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ORIGINAL ARTICLE

Dihydromyricetin inhibits migration and invasion of hepatoma cells through regulation of MMP-9 expression

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Abstract

AIM: To investigate the effects of dihydromyricetin (DHM) on the migration and invasion of human hepatic cancer cells.

METHODS: The hepatoma cell lines SK-Hep-1 and MHCC97L were used in this study. The cells were cultured in RPIM-1640 medium supplemented with 10% fetal bovine serum at 37 ℃ in a humidified 5% CO_2 incubator. DHM was dissolved in dimethyl sulfoxide and diluted to various concentrations in medium before applying to cells. MTT assays were performed to measure the viability of the cells after DHM treatment. Wound healing and Boyden transwell assays were used to assess cancer cell motility. The invasive capacity of cancer cells was measured using Matrigel-coated transwell chambers. Matrix metalloproteinase (MMP)-2/9 activity was examined by fluorescence analysis. Western blot was carried out to analyze the expression of MMP-2, MMP-9, p-38, JNK, ERK1/2 and PKC-δ proteins. All data were analyzed by Student's *t* tests in GraphPad prism 5.0 software and are presented as mean ± SD.

RESULTS: DHM was found to strongly inhibit the migration of the hepatoma cell lines SK-Hep-1 (without DHM, 24 h: 120 ± 8 μmol/L *vs* 100 μmol/L DHM, 24 h: 65 ± 10 μmol/L, P < 0.001) and MHCC97L (without DHM, 24 h: 126 ± 7 μmol/L *vs* 100 μmol/L DHM, 24 h: 74 ± 6 μmol/L, P < 0.001). The invasive capacity of the cells was reduced by DHM treatment (SK-Hep-1 cells without DHM, 24 h: 67 ± 4 μmol/L *vs* 100 μmol/L DHM, 24 h: 9 ± 3 μmol/L, P < 0.001; MHCC97L cells without DHM, 24 h: 117 ± 8 μmol/L *vs* 100 μmol/L DHM, 24 h: 45 ± 2 μmol/L, P < 0.001). MMP2/9 activity was also inhibited by DHM exposure (SK-Hep-1 cells without DHM, 24 h: 600 ± 26 μmol/L *vs* 100 μmol/L DHM, 24 h: 100 ± 6 μmol/L, P < 0.001; MHCC97L cells without DHM, 24 h: 504 ± 32 μmol/L *vs* 100 μmol/L DHM 24 h: 156 ± 10 μmol/L, P < 0.001). Western blot analysis showed that DHM decreased the expression level of MMP-9 but had little effect on MMP-2. Further investigation indicated that DHM markedly reduced the phosphorylation levels of p38, ERK1/2 and JNK in a concentration-dependent manner but had no impact on the total protein levels. In addition, PKC-δ protein, a key protein in the regulation of MMP family protein expression, was up-regulated with DHM treatment.

CONCLUSION: These findings demonstrate that DHM inhibits the migration and invasion of hepatoma cells and may serve as a potential candidate agent for the prevention of HCC metastasis.

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Key words: Dihydromyricetin; Migration; Invasion; He-

patic cancer; Matrix metalloproteinase-9

Core tip: The novel findings of this report are that dihydromyricetin (DHM) can strongly inhibit the migration and invasion (in transwell experiments) of the hepatic cancer cell lines SK-Hep-1 and MHCC97L. Western blot analysis showed that DHM down-regulated the level of matrix metalloproteinase 9 (MMP-9) protein but had little effect on the expression of MMP-2. Further investigation indicated that this reduction of MMP-9 expression underlies the inhibitory effects of DHM on cell migration and invasion and that this decrease was associated with an increase in the total protein levels of PKC-δ and decrease in the protein phosphorylation levels of members of the MAPK signaling pathway.

Zhang QY, Li R, Zeng GF, Liu B, Liu J, Shu Y, Liu ZK, Qiu ZD, Wang DJ, Miao HL, Li MY, Zhu RZ. Dihydromyricetin inhibits migration and invasion of hepatoma cells through regulation of MMP-9 expression. *World J Gastroenterol* 2014; 20(29): 10082-10093 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i29/10082.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i29.10082

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers and the third leading cause of cancer-related deaths worldwide; approximately 700000 deaths due to HCC are reported annually[1,2]. The incidence of HCC has demonstrated a particularly dramatic increase in China. However, liver transplantation and surgical resection are limited treatment options for HCC. Chemoprevention is still an important approach to decreasing the mortality rate of patients with liver cancer. Therefore, there is a special need to develop new agents with strong efficacy in the treatment of HCC.

Cancer metastasis is a multistep process that accounts for more than 90% of all cancer-related deaths[3,4]. Cancer invasion is an indispensable step in successful metastasis, which involves the proteolytic degradation of the extracellular matrix (ECM) and basement membrane of normal surrounding tissues[5]. Matrix metalloproteinases (MMPs), a family of zinc-dependent proteolytic enzymes, can degrade various components of the ECM and basement membrane. Among MMPs, MMP-2 and MMP-9 are believed to play a pivotal role in the degradation of laminin, type Ⅳ collagen and gelatin, components of the ECM and basement membrane[6]. Additionally, high levels of MMP-2 and MMP-9 expression have been shown to be related to inflammation, tissue repair, cancer progression, invasion, and metastasis[7]. Therefore, the inhibition of cancer cell invasion and metastasis by some pharmacological agents can be partially attributed to the down-regulation of protein levels of MMP-2 and MMP-9[8, 9].

