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Inhibitory effect of HMGN2 protein on human hepatitis B virus expression and replication in the HepG2.2.15 cell line

Yun Feng[a], Fang He[b], Ping Zhang[a], Qi Wu[c], Ning Huang[c], Hong Tang[b], Xiangli Kong[a], Yan Li[a], Junju Lu[a], Qianming Chen[a,*], Boyao Wang[c,*]

[a] State Key Laboratory of Oral Diseases, Sichuan University, Chengdu 610041, China
[b] Division of Molecular Biology of Infection Diseases, National Key Laboratory of Biotherapy, Sichuan University, China
[c] Research Unit of Infection and Immunity, West China Medical Center, Sichuan University, China

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ABSTRACT

Natural killer (NK) cells and cytolytic T lymphocytes (CTL) have been implicated as important effectors of antiviral defense. We previously isolated a novel antibacterial polypeptide, which was identified as high mobility group nucleosomal-binding domain 2 (HMGN2), from human mononuclear leukocytes. This study examined the antiviral activity of HMGN2 against human hepatitis virus B. HMGN2 was isolated and purified from the acid soluble proteins of the human THP-1 cell line, and identified by mass spectrum, Western blot and antibacterial assay. The hepatitis B virus (HBV)-transfected HepG2.2.15 cell line was used in the in vitro assay system. In the range of 1–100 μg/ml HMGN2, no cytotoxicity for HepG2.2.15 cells was detected by MTT assay. When incubated with HMGN2 at 1–100 μg/ml for 72 or 144 h, there was a significant reduction in hepatitis B e antigen (HBeAg) and hepatitis B surface antigen (HBsAg) expression, which were detected by ELISA, and a significant reduction in HBV DNA copies, which was determined by the real time quantitative PCR, in the supernatant of HepG2.2.15 cells. Northern and Southern blot analysis also showed that the levels of the HBV 3.5 kb and the 2.4/2.1 kb mRNA species and HBV replicative intermediate DNA were significantly reduced in the HMGN2-treated HepG2.2.15 cells. These results indicated that HMGN2 protein could markedly inhibit HBV protein expression and replication in vitro.

1. Introduction

Natural killer (NK) cells and cytolytic T lymphocytes (CTL) have been implicated as important effectors of antiviral defense. The hepatitis B virus (HBV) is a non-cytopathic DNA virus, which can cause acute and chronic viral hepatitis and other liver diseases including cirrhosis and hepatocellular carcinoma. The classical concept that viral clearance is mediated by CTL, which destroy the infected hepatocytes, has been challenged by recent studies which revealed that virus specific CTL can abolish HBV gene expression and replication without killing the infected hepatocytes (Chisari, 1995; Guidotti et al., 1996).

In our previous study, an antimicrobial polypeptide was isolated and purified from interleukin (IL)-2-stimulated human peripheral blood mononuclear leukocytes and shown to contain the high mobility group nucleosomal-binding domain 2 (HMGN2). When cultured mononuclear leukocytes were stimulated with IL-2, HMGN2 was expressed in the cytoplasm and then secreted (Feng et al., 2005).

In this study, we prepared a bulk of HMGN2 protein from THP-1, a human monocyte cell line, and examined its antiviral activity against human hepatitis B virus in the HBV-transfected HepG2.2.15 cell line. When incubated with 1–100 μg/ml HMGN2 protein for 72 or 144 h, there was a significant reduction in hepatitis B e antigen (HBeAg) and hepatitis B surface antigen (HBsAg) expression, and in HBV DNA copies, in the supernatant of HBV-transfected HepG2.2.15 cells.

2. Materials and methods

2.1. Isolation and identification of HMGN2 proteins from the human THP-1 cell line

THP-1 cells, a monocyte cell line from ATCC, were cultured in RPMI-1640 medium in the presence of LPS (Sigma Chemical Co., St. Louis, MO) at the concentration of 10 ng/ml for 2 days, and then collected and washed with phosphate-buffered saline (PBS). The cell pellet was dissolved in 5% acetic acid solution containing 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, and 10 μM pep-

* Corresponding authors at: State Key Laboratory of Oral Diseases, Sichuan University, Chengdu, Sichuan 610041, China. Tel.: +86 28 85501484; fax: +86 28 85582167.
E-mail address: fengyunxixi@yahoo.com.cn (Q. Chen).

