Dual effects of interleukin-18: inhibiting hepatitis B virus replication in HepG2.2.15 cells and promoting hepatoma cells metastasis

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Zhang Y, Li Y, Ma Y, Liu S, She Y, Zhao P, Jing M, Han T, Yan C, Wu Z, Gao J, Ye L. Dual effects of interleukin-18: inhibiting hepatitis B virus replication in HepG2.2.15 cells and promoting hepatoma cells metastasis. Am J Physiol Gastrointest Liver Physiol 301: G565–G573, 2011. First published June 30, 2011; doi:10.1152/ajpgi.00058.2011.—Interleukin-18 (IL-18) has been reported to inhibit hepatitis B virus (HBV) replication in the liver of HBV transgenic mice; however, the molecular mechanism of its antiviral effect has not been fully understood. In the present study, it was shown that IL-18 and its receptors (IL-18R) were constitutively expressed in hepatoma cell lines HepG2 and HepG2.2.15 as well as normal liver cell line HL-7702. We demonstrated that IL-18 directly inhibited HBV replication in HepG2.2.15 cells via downregulating the activities of HBV core and X gene promoters. The suppressed HBV replication by IL-18 could be rescued by the administration of BAY11-7082, an inhibitor of transcription factor NF-κB. On the other hand, it was of interest that IL-18 promoted HepG2 cell metastasis and migration dose dependently in both wound-healing assays and Transwell assays. The underlying mechanism could be partially attributable to the increased activities of extracellular matrix metalloproteinase (MMP)-9, MMP-3, and MMP-2 by IL-18, which upregulated the mRNA levels of MMP-3 and MMP-9 in a NF-κB-dependent manner. Furthermore, it was confirmed that expression of IL-18/IL-18R and most MMPs were remarkably upregulated in hepatocellular carcinoma (HCC) liver cancer tissue specimens, suggesting that IL-18/IL-18R-triggered signaling pathway was closely related to HCC metastasis in vivo. Therefore, we revealed the dual effects of IL-18 in human hepatocytes: it not only inhibited HBV replication but also promoted hepatoma cells metastasis and migration. NF-κB played a critical role in both effects. Our work contributed to a deeper understanding of the biological function of IL-18 in human hepatocytes.

Interleukin-18, hepatitis B virus, hepatoma cells; metastasis and migration, extracellular matrix metalloproteinases

IL-18 is a uniquely pleiotropic cytokine belonging to the IL-1 family. It is expressed as a 24-kDa precursor protein that can be proteolytically cleaved into an 18-kDa mature form by caspase-1 and then secreted (17). IL-18 is produced mainly by immune cells, such as monocytes, macrophages, and dendritic cells, and it effectively enhances innate and adaptive immune responses (1, 14). Unlike other cytokines, IL-18 has recently been reported to be also produced by nonimmune cells including osteoblasts, keratinocytes, intestinal epithelial cells and some cancer cells (17). This attracts people’s strong interests in the roles of IL-18 in these cells, especially in cancer cells. It was found that IL-18 played a pathogenic role in gastrointestinal injury and cancer progression (6, 16). IL-18 works by binding to IL-18 receptor (IL-18R), which consists of two chains: IL-18Rα, an IL-18-binding chain, and IL-18Rβ, a signal-transducing chain. The recognition of IL-18 by IL-18R triggers the recruitment of MyD88 and finally activates transcription factors NF-κB and activator protein-1 (AP-1) (19).

IL-18 has been demonstrated to be an effective adjuvant in HBV vaccination (2, 3, 20). Epidemic studies in the Korean population revealed that IL-18 gene polymorphism was likely associated with hepatitis B virus (HBV) clearance (4, 7). Furthermore, animal studies also demonstrated that administration of recombinant mouse IL-18 rapidly and noncytopathically inhibited HBV replication in the liver of HBV transgenic mouse (10). Therefore, IL-18 has been considered as a promising treatment for chronic HBV infection. However, the underlying mechanism by which IL-18 inhibits HBV replication has not been fully understood.