Dihydromyricetin (DHM) is one of many active flavonoids that were reported to possess anti-

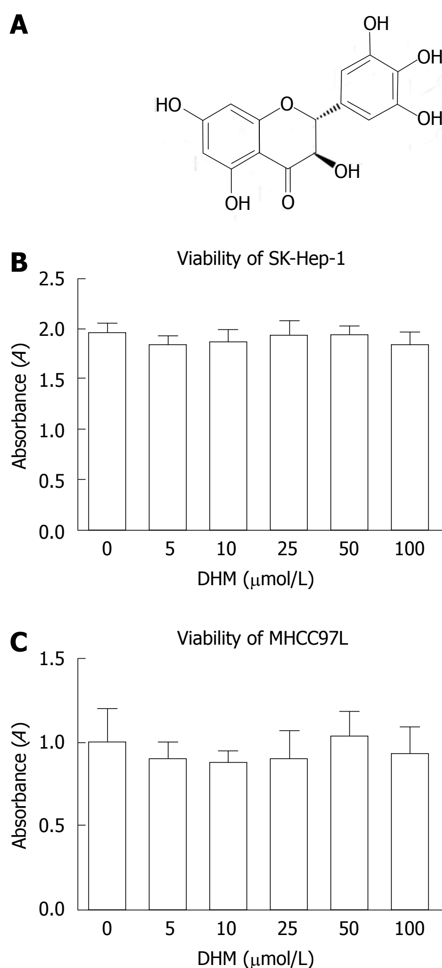


Figure 1 Chemical structure of dihydromyricetin and cell viability after dihydromyricetin treatment. A: The chemical structure of dihydromyricetin (DHM); B, C: The effects of DHM treatment on cell viability of SK-Hep-1 (B) and MHCC97L (C) cells were tested by measuring cell proliferation. After the cells were treated with DHM in a concentration range from 0 to 100 μmol/L for 24 h, MTT assay was used to determine cell proliferation. The data showed DHM treatment at the indicated concentrations (0-100 μmol/L) did not apparently affect cell proliferation (n = 6, Student's t test).

cancer activities[10,11]. DHM is also known as ampelopsin [(2R,3R)-3,5,7-trihydroxy-2-(3,4,5--trihydroxyphenyl)-2,3-dihydro--chromen-4-one], and its chemical structure is shown in Figure 1A. DHM is one of the active components isolated from the classical Chinese herb *Ampelopsis grossedentata*. DHM was reported to have various pharmacological activities such as hepatic protection[12,13], antioxidation[14,15], anti-inflammation[16] and hypoglycemic effects[17].

In addition, previous studies indicated that DHM could inhibit the growth of xenograft tumors from the human lung cancer cell line GLC-82 in nude mice[18]. DHM sodium was reported to cause a cell cycle arrest and suppress the proliferation of bladder carcinoma[19]. Moreover, DHM could reverse multidrug resistance in the human leukemia cell line K562/ADR by increasing the intracellular accumulation of chemotherapeutic drugs and inhibiting the efflux of drugs mediated by p-glycoprotein[20]. In recent years, it has been reported that DHM can inhibit angiogenesis in hepatocellular car-

cinoma cells by down-regulating the expression and production of vascular endothelial growth factor and basic fibroblast growth factor[21,22]. The potential mechanisms of its anti-cancer effects may be attributed to inhibiting cell proliferation, causing cell cycle arrest or apoptosis, or anti-oxidant and anti-angiogenesis activities. However, whether DHM can inhibit the migration and invasion of liver cancer cells remains vague and needs to be clarified.

In this study, human hepatocellular carcinoma SK-Hep-1 and MHCC97L cells were used to elucidate the potential effects of DHM on cell migration, adhesion, and invasiveness *in vitro*; the mechanisms underlying these activities were analyzed by Western blot to examine the expression levels of endogenous MMP-2 and MMP-9 and the possible roles of DHM in the MAPK and protein kinase C (PKC)δ signaling pathways.

MATERIALS AND METHODS

Chemicals and reagents

DHM was purchased from Sigma-Aldrich Biotechnology (St. Louis, MO, United States), dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mmol/L as a primary stock solution and stored at -20 ℃ for less than three months. The purity of DHM used in the present study was greater than 99.90%. The desired final concentrations of DHM for cell treatment were obtained by dilution with the appropriate culture medium, and the final content of DMSO was kept less than 0.3% throughout all experiments. Primary antibodies against PKC-δ, c-Jun N-terminal kinase (JNK), P-JNK, MMP-2, MMP-9, extracellular signal-regulated kinase (ERK1/2), p-ERK1/2, p38, and P-p38 were obtained from Cell Signal Technology (Beverly, MA, United States). The secondary antibody was provided by Earthox (Cat: E030120-01) and used at a dilution of 1:3000. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was purchased from Sigma-Aldrich Biotechnology (St. Louis, MO, United States).

Cell lines and culture

The human hepatic cancer cell lines SK-Hep-1 (human liver adenocarcinoma cells) and MHCC97L (human hepatocellular carcinoma) were obtained from the Shanghai Cell Bank of Chinese Academy of Sciences and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gibco, United States), 100 U/mL penicillin and 100 mg/L streptomycin at 37 ℃ in a humidified 5% CO_2 incubator.

