Hepatitis B virus X protein induces TNF-α expression via down-regulation of selenoprotein P in human hepatoma cell line, HepG2

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Received 30 December 2002; received in revised form 21 May 2003; accepted 23 May 2003

Abstract

Human hepatitis B virus X protein (HBx) is associated with the induction of oxidative stress, which is considered significant in the development of liver damage. In this study, we investigated the molecular mechanisms by which HBx induced lipid peroxidation and tumor necrosis factor-α (TNF-α) expression through regulation of selenoprotein P (SeP) expression in the human hepatoma cell line, HepG2. Forced expression of HBx significantly down-regulated the expression of SeP mRNA and protein in both the cell lysates and the culture medium. Lipid peroxidation increased 2.5-fold when expression of the SeP protein was blocked with a SeP antisense vector. Also, HBx transfection increased lipid peroxidation by 3.0-fold, whereas the hepatitis B virus core protein (HBc) had no significant effects. The induction of lipid peroxidation due to the block in SeP protein expression or treatment with ferric chloride (FeCl3) up-regulated the expression levels of TNF-α mRNA and protein. The pattern of HBx-induced lipid peroxidation and TNF-α up-regulation was reversed by SeP introduction. These results suggest that HBx induces lipid peroxidation via down-regulation of SeP expression, resulting in increased expression of TNF-α in the human hepatoma cell line, HepG2.

Keywords: HBx; SeP; Antisense; Lipid peroxidation; TNF-α; HepG2

1. Introduction

Human hepatitis B virus (HBV) is the major causative factor for hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. HBV encodes four viral proteins—polymerase (P), core protein (C), surface protein (S), and X protein (X). Among these proteins, X protein (HBx) is known to have various functions in transcriptional regulation [2], cell growth [3], apoptosis [4], and hepatocarcinogenesis [5]. Participation of HBx in induction of oxidative stress along with other host factors [6] leads to apoptosis of hepatocytes [7]. Oxidative stress is considered significant in the development of liver damage. Selenoprotein P (SeP) is an extracellular, monomeric glycoprotein which contains 10 selenocysteines in its polypeptide chain [8]. SeP is known to be secreted into medium. Many previous reports show the detection of SeP in culture medium [9–11]. Human SeP mRNA has two selenocysteine insertion sequences (SECIS) in the 3'-untranslated region essential for expression of selenium-containing proteins [12]. SeP is known to be an antioxidant protein which protects cells against lipid peroxidation by reducing phospholipid hydroperoxide [8].

SeP expression can be down-regulated by many factors. It is reported that selenium deficiency down-regulates both SeP protein and its mRNA expression in HepG2 cells [10]. SeP expression is also down-regulated by transforming growth factor-β1 (TGF-β1) and alcohol [11,13]. SeP being an antioxidant protein against lipid peroxidation, the down-regulation of SeP expression would allow induction of lipid peroxidation. Burk et al. [14] reported that SeP down-regulation due to selenium deficiency induces lipid peroxidation.

Induction of lipid peroxidation stimulates the secretion of many cytokines in liver tissues and hepatocytes [15].
Cytokines, tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6 are well-known pro-inflammatory cytokines that induce liver damage or hepatic cell death [16]. Among these cytokines, TNF-α is the one well studied in relation to apoptosis and liver injuries [17]. Iimuro et al. [18] reported that TNF-α triggered apoptosis or necrosis of hepatocytes and anti-TNF-α antibodies clearly reduced liver cell damage. Su and Schneider [19] also reported that HBx sensitized hepatocytes to apoptosis in presence of TNF-α. Based on these reports, it is clear that TNF-α is an important cytokine in liver damage and apoptosis. Some routes through which HBx up-regulates the expression of TNF-α cytokine in liver damage and apoptosis. Some routes have been reported [20,21]. Lipid peroxidation also directly

Based on these reports, it is clear that TNF-α up-regulates TNF-α expression in hepatocytes [22]. Although it is known that HBx is associated with the induction of oxidative stress which leads to liver damage and that SeP is an antioxidant protein against lipid peroxidation which is a type of oxidative stress, the relationship between HBx and SeP in oxidative stress and TNF-α expression has not been studied yet. In this report, we examined the role of HBx in regulation of SeP expression and its effects on HepG2 cells related to lipid peroxidation and TNF-α expression.