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statin, homogenized at 4 °C, and centrifuged. The supernatant was collected and dialyzed against water at 4 °C for 48 h, lyophilized, and stored at −70 °C.

The purification of the HMGN2 proteins included preparative acid-urea polyacylamide gel electrophoresis (AU-PAGE) elution and reverse-phase high-performance liquid chromatography (RP-HPLC). Briefly, the preparative acid-soluble proteins isolated from the THP-1 cell line were purified by preparative AU-PAGE elution first following the protocol of Harwig et al. (1993). The eluates of preparative AU-PAGE were lyophilized, reconstituted in 0.01% acetic acid and then subjected to a 4.6 mm × 250 mm Vydac C18 column (Agilent Inc., Santa Clara, CA). Bound materials were eluted with a linear gradient of acetonitrile (ACN) in acidified water [0.1% trifluoroacetic acid (TFA) in water to 60% ACN with 0.1% TFA] for 60 min at a flow rate of 1.0 ml per minute. The elution profile was monitored at 214 nm. All fractions were collected, lyophilized, reconstituted with 0.01% acetic acid and stored at −70 °C.

Protein concentrations were measured by the bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL). Bovine serum albumin (BSA) was used as the standard for the protein assay. Acid-urea polyacylamide gel electrophoresis (AU-PAGE) (Panyim and Chalkley, 1969) and tricine sodium dodecyl sulfate (SDS)-PAGE (Schägger and von Jagow, 1987) were performed in mini-gel format, using the Modular Mini-Protein II electrophoresis system (Bio-Rad, Hercules, CA). The gels were stained with 0.1% Coomassie brilliant blue R-250.

Mass spectrometric measurement was performed by Shanghai Genecore Bio-technologies Company (Shanghai, PRChina). The method is as follows: Samples were eluted in 1 ml 50% (v/v) acetonitrile/0.1% TFA and mixed 1:1 with a saturated solution of sinapinic acid in 50% (v/v) acetonitrile/0.3% TFA. Samples (1 μl) were applied onto a stainless steel 96 × 2 target matrix-assisted laser desorption–ionization plate and air-dried before analysis in the mass spectrometer, where mass spectrometry was performed using an Applied Biosystems Voyager DE-PRO (ABI, Foster City, CA), equipped with a nitrogen laser (337 nm, 3 ns pulse width, 3.0 Hz REP rate). Mass spectra were obtained in the liner-positive mode with an accelerating voltage of 20 kV, a grid voltage setting of 95%, and a 400-ns delay using ~150 laser shots.

Western blot analysis was performed using an anti-HMGN2 polyclonal antiserum prepared in our laboratory (Wenbi et al., 2005). The proteins were run on a 15% gel by SDS-PAGE at 80 V for 3 h and blotted onto polyvinylidene difluoride membranes in transfer buffer at 30 V overnight at 4 °C. The membranes were blocked in 1 × tris-buffered saline (TBS), 5% (w/v) non-fat dried milk, and 0.1% Tween-20 (v/v) for 2 h at room temperature. The 1:500 dilution of anti-HMGN2 polyclonal antibody in primary antibody buffer (1 × TBS, 5% BSA, 0.1% Tween-20) was added and incubated for 1 h at room temperature. The membrane was then incubated with goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody at 1:5000-fold dilution in blocking buffer for 1 h at room temperature with gentle agitation. The ECL detection kit from Amersham Co. (Buckinghamshire, UK) was used in the experiments.

The agar radial diffusion assay developed by Lehrer et al. (1991), was used to detect the antibacterial activity of the purified protein. Underlay bacterial agar (10 ml) contained 1% agarose (Sigma A-6013), 0.3 mg/ml trypticase soy broth, 10 mM phosphate buffer (pH 7.2), and 1×10^5 mid-logarithmic-phase bacteria. A series of wells, each 3 mm in diameter, were punched into the solidified underlay gel. About 5 μl of samples (1 μg) or positive control (lysozyme 10 μg) was added into the designated wells. Overlay nutrient agar (10 ml) contained 30 g/l tripticase soy broth (Sigma Chemical Co., St. Louis, MO) and were poured on the underlay agars following 3 h incubation of the latter. After overnight incubation at 37 °C, the clearing zones were observed. The clear zones around the holes indicating no growth of bacteria were seen when the samples contained antimicrobial effectors. The microbes we used were *Escherichia coli* ML-35p, and *Pseudomonas aeruginosa* ATCC27853.