In addition to the antiviral effect, recent data indicated a pathogenic role of IL-18 in cancer progression. Elevated serum levels of IL-18 were found to be correlated with the carcinogenesis of several cancers including breast cancer, lung cancer, gastrointestinal carcinoma, and skin cancer (16). Tangkijvanich et al. (21) reported that plasma levels of IL-18 were significantly elevated in patients with hepatocellular carcinoma (HCC) compared with healthy controls, implying a pathogenic role of IL-18 in HCC progression. However, few studies investigated the function of IL-18 on HCC progression. Since chronic HBV infection plays an important role in the development of HCC, investigation about the role of IL-18 in HCC progression is essential for its clinical application in the future.

In the present study, we investigated the effects of IL-18 on HBV replication in HepG2.2.15 cell line and hepatoma cell metastasis and migration. Our results suggested that IL-18 dramatically inhibited HBV replication through selectively suppressing HBV genes transcription in HepG2.2.15 cells. On the other hand, IL-18 significantly promoted the metastasis and migration of hepatoma cells via increasing the activities of MMP-9, MMP-3, and MMP-2. The dual effects of IL-18 on HBV replication and hepatoma cell metastasis were both mediated by NF-κB.

MATERIALS AND METHODS

Plasmid construction. The coding sequence of mature human IL-18 was amplified from plasmid pET-IL18, which was kept in our laboratory, by use of primers for IL-18 listed in Table 1. After digestion with XhoI and BamHI, it was inserted into pcDNA3.1 (−) vector (pcDNA3.1-mIL18). pNF-κB-luc and pNFAT-luc plasmids (Clontech) were gifts from Dr. Weilin King (University of Shanghai Jiao
Tong, Shanghai, China). HBV promoter reporter vectors pSIP-luc, pmSP-luc, pCP-luc, and pXP-luc were gifts from Dr. Ying Zhu (Wuhan University, Wuhan, Hubei, China).

Cell culture. HepG2.2.15 cells were cultured in 1640 medium (Invitrogen) with 10% fetal bovine serum (FBS, Gibco), 100 U/ml penicillin, 100 μg/ml streptomycin (Hyclone), and 250 μg/ml G418 (Sigma). HepG2 cells were cultured in DMEM medium (Invitrogen) with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Both cells were incubated in a humidified 5% CO2 atmosphere at 37°C.

Western blotting. HepG2.2.15 cells were transiently transfected with expression plasmids or small interfering RNA (siRNA) oligos by using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. siRNA oligos for IL-18 (siIL-18) or for negative control (siNC) were synthesized in GenePharma Biotechnology (China). The sequences are shown in Table 1. At 48 h after transfection, the cells lysates were collected and analyzed by Western blot (Boster) with use of mouse anti-IL-18 antibody (Santa Cruz Biotechnology) or mouse anti-GADPH antibody (loading control, Boster), followed by horseradish peroxidase-coupled goat anti-mouse secondary antibody (KPL), and finally visualized via enhanced chemiluminescence (ECL) system (Thermo Scientific).

ELISA. HepG2.2.15 cells were transiently transfected with plasmid pcDNA3.1-mIL18 or sil-18 with use of Lipofectamine 2000 or treated with recombiant human IL-18 (rhIL-18, active form, Prospec) for 24 h. The levels of HBV surface antigen (HBsAg) and e antigen (HBeAg) in the supernatant were determined by ELISA kits (Promega) according to the manufacturer’s instructions. Cells were washed twice with cold PBS and lysed with 50 μl PBS by repeated freeze-thaw cycles. The levels of cytosolic HBsAg were also determined by ELISA kits. The absorbance values were measured at 450/630 nm via a microplate reader (Thermo Electron).

HBV RI-DNA extraction. HepG2.2.15 cells were washed twice with ice-cold PBS (pH 7.4) and lysed with cell lysis buffer (Beyotime) for 30 min. After centrifugation at 13,000 rpm for 1 min, the supernatant was collected and digested extensively with DNase I (Promega) for 3 h at 37°C. The reaction was terminated by adding the stop solution. Then the sample was digested with 200 μg/ml protease K (Sigma) for another 3 h at 37°C. After phenol-chloroform extraction, HBV replicative intermediate DNA (RI-DNA) was precipitated with two volumes of 100% ethanol, washed once with 75% ethanol, and finally resolved in sterile double-distilled H2O (ddH2O).