Cell viability assay

MTT assay was used to evaluate cell viability and the effects of DHM treatment on the viability of SK-Hep-1 and MHCC97L cells. Briefly, the cells were seeded at a density of 1 × 10^4 cells/well into 96-well plates. After 24 h of incubation, the cells were treated with various concentrations (5, 10, 25, 50 or 100 μmol/L) of DHM for 24 h at 37 ℃ in a 5% CO_2 incubator; untreated cells served as a control. Next, 20 μL of MTT solution (5 mg/mL) was added to each well and the incubation was continued for 4 h. The medium was subsequently removed, and 150 μL DMSO was added. The absorbance of each well was measured at 570 nm with an automated spectrophotometric plate reader (PerkinElmer, United States).

Wound healing assay

The effect of DHM on wound healing ability was examined *in vitro* as previously reported, with some modifications[23,24]. Briefly, the cells were plated in 6-well plates and grown to 80%-90% confluence. A wound was created in the adherent cells using a pipette tip. The cells were then washed twice with phosphate buffered saline (PBS) to remove cell debris and floating cells. After this, the cells were incubated in the absence or presence of 50 or 100 μmol/L DHM for 12 or 24 h. The wounds were subsequently observed under an inverted microscope, and images covering the entire width of the wounds were captured at various time points using a 10 × objective. The number of cells was analyzed using Image J software *via* a tool named "analyze particles". At least three randomly selected areas were assayed for each well.

Cell motility assay

Cell motility was assayed *in vitro* as previously described[25,26], with some modifications. A total of 1 × 10^5 cells in 100 μL of DMEM growth medium in the absence or presence of 50 or 100 μmol/L DHM were seeded into the top inserts (chambers) above porous polycarbonate membranes with a pore size of 8.0 μm; these inserts fit into the bottom wells of the Boyden transwell system in 24-well plates (BD Biosciences). Subsequently, 600 μL of growth medium containing 10% FBS was added to the bottom wells. After incubation for 24 h, the cells that passed through membrane into the bottom wells were fixed in 75% ethanol and then stained with hematoxylin and eosin (HE) solution. Subsequently, the cells in more than four fields of each bottom well were randomly photographed and counted for each assay. The experiments were carried out three times, and the means and standard deviations of numerous values were calculated.

Cell invasiveness assay

Cell invasion was measured *in vitro* using a method similar to the cell motility assay, except that the inserts were coated with Matrigel. Briefly, transwell inserts (chambers) were coated with 100 μL of Matrigel (BD Biosciences) diluted with serum-free DMEM medium and placed in an incubator at 37 ℃ to solidify the Matrigel for 3-4 h. Subsequently, SK-Hep-1 cells were harvested, and the cell density was adjusted to 1 × 10^6 cells/mL. Similarly, the cell density of MHCC97L was adjusted to 5 × 10^6 cells/mL. Then, 100 μL of cells suspended in DMEM growth medium in the absence or presence of DHM was immediately added to each top insert above the polycarbonate membrane. Six hundred microliters of growth medium containing 10% FBS was added to the

lower chambers. After incubation for 24 h, the cells that invaded through the filter (membrane) to the bottom wells were fixed in 75% ethanol and then stained with HE solution. The cells in more than four fields of each bottom well were randomly photographed and counted for each assay. The experiments were performed three times, and the means and standard deviations of numerous values were calculated.

Cell adhesion assay

The cell adhesion assays were carried out as previously reported[8], with some modifications. Each well of 96-well plates was coated with 10 μL of fibronectin (RD systems, United States); the plates were then placed in an incubator to dry at 37 ℃ for two days and washed twice with PBS before use. After pre-treatment of SK-Hep-1 and MHCC97L cells with 50 or 100 μmol/L DHM for 24 h, cells were harvested, and then they were resuspended in serum-free DMEM medium. One hundred microliters of a cell suspension at a density of 5×10^5 cells/mL was added to each well, and the plates were incubated for 1 h. Subsequently, the growth medium and non-adherent cells were gently removed, and the adherent cells were counted using an MTT assay. The experiments were carried out at least three times.

Western blot analysis

After treatment with various concentrations of DHM for 24 h, the cells were harvested and lysed in lysis buffer (0.5 mol/L Tris-HCl, pH 7.4, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1.5 mol/L NaCl, 2.5% deoxycholic acid, 1% NP-40, 10 mmol/L ethylenediaminetetraacetate (EDTA), 1 mmol/L sodium orthovanadate, and protease inhibitor cocktail). After 30 min of incubation on ice, total cell lysates were prepared by centrifugation at 4 ℃ for 10 min at 12000 × *g*. The supernatant containing the soluble protein was collected, and its concentration was determined using BCA assays (Beyotime, China). Equal amounts of protein (30 μg) of each sample were prepared, separated on 10% SDS-PAGE gels, and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were then blocked with 5% skim milk in 1 × TBST containing 0.1% Tween 20 for 1 h at room temperature and then incubated with primary antibodies (such as MMP-2, MMP-9, p38, P-p38, ERK1/2, P-ERK1/2, PKC-δ, JNK and P-JNK) for 8-12 h at 4 ℃. The membranes were subsequently washed and then incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibodies for 1 h at 37 ℃. The bands were detected using an enhanced chemiluminescence reagent and system (Amersham Biosciences Corp., NJ, United States).

