

CHM-1 inhibits hepatocyte growth factor-induced invasion of SK-Hep-1 human hepatocellular carcinoma cells by suppressing matrix metalloproteinase-9 expression

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Abstract

Clinical observations suggest that hepatocyte growth factor (HGF) can promote invasion and metastasis in hepatocellular carcinoma. In this study, we found that HGF-stimulated invasion of SK-Hep-1 cells, together with increased expression of matrix metalloproteinase (MMP)-9. CHM-1 was identified from 2-phenyl-4-quinolone derivatives to potentially inhibit HGF-induced cell invasion, proteolytic activity, and expression of MMP-9. CHM-1 significantly inhibited tyrosine autophosphorylation of c-Met induced by HGF. CHM-1 also suppressed HGF-induced Akt phosphorylation, and NF- κ B activation, the downstream regulators of HGF/c-Met signaling, resulting in the inhibition of MMP-9. Thus, we suggest that CHM-1 is a potential therapeutic agent against tumor invasion.

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1. Introduction

The lethality of most malignant tumors is the result of local invasion and metastasis from the primary tumors to other tissues. Invasion is a characteristic feature of hepatocellular carcinoma (HCC), which frequently shows early invasion into blood

vessels as well as intrahepatic metastasis and extrahepatic metastasis occurs subsequently [1]. Thus, the discovery and subsequent development of novel small-molecule agents to block HCC invasion are the goals of cancer researchers.

Hepatocyte growth factor (HGF), a pleiotropic modulator, is produced by nonparenchymal liver cells, and its serum levels are elevated in a variety of liver diseases, including HCC [2]. The receptor for HGF is a receptor type tyrosine kinase encoded

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by the c-Met proto-oncogene. c-Met is normally expressed by epithelial cells and has been found to be overexpressed and amplified in various human tumor tissues [3]. It has been reported that overexpression of c-Met is detected in some cases of HCC and that elevated levels of c-Met expression in HCC correlate with increased incidence of liver metastasis [4]. These findings suggest that the HGF/c-Met signaling plays a pivotal role in the invasion and metastasis of HCC cells. Activation of c-Met by HGF can induce cell proliferation, survival, motility, invasion, and changes in morphology. Kinase activation is achieved through autophosphorylation of tyrosines 1234 and 1235 in the catalytic domain, subsequently results in binding and/or phosphorylation of adaptor proteins including Grb2, Src, and Gab-1, which in turn, are capable of activating downstream pathways including PI3K/Akt, Ras/MAPK, FAK, and STAT signaling [5].

Cell invasion is a major component of the complex multistep process of tumor metastasis. Invasion of malignant tumor cells requires destruction of basement membranes and proteolysis of extracellular matrix (ECM) [6]. Of the several families of ECM-degrading enzymes, the most extensive are the matrix metalloproteinases (MMPs), which are a large family of structurally related zinc-endopeptidases that collectively degrade most of the components of ECM [7]. Among previously reported human MMPs, MMP-9 (gelatinase B) is thought to be a key enzyme for degrading type IV collagen. MMP-9 is abundantly expressed in diverse malignant tumors and is postulated to play an important role in HCC invasion and metastasis [8]. Therefore, the inhibition of invasion mediated by MMP-9 may be critical for the prevention of cancer metastasis.

The 2-phenyl-4-quinolones and related compounds, a series of synthetic quinolone derivatives, have been reported to against a broad spectrum of human cancer cell lines [9–11]. However, the anti-invasion property of the 2-phenyl-4-quinolone series has not been demonstrated. In this study, we used HGF to induce invasion in SK-Hep-1 cells, a highly invasive human HCC cell line, as the screen system in studying anti-invasive effects of 2-phenyl-4-quinolone derivatives. We identified that 2'-fluoro-6,7-methylenedioxy-2-phenyl-4-quinolone (CHM-1, Fig. 1) potently inhibited HGF/c-Met-mediated cell invasion in SK-Hep-1 cells. The mechanism of CHM-1 to inhibit HGF-induced invasion in HCC cells was also investigated in this study.

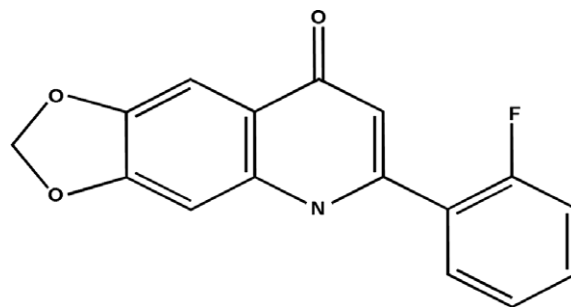


Fig. 1. Structure of CHM-1 (2'-fluoro-6,7-methylenedioxy-2-phenyl-4-quinolone).