Semiquantitative PCR and real-time PCR. Total RNA was extracted from HepG2.2.15, HepG2, or HL-7702 cells by use of TRIzol reagent (Invitrogen) and was reverse transcribed into cDNA by use of Maloney-murine leukemia virus transcriptase (Promega) and random primer (Promega). After the DNA fragments of IL-18, IL-18Rα, IL-18Rβ, and β-actin were amplified from the cDNA with Taq DNA polymerase (Takara) and primers listed in Table 1, they were analyzed on 1.2% agarose gel containing 0.5 μg/ml ethidium bromide with β-actin as the loading control and photographed under UV light. The cDNA, HBV RI-DNA, or culture medium of HepG2.2.15 was used as the template for real-time PCR, which was performed by using a Lightcycler 480II (Roche) with Thunderbird SYBR qPCR mix (Toyobo). The primers for HBV fragment were also list in Table 1. Dual-luciferase assay. HepG2 cells were transiently transfected with reporter vectors along with expression plasmid pcDNA3.1-

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<tr>
<th>Sense Primer (5'-3')</th>
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<tr>
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IL-18Rs and IL-18Rβ, IL-18 receptor chains α and β; MMP, matrix metalloproteinase; HBV, hepatitis B virus.

**Table 1. The sequences of primers used for PCR reaction**

**Fig. 1. Analysis about the expression of IL-18 and its receptors (IL-18Rα and IL-18Rβ) in liver cells. A: mRNA levels of IL-18, IL-18Rα, and IL-18Rβ in HL-7702. HepG2, and HepG2.2.15 cells were determined by semiquantitative RT-PCR, with β-actin as an internal control. B–D: HepG2.2.15 cells in 6-well plates were transiently transfected with plasmid pcDNA3.1-mIL18 [pcDNA3.1(−) as empty control] or small interfering RNA (siRNA) oligo for IL-18-si (siIL-18) [small interfering oligo for negative control (siNC) as negative control]. At 48 h after transfection, the cells lysates were collected and analyzed by Western blot (B) or by real-time PCR (C and D). Data shown represent mean values (± SD) of 3 independent experiments. ***P < 0.001.**
mIL18 or siIL-18. At 48 h after transfection, cells were collected for luciferase assay, which was performed by using the dual-luciferase reporter assay system (Promega) according to the protocol.

Wound healing assay. HepG2 cells with a 90% confluence were transiently transfected with plasmid pcDNA3.1-mIL18 or empty vector. At 24 h after transfection, cells were scraped with a pipette tip and cultured in DMEM containing 5% FBS. Photographs were taken at the indicated time points by using an Olympus IX81 microscope with software Qcapture.

Transwell assay. The 8-μm-pore-size polycarbonate membrane, which separates the two chambers of a 6.5-mm Transwell (Costar), was coated with or without 1:2 DMEM-diluted Matrigel (Sigma). HepG2 cells were transiently transfected with plasmid pcDNA3.1-mIL18 or empty vector. At 24 h after transfection, cells were collected and adjusted to a density of 3 × 10^5 cells/ml with serum-free DMEM containing 0.2% BSA, and 100 μl cell suspension was added to the upper chamber. The lower chamber was filled with 600 μl DMEM containing 10% FBS. After incubation at 37°C for 24 h, the transmembrane cells were stained with 0.1% crystal violet. Five fields were randomly selected by use of an Olympus IX81 microscope, and the numbers of stained cells were counted.