2. Materials and methods

2.1. Vector constructions

The pCMV/X encoding HBx was constructed by cloning the polymerase chain reaction (PCR)-amplified product of the entire HBx gene into the HindIII and Apal sites of the mammalian expression vector, pRC/CMV (Promega). The HBx gene was amplified using forward (5'-AAAGCTTCTGAGTGGCTGCTGGTG-3') and reverse (5'-AGGGGCCCTTAGGGACAGGTGAAAAG-3') primers. PCR was performed first at 94 °C for 5 min and then at 94 °C for 50 s, 57 °C for 50 s, and 72 °C for 30 s, repeating for 30 cycles. The last extension step was performed at 72 °C for 7 min. The pCMV/C encoding hepatitis B virus core protein (HBc) was constructed by cloning the PCR amplified product of the entire HBc gene into the HindIII and Apal sites of the pRC/CVM vector. The HBc gene was amplified using forward (5'-AAAGCTTGGCCATGGACATTGAC-3') and reverse (5'-AGGGCCCCCTAAGACATTGACATCCCC-3') primers. PCR was performed first at 94 °C for 5 min and then at 94 °C for 50 s, 57 °C for 50 s, and 72 °C for 30 s, repeating for 30 cycles. The last extension step was performed at 72 °C for 7 min. The pCMV/SeP encoding SeP was constructed by cloning the PCR amplified product of the entire SeP gene into the HindIII and XbaI sites of the pRC/CVM vector. The SeP gene was amplified using forward (5'-AAAGCTTTAGGTGGAGAAGCCTGGGCTTGG-3') and reverse (5'-ATCTAGATTAGTTGAGGTCTATCC-3') primers. PCR was performed first at 94 °C for 5 min and then at 94 °C for 50 s, 57 °C for 50 s, and 72 °C for 30 s, repeating for 30 cycles. The last extension step was performed at 72 °C for 7 min. The pCMV/SeP, SeP antisense vector, was constructed by cloning a SeP gene fragment of 282 bp into the XmnI and HindIII sites of pRC/CVM vector. The SeP gene fragment was prepared by cutting the SeP gene from the start site to 282th bp XmnI site. The SeP gene fragment was placed downstream from the driving promoter in antisense orientation.

2.2. Cell culture and transfection

The HepG2 cells were cultured in minimal essential medium (MEM; Sigma) supplemented with 10% fetal bovine serum (FBS; Bio Whittaker) and incubated in 5% CO2 at 37 °C. Twenty-four hours before transfection, the HepG2 cells were plated in MEM supplemented with 10% FBS and incubated in 5% CO2 at 37 °C. The HepG2 cells were then transfected with pCMV and pCMV/X using Lipofectamin™ 2000 (Invitrogen) according to the manufacturer’s instructions. HepG2 cells were also transfected with pCMV/C, pCMV/SeP, and pCMV/SePa.s as described above. Forty-eight hours post-transfection, the transfected HepG2 cells were washed with 1 × phosphate buffered saline (PBS) twice and harvested for further research.

2.3. RT-PCR

Forty-eight hours post-transfection, total RNA was extracted from HepG2 cells using TRizol® Reagent (Invitrogen) according to the manufacturer’s instructions. Total RNA extract was treated with DNase I (Promega) at 37 °C for 30 min and 1.0 µg of the total RNA was reverse-transcribed by AMV-RT (Promega) with an oligo-dT14 at 42 °C. AMV-RT was inactivated at 75 °C for 15 min. TNF-α and glyceraldehyde 3′-phosphate dehydrogenase (GAPDH) were co-amplified using one fifth of the total cDNA. To amplify the TNF-α gene, TNF-α-specific forward (5′-AAAGCTTATGACGTGAAGAAGACATGAT-3′) and reverse (5′-ATGTTAGATCAAGGGCAATGATCC-3′) primers were used, and to amplify the 257 bp of partial GAPDH fragment, GAPDH-specific forward (5′-ATCATCCCTGCTCTACTGG-3′) and reverse (5′-TGGGTGTCGTGTTGAAGTC-3′) primers were used. PCR was performed first at 95 °C for 5 min and then at 95 °C for 50 s, 57 °C for 50 s, and 72 °C for 45 s, repeating for 30 cycles. The last extension step was performed at 72 °C for 7 min. The amplified products were electrophoresed on a 1.5% agarose gel.

