genomic sites directly targeted by Tax, we isolated DNA fragments associated with protein-DNA complexes containing protein Tax using a Tax-specific antibody through ChIP method from the Tax-T-cell line (TaxP). Using the specific primer, amplification was conducted with ChIPed DNA to probe the HMGB1 gene region bound by Tax protein. Small amounts of nonspecific protein-DNA complexes may be co-purified to lead to a background signal detected by qRT-PCR in ChIP assays. To compare the levels of retained Tax, we quantified the fold enrichment levels of Tax protein in two separate portions of the HMGB1 gene through qRT-PCR amplification. The regions were centered at -1103 and -797 with respect to the site of Tax-enhancing HMGB1 transcription. We observed stronger Tax enrichment at the -1103 site (the fragment encompassing -1163 to -1043) than that at the -797 site (the fragment spanning -848 to -746) (Figure 4). Therefore, the overall results suggest that Tax is recruited at the -1103 site, but not for any known Tax-binding partners.

Tax is involved in the potentiation of HMGB1 expression by C/EBP

The above ChIP analysis indicates that Tax is recruited at the region of HMGB1 gene. To clarify this finding, we used bioinformatics and found that the region from -1163 to -975 (pHLuc6) revealed cis-elements for the following transcription factors: CdxA, AP-1, AML-1a, USF, v-Myb, and C/EBP (Figure 5A). To further confirm the involvement of these transcription factors in HMGB1
promoter activation, we each performed a deletion mutation by using wt pHLuc6 to generate six mut reporter vectors for Luc analysis, each deleting the corresponding cis-element designated as mLuc6-AM for AML-1a, mLuc6-AP for AP-1, mLuc6-Cd for CdxA, mLuc6-U for USF, mLuc6-M for v-Myb, and mLuc6-C for C/EBP. As shown by the Luc assays, mutation of the C/EBP-motif (mLuc6-C) reduced the Luc activity to 52% of the pHLuc6 in TaxP cells (Figure 5B). Tax did not affect mut HMGB1 (mLuc6-C) transcription in Jurkat cells (Figure 5C). Taken together, these findings have demonstrated the novel mechanism through which Tax is involved in the potentiation of HMGB1 expression inside the nucleus by C/EBP.

Discussion

HMGB1 is a chromatin-binding non-histone protein that participates in chromatin-modulating processes in eukaryotes (Bustin, 1999), such as transcriptional regulation, DNA repair, and recombination by binding to the chromatin site in the nucleus (Bianchi and Agresti, 2005; Gerlitz et al., 2009). Generally, HMGB1 is expressed at a basal level as an architectural chromatin-binding protein, but at a slightly elevated level via passive release from damaged or necrotic cells (Beyer et al., 2012; Yi et al., 2013) or active secretion (Akirav et al., 2012; Kang et al., 2013; Mohammad et al., 2013). Moreover, overexpression of HMGB1 protein is observed in breast, colon, and gastrointestinal cancers, as well as in leukemia and other diseases (Kostova et al., 2010; Ohmori et al., 2011; Jube et al., 2012; Lee et al., 2012; Liu et al., 2012; Xing et al., 2012; Yu et al., 2012; Stoezter et al., 2013). ATL is an acute T-cell malignancy modulated by an oncogenic retrovirus, and the virally encoded Tax protein is believed to be critically involved in the development of ALT. To initially understand the regulatory mechanism of HMGB1 gene in ATL and to observe whether oncprotein Tax affects HMGB1 regulation in T lymphocytes, we extracted the total RNA and HMGB1 protein from Tax-T cells (TaxN) and TaxX-T cells (TaxP). qRT-PCR and Western blot assays showed that HMGB1 transcriptional activity and protein level increased in TaxP cells, which demonstrated that virally encoded Tax protein enhanced HMGB1 expression.

To further analyze the transcription of HMGB1 gene in different T cells, we constructed various recombinant Luc reporter vectors (pGL3-HMGB1-luc) containing the 5′-upstream region of the human HMGB1 gene. We transiently transfected these reporter vectors into TaxN and TaxP cells. The Luc assay showed that the transcription regulation trend was slightly similar but not identical in diverse T cells, with an increase of 30 to 130-fold that of the pGL3-neo-luc and maximal promoter activity in pHLuc3 containing -504 to +83 fragment. Interestingly, the Luc assay of pHLuc6 showed that HMGB1 promoter activity was strengthened in TaxP cells, and the result was confirmed by transient transfection with pCMV-Tax together with pHLuc vectors into Jurkat cells. Tax (active in CREB/ATF and NF-κB pathways), mut M22 (inactive in NF-κB pathway), and mut M47 (inactive in CREB/ATF pathway) (Kwon et al., 2005) also enhanced HMGB1 promoter activity by 2-fold between -1163 to +83. However, BAY11-7082 (NF-κB inhibitor) could not inhibit the enhanced effect by Tax protein. These findings suggest the existence of genomic sites targeted by Tax is between -1163 to -975, and that the enhancement effect mediated by Tax is through the non-NF-κB pathway.

As described in numerous papers, various transcription reactions occurring in the chromatin are mainly triggered by gene specific regulatory factors and chromatin remodeling factors in the transcriptional regulation of genes via their interaction with transcription regulators and chromatin (Bianchi and Agresti, 2005; Nicholas et al., 2011). These properties enable the Tax protein to associate with specific DNA-bound protein complexes and influence HMGB1 gene expression. To verify the above hypothesis, we performed ChIP assays using Tax-DNA complexes from TaxP cells and found that the viral Tax protein was enriched at the -1103 site of the HMGB1 gene (Figure 4). The overall results suggested that the region of the HMGB1 gene that recruited stronger Tax signal was a possible Tax-binding region, but not for any known Tax-binding partners. To further clarify this finding, we used TRANSFEC database search to identify six putative cis-elements between -1163 to -975 for the following transcription factors: AM-1a, CdxA, AP-1, USF, v-Myb, and C/EBP. Previous study showed that transcription factor C/EBP could regulate Tax-mediated transactivation of the HTLV-1 long-terminal repeat (Christian et al., 2006). Significantly, in the current study, we found that mLuc6-C for C/EBP could effectively reduce the HMGB1 transcription up-regulated by Tax protein. These data demonstrate a novel mechanism through which Tax is involved in the potentiation of HMGB1 expression inside the nucleus by C/EBP.
Acknowledgements

This work was supported partly by grants from the National Natural Science Foundation of China (No. 81273241) awarded to Hui Wang, the key project of science and technology of Henan Province Education Department (No. 12A310006) and the key research project fund of Xinxiang Medical University (No. ZD2011-13) awarded to Chen-guang Zhang.

The authors declare that they have no conflict of interests with the publication of this manuscript. We are most grateful to Ms. Wen Zhang for assistance in the preparation of this manuscript. We are most grateful to Chen-guang Zhang.

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