MMP-2/MMP-9 activity assay

The activity of MM-2/MMP-9 was measured using a kit from Merck Millipore (CBA003). According to the manufacturer's protocol, the cells were first treated with different concentrations of DHM (0, 10, 50, or 100 μmol/L) for 24 h. Next, 90 μL of medium was removed and added to 10 μL of substrate working solution. This mixture was incubated for 18 h at 37 ℃ in a 5% CO_2 incubator; fluorescence was then measured using a microplate reader with a filter set for excitation/emission of 320/405 nm.

Statistical analysis

The data are presented as mean ± SD. Statistical comparisons between two groups were performed using Student's *t* tests; the significance levels were defined as $P < 0.05$, $P < 0.01$ and $P < 0.001$.

RESULTS

DHM inhibits the migration of SK-Hep-1 and MHCC97L cells

To evaluate the effects of DHM on cell viability, MTT assays were used to analyze the effects of DHM on the viability of SK-Hep-1 and MHCC97L cells treated with various concentrations of DHM. First, we tested the acute toxicity of DHM at concentrations ranging from 150 mg/kg (500 μmol/L) to 1.5 g/kg (5000 μmol/L) body weight in mice; we observed no significant effects at these doses on body weight or tissue morphology. Because body fluid only accounts for 60% of body weight and taking bioavailability into account, we estimated that 100 μmol/L DHM is a safe concentration. Second, we have demonstrated that DHM inhibits liver cancer cell (HepG2) proliferation and induces apoptosis at concentrations of 10-150 μmol/L[27]. Therefore, we used concentrations from 5 μmol/L to 100 μmol/L in this study. Our data showed that the viability of SK-Hep-1 and MHCC97L cells was not affected by treatment with DHM in a concentration range of 5 to 100 μmol/L (Figure 1B and C). Therefore, we carried out subsequent experiments in this concentration range.

Wound healing assays were performed to determine the effects of DHM on cell migration. When cell monolayers with 80%-90% confluence were scraped and treated without or with 50 and 100 μmol/L DHM for 12 or 24 h, the untreated cells (control cells) migrated to the gap created by a pipette tip, whereas the cells that were treated with 50 or 100 μmol/L DHM did not migrate (Figure 2). These data clearly demonstrated that DHM can suppress the migration of SK-Hep-1 cells and MHCC97L cells.

To further quantify the effects of DHM on the migration of SK-Hep-l and MHCC97L cells, we used the Boyden transwell system with porous polycarbonate membranes to determine cell motility. After SK-Hep-1 cells were treated with 50 or 100 μmol/L DHM for 24 h, the number of SK-Hep-1 cells that passed through the membrane per field under an inverted microscope at 100 × magnification was reduced from 122.71 ± 13.44 to 75.13 ± 11.48 and 58.63 ± 11.26, respectively, compared with the control (Figure 3A and B). Similarly, the number of migrated MHCC97L cells per field was reduced from