2.4. Northern blot analysis

Total RNA was extracted from HepG2 cells as described in Section 2.3. Total RNA extract, 20 µg, was electrophoresed on a 1.2% agarose-formaldehyde gel and transferred onto a nylon membrane (NEN). After baking the membrane at 80°C for 2 h, it was hybridized with probes
labeled with \( \alpha^{-32}\text{P} \)dCTP (NEN) at 42 °C for 20 h. Membrane was washed twice with washing buffer (1 × SSC/0.1% SDS and 0.5 × SSC/0.1% SDS) at 68 °C for 20 min and exposed on a X-ray film at −70 °C for 3 days.

2.5. Immunoprecipitation and Western blot analysis

For the immunoprecipitation of HBx, the HepG2 cells transfected with pCMV/X were lysed with lysis buffer [150 mM NaOH, 1% NP-40, and 50 mM Tris–HCl (pH 8.0)] and HBx was immunoprecipitated using 1-μg monoclonal anti-HBx antibodies (Serotech). Immunoprecipitated HBx was electrophoresed on a 15% SDS-PAGE gel. After transferring onto a PVDF membrane (NEN), Western blot analysis of HBx was performed using monoclonal anti-HBx antibodies diluted to a concentration of 1:1000. SeP was detected by immunoprecipitation and Western blot analysis with both culture medium and cell lysates of the HepG2 cells transfected with pCMV, pCMV/X, pCMV, and pCMV/SePa.s. In the culture medium, SeP was immunoprecipitated using 1-μg monoclonal anti-SeP antibodies (Transduction Laboratories) as described above. Immunoprecipitated SeP was electrophoresed on a 7.5% SDS-PAGE gel and Western blot analysis of SeP was performed using monoclonal anti-SeP antibodies diluted to a concentration of 1:1000. In the cell lysates, the cell lysates were electrophoresed on a 10% SDS-PAGE gel.
PAGE gel and Western blot analysis was performed as described above. Immunocomplexes were visualized using an enhanced chemiluminescence system (ECL, NEN) according to the manufacturer’s instructions. To ensure that equal amounts of proteins were used, the membrane was stripped using a stripping buffer [62.5 mM Tris–HCl (pH 6.8), 2% SDS, and 100 mM 2-mercaptoethanol] at 70 °C for 1 h and β-actin was detected by Western blotting using the same membrane. We did not use β-actin when performing Western blot analysis of the culture medium. However, an appropriate amount of the culture medium corresponding to the same number of cells was used as the control.

2.6. Lipid peroxidation assay

As an index of lipid peroxidation, the concentrations of 2-thiobarbituric acid reactive substances (TBARS) were measured based on a previously reported method [23]. HepG2 cells were thoroughly lysed with 200-μg lysis buffer and mixed with 400-μg TCA-TBA-HCl reagent (15% w/v trichloroacetic acid, 0.375% w/v 2-thiobarbituric acid, and 0.25 N hydrochloric acid). Samples were heated at 95 °C for 15 min and cooled. After centrifugation at 1000 × g for 10 min, the absorbance of the resulting colored layer containing TBARS was measured at 535 nm against a blank that contained all of the reagents except the sample. 1,1,3,3-Tetraethoxypropane (Sigma) was used as the internal standard and lipid peroxidation was shown in nanomole per milligram of proteins. All of the experiments were repeated three times.

2.7. TNF-α quantification

Enzyme-linked immunosorbent assay (ELISA) was used to quantify the level of secreted TNF-α protein from HepG2 cells after induction of lipid peroxidation. TNF-α quantification was performed with 100-μg medium from each sample using human TNF-α OptEIA™ Set (Pharmigen). All quantification assays were performed according to the manufacturer’s instructions. Complete medium was used as a blank and a standard curve was generated with the human TNF-α standard provided by the ELISA assay kit. Amounts of TNF-α were quantified at 450 nm with an Emax precision microplate reader (Molecular Devices). The TNF-α levels were shown in picogram per million of cells and all of the samples were assayed twice.

2.8. Statistics

Band intensities from the Northern and Western blot analyses were measured with Image Station 440 CF (Kodak). All of the data were shown as means with error bars indicating the standard deviation (S.D). *Statistical significance ($P < 0.05$).