2.2. Purification of the HNP1-3 peptides

HNP1–3 was used as the control and prepared as described elsewhere (Ganz et al., 1985).

2.3. Cell culture

The HepG 2.2.15 cells were cultured in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (Gibco-Invitrogen, Carlsbad, CA) and 200 mg/l G418 (Sigma) under 5% CO_2 atmosphere at 37 °C. Subconfluent monolayer cells of HepG 2.2.15 were detached from the culture dishes by trypsin treatment, then centrifuged at 90 × *g* for 5 min and resuspended in the fresh media. Cells were planted onto 12-well flat bottom plates at a density of 2.0×10^4 cells per well for cell viability assay, or onto 24-well flat bottom plates at a density of 5.0×10^4 cells per well for ELISA and real-time polymerase chain reaction (PCR). After plating for 24 h, cells were treated with the respective reagents at the indicated concentrations for 72 or 144 h, then the cell viability was analyzed, or the culture media and cells were harvested for detection.

2.4. Cytotoxicity assay

The MTT [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide] (Sigma) assay was used to measure the viability of cultured cells and for further identification of the non-toxic concentrations of different reagents to the culture cells. The HepG 2.2.15 cells were treated with different concentrations of HMGN2 protein (100, 50, 20, 10, 5, 2 and 1 μg/ml) for 72 or 144 h. 5-Fluorouracil (5-FU) (50 μg/ml) was used as a positive cytotoxicity control. Then incubated medium was removed and 200 μl of fresh medium containing MTT (2.5 mg dissolved in 50 μl of dimethylsulfoxide) was added to each well. After incubation for 4 h at 37 °C, the culture medium containing MTT was removed and 200 μl of dimethylsulfoxide was added to each well, and then viable cells were detected by measuring absorbance at 570 nm. The cell viability was expressed as a percentage of the control. Reagents concentrations were considered non-toxic if the corresponding cell viability was greater than 95%.

2.5. HBsAg and HBeAg assay

The levels of HBV surface antigen (HBsAg) and e antigen (HBeAg) in supernatant of the HepG 2.2.15 cells were determined by the enzyme-linked immunosorbent assay (ELISA) according to the Manufacturer's protocol (Shanghai Siic Kehua Biotech Co., Ltd.). The absorbance was measured at 450/630 nm using a microplate reader (Bio-Rad).

2.6. Real-time PCR analysis

Real-time fluorescence quantitative PCR was performed to quantify the levels of HBV viral genomic DNA by using an Applied Biosystems Prism 7000 instrument and an Applied Biosystems SYBR® green master mix reagent. The Manufacturer's protocol was followed in detail (Finnzymes, Finland). The quantitative PCR was performed in a 25 μl reaction solution containing 3 μl of supernatant from normal 2215 cells and from 2215 cells in the presence of HMGN2 protein using the SYBR green master mix, and the primers 5′-AGGAGGCTGTAGGCATAAATTGG-3′ (sense) and 5′-CAGCTTGGAGGCTTGAACAGT-3′ (antisense) of HBV gene (Ren et al., 2005). A series of dilutions of Topo-HBV plasmid containing HBV genes were used to create a standard curve for quantifying HBV

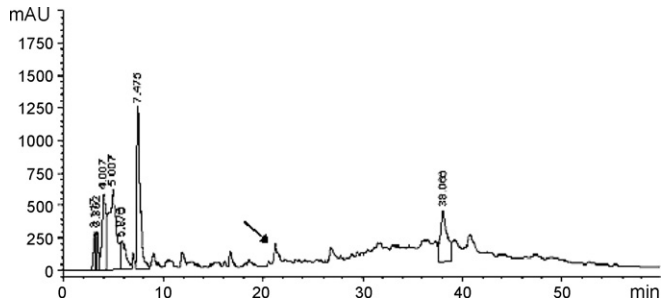

Fig. 1. HPLC profile of the acid-soluble proteins of the human THP-1 cell line. The acid-soluble protein of human THP-1 cell was subjected to RP-HPLC on a 4.6 mm × 250 mm Vydac C18 column and eluted with a 0–60% linear gradient of solvent B (0.1% TFA, 60% actonitrile, 40% water) in 60 min at a flow rate of 1.0 ml/min. Arrow indicates the HMGN2 protein.