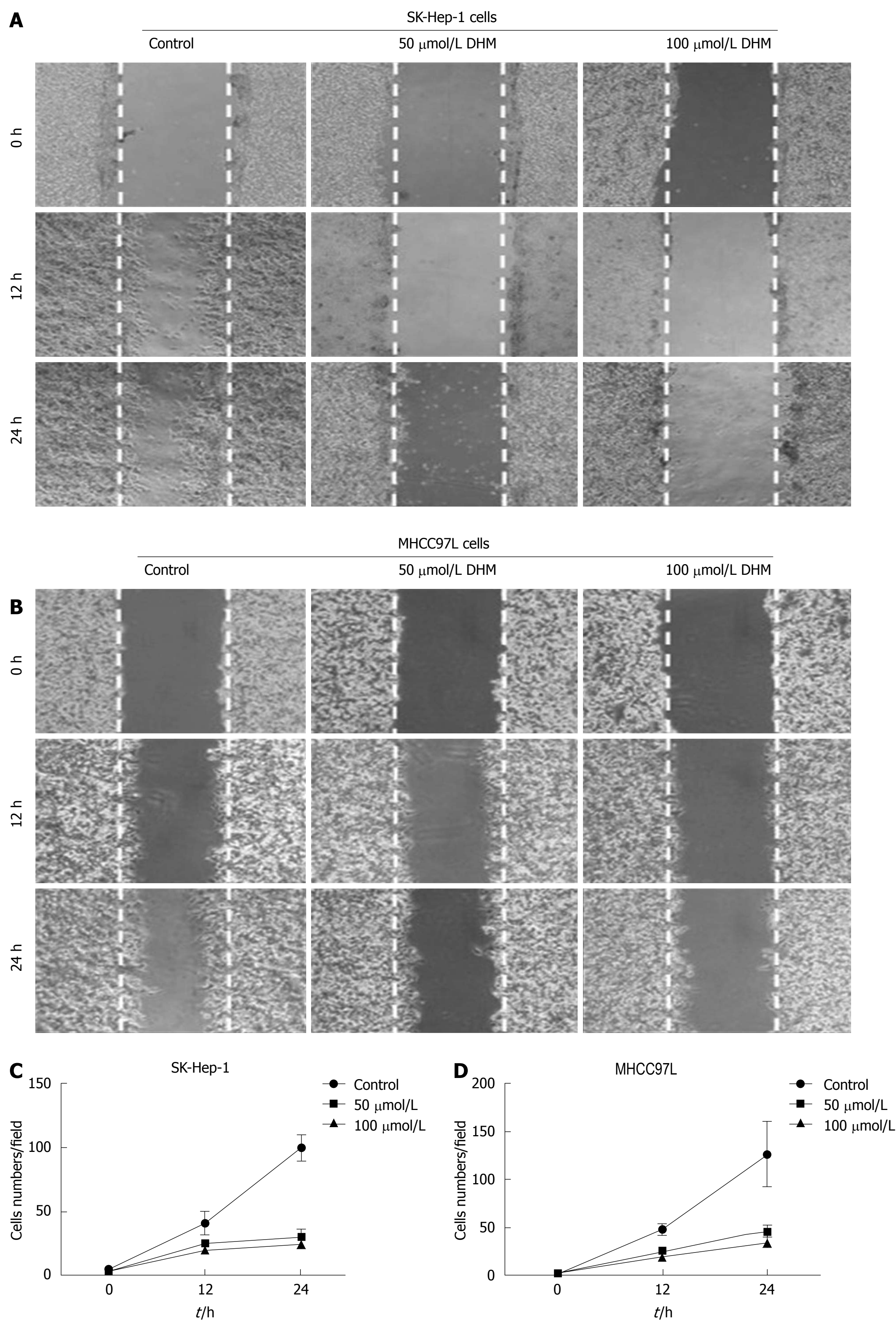


Figure 2 Effect of dihydromyricetin on wound healing *in vitro*. Cell monolayers of 80%-90% confluence were scraped with a pipette tip and treated without or with 50 or 100 μmol/L dihydromyricetin (DHM) for 12 or 24 h. Wound healing was evaluated in photographs taken at the indicated time point by assaying the migration of cells into the wound. DHM treatment inhibited SK-Hep-1 (A, C) and MHCC97L (B, D) cell migration compared to the corresponding controls.

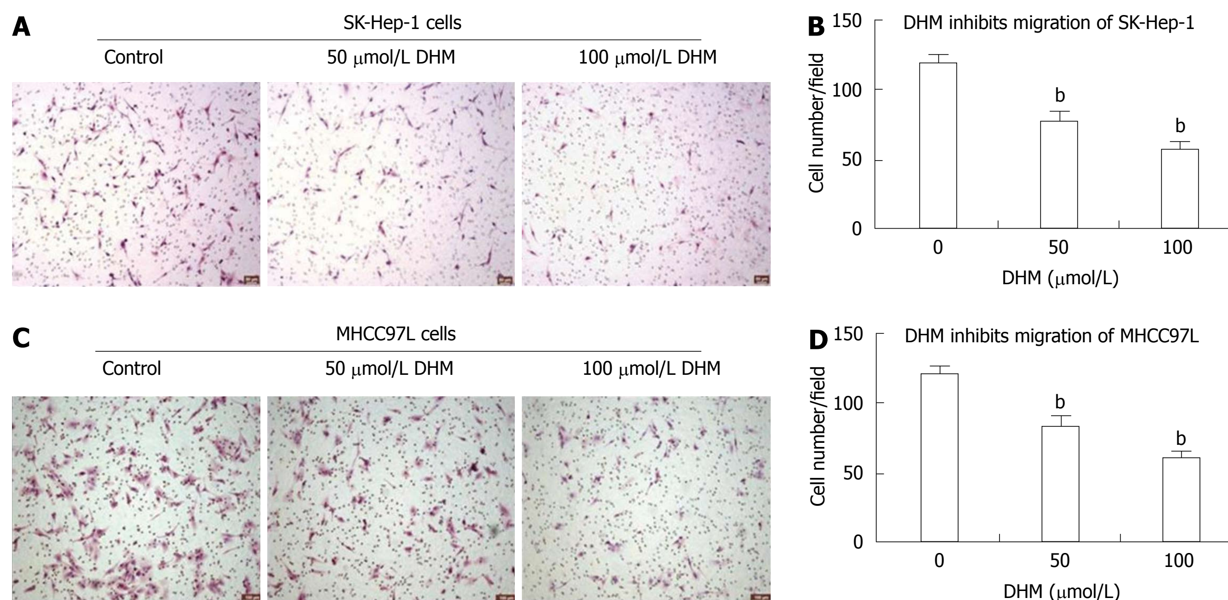


Figure 3 Dihydromyricetin inhibits cell migration *in vitro*. The Boyden transwell system was used to assay cell migration as described in the "MATERIALS AND METHODS". SK-Hep-1 (A) and MHCC97L (C) cells that passed through polycarbonate membranes with an 8.0-μmol/L pore size were fixed in 75% ethanol and stained with hematoxylin and eosin solution. The number of SK-Hep-1 (B) and MHCC97L (D) cells that migrated through the membrane was quantified by determining the number of cells per field under an inverted microscope at 100 × magnification. Dihydromyricetin (DHM) significantly suppressed the migration of SK-Hep-1 and MHCC97L cells. Each experiment was carried out at least three times. ^bP < 0.01 *vs* control, Student's *t* test.

119.56 ± 16.62 to 83.50 ± 11.98 and 67.45 ± 4.87, respectively (Figure 3C and D). These results further confirmed that DHM significantly decreased the migration of SK-Hep-1 and MHCC97L cells.