Fig. 2. SeP down-regulation and the effects of SeP down-regulation and HBx on lipid peroxidation. (a) SeP protein was detected by Western blot analysis in HepG2 cells transfected with pCMV (left lane), and pCMV/SePa.s (right lane) in the cell lysates. (b) β-actin was used as the internal control and was detected using polyclonal anti-β-actin antibodies. (c) SeP protein was detected by Immunoprecipitation and Western blot analysis in the HepG2 cells transfected with pCMV (left lane), and pCMV/SePa.s (right lane) in the culture medium. (d) SeP mRNA was detected by Northern blot analysis using a SeP probe labeled with [α-32P]dCTP. GAPDH was used for the internal control. (e) TBARS levels as the index of lipid peroxidation were measured in the HepG2 cells transfected with pCMV and pCMV/SePa.s and (f) in the HepG2 cells transfected with pCMV, pCMV/X, and pCMV/C. The absorbance of TBARS was determined at 535 nm. Lipid peroxidation values shown are means ($n = 3$) with error bars indicating the standard deviation (S.D). *Statistical significance ($P < 0.05$).
bars indicating standard deviation (S.D.). The significance of the difference between the assayed samples was determined using a Student’s t test. \( P < 0.05 \) was considered significant.

3. Results

3.1. Expression of HBx in HepG2 cells

HepG2 cells were transfected with the HBx expression vector (pCMV/X), and the expression of both HBx mRNA and protein was verified prior to the investigation of HBx-induced effects. The expression of HBx mRNA was confirmed by Northern blot analysis (Fig. 1a) and the RNA controls of corresponding blots are shown (Fig. 1b). Furthermore, the expression of HBx protein was also confirmed by both immunoprecipitation and Western blot analysis using monoclonal anti-HBx antibodies (Fig. 1c).

3.2. Down-regulation of SeP by HBx

To investigate the effects of HBx on SeP expression, HepG2 cells were transfected with pCMV/X and the expression levels of SeP mRNA and protein were determined. Northern blot analysis revealed that the expression levels of SeP mRNA were down-regulated in HepG2 cells transfected with pCMV/X compared to the control (data not shown). Since SeP is known to be a secretory protein \([9–11]\), it was detected in both the cell lysates and the culture medium. Western blot analysis revealed that in the cell lysates, the expression level of SeP protein was down-regulated in the HepG2 cells transfected with pCMV/X compared to the control (Fig. 1d), whereas the expression of the \( \beta \)-actin protein was not changed (Fig. 1e). Immunoprecipitation and Western blot analysis revealed that in the culture medium, the expression level of SeP protein was also down-regulated in the HepG2 cells transfected with pCMV/X compared to the control (Fig. 1f). The expression level of SeP protein decreased 6.4-fold in the cell lysates (Fig. 1g) and 6.3-fold in the culture medium (Fig. 1h) compared to the control. The relative ratio of SeP expression level was almost the same.

3.3. Induction of lipid peroxidation by SeP down-regulation and HBx

To investigate subsequent effects due to SeP down-regulation, a block in translation of SeP was brought about

Fig. 3. The effect of lipid peroxidation on TNF-\( \alpha \) expression. (a) The expression level of TNF-\( \alpha \) mRNA was detected by RT-PCR using one fifth of cDNA reverse-transcribed from 1.0 \( \mu \)g of total RNA extracted from the HepG2 cells transfected with pCMV (left lane) and pCMV/SePa.s (right lane) vectors. GAPDH cDNA was co-amplified with TNF-\( \alpha \) cDNA as the internal control. (b) TNF-\( \alpha \) protein secreted into the medium from the HepG2 cells transfected with pCMV and pCMV/SePa.s vectors was quantified by ELISA at 450 nm. (c) HepG2 cells were treated with 0, 0.05, and 0.1 mM FeCl\(_3\) for 2 h and the expression levels of TNF-\( \alpha \) mRNA were detected by RT-PCR using one fifth of the cDNA reverse-transcribed from 1.0 \( \mu \)g of total RNA. GAPDH cDNA was co-amplified with TNF-\( \alpha \) cDNA as internal control. (d) The amount of TNF-\( \alpha \) protein secreted from the HepG2 cells treated with 0, 0.05, and 0.1 mM FeCl\(_3\) was quantified by ELISA at 450 nm. The secreted levels of TNF-\( \alpha \) protein shown are means (\( n = 2 \)) with error bars indicating the S.D. *Statistical significance (\( P < 0.05 \)).
using the SeP antisense vector (pCMV/SePa.s) in HepG2 cells. We performed Western blot analysis with the cell lysates and the culture medium. In the cell lysates, the expression levels of SeP protein decreased in the HepG2 cells transfected with pCMV/SePa.s compared to the control (Fig. 2a), whereas the expression of the β-actin protein was not changed (Fig. 2b). In the culture medium, the expression levels of SeP protein also decreased in the HepG2 cells transfected with pCMV/SePa.s compared to the control (Fig. 2c). The expression level of SeP mRNA, however, was not changed (Fig. 2d). Lipid peroxidation was confirmed in the HepG2 cells transfected with pCMV/SePa.s because SeP is an antioxidant protein for lipid peroxidation [8]. Transfection with pCMV/SePa.s increased lipid peroxidation by 2.5-fold compared to the control (Fig. 2e). Given that SeP down-regulation by HBx induced lipid peroxidation, the direct effect of HBx on lipid peroxidation was investigated. After transfection of HepG2 cells using pCMV/X, the lipid peroxidation was confirmed. HBx induced a 3.0-fold increase in lipid peroxidation compared to the control (Fig. 2f). HepG2 cells were also transfected using the HBc expression vector (pCMV/C) and the effect of HBc, another viral protein of HBV, on lipid peroxidation was checked. HBc expression was confirmed (data not shown) and no significant induction ($P < 0.05$) of lipid peroxidation was shown in the HepG2 cells transfected with pCMV/C compared to the control (Fig. 2f).