Zymography. HepG2 cells were seeded in 6-cm dishes and incubated overnight in DMEM containing 10% FBS at 37°C. Then cells were washed and incubated in 2 ml fresh DMEM containing 20 ng/ml rhIL-18 at 37°C. Medium was collected after 24 h incubation and centrifuged at 1,000 rpm for 5 min to remove cellular debris. After lyophilization in a vacuum freezing dryer (Beijing Boyikang Laboratory Instruments), the sample was re-solved with 100 μl ddH2O and the total protein concentration was determined by the Bradford method. Equal amounts of protein were separated by a 10% nonreductive SDS-PAGE gel containing 1 mg/ml gelatin (Sigma) or 0.5 mg/ml casein (Sigma) to detect the activities of MMP-2 and MMP-9 in gelatin zymography or the activity of MMP-3 in casein zymography, as previously described (5). Briefly, after electrophoresis, the gel was incubated twice in renaturing buffer [50 mM Tris, 5 mM CaCl2, 1 μM ZnCl2, 2.5% (vol/vol) Triton X-100, pH 7.6] for 30 min, washed twice in the same buffer without Triton X-100 for 20 min, and finally incubated at 37°C in developing buffer (50 mM Tris, 5 mM CaCl2, 1 μM ZnCl2, 0.02% Brij, pH 7.6) for 18 h. The gels were stained with 0.5% Coomassie blue for 30 min and washed with 10% acetic acid and 50% methanol in water for 1 h.

MTT assay. HepG2 cells with a 30–40% confluence were treated with rhIL-18 for 24 h and incubated for another 4 h with 20 μl 5 mg/ml MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma). After the culture medium was removed, 150 μl DMSO was added to each well. The number of viable cells was evaluated by measuring the absorbance values at 490 nm.

Statistical analysis. All experiments were repeated at least three times. A Student’s t-test was used to assess statistical significance with P < 0.05.

Fig. 2. Effects of IL-18 on the protein levels of hepatitis B virus (HBV) surface antigen (HBsAg) and e antigen (HBeAg) in HepG2.2.15 cells. A and D: HepG2.2.15 cells in 96-well plates were transiently transfected with plasmid pcDNA3.1-mIL18 [pcDNA3.1(−) as empty control] or siIL-18 (siNC as negative control). At 48 h after transfection, the levels of HBsAg and HBeAg in supernatant and cytosolic HBsAg were examined by using ELISA kits according to the manufacturer’s instructions. The levels of supernatant HBsAg were assayed in HepG2.2.15 cells, which were treated with increasing concentrations of recombinant human IL-18 (rhIL-18) for 24 h (C) or transiently transfected with increasing amounts of plasmid pcDNA3.1-IL18 (B) or siIL-18 (E). The absorbance values were measured at 450/630 nm via a microplate reader. Data shown represent the mean values (± SD) of 3 independent experiments. *P < 0.05, **P < 0.01.
RESULTS

**IL-18 inhibited HBV replication in HepG2.2.15 cells.** IL-18 has been reported to function as a proinflammatory cytokine in a receptor-dependent manner. We first examined the expression of IL-18 and IL-18R in hepatoma cell line HepG2 and HBV-replicating hepatoma cell line HepG2.2.15 as well as normal liver cell line HL-7702. RT-PCR analysis showed that IL-18, IL-18Rα, and IL-18Rβ mRNA were constitutively expressed in all the three cell lines (Fig. 1A). Compared with HL-7702, the levels of IL-18Rβ mRNA increased significantly in both hepatoma cells lines, suggesting that IL-18/IL-18R-triggered signaling pathway might be upregulated in hepatoma cells.