DHM inhibits cell invasion in vitro

It is well known that the basement membrane underlies epithelial and endothelial cell layers and acts as a dense meshwork, thereby providing a physical boundary and signaling substrate to orient cells through integrin-base adhesion[14]. Because the proteolytic disruption of the basement membrane is a key step in the invasiveness and metastasis of cancer cells, we used assays that were similar to the cell motility assays but added a Matrigel coating to the transwell chambers to mimic the basement membrane; these adapted transwell chambers were used to test the effects of DHM on the invasiveness of SK-Hep-1 and MHCC97L cells. After incubation for 24 h in the presence of 50 and 100 μmol/L DHM, we found that the number of cells that invaded through the Matrigel and membrane was decreased (Figure 4), whereas the control cells were strongly able to invade through the Matrigel and membrane. As shown in Figure 4A and B, the number of SK-Hep-1 cells per field under an inverted microscope at 100 × magnification was decreased from 69.6 ± 26.60 to 19.60 ± 5.59 and 7.80 ± 5.22 after treatment with 50 and 100 μmol/L DHM for 24 h, respectively, compared to the control. Similarly, the number of MHCC97L cells per field was decreased from 122.67 ± 8.09 to 84.89 ± 5.16 and 44.22 ± 5.41, respectively (Figure 4C and D). These results demonstrated that the invasion of SK-Hep-1 and MHCC97L cells could be significantly suppressed by DHM treatment.

DHM inhibits cell adhesion in vitro

Cancer metastasis is a complex process involving cell migration, adhesion and invasiveness. Cancer cell adhesion to the extracellular matrix or basement membrane is a crucial step during cancer invasiveness. Therefore, we determined the effect of DHM on cell adhesion. The data demonstrated that pre-treatment of SK-Hep-1(Figure 4E) and MHCC97L (Figure 4F) cells with DHM for 24 h significantly inhibited cell adhesion. Fewer DHM-treated cells than control cells adhered to fibronectin, which indicates that the adhesion ability of SK-Hep-1 and MHCC97L cells was inhibited by DHM treatment.

DHM down-regulates MMP-9 rather than MMP-2

It is well-known that MMPs are the key enzymes for degrading the extracellular matrix; in particular, MMP-2 and MMP-9 are considered to play an important role in cancer invasion and metastasis[28]. We initially demonstrated that MMP2/9 activity was inhibited by DHM via fluorescence analysis (Figure 5). Accordingly, to explore the mechanisms underlying the anti-invasive effect of DHM, we examined MMP-2 and MMP-9 protein expression in SK-Hep-1 and MHCC97L cells. After incubation in the absence or presence of DHM at various concentrations for 24 h, the cells were harvested, and the total protein was examined by Western blot. We found that the production of MMP-9 protein in SK-Hep-1 and MHCC97L cells was down-regulated by DHM in a concentration-dependent manner. However, MMP-2 protein was not down-regulated in SK-Hep-1 and MHCC97L cells. (Figure 5A, B, D and E). These results demonstrated that DHM treatment could suppress the expression of