3.4. Up-regulation of TNF-α expression by induction of lipid peroxidation

To investigate the effect of lipid peroxidation induction on TNF-α expression, the expression levels of TNF-α mRNA and protein were verified in HepG2 cells after induction of lipid peroxidation. After transfection of HepG2 cells using pCMV/SePa.s, total RNA was extracted and RT-PCR analysis of TNF-α mRNA was performed. The expression level of TNF-α mRNA was up-regulated in the HepG2 cells transfected with pCMV/SePa.s compared to the control (Fig. 3a). Furthermore, the level of TNF-α protein secreted into the medium showed a 6.7-fold increase compared to the control (Fig. 3b).

To confirm that the induction of lipid peroxidation up-regulated TNF-α expression, lipid peroxidation was directly induced by treatment with ferric chloride (FeCl₃) [24] and the expression levels of TNF-α mRNA and protein were checked. HepG2 cells were treated with 0, 0.05, 0.1, 0.2, and 0.3 mM FeCl₃ for 2 h based on a previous report [25]. Concentrations of FeCl₃ up to 0.1 mM showed gradual induction of lipid peroxidation, but it deceased in concentrations higher than 0.1 mM (data not shown). TNF-α expression was confirmed using the HepG2 cells treated with 0, 0.05, and 0.1 mM FeCl₃ as described above. The expression levels of TNF-α mRNA (Fig. 3c) and the secreted levels of TNF-α protein (Fig. 3d) showed a clear-cut induction as the concentration of FeCl₃ increased. The levels of TNF-α protein secreted into the medium from the 0.05 and 0.1 mM FeCl₃-treated HepG2 cells showed 3.6-fold and 5.2-fold increase, respectively, compared to the control.

3.5. Reversal of HBx-induced lipid peroxidation and TNF-α up-regulation by SeP introduction

We hypothesized that SeP introduction to the HepG2 cells transfected with pCMV/X would reverse HBx-induced lipid peroxidation and TNF-α up-regulation. To investigate this, we prepared three kinds of samples: HepG2 cells transfected with pCMV as the negative control, HepG2 cells transfected with pCMV/X as the positive control, and HepG2 cells co-transfected with pCMV/X and pCMV/SePa.s. pCMV/X transfection induced a 3.0-fold increase in lipid peroxidation compared to the negative control, while lipid peroxidation in the HepG2 cells co-transfected with pCMV/SeP and pCMV/X decreased by as much as that of the negative control (Fig. 4a). Secreted levels of TNF-α showed a similar pattern to that of lipid peroxidation. pCMV/X transfection increased the TNF-α secretion level

![Fig. 4. Reversal of HBx-induced lipid peroxidation and TNF-α up-regulation by SeP introduction. (a) Lipid peroxidation was measured in the HepG2 cells transfected with pCMV, HepG2 cells transfected with pCMV/X, and HepG2 cells co-transfected with pCMV/X and pCMV/SeP. The quantification of lipid peroxidation was performed three times. (b) The amount of TNF-α protein secreted from the same cells was quantified by ELISA at 450 nm. TBARS levels as the index of lipid peroxidation ($n = 3$) and amounts of secreted TNF-α protein ($n = 2$) shown are means with error bars indicating the S.D. *Statistical significance ($P < 0.05$).]
5.7-fold compared to the negative control and the TNF-α secretion level of the HepG2 cells co-transfected with pCMV/SeP and pCMV/X was also reversed (Fig. 4b).