2. Materials and methods

2.1. Materials

CHM-1 was synthesized at the Graduate Institute of Pharmaceutical Chemistry, School of Medicine, China Medical University (Taichung, Taiwan). Recombinant human HGF was purchased from R&D Systems, Inc. (Minneapolis, MN). DMEM, fetal bovine serum (FBS), and all the other cell culture reagents were obtained from Gibco-BRL life technologies (Grand Island, NY). Antibodies to phospho-c-Met (Tyr1234/1235), phospho-Akt (Ser473), Akt, phospho-ERK1/2 (Thr202/Tyr204), ERK1/2, phospho-JNK (Thr183/Tyr185), JNK, phospho-p38 (Thr180/Tyr182), p38, and phospho-I κ B α (Ser32) were purchased from Cell Signaling Technologies (Boston, MA). Antibodies to c-Met, I κ B α , NF- κ B/p65, nucleolin, anti-mouse immunoglobulin (Ig) G, and anti-rabbit IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody to GAPDH was purchased from Abcam (Cambridge, UK). Anti-MMP-2 polyclonal antibody, anti-MMP-9 polyclonal antibody, and GM6001 were purchased from Chemicon International (Temecula, CA). SU11274 and other chemical agents were obtained from Sigma Chemical Co. (St Louis, MO).

2.2. Cell culture

The human HCC cell line SK-Hep-1 was obtained from American Type Culture Collection (ATCC, Manassas, VA) and cultured in DMEM containing supplements (10% FBS, penicillin/streptomycin, and L-glutamine). Cells were maintained in humidified air containing 5% CO₂ at 37 °C.

2.3. Invasion assay

Invasion assays were performed in Transwell chambers (Corning, Corning, NY). The upper side of the filters was coated with Matrigel (BD Biosciences, Bedford, MA) at a concentration of 125 μ g/cm². Cells were seeded (5×10^4 cells/well) onto the upper chamber with serum-

free medium, then incubated in the bottom chamber with serum-free medium containing recombinant HGF as a source of chemoattractants. After 6 h of treatment, cells on the upper side of the filters were mechanically removed, and those migrated on the lower side were fixed with 4% formaldehyde, then stained with 0.5% crystal violet for 10 min. Finally, invaded cells were counted at 200 \times magnification in 10 different fields of each filter.

2.4. Cell viability assay

Cells were incubated in 96-well plates at a density of 10⁴ cells per well, and the percentage of cell survival was assessed using MTT colorimetric assay after drug treatment.

2.5. Western blot analysis

Cells were lysed with lysis buffer and nuclear fractionation was performed as described previously [12]. Cell homogenates were diluted with loading buffer and boiled for 5 min for detecting phosphorylation, and protein expression. Total protein was determined and equal amounts of protein were separated by 8–12% SDS-PAGE and immunoblotted with specific primary antibodies. Horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) were used, and the signal detected using an enhanced chemiluminescence detection kit (Amersham, Buckinghamshire, UK).

2.6. Gelatin zymography

The supernatant of SK-Hep-1 cells was electrophoresed for the analysis in 10% SDS-PAGE gels containing gelatin (1 mg/ml). The gels were washed twice with 2.5% Triton X-100 for 30 min to remove SDS. The gels were incubated at 37 °C in 50 mM Tris-HCl (pH 7.4), containing 10 mM CaCl₂ and 150 mM NaCl for 24 h. Following incubation, the gels were stained with 0.25% Coomassie Blue for 1 h, and then destained with de-staining buffer until bands became clear.

2.7. MMP-2 and MMP-9 activity assay

Substrate-linked enzyme-linked immunosorbent assay (ELISA) techniques (Amersham, Buckinghamshire, UK) were used to quantify enzymatic activity of individual MMPs. The samples were thawed on ice, and all reagents needed for the assay were brought to room temperature. The MMP-2 and MMP-9 activities were performed according to the manufacturer's instructions.

2.8. RT-PCR analysis

RNA was extracted from homogenized tissue with TRIzol reagent by a standard protocol (Invitrogen, Carlsbad, CA). Reverse transcription was performed with 5 μ

of mRNA and random primer at 65 °C for 5 min and then mixed with Moloney murine leukemia virus (M-MLV) reverse transcriptase to react at 37 °C for 1 h to obtain cDNA. Gene amplification was followed with reverse transcriptase-polymerase chain reaction. Primers used in this study were synthesized as follows, MMP-2 primers: sense primer, 5'-GGCCCTGTCACCTCTGAGAT-3'; anti-sense primer, 5'-GGCATCCAGTTATCGGGGA-3', MMP-9 primers: sense primer, 5'-TGGGCTACGTGACCTATGAC-3'; anti-sense primer, 5'-CAAAGGTGAGAAGAGAGGGC-3'; GAPDH primers: sense primer, 5'-TGATGACATCAAGAAGGTGGTGAAG-3'; anti-sense primer, 5'-TCCTTGGAGCCATGTGGGCAT-3'. The PCR consisted of an initial denaturation at 94 °C for 5 min; 30 three-step cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. PCR products were analyzed on 1.5% agarose gel in the presence of 1 μ g/ml ethidium bromide. The gels were photographed using a digital imaging system (Gel DOC 2000, Bio-Rad, Hercules, CA).

2.9. Analysis of NF- κ B/p65 activity

The NF- κ B/p65 transcription factor ELISA kits purchased from Active Motif Inc. (Carlsbad, CA) were used for the detection of DNA binding activity of NF- κ B/p65 subunit in commercial protocol.