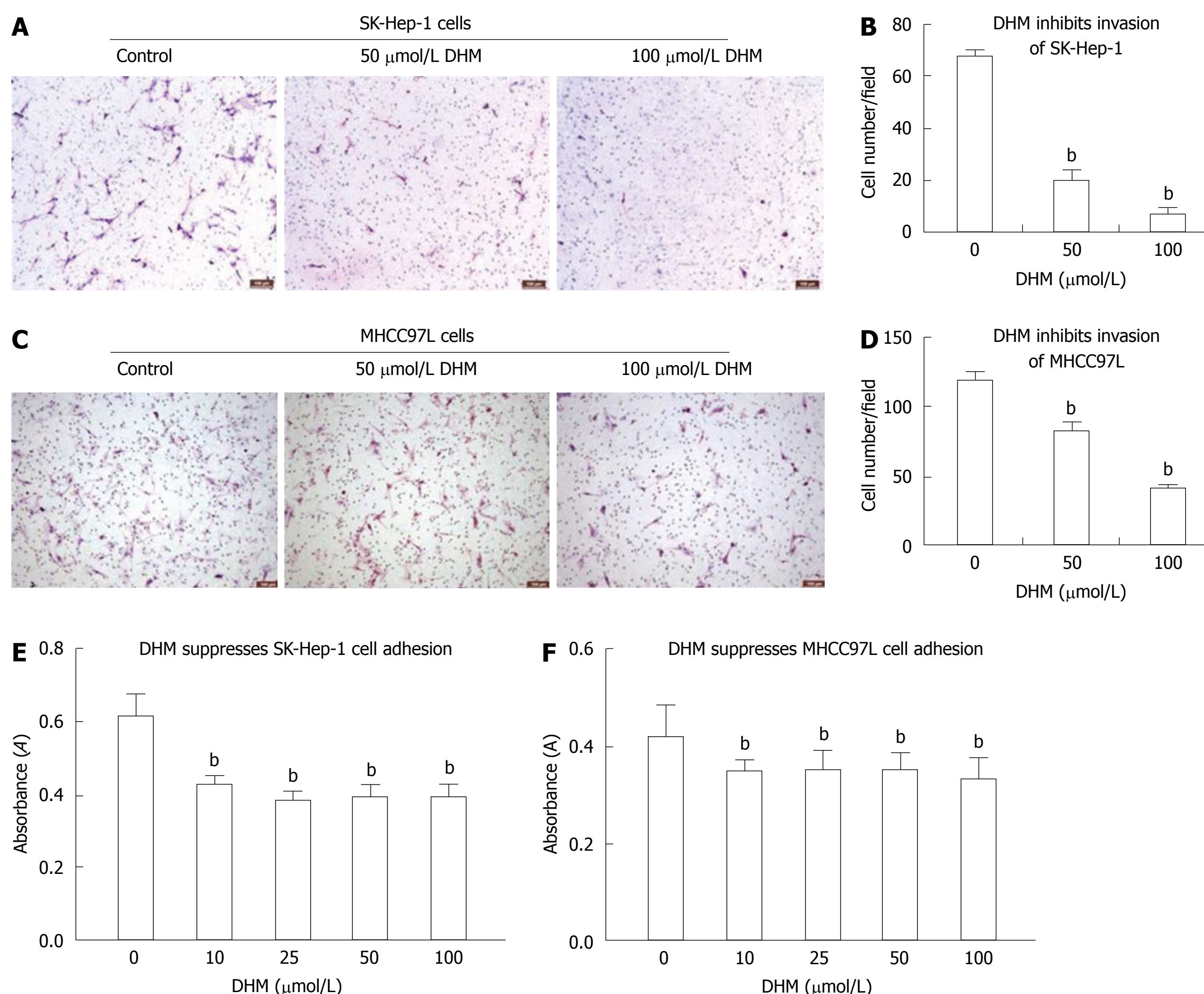


Figure 4 Dihydromyricetin inhibits cell invasion and adhesion *in vitro*. Cell invasion assays were performed using a method similar to the cell motility assays, but using 8.0-μm pore size polycarbonate membranes that were pre-coated with 100 μL of Matrigel and allowed to solidify in an incubator at 37 ℃ for 3-4 h as described in the "MATERIALS AND METHODS". The SK-Hep-1 (A) and MHCC97L (C) cells that passed through the Matrigel and membrane were fixed in 75% ethanol and stained with hematoxylin and eosin solution. The number of SK-Hep-1 (B) and MHCC97L (D) cells that passed through Matrigel and membrane was quantified by determining the number of cells per field under an inverted microscope at 100 × magnification. The cells in at least four fields of each bottom of chamber were randomly photographed and counted for each assay. DHM treatment significantly inhibited the invasion of SK-Hep-1 and MHCC97L cells. Each experiment was performed at least three times. To measure the effect of DHM treatment on cell adhesion, SK-Hep-1 and MHCC97L cells were pre-treated with or without DHM at the indicated concentrations for 24 h. The cells were then resuspended in serum-free culture medium and added to 96-well plates coated with fibronectin. After incubation for 1 h, the adherent cells were measured using MTT assays. Dihydromyricetin (DHM) treatment decreased the number of SK-Hep-1 (E) and MHCC97L (F) cells adhered to fibronectin compared to the control. $^bP < 0.01$ *vs* control, Student's *t* test.

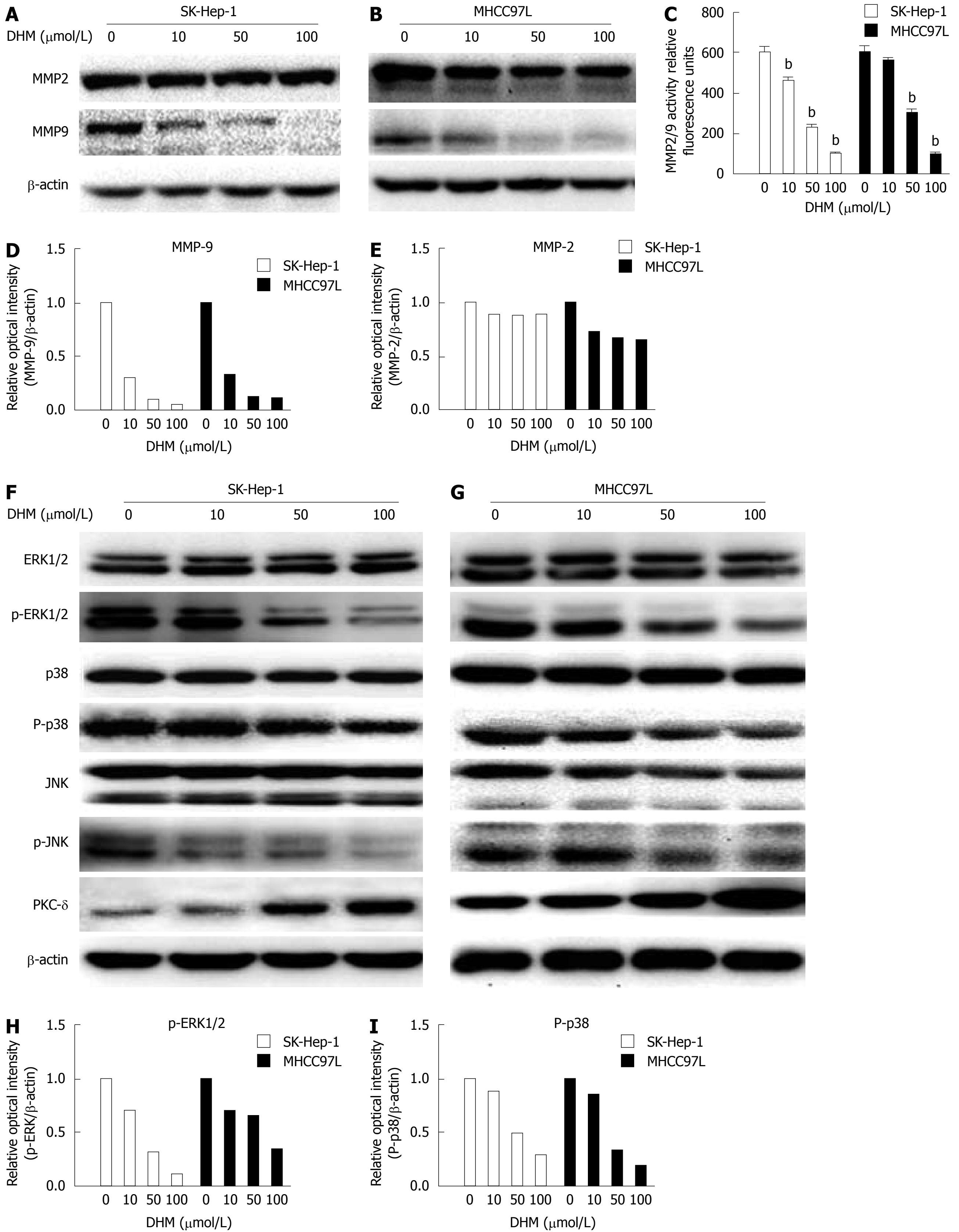
MMP-9 in SK-Hep-1 and MHCC97L cells but had little effect on MMP-2.