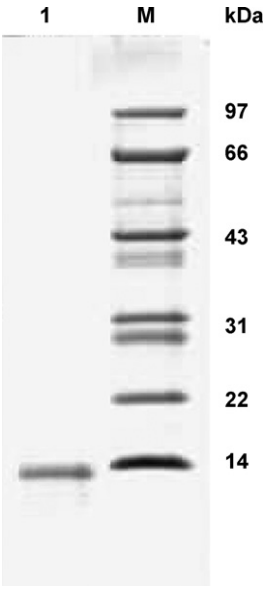

Fig. 2. SDS-PAGE of the isolated protein.

DNA levels. The Topo-HBV plasmid concentrations were as follows (copy μl^{-1}): 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7. The reactions were denatured at 95 °C for 10 min, followed by 40 cycles of 30 s at 95 °C and 60 s at 60 °C.

2.7. Southern blot analysis

After treatment with the respective reagents, cells were washed twice with chilled PBS and lysed in 650 μl of lysing buffer (10 mM Tris–HCl, pH 7.9, 1 mM EDTA, 1% NP-40, 8% sucrose) and centrifuged at 12,000 × *g* for 2 min at 4 °C to remove nuclei and debris. The supernatants were collected. Intracellular core particles were purified from the supernatants, and HBV replicative intermediate DNA was extracted from the core particles as described previously (Lin et al., 2001). The viral replicative intermediate DNA samples were electrophoresed onto 1% agarose gel and blotted onto a positive nylon membrane (Roche). After fixing at 120 °C for 30 min, the membrane was prehybridized for 6 h at 42 °C in 5× SSC, 5× Denhardt's solution, 1% SDS, 50% formamide, 0.1 mg/ml salmon sperm DNA, and then hybridized with full-length HBV DNA probes labeled with [α-^{32}P]dCTP by an hexamer random labeling kit (Roche) under the same condition of prehybridization at 42 °C for 16 h. After stringent washing at 68 °C, signals were detected by autoradiography. The experiment was repeated three times and blots were quantified by densitometry.

2.8. Northern blot analysis

Total RNAs were extracted from HepG2.2.15 cells using the Trizol method according to the Manufacturer's instructions (Invitrogen). RNAs were separated by electrophoresis on a 1.5% agarose-formaldehyde gel and transferred to a NC+ membrane. Blots were probed using a PCR-generated ^{32}P-labeled HBV DNA (an S fragment from Topo-HBV spanning the entire HBV genome) and ^{32}P-labeled GAPDH fragments (endogenous gene, as a control) using the random primer labeling mix (TaKaRa Biotechnology, Tokyo, Japan) at 65 °C overnight. After washing, the membranes were analyzed using a phosphor imaging system.

2.9. Statistic analysis

All the detection items in this study were repeated at least three times, and the results were expressed as mean ± S.D.

3. Results

3.1. Purification and identification of the HMGN2 proteins from the THP-1 cell line

The AU-PAGE elutes were subjected to RP-HPLC for purification. Fraction 21 (shown in Fig. 1) was analyzed by SDS-PAGE. Fraction 21