2.10. Transfection and reporter gene assay

Reporter plasmid pNF- κ B-Luc and pMMP-9-Luc were kindly provided by Dr. J.C.-H. Cheng (National Taiwan University Hospital and College of Medicine, Taipei, Taiwan). A dominant-negative I κ B α mutant (I κ B α M) was a kind gift from Prof. C.-H. Lin (Taipei Medical University, Taipei, Taiwan). *Renilla* luciferase reporter vector (phRG-TK) was purchased from Promega (Madison, WI). SK-Hep-1 cells (2 \times 10⁵) were seeded into 12-well plates and grown overnight. Cells were transiently transfected with 0.4 μ g of NF- κ B promoter plasmid or 2.5 μ g MMP-9 promoter plasmid using Lipofectmine 2000 (Invitrogen) according to the manufacturer's protocol. The phRG-TK was cotransfected with the above plasmids as an internal control. To assay the affect of dominant-negative mutant, I κ B α M (1 μ g) was cotransfected with pNF- κ B-Luc or pMMP-9-Luc in this study. The luciferase activity was measured in the cellular extracts using dual-luciferase reporter assay system (Promega).

2.11. Statistical analyses

Data are presented as the mean \pm SEM for the indicated number of separate experiment. Statistical analyses of data were performed with one-way ANOVA followed by Student's *t*-test, and *p*-values less than 0.05 were considered significant.

3. Results

3.1. CHM-1 inhibits HGF-induced invasion of SK-Hep-1 cells

By Transwell chamber assay, 50 ng/ml of HGF-induced *in vitro* invasion of SK-Hep-1 cells. CHM-1 (0.1–10 μ M) significantly inhibited HGF-induced cell invasion in a concentration-dependent manner (Fig. 2a). Next, we determined the cytotoxicity of CHM-1 using MTT assay. CHM-1 did not affect cell viability at the indicated concentrations. These results indicate that inhibitory effect of CHM-1 on cell invasion is independent of cellular cytotoxicity.

3.2. CHM-1 inhibits HGF/c-Met signaling of SK-Hep-1 cells

We next investigated whether the antagonistic effect of CHM-1 on HGF-induced invasive activity could be attributed to the inhibition of tyrosine autophosphorylation of c-Met induced by HGF. The c-Met of SK-Hep-1 cells were strongly phosphorylated in response to stimulation with HGF for 15 min. CHM-1 significantly inhibited HGF-stimulated c-Met phosphorylation (Fig. 3a). SU11274 was used as a positive control for the specific inhibition on HGF/c-Met signaling. To examine HGF-induced downstream signaling of c-Met activation, the phosphorylation of Akt, ERK1/2, JNK, and p38 in the presence of CHM-1 was evaluated. CHM-1 substantially inhibited HGF-induced phosphorylation of Akt (Fig. 3b). However, CHM-1 did not suppress the phosphorylation of ERK1/2, JNK, or p38 induced by HGF (Fig. 3c).

3.3. CHM-1 inhibits HGF-induced NF- κ B activation through inhibition of I κ B α phosphorylation

Nuclear factor- κ B (NF- κ B) is a transcription factor that plays an important regulator in invasion and metastasis [13]. The effect of CHM-1 on activation of NF- κ B was examined in HGF-treated cells. As shown in Fig. 4a, HGF-induced NF- κ B/p65 translocation was significantly inhibited by CHM-1 in a 2 h treatment. Furthermore, HGF potently increased the phosphorylation of I κ B α at the indicated times, and treatment with CHM-1 for 2 h substantially suppressed HGF-induced I κ B α phosphorylation in SK-Hep-1 cells (Fig. 4b). The NF- κ B activity of SK-Hep-1 cells treated with CHM-1 for 2 h was then measured by ELISA-based Trans-AMTM NF- κ B p65 kit. As illustrated in Fig. 5a, CHM-1-treatment suppressed HGF-induced NF- κ B activation in a concentration-dependent manner. To further confirm this result, NF- κ B-binding site-driven luciferase activity assay was performed. The pNF- κ B-Luc was transfected to measure the binding of