4. Discussion

HBx induces oxidative stress which is the cause of apoptotic or necrotic death of hepatocytes leading to liver damage [6]. This implies that HBx contributes to liver disease through oxidative stress when infected by HBV. SeP is a well-known antioxidant protein that suppresses lipid peroxidation [8]. However, there are no reports concerning the relationship between HBx and SeP.

In our previous study, we generated a cDNA microarray to investigate gene regulation by HBV infection in human primary hepatocytes (S.G. Park and G. Jung, unpublished data). We found that SeP is one of the genes being down-regulated. So we targeted SeP to investigate the relationship between its down-regulation and HBV infection and to investigate the effects of its down-regulation on HepG2 cells. We also confirmed down-regulation of SeP expression by HBV in the human hepatoma cell line, HepG2 (data not shown). Considering the transcriptional regulatory function of HBx, we hypothesized that HBx is responsible for SeP down-regulation in HepG2 cells. We examined the expression of SeP after transfecting HepG2 cells using pCMV/X. The mRNA level of SeP expression was down-regulated considerably by HBx. Based on the previous reports that SeP is secreted into the medium [9–11], and that it works by binding to heparin and the cell membrane [8], the SeP secreted into the medium and the SeP included in the cell lysates were analyzed by immunoprecipitation and Western blot analysis. As expected, the protein levels of SeP were down-regulated by HBx in both cases. Because we found that SeP existed in the cell lysates and was secreted into the culture medium and that the ratio of SeP down-regulation in both culture medium and cell lysates detected by Western blot analyses were almost the same, we concluded that transfection of HBx did not inhibit ER and Golgi processes of SeP and SeP secretion into the medium. As SeP is an antioxidant protein for lipid peroxidation [8], the down-regulation of SeP expression implies a decrease in the defense ability against lipid peroxidation, a type of oxidative stress generated continuously by environmental and other factors.

To investigate the subsequent effects of SeP down-regulation, we excluded other possible influences of HBx than down-regulation of SeP by transfecting pCMV/SePa.s. We first confirmed a translation block of SeP using pCMV/SePa.s. Lipid peroxidation was induced in the HepG2 cells transfected with pCMV/SePa.s compared to the control. From this, it can be inferred that SeP was down-regulated by HBx and that this down-regulation induced lipid peroxidation. Therefore, we hypothesized that direct transfection of pCMV/X can induce lipid peroxidation. To confirm the effects of HBx on lipid peroxidation, we checked lipid peroxidation in the HepG2 cells transfected with pCMV/X. As expected, lipid peroxidation was induced by HBx. We also introduced SeP by transfecting pCMV/SeP to the HepG2 cells transfected with pCMV/X and confirmed the reversal of lipid peroxidation compared to the positive control. These results imply that SeP is an antioxidant protein defending the hepatocytes from lipid peroxidation and that HBx induces lipid peroxidation by down-regulating SeP expression.

We examined the effect of the induction of lipid peroxidation on TNF-α expression and the results showed the up-regulation of TNF-α expression in HepG2 cells after the induction of lipid peroxidation. Secreted levels of TNF-α from HepG2 cells transfected with pCMV/X were also increased. We introduced SeP to the HepG2 cells transfected with pCMV/X and confirmed a reverse in the TNF-α secretion level compared to the positive control. These results imply that HBx induces lipid peroxidation via SeP down-regulation and this leads to up-regulation of TNF-α expression. As mentioned previously, TNF-α is a critical factor in the development of liver damage [17] and, when functioning with HBx, they exhibit a synergic effect [18]. Therefore, it can be inferred that up-regulation of TNF-α expression by HBx exposes hepatocytes or hepatic tissue to liver damage.

In this report, we first showed that HBx down-regulated SeP expression and we also established a novel pathway in which this down-regulation of SeP by HBx led to lipid peroxidation which up-regulated TNF-α expression. These data would be useful in understanding the relationship between up-regulation of TNF-α by oxidative stress and liver damage due to chronic and acute HBV infections.

Acknowledgements

A grant from the Korean Ministry of Science and Technology (Critical Technology 21 on “Life Phenomena and Function Research”) [M1-0016-00-0022] is acknowledged. Young-Su Yi and Sung Gyoo Park are supported by Brain Korea 21 Research Fellowship from the Ministry of Education and Human Resources Development.

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