To investigate the effect of IL-18 on HBV replication, HepG2.2.15 cells, which constitutively secreted HBsAg-containing particles and HBeAg, were transiently transfected with expression plasmid pcDNA3.1-mIL18 or IL-18 siRNA oligos (siIL-18). The level of mature IL-18 was confirmed to dramatically increase in pcDNA3.1-mIL18-transfected cells and decrease in siIL-18-transfected cells at 48 h after transfection by use of Western blot assay (Fig. 1B) and real-time PCR (Fig. 1, C and D). Moreover, more IL-18 was secreted into medium by IL-18-overexpressed cells between 24–60 h after transfection compared with the control (Supplemental Fig. S1A; the online version of this article contains supplemental data). Then we examined the effects of IL-18 on the levels of HBsAg in culture medium, which indicated the levels of viral particle secretion, cytosolic HBsAg, and supernatant HBeAg. Overexpressed IL-18 was shown to significantly inhibit the HBsAg level in culture medium between 24 and 56 h after transfection with a peak inhibition around 48 h, but it had no significant effects on the levels of cytosolic HBsAg and supernatant HBeAg (Fig. 2D and Supplemental Fig. S1B). The HBsAg level in culture medium gradually decreased along with the increasing amount of plasmid pcDNA3.1-mIL18 (Fig. 2B) or rhIL-18 (Fig. 2C). In contrast, IL-18 knockdown with siIL-18 oligos increased the HBsAg level in culture medium dose dependently (Fig. 2E). Consistently, IL-18 knockdown had no apparent effect on the levels of cytosolic HBsAg and supernatant HBeAg. These results indicated that IL-18 inhibited HBV viral particles production in HepG2.2.15 cells in a dose-dependent manner.

Since IL-18 binding protein (IL-18BP) is a natural inhibitor of IL-18 (15), we also studied the effect of IL-18BP on supernatant HBsAg and HBeAg levels in HepG2.2.15 cells. There was no constitutive expression of IL-18BP in HepG2 and HepG2.2.15 cells (data not shown). We transiently transfected HepG2.2.15 cells with expression plasmid pCMV-tag2A-IL18BP and confirmed the high level of IL-18BP expression at 48 h after transfection using Western blot assay (Supplemental Fig. S1E). We found that IL-18BP significantly increased the supernatant HBsAg level but had no impact on supernatant HBeAg level (Supplemental Fig. S1, C and D), indicating that IL-18BP antagonized IL-18 activity.

Fig. 3. Effects of IL-18 on HBV nucleic acid levels in HepG2.2.15 cells. HepG2.2.15 cells in 6-well plates were transiently transfected with plasmid pcDNA3.1-mIL18 [pcDNA3.1(−) as empty control] or siIL-18 (siNC as negative control). The levels of HBV mRNA (A and D) and supernatant DNA copies (B and E) were quantified by real-time PCR at 48 h after transfection. At 24 h after transfection, cells were treated with 10 μM BAY11-7082 for 12 h or 10 ng/ml rhIL-18 for 24 h. Then, HBV replicative intermediate DNA (RI-DNA) levels were quantified by real-time PCR (C and F). The results in B and E were expressed as fold change compared with control. The rest results were calculated on the basis of their Ct value with β-actin as an internal control. Data shown represent mean values (± SD) of 3 independent experiments. **P < 0.01, ***P < 0.001.
Furthermore, we examined the levels of HBV nucleic acids in HepG2.2.15 cells. The levels of HBV mRNA, viral DNA copies in culture medium, and HBV RI-DNA were decreased by IL-18 overexpression by 50% (Fig. 3A), 70% (Fig. 3B), and 66% (Fig. 3C), respectively, and conversely were increased by IL-18-knockdown by threefold (Fig. 3D), threefold (Fig. 3E), and eightfold (Fig. 3F), respectively. The administration of rhIL-18 markedly diminished the increased level of HBV DNA replication in HepG2.2.15 cells was almost completely rescued by the administration of BAY11-7082, a NF-κB inhibitor (Fig. 3C), suggesting NF-κB played a critical role in IL-18-mediated inhibition of HBV replication. Since IL-18 did not induce the production of other inflammatory cytokines including IL-6, TNF-α, and IFN-γ in HepG2.2.15 cells (Supplemental Fig. S2A), NF-κB activation and HBV replication inhibition were mainly mediated by IL-18/IL-18R-triggered signal transduction cascades.

**IL-18 suppressed the activities of HBV core gene and X gene promoters.** To study how IL-18 regulated HBV transcription, we transiently transfected HepG2 cells with the reporter vectors of HBV promoters pS1P-luc, pmSP-luc, pCP-luc, or pXP-luc along with plasmid pcDNA3.1-IL18 [pcDNA3.1(−) as empty control] (A), or siIL-18 (siNC as negative control) (B). At 48 h after transfection, the luciferase assay was performed according to the protocol. The results were expressed as fold change compared with control. Data shown represent mean values (± SD) of 3 independent experiments. **P < 0.01, ***P < 0.001.