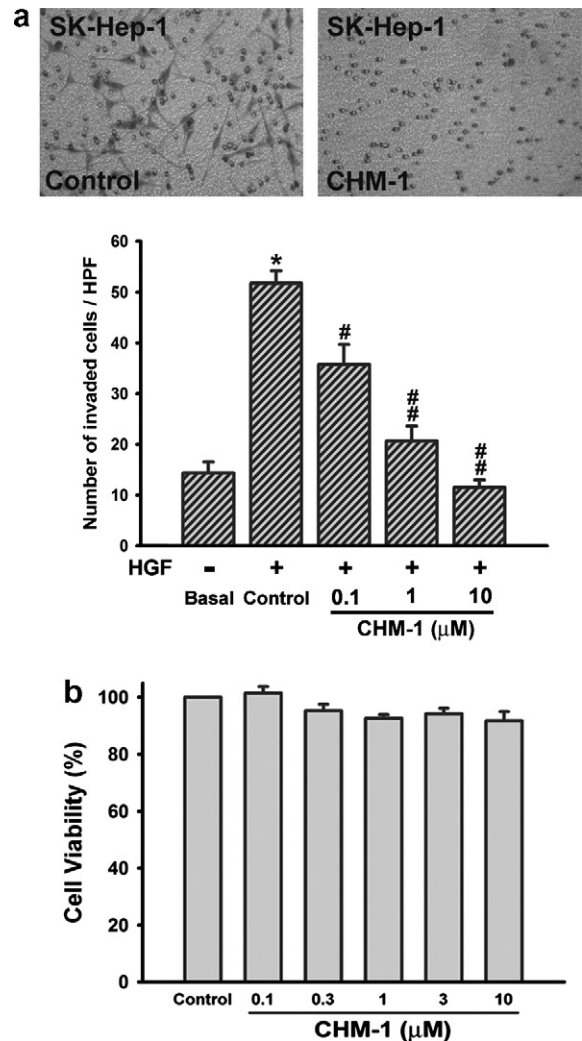


Fig. 2. Effect of CHM-1 on HGF-induced cell invasion in SK-Hep-1 cells. (a) Cells were seeded onto the upper chamber consisting of 8 μ m pore-size filters coated with Matrigel, then treated without or with CHM-1 (0.1, 1, 10 μ M) for 6 h in the absence or presence of HGF (50 ng/ml) as a chemoattractant in the lower chamber. Cells that invaded the filter were counted as means \pm SEM of five independent experiments. * p < 0.001 compared with the basal group; # p < 0.01, ## p < 0.001 compared the control group. (b) Cells were treated with the indicated concentrations of CHM-1 (0.1–10 μ M) for 6 h in the presence of HGF (50 ng/ml), and the cell viability was determined using MTT assay. Data are expressed as means \pm SEM of four independent experiments.

transcription factors to the κ enhancer, providing a direct measurement of NF- κ B activation. The results showed that HGF-induced κ B-luciferase activity was

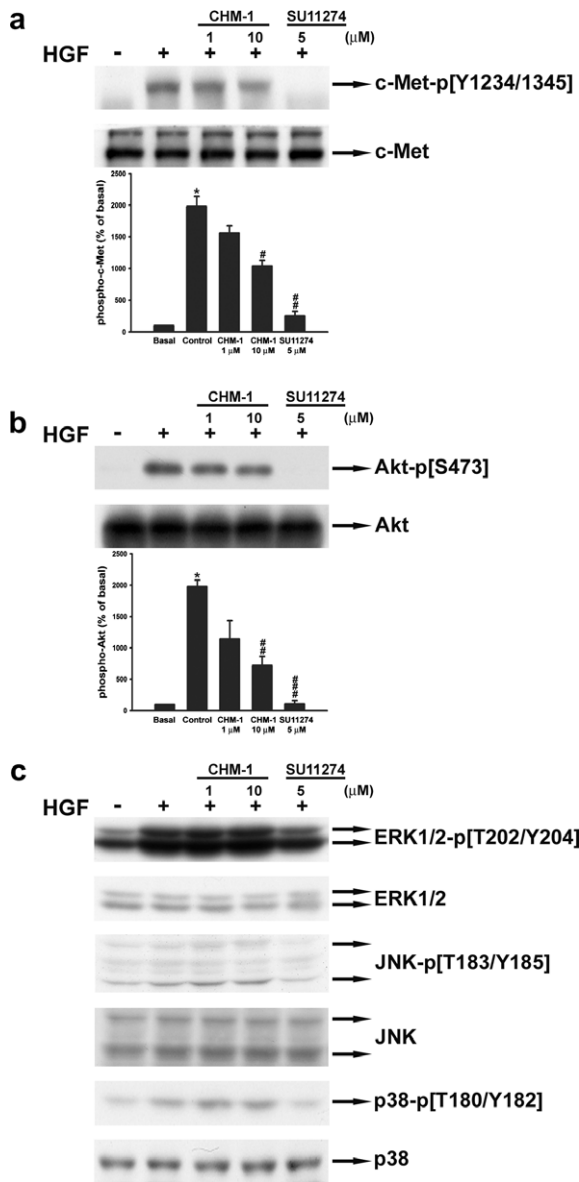


Fig. 3. Effect of CHM-1 on c-Met phosphorylation and signal transduction in SK-Hep-1 cells. Serum-starved cells were pre-treated 3 h without or with CHM-1 (1, 10 μ M) or SU11274 (5 μ M), then cells were stimulated with 50 ng/ml HGF for 10 min. Cells were harvested and lysed for the detection of phospho-Tyr^{1234/1235}-c-Met and c-Met (a), phospho-Ser⁴⁷³-Akt and Akt (b), and phospho-Thr²⁰²/Tyr²⁰⁴-ERK1/2, ERK1/2, phospho-Thr¹⁸³/Tyr¹⁸⁵-JNK, JNK, phospho-Thr¹⁸⁰/Tyr¹⁸², and p38 (c) protein expressions by Western blot analysis. The quantitative densitometry of the relative level of protein was performed with Image-Pro Plus. Data are expressed as means \pm SEM of five independent experiments. * p < 0.001 compared with the basal group; # p < 0.05, ## p < 0.01, ### p < 0.001 compared the control group.

inhibited by CHM-1, and the I κ B α phosphorylation inhibitor BAY 11-7082 was used as a positive control (Fig. 5b).