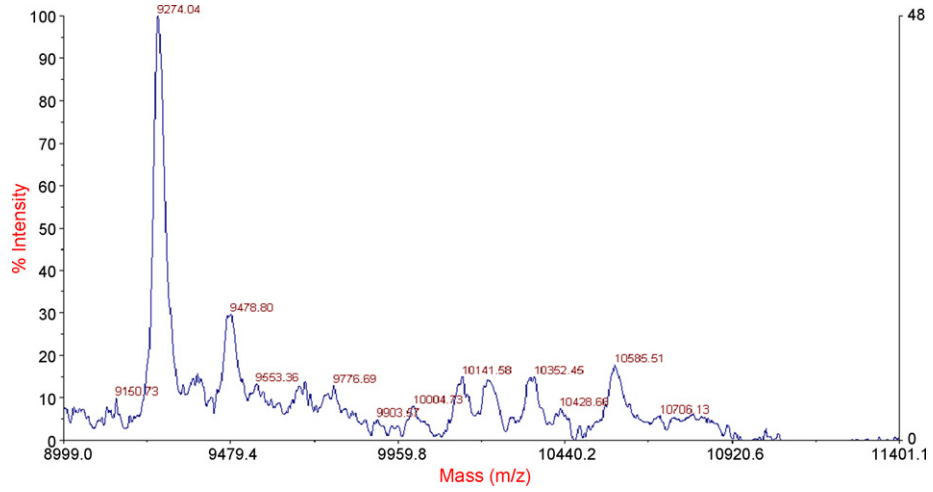

Fig. 3. Mass spectrum of the isolated protein. Mass spectrometric analysis of the isolated peotein revealed the same molecular mass (m/z = 9274.04) as HMGN2.

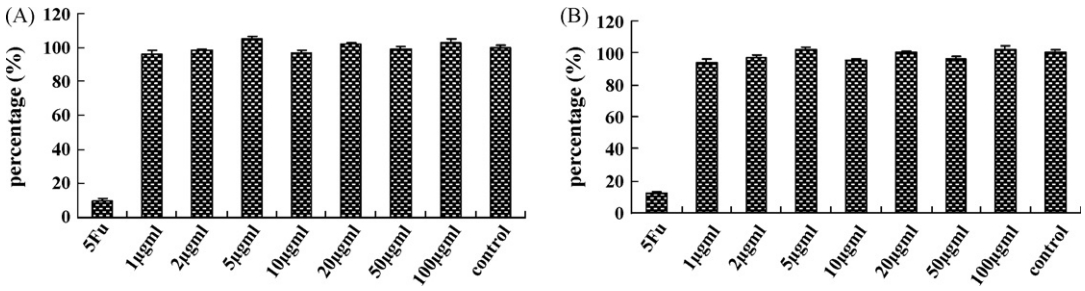

Fig. 4. MTT cytotoxicity assay results. (A) The MTT results of 72 h. (B) The MTT results of 144 h. The HepG 2.2.15 cells were treated at different concentrations of HMGN2 protein (100, 50, 20, 10, 5, 2 and 1 μg/ml) for 72 or 144 h, 5-fluorouracil (5-FU) (50 μg/ml) was used as a positive cytotoxicity control. The cell viability was expressed as a percentage of the control. Reagent concentrations were considered non-toxic if the corresponding cell viability was greater than 95%.

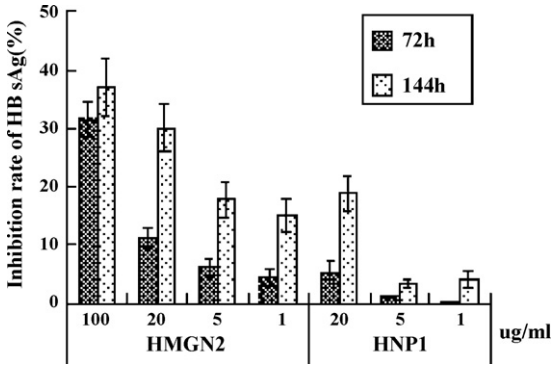

Fig. 5. Inhibition of HBsAg expression by HMGN2 and HNP1-3 protein in HepG 2.2.15 cells. The amount of HBeAg in the culture medium was measured by ELISA and is presented as a percentage of the amount secreted by the mixed cell pools (control). The data shown represent the mean values (±S.D.) based on three independent experiments.

displayed only one protein band with a molecular mass of approximately 14 kD (Fig. 2).

Mass spectrometric analysis of the HMGN2 protein revealed the same molecular mass (*m*/*z* 9274.04) as HMGN2 (Fig. 3). A strong HMGN2 antibody-binding signal at the protein migration position was detected by Western blot (data not shown). Gel overlay antimicrobial testing showed that the protein had antibacterial activity against *E. coli* ML-35P and *P. aeruginosa* ATCC27853 (data not shown).