The down-regulation of MMP-9 is associated with the MAPK and PKC-δ signaling pathways

To further study whether the inhibition of MMP-9 expression by DHM occurs through the MAPK or PKC-δ signaling pathways, the total protein levels of ERK1/2, p38, JNK, their phosphorylated forms, and PKC-δ protein were measured by Western blot. DHM treatment for 24 h had little effect on ERK1/2 expression in SK-Hep-1 and MHCC97L cells. However, the phosphorylated forms of ERK1/2 in SK-Hep-1 and MHCC97L cells were down-regulated by DHM in a concentration-dependent manner. Moreover, DHM treatment markedly reduced P-p38, but p38 protein levels were constant. In addition, JNK levels were found to be decreased by DHM treatment in SK-Hep-1 and MHCC97L cells, and the phosphorylated forms of JNK were significantly down-regulated in SK-Hep-1 and MHCC97L cells in a concentration-dependent manner. In addition, we found that DHM treatment for 24 h increased the expression of PKC-δ in SK-Hep-1 and MHCC97L cells in a concentration-dependent manner (Figure 5F-K).

DISCUSSION

HCC is a highly malignant type of solid tumor that is associated with frequent metastasis. Currently, there is no effective treatment for this malignancy[29]. Previous stud-



A
SK-Hep-1
DHM (μmol/L) 0 10 50 100
MMP2
MMP9
β-actin
B
MHCC97L
0 10 50 100
C
MMP2/9 activity relative fluorescence units
800
600
400
200
0
SK-Hep-1
MHCC97L
b
0 10 50 100 0 10 50 100
DHM (μmol/L)
D
MMP-9
Relative optical intensity (MMP-9/β-actin)
1.5
1.0
0.5
0.0
E
MMP-2
Relative optical intensity (MMP-2/β-actin)
F
SK-Hep-1
ERK1/2
p-ERK1/2
p38
P-p38
JNK
p-JNK
PKC-δ
G
MHCC97L
H
p-ERK1/2
Relative optical intensity (p-ERK/β-actin)
I
P-p38
Relative optical intensity (P-p38/β-actin)

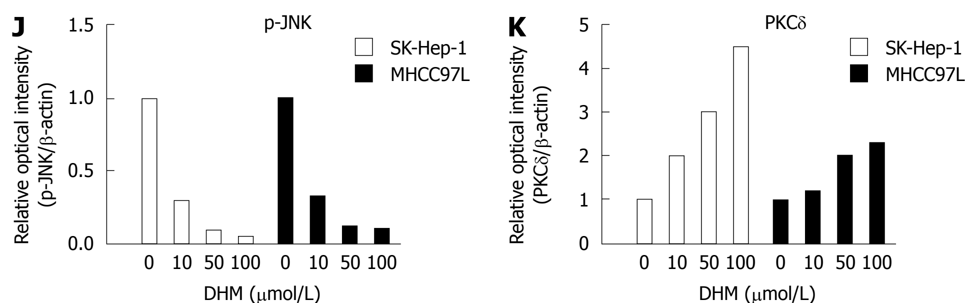


Figure 5 Effect of dihydromyricetin on matrix metalloproteinase-2/matrix metalloproteinase-9 expression and the signaling pathways upstream of matrix metalloproteinase (MMP)-9. SK-Hep-1 and MHCC97L cells were treated with or without dihydromyricetin at the indicated concentrations for 24 h. The cells were then collected and lysed in lysis buffer. The total cell lysates were examined by Western blot. Matrix metalloproteinase (MMP)-2 and MMP-9 protein expression was detected by Western blot in SK-Hep-1 (A, D) and MHCC97 L (B, E) cells after dihydromyricetin (DHM) treatment for 24 h. MMP2/9 activity was measured by fluorescence analysis (C). The levels of ERK1/2, p38, JNK, PKC-δ, p-ERK1/2, p-p38, and p-JNK in SK-Hep-1 (F) and MHCC97L (G) cells were also measured by Western blot assays. A bicinchoninic acid (BCA) assay was used to measure the protein concentration of each sample. DHM regulated p-ERK1/2 (H), P-p38 (I), p-JNK (J) and PKC-δ (K) levels in a concentration-dependent manner. $^b