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**IL-18 directly upregulated NF-κB and AP-1 activity in hepatoma cells.** The transcription of HBV genes is regulated by some transcription factors. Here, we analyzed the effects of IL-18 on the activation of three common transcription factors including NF-κB, AP-1, and nuclear factor of activated T cells (NFAT). Data showed that IL-18 overexpression increased the activity of NF-κB and AP-1 by 75 and 80%, respectively, and, conversely, IL-18-knockdown decreased NF-κB and AP-1 activity by 66 and 55%, respectively (Fig. 5A and Supplemental Fig. S2A). However, NFAT was not activated by IL-18 (Fig. 5B). Suppression of HBV DNA replication by IL-18 overexpression in HepG2.2.15 cells was almost completely rescued by the administration of BAY11-7082, a NF-κB inhibitor (Fig. 3C), suggesting NF-κB played a critical role in IL-18-mediated inhibition of HBV replication. Since IL-18 did not induce the production of other inflammatory cytokines including IL-6, TNF-α, and IFN-γ in HepG2.2.15 cells (Supplemental Fig. S2B), NF-κB activation and HBV replication inhibition were mainly mediated by IL-18/IL-18R-triggered signal transduction cascades.

**IL-18 induced hepatoma cells metastasis and migration in vitro.** We examined the effect of IL-18 on hepatoma cells migration in wound healing assay. As shown in Fig. 6, A and B, overexpressed IL-18 promoted more HepG2 cells to migrate into the wound area at the indicated time points compared with...
control and the number of cells in the wound area increased in an IL-18 dose-dependent manner. Since it was demonstrated by MTT assay that IL-18 overexpression did not induce HepG2 cells proliferation (Fig. 6C), the increased number of migrated cells in the wound area was due to the enhanced mobility of hepatoma cells by IL-18. Furthermore, we performed metastasis and migration assays using extracellular matrix (ECM)-coated and non-ECM-coated Transwells, respectively. It was revealed that IL-18 overexpression promoted the metastasis and migration of HepG2 cells by 2.4-fold (Fig. 6, D and E) and 1.4-fold (Fig. 6, F and G), respectively. Hence, IL-18 significantly promoted hepatoma cells metastasis and migration in vitro.

**IL-18 induced hepatoma cells metastasis and migration mainly through upregulating the expression and activities of MMPs.** Since MMPs expression is closely related to cell metastasis, we examined the effect of IL-18 on the mRNA levels of MMPs, including MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, and MMP-13. As shown in Fig. 7A, most of the MMPs were moderately expressed in HepG2 cells except MMP-8. Overexpression of IL-18 upregulated the mRNA levels of MMP-3, MMP-9, and MMP-13 by 1.3-fold, 2-fold and 1-fold, respectively, but downregulated MMP-2 level by 30%. The effects of rhIL-18 administration on the mRNA levels of MMP-9, MMP-3, and MMP-2 were consistently with the results from IL-18 overexpression (Fig. 7B). In contrast, IL-18 knockdown significantly downregulated the mRNA levels of MMP-3 and MMP-9, but upregulated MMP-2 level by half (Fig. 7D). BAY11-7082 administration almost counteracted the effects of IL-18 on MMPs levels (Fig. 7C), indicating that IL-18 regulated MMPs expression also in a NF-κB-dependent manner.

We next investigated the effects of IL-18 on extracellular MMPs activities by the classic zymography method. As shown in Fig. 8, rhIL-18 increased the secretion of pro-MMP-9 from HepG2 cells, which is consistent with its mRNA levels. What is more, rhIL-18 increased the activities of extracellular MMP-9 and MMP-3. Interestingly, rhIL-18 had no apparent effect on the secretion of pro-MMP-2 but increased extracellular MMP-2 activity. The exact mechanism involved is unclear.