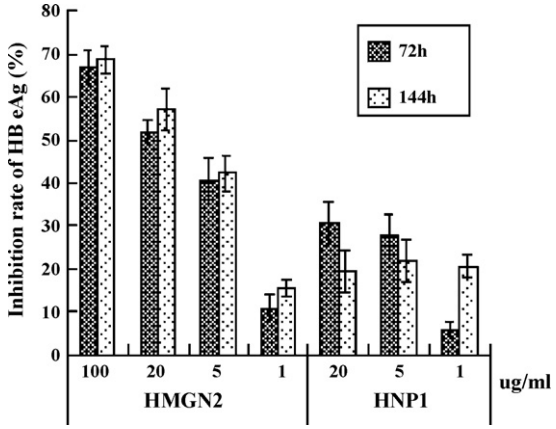

Fig. 6. Inhibition of HBeAg expression by HMGN2 and HNP1-3 protein in HepG 2.2.15 cells. The amount of HBsAg in the culture medium was measured by ELISA and is presented as a percentage of the amount secreted by the mixed cell pools (control). The data shown represent the mean values (±S.D.) based on three independent experiments.

3.2. HMGN2 protein reduces the synthesis of HBsAg and HBeAg

To investigate the effect of the HMGN2 protein on HBV gene expression, the ELISA assay was performed to measure HBsAg and HBeAg levels in supernatants of HepG2.2.15 cells. The MTT assay proved that the detected concentrations (100, 50, 20, 10, 5, 2 and 1 μg/ml) of the HMGN2 protein were non-toxic to HepG 2.2.15 cells (Fig. 4). As shown in Figs. 5 and 6, the expression levels of HBsAg and HBeAg were significantly reduced in the HepG 2.2.15 cell pools in the presence of the HMGN2 protein. At the concentrations of HMGN2 protein (100, 20, 5, and 1 μg/ml), the reduction of HBsAg was after 144 h 31.6 ± 3.0%, 11.2 ± 1.8%, 6.0 ± 1.6%, 4.44 ± 1.3% after 72 h, and 36.9 ± 5.0%, 30.1 ± 4.1%, 17.8 ± 2.9%, 14.9 ± 2.8%, respectively. HBeAg decreased after 72 h to 66.7 ± 4.1%, 52 ± 2.8%, 40.8 ± 4.9% and 10.8 ± 2.7%, respectively, after 144 h to 68.6 ± 3.1%, 57.1 ± 4.9%, 42.1 ± 3.8% and 15.5 ± 1.8%, respectively. The HMGN2 protein was more efficient than HNP1-3 peptide in inhibiting HBsAg and HBeAg. These data demonstrated that the inhibition of HBV gene expression by the HMGN2 protein was efficient and persistent.

3.3. Inhibition of HBV replication by HMGN2 protein

During HBV replication, pgRNA is reversely transcribed and the newly synthesized DNA is encapsulated and later secreted as mature virions. Real-time PCR analysis was performed with virus DNA in the supernatants obtained from the HepG 2.2.15 cell pools to determine whether HMGN2 protein could inhibit HBV DNA replication. To quantitate the HBV DNA levels, a series of dilutions of Topo-HBV plasmids containing the HBV genes were run as a standard curve in parallel with the HBV DNA from the supernatants of the HepG 2.2.15 cells. The standard curve was made based on

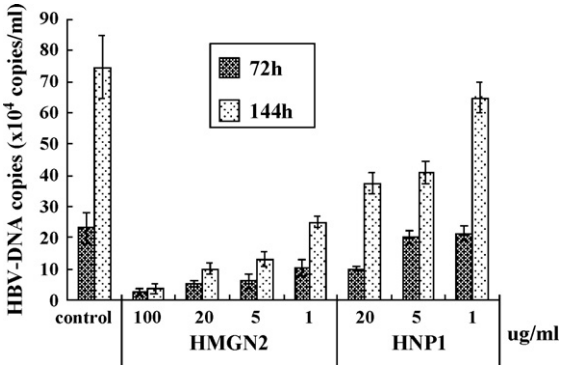

Fig. 7. Inhibition of HBV DNA replication by HMGN2 and HNP1 protein in HepG 2.2.15 cells. The HBV DNA in supernatants of the HepG 2.2.15 cells were harvested and analyzed by real-time PCR. The copies of the HBV DNA from the HepG 2.2.15 cells were calculated based on their Ct value and the standard curves. Data shown represent the mean values (±S.D.) based on three independent experiments.