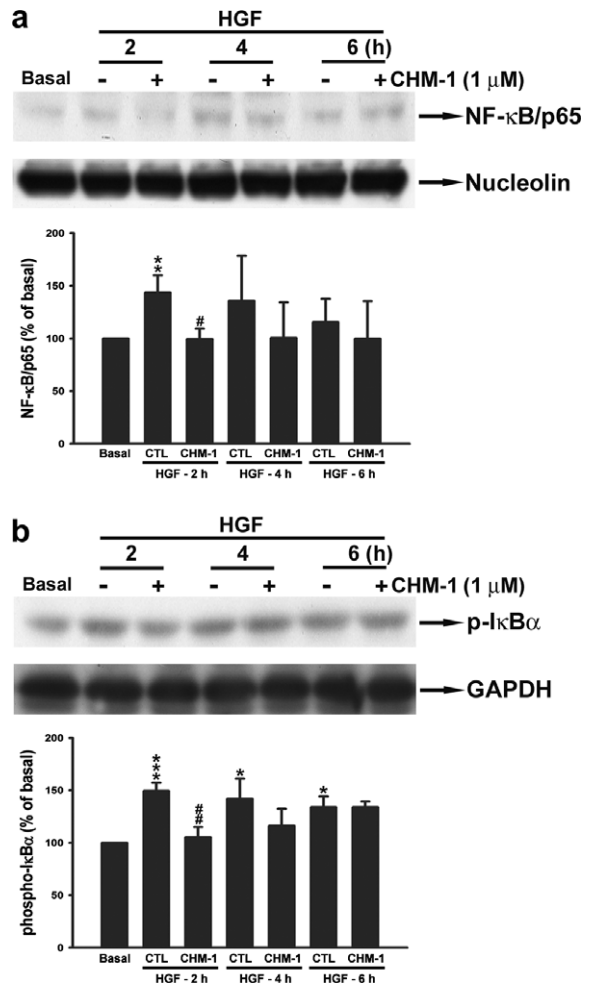


Fig. 4. Effect of CHM-1 on HGF-induced NF- κ B/p65 translocation and I κ B α phosphorylation in SK-Hep-1 cells. Cells were treated without or with CHM-1 (1 μ M) for the indicated times in the absence or presence of HGF (50 ng/ml). Cells were harvested and lysed for the detection of NF- κ B/p65, and nucleolin (a), and phospho-Ser³²-I κ B α and GAPDH (b) protein expressions by Western blot analysis. The quantitative densitometry of the relative level of protein was performed with Image-Pro Plus. Data are expressed as means \pm SEM of five independent experiments. * p < 0.05, ** p < 0.01, *** p < 0.001 compared with the basal group; # p < 0.05, ## p < 0.01 compared the control (CTL) group.

3.4. CHM-1 inhibits HGF-activated MMP-9 expression and enzyme activity

We then investigated the mechanism of HGF-mediated cell invasive phenotype by looking at the involvement of MMP-2 and MMP-9. Gelatin zymography was firstly used to analyze the effects of CHM-1 on MMP-2 and MMP-9 activities for 6 h of treatment. As shown in Fig. 6a, we found that SK-Hep-1 cells constitutively secreted high levels of MMP-9 and low levels of MMP-2, and the proteolytic activity of MMP-9 was dramatically

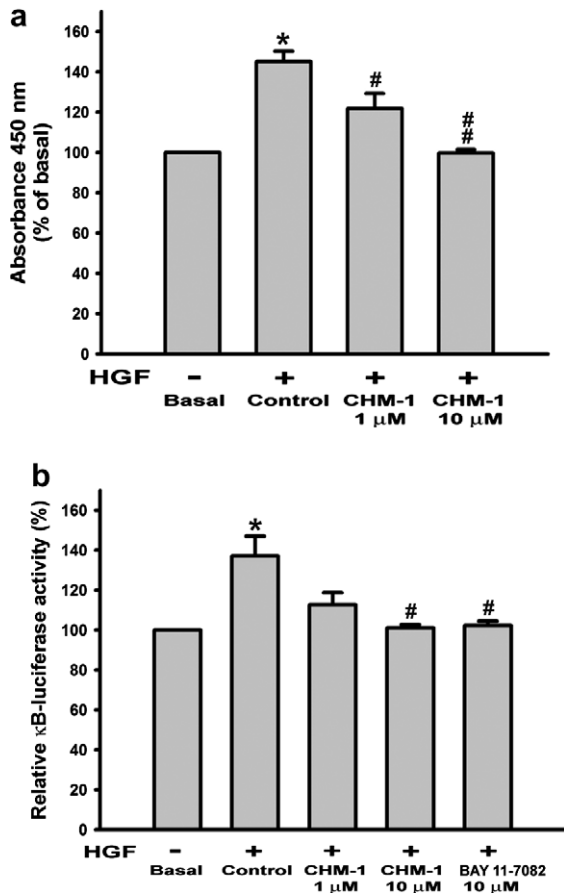


Fig. 5. Effect of CHM-1 on HGF-induced NF- κ B activation in SK-Hep-1 cells. (a) ELISA assay was performed after 2 h of incubation with CHM-1 (1, 10 μ M) in HGF-treated cells. (b) Cells cotransfected with pNF- κ B-Luc and pHRG-TK vector were treated with CHM-1 (1, 10 μ M) or BAY 11-7082 (10 μ M) in the absence or presence of HGF (50 ng/ml). After 2 h of treatment, the promoter luciferase activities were detected using a luminometer. Data are expressed as means \pm SEM of four independent experiments. * p < 0.001 compared with the basal group; # p < 0.01, ## p < 0.001 compared the control group.