To confirm the roles of MMPs in IL-18-promoted hepatoma cells metastasis and migration, we performed the Transwell assays by use of the MMP inhibitor GM6001. We found the enhanced metastasis and migration abilities of hepatoma cells were both impaired by GM6001 treatment (Supplemental Fig. S3). Taken together, these results suggested that IL-18 induced hepatoma cells metastasis and migration mainly via increasing the activities of extracellular MMP-9, MMP-3, and MMP-2.

**Expression of IL-18/IL-18R and MMPs were dramatically increased in HCC cancer tissue specimens.** We have demonstrated that IL-18 promoted hepatoma cells migration and metastasis in vitro through increasing MMPs activities. To validate the in vivo role of IL-18 in HCC progression, we performed immunohistochemical analysis on liver sections of cancer tissues and normal tissues from HCC patients. As shown in Supplemental Figs. S4A and S5, IL-18 was expressed...
at a much higher level in all the cancer tissues from four HCC patients than in normal tissues. We analyzed the mRNA levels of IL-18R and MMPs in liver cancer tissues. It was shown that the mRNA levels of both IL-18R<sub>α</sub> and IL-18R<sub>β</sub> were strongly upregulated in cancer tissues compared with normal tissues (Supplemental Fig. S4B). These results indicated that IL-18/IL-18R-triggered signaling pathway was closely associated with HCC progression in vivo. Furthermore, we found that the mRNA levels of most MMPs including MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, and MMP-13 were remarkably upregulated in cancer tissues by 2-fold to 160-fold compared with normal tissues. It suggested the IL-18/IL-18R-rich liver cancer cells were highly metastatic.

**DISCUSSION**

IL-18 has been reported to be produced in many nonimmune cells including cancer cells. Characterization of its role in these cells is of benefit for us to deeply understand the biological function of IL-18 and find some clues for cancer therapy. We found that IL-18 and IL-18R were constitutively expressed in both normal liver cell line and hepatoma cell line, implying that IL-18 may play a role in human hepatocyte function. In the present study, we investigated the effects of IL-18 on HBV replication and hepatoma cells metastasis and migration. We revealed the dual effects of IL-18 in human hepatocytes for the first time: it inhibited HBV replication in HepG2.2.15 cells; on the other hand, it promoted hepatoma cell metastasis and migration. Further studies demonstrated that IL-18 inhibited HBV replication through downregulating the activities of HBV

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**Fig. 7. Effects of IL-18 on matrix metalloproteinase (MMP) mRNA levels.**

A: HepG2 cells in 6-well plates were transfected with plasmid pcDNA3.1-IL18 or empty vector. At 48 h after transfection, the mRNA levels of MMPs were analyzed by real-time PCR. B–D: HepG2 cells were treated with 10 ng/ml rhIL-18 for 24 h or transiently transfected with plasmid pcDNA3.1-mIL18 (pcDNA3.1 (−) as empty control) or siIL-18 (siNC as negative control). After 36 h of transfection, cells transfected with plasmid pcDNA3.1-mIL18 were treated with 10 μM BAY11-7082 for 12 h. The mRNA levels of MMP-2, MMP-3, and MMP-9 were analyzed by real-time PCR. The results were calculated on the basis of their Ct value by using β-actin as an internal control. Data shown represent mean values (± SD) of 3 independent experiments. *P < 0.05, ***P < 0.001.

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**Fig. 8. Effects of IL-18 on the activities of MMPs.**

HepG2 cells were incubated with serum-free DMEM containing 20 ng/ml rhIL-18 for 24 h. Medium was collected, lyophilized, and resolved in 100 μl double-distilled H<sub>2</sub>O. Equal amounts of protein were separated by 10% nonreductive SDS-PAGE containing 1 mg/ml gelatin (A) or 0.5 mg/ml casein (B). After electrophoresis, zymography assay was performed.
precore/pregenomic and X gene promoters. The pregenomic mRNA serves as the template not only for the reverse transcription into the minus strand DNA but also for the synthesis of core and polymerase proteins. Therefore, IL-18-mediated reduction of pregenomic mRNA level could directly lead to the decrease of HBV DNA replication level and viral nucleocapsid formation. Interestingly, although precore mRNA, the template for translation of the precursor of HBeAg, is also regulated by HBV precore/pregenomic promoter element, the level of HBeAg in HepG2.2.15 culture medium was not affected by IL-18. As a matter of fact, the precore/pregenomic promoter element consists of two partly overlapping promoters that control the transcription of pregenomic mRNA and precore mRNA, respectively. Since the two promoters are regulated by different nuclear factors (24), the levels of pregenomic mRNA and HBeAg are differently affected by IL-18.