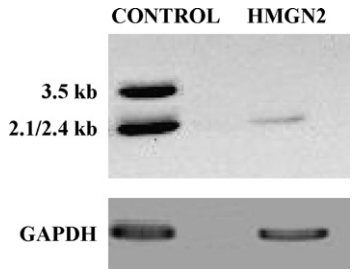

Fig. 8. Inhibition of HBV total RNAs by the HMGN2 protein. Total RNAs were extracted 48 h after HMGN2 (5 μg/ml) protein treated and analyzed by Northern blot using [α-^{32}P] dCTP-labeled full-length HBV DNA probes. The blots were stripped and rehybridized with [α-^{32}P] dCTP-labeled GAPDH probes to control for gel loading. The arrows indicated the location of the 3.5-, 2.4-, and 2.1-kb viral RNA bands. Quantification of three independent experiments was obtained by densitometry and is shown at the bottom. Data were normalized to the level of the corresponding GAPDH.

Log quantity and C_t value of a series of different concentrations of Topo-HBV plasmid. The copies of HBV DNA from the supernatants of the HepG 2.2.15 cells were calculated based on their C_t value and the standard curves of Topo-HBV plasmid. Results showed that the HBV DNA was markedly reduced when HepG 2.2.15 cell pools were incubated with HMGN2 protein, as compared to controls, even at a protein concentration of 1 μg/ml (Fig. 7). The results revealed that HMGN2 protein could markedly inhibit the HBV replication.

3.4. HMGN2 protein depressed HBV mRNA expression in the HepG2.2.15 cells

To determine if the effect of HMGN2 peptide on HBsAg and HBeAg expression was caused by a reduction in the HBV RNA levels, Northern blot analysis was performed using total RNAs extracted from the HepG2.2.15 cell pools. The levels of the HBV 3.5 kb and the 2.4/2.1 kb mRNA species were noticeably reduced in the cell pools treated with the HMGN2 peptide (Fig. 8). When treated with HMGN2 at the concentration of 5 μg/ml, more than 50% of reduction in the density of specific HBV mRNAs was observed compared with controls. Meanwhile, as shown in Fig. 8, it seems that HMGN2 did not interfere with the expression of the host cell mRNAs, such as GAPDH mRNA. These results suggested that the inhibition of the HBsAg and HBeAg expression by HMGN2 in the HepG2.2.15 cells would result from the specific depression of the major viral mRNA species.

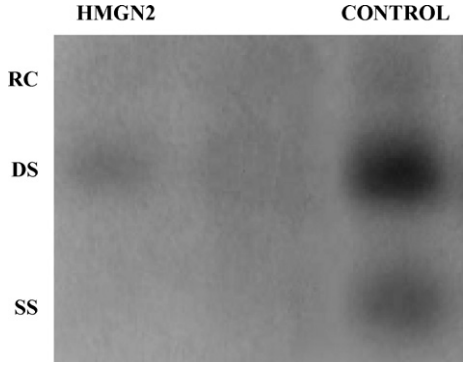

Fig. 9. HMGN2 protein efficiently inhibits HBV replication. Cells were harvested 48 h after treatment with HMGN2 (5 μg/ml). HBV replicative intermediates DNA were extracted from core particles and analyzed by Southern blot. The HBV DNA was loaded onto 1.0% agarose gel, and then transferred to nylon membranes. For hybridization, full-length HBV DNA probes labeled with [α-^{32}P] dCTP were used. The relaxed circular (RC), double-stranded linear (DS), and single-stranded linear (SS) HBV DNA forms were visualized.

3.5. HMGN2 protein reduced the synthesis of HBV replicative intermediates

When incubated with HMGN2 at the concentration of 5 μg/ml for 2 days, Southern blot analysis (Fig. 9) showed that of the HBV replicative forms were markedly reduced by about 80% in the HMGN2-treated HepG2.2.15 cells. Thus, in addition to reducing the synthesis of viral proteins, the HMGN2 protein also effectively depressed HBV DNA replication.