activated by HGF. CHM-1 significantly inhibited HGF-activated MMP-9 proteolytic activity in a concentration-dependent manner. We further confirmed the inhibition of CHM-1 on MMP-9 activity by ELISA assay, and

pan-MMP inhibitor GM6001 was used as a positive control. We found that CHM-1 substantially inhibited HGF-induced MMP-9 activity, but did not affect MMP-2 activity in SK-Hep-1 cells (Fig. 6b). As the MMP-9 expression in CHM-1-treated cells, we demonstrated that CHM-1 caused an inhibition of HGF-induced MMP-9 protein expression after 6 h of treatment (Fig. 6c), and suppressed HGF-induced MMP-9 mRNA expression at the earlier time point (4 h) (Fig. 6d). For further confirming transcriptional inhibition of CHM-1 to MMP-9 expression, cells were transfected with MMP-9 promoter containing reporter constructs and treated with CHM-1. As shown in Fig. 7a, CHM-1 profoundly inhibited HGF-induced MMP-9 promoter activity.

3.5. NF- κ B activation is involved in HGF-induced MMP-9 expression

To investigate the involvement of NF- κ B in HGF-induced MMP-9 expression, cells were transiently transfected with I κ B α M. This I κ B α mutated form contains serine-to-alanine mutations at residues 32 and 36 and do not undergo signal-induced phosphorylation; therefore, cell expressing I κ B α M block the NF- κ B pathway [14]. As shown in Fig. 7b, cells transfected with I κ B α M almost completely abolished the HGF-induced increase in κ B-luciferase activity. The HGF-induced MMP-9 promoter activity was also significantly attenuated by I κ B α M (Fig. 7c). These results suggest that NF- κ B appears to serve as an upstream signal for induction of MMP-9 expression by HGF.

4. Discussion

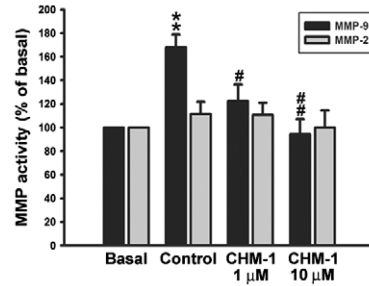
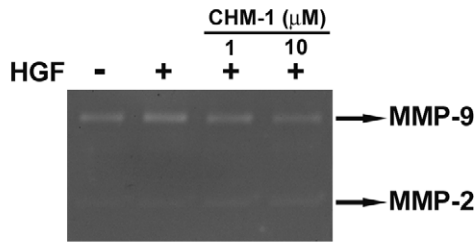
The high recurrence rate with intrahepatic metastatic spread is major obstacle for improving survival of patients with HCC [1]. The development of novel therapeutic agents targeting the malignant behavior of HCC cells, especially their invasiveness, is important to improve the prognosis of patients. 2-Phenyl-4-quinolone derivatives have been demonstrated with potent anti-mitotic anti-tumor effects by inhibiting tubulin polymerization in a wide variety of human cancer cells [9–11]. CHM-1 is a small-molecule compound that was derived from

Fig. 6. Effect of CHM-1 on HGF-increased MMP-9 expression and activity in SK-Hep-1 cells. (a) Cells were treated without or with CHM-1 (1, 10 μ M) in the absence or presence of HGF (50 ng/ml). After 6 h of treatment, each conditioned medium was collected for analysis of proteolytic activities of MMP-2 and MMP-9 by gelatin zymography. (b) Cells were treated with CHM-1 (1, 10 μ M) or GM6001 (10 μ M) for 6 h in the absence or presence of HGF (50 ng/ml). Then, each conditioned medium was collected and subjected to ELISA assay for MMP-2 and MMP-9 activities. (c) Cells were treated with CHM-1 (1, 10 μ M) or GM6001 (10 μ M) for 6 h in the absence or presence of HGF (50 ng/ml). Cells were harvested and lysed for the detection of MMP-9 and GAPDH protein expressions by Western blot analysis. (d) Cells were treated without or with CHM-1 (1, 10 μ M) in the absence or presence of HGF (50 ng/ml). After 4 h of treatment, the mRNA level of MMP-9 was detected by RT-PCR. Imaging was performed using quantitative densitometry with Image-Pro Plus. Data are expressed as means \pm SEM of four independent experiments. * p < 0.05, ** p < 0.01 compared with the basal group; # p < 0.05, ## p < 0.01 compared the control group.