The antiviral effect of IL-18 was commonly thought to be dependent on the induction of inflammatory cytokines, such as IFN-γ and TNF-α. In our study, it was confirmed that IL-18 does not induce the production of cytokines including IL-6, IFN-γ, and TNF-α in HepG2.2.15 cells, indicating that the antiviral effect was mediated directly by IL-18/IL-18R-triggered signal transduction cascades. We demonstrated that IL-18R expression was significantly increased in HepG2 and HepG2.2.15 cells, and that IL-18/IL-18R complex triggered NF-κB activation. Since many recent studies reported that MyD88 inhibited HBV replication in NF-κB-dependent manner (12, 23), IL-18 might directly inhibit intracellular HBV replication through MyD88 recruitment and NF-κB activation. We further confirmed the essential role of NF-κB in IL-18 suppressed HBV DNA replication using a NF-κB inhibitor. Therefore, we could conclude that IL-18 directly inhibited HBV replication in HepG2.2.15 cells in a NF-κB-dependent manner. Transcription factor Sp1 has been reported to positively regulate HBV genes expression via binding to the two Sp1 binding sites located in HBV precore/pregenomic promoter (11). Lin et al. (13) reported that NF-κB interacted with Sp1 and interfered with Sp1-mediated transcription activation. Therefore, the interaction between activated NF-κB and Sp1 might contribute to the inhibitory effects of IL-18 on HBV genes transcription.

Every coin has two sides. Despite the antiviral function, the pathogenic effect of IL-18 has been reported in several cancers, such as gastric cancer, breast cancer, lung cancer, and melanoma (16). It has been shown that the level of IL-18 was significantly elevated in these cancer patients compared with health controls. Moreover, the levels of IL-18 were generally higher in cancer patients with metastasis than those in cancer patients without metastasis (22). Proteomic analysis between highly and poorly metastatic cell lines PLA810D and PLA810C suggested that IL-18 was significantly upregulated in highly metastatic cells (8). These data suggested that IL-18 was closely related to the metastasis of cancer cells. It has been reported that plasma levels of IL-18 are elevated in patients with HCC. Maybe IL-18 plays a role in HCC progression. In our study, IL-18 was demonstrated to promote hepatoma cells metastasis and migration dose dependently via increasing the expression of MMPs including MMP-9, MMP-3, and MMP-2, which are responsible for degrading ECM components (9). The increased activities of MMPs were confirmed to contribute to the enhanced metastasis and migration of hepatoma cells.

Furthermore, it was confirmed that the expression of IL-18/IL-18R and most MMPs were notably increased in HCC liver cancer tissues, indicating a consistent role of IL-18 in HCC progression with the in vitro data. NF-κB was found to be essential for the IL-18-mediated upregulation of MMP-3 and MMP-9 expression. It could bind to its specific binding site located in the promoter of MMP-9 and directly activate the transcription (18). However, there were no NF-κB binding sites in MMP-3 promoter. So NF-κB might indirectly regulate MMP-3 transcription via activating other transcription factors. The exactly mechanism involved awaits further characterization.

In summary, we demonstrated the dual effects of IL-18 that it not only inhibited HBV replication and viral particles production in human hepatocytes but also promoted hepatoma cells metastasis and migration. NF-κB mediated the dual effects of IL-18, suggesting there was a cross talk between the signaling pathways involved. Our work contributed to a better understanding of the biological functions of IL-18 in human hepatocytes and also provided theoretical basis for the application of IL-18 in liver diseases therapies. However, further researches into the biological functions of IL-18 are required for its clinical application in the future.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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