4. Discussion

HMG proteins have been described to be an abundant family of non-histone proteins in the cell nucleus of vertebrate and invertebrate organisms (Bustin, 1999). In the narrowest, traditional sense, this HMG protein family consists of six proteins and is subdivided into three subfamilies: the HMGB (formerly HMG-1/-2), the HMGA (formerlyHMG-I/-Y/-C), and the HMGN (formerly HMG-14/-17) subfamilies. Each of these classes seems to have a distinct type of function in the nucleus (Bustin, 1999). However, it is now well known that peptides in the HMG protein family have additional functions. HMGbox1 (HMGB1) is the first example. HMG-1, an abundant, highly conserved cellular protein, is widely known as a nuclear DNA-binding protein, which stabilizes nucleosome formation, facilitates gene transcription, and regulates the activity of a steroid hormone receptor (Czura et al., 2001; Yang et al., 2001). A decade-long search has culminated in HMGB1 as a late toxic cytokine of endotoxemia. HMGB1, released by macrophages upon exposure to endotoxin, activates many other proinflammatory mediators and is lethal to otherwise healthy animals (Czura et al., 2001; Yang et al., 2001). It was demonstrated that HMGB1 possesses potent bactericidal activity (Zetterström et al., 2006). More recently, Fernandes et al. (2003) have described a potent antimicrobial peptide isolated from skin mucus secretion of fish, which is a member of the HMG protein family.

The HMGN2 gene is located in chromosome 1p36.1 (Landsman et al., 1989; Popescu et al., 1990), and it contains six exons, with an extremely high GC content and an “HpaII tiny fragment” island, indicative of a housekeeping gene that could be crucial for the regular functioning of cells (Landsman et al., 1986; Srikantha et al., 1987). However, until now, the biological role of this protein has not been fully defined. A variety of experiments have shown that HMGN2 is preferentially associated with chromatin subunits (Hock et al., 1998; Paranjape et al., 1995; Tremethick and Hyman, 1996; Vestner et al., 1998). Furthermore, the abnormal gene or protein expression of HMGN2 is related to some diseases such as neoplasms (Okamura et al., 1999; Spieker et al., 2000) and autoimmune diseases (Ayer et al., 1994; Bustin et al., 1982; Vlachoyiannopoulos et al., 1994). The significance of HMGN2 in the host defense against infection is unclear. In our previous study, we found that HMGN2 is released by human peripheral blood mononuclear leukocytes in the presence of interleukin (IL)-2, and it has an antimicrobial activity against *E. coli, P. aeruginosa* and *Candida albicans*. Recently our co-workers (Ming et al., 2007) isolated an antimicrobial polypeptide from human female cervical mucus and demonstrated it to be HMGN2. These data made us think that the physiological role of HMGN2 is not so simple. The present study provided some evidence indicating that HMGN2 had an antiviral activity against HBV, including inhibitory effects on HbeAg, HbsAg, the HBV 3.5 kb and the 2.4/2.1 kb mRNA species expression and HBV DNA replication in HBV-transfected HepG2.2.15 cells. Thus, we proposed from these preliminary data that HMGN2 might preferentially bind to some HBV-specific DNA sequences and interfere with viral DNA replication and protein expression. Further study of the antiviral mechanisms is underway.

HBV is a typical non-cytopathic virus that can induce variable severity of tissue damage by stimulating a protective immune response that can simultaneously cause damage and protection. Elimination of intracellular viruses is through the immune destruction of virus-infected cells (Carlo et al., 2003). Therefore, immune destruction of infected cells can lead to the termination of infection when it is efficient, or to a persistent disease when it is not. Destruction of infected cells, however, is not the only mechanism implicated in the elimination of intracellular virus, as demonstrated by studies carried out in animal models of HBV infection and in human hepatitis B showing the importance of cytokine-mediated, non-cytolytic mechanisms of anti-viral protection (Carlo et al., 2003).

In 1986, Walker et al. (1986) first described the CD8 antiviral factor (CAF), a diffusible molecule secreted by stimulated CD8 T cells from certain HIV-1 infected individuals. Unlike the activity of CTLs, the antiviral activity of CAF is non-cytolytic and does not require restriction by major histocompatibility complex class I molecules or cell-to-cell contact (Zhang et al., 2002). Instead, the activity is believed to be mediated by a heat-stable, acid-stable protein (Levy et al., 1996) with a molecular mass of 20 kD (Lacey et al., 1998) or 10 kD (Mosoian et al., 2000). The molecular characteristics of CAF are still not clear, decades after its presence was first inferred. HMGN2 protein is a small, heat- and pH-stable molecule. Based on the finding of its anti-hepatitis B virus activity from the present study, HMGN2 protein might presumably be implicated in the molecular basis of action of CAF.

Acknowledgments

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