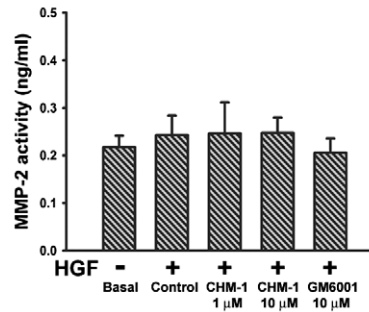
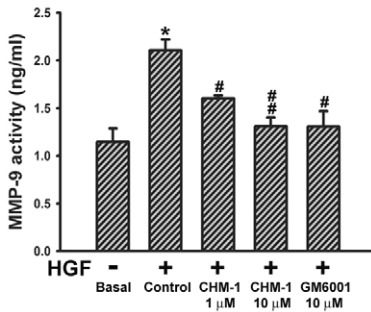
2-phenyl-4-quinolones. In this study, we identified CHM-1 as a potential lead base on anti-invasive activity in HCC cells with good pharmacological properties. CHM-1 induced a significant concentration-dependent inhibition of HGF-activated cell

invasion, and dramatically inhibited HGF-induced MMP-9 expression and enzyme activity in SK-Hep-1 cells. Thus, CHM-1 is a promising chemotherapeutic agent worthy of further development for treatment of human HCC.

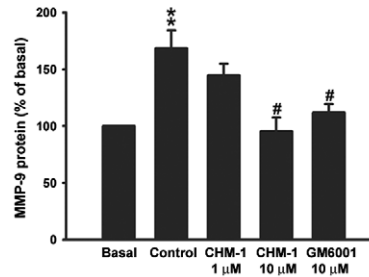
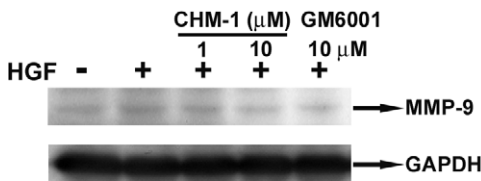
a Zymography



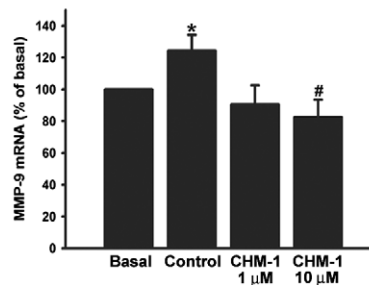
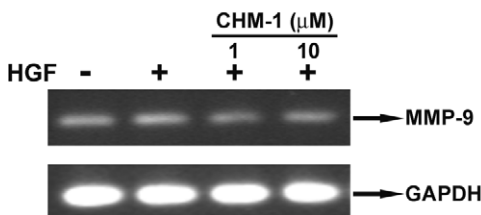
b ELISA assay



c Western blot



d RT-PCR



HGF/c-Met signaling is implicated in numerous human malignancies, including colon, gastric, ovarian, lung, and liver cancer. This pathway activates a

program cell dissociation and motility coupled with increased protease production that has been shown to promote cellular invasion through ECM and that closely resembles tumor metastasis *in vivo* [15]. These suggest that strategies targeting c-Met represent an attractive novel therapeutic approach. In the present study, we found that CHM-1 could inhibit HGF-induced invasive activity through suppressing tyrosine phosphorylation of c-Met. Previous evidence reports that HGF-induced response appears to work through both Ras/MAPK and PI3K/Akt signaling pathways [16,17]. Our results showed that CHM-1 profoundly inhibited HGF-induced Akt phosphorylation, but not MAPK phosphorylation. Thus, we suggest that the PI3K/Akt pathway may play an important role in the inhibition of CHM-1 on HGF/c-Met-mediated invasion of SK-Hep-1 cells.

NF- κ B regulates a variety of genes whose products are involved in many biological processes, including inflammation, apoptosis, cell growth, invasion, and metastasis [18]. In resting cells, heterodimeric NF- κ B complexes are located in the cytoplasm of most cell type by the inhibitory protein of I κ B α . NF- κ B activation normally proceeds through a pathway involving phosphorylation and subsequent degradation of I κ B α , resulting in the translocation of NF- κ B from the cytoplasm to the nucleus. In previous studies, CA and its derivative CAPE reduced the liver metastasis through suppression of MMP-9 gene expression by inhibiting NF- κ B activation [19]. Blocking NF- κ B activity by transfection with a mutated I κ B α caused suppression of angiogenesis, invasion, and metastasis in prostate

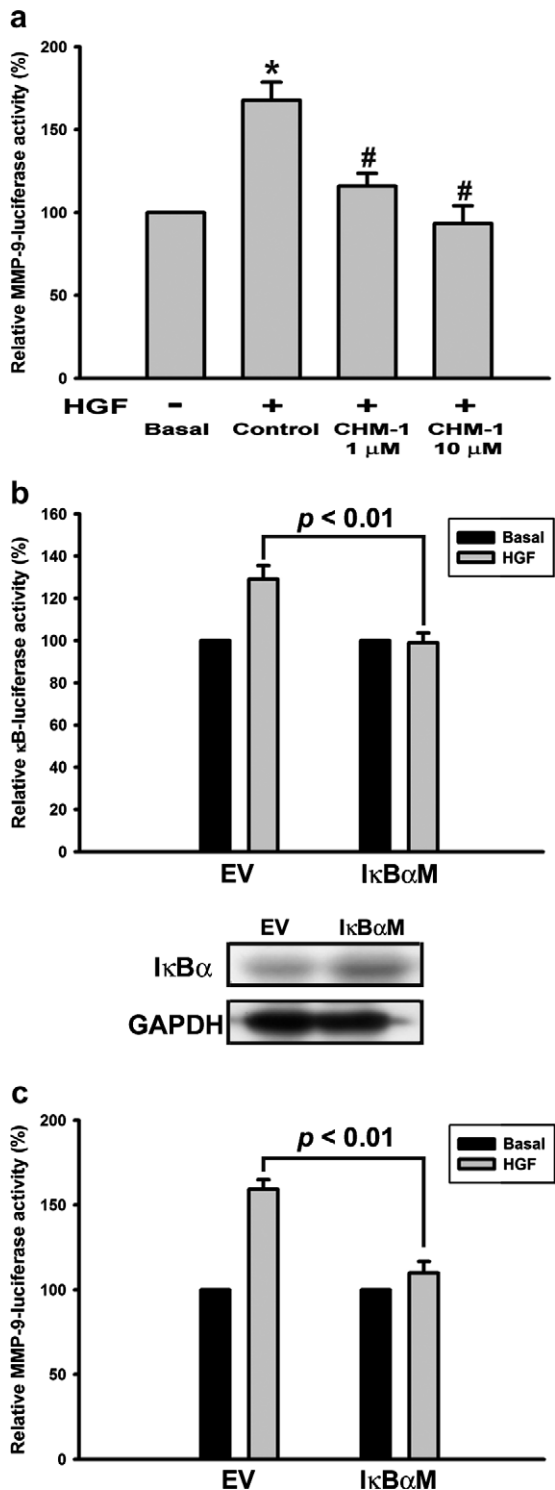


Fig. 7. Role of NF- κ B in HGF-induced MMP-9 gene transcription in SK-Hep-1 cells. (a) Cells cotransfected with pMMP-9-Luc and phRG-TK vector were treated without or with CHM-1 (1, 10 μ M) in the absence or presence of HGF (50 ng/ml). After 4 h of treatment, the promoter luciferase activities were detected using a luminometer. Data are expressed as means \pm SEM of four independent experiments. * p < 0.001 compared with the basal group; # p < 0.01 compared the control group. (b) Cells cotransfected with pNF- κ B-Luc and I κ B α M were incubated in the absence or presence of HGF (50 ng/ml). After 2 h of incubation, the promoter luciferase activities were detected using a luminometer. The content of I κ B α was determined in I κ B α M-transfected cells by Western blot analysis. (c) Cells cotransfected with pMMP-9-Luc and I κ B α M were incubated in the absence or presence of HGF (50 ng/ml). After 4 h of incubation, the promoter luciferase activities were detected using a luminometer. Data represent the percentage in luciferase expression relative to that of the empty vector (EV) control in the presence of the vehicle.

cancer cells [20]. In this study, we demonstrated that CHM-1 inhibited HGF-induced NF- κ B activation through inhibition of I κ B α phosphorylation. These results suggest that CHM-1 suppress the function of NF- κ B by blocking the nuclear translocation of NF- κ B. Accordingly, CHM-1 may be useful to suppress metastasis of liver cancer.

Many studies have revealed that growth factors and cytokines secreted by tumor cells will induce the production of MMPs. It has shown that elevated serum levels of MMP-9 in HCC patients and over-expression of MMP-9 in HCC tissues are related to hematogenous invasion or capsular infiltration of HCC cells [8]. On the other hand, growth factors and cytokines can control the expression of MMP-9 by modulating the activation of transcription factors such as NF- κ B and AP-1 through Ras/MAPK and PI3K/Akt signal pathways. The NF- κ B and AP-1 elements of MMP-9 promoter are centrally involved in the induction of MMP-9 gene associated with the invasion of tumor cells [21–23]. In this study, CHM-1 inhibited the enzymatic activity of the MMP-9 protein secreted from SK-Hep-1 cells via induction by HGF. We further showed that CHM-1 inhibited HGF-induced MMP-9 protein expression and gene transcription. Additionally, we found that induction of MMP-9 expression by HGF was a direct result of NF- κ B activation, as HGF did not induce MMP-9 gene expression in I κ B α M-transfected cells. We demonstrated that suppression of NF- κ B by CHM-1 down-regulated the expression of MMP-9. Recently, Abiru et al. showed that HGF-stimulated invasion in HCC cells through induction of NF- κ B target gene MMP-9, and the inhibition of NF- κ B activity using aspirin and NS-398 led to suppression of HGF-induced invasion through down-regulation of MMP-9 gene expression [24]. Moreover, the PI3K/Akt pathway plays an important role in the activation of NF- κ B, and Agarwal et al. found that PI3K/Akt/I κ B kinase pathway positively regulated NF- κ B to promote metastatic gene expression in colorectal cancer [25,26]. Thus, we suggest that anti-invasive activity of CHM-1 may be through the selective suppression of MMP-9 regulated by PI3K/Akt/NF- κ B signal transduction.

In conclusion, we demonstrate that CHM-1 inhibits HGF/c-Met-mediated cell invasion via the down-regulation of MMP-9 in SK-Hep-1 cells. The Akt/NF- κ B signaling pathway may be coordinately involved in CHM-1's anti-invasive effect. The further study of CHM-1 on the downstream

signal of HGF/c-Met activation in HCC cells is needed to investigate in the future. Based on the findings herein, we suggest that CHM-1 could be effective candidate for prevention of HCC cell invasion associated with the HGF/c-Met system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.canlet.2007.07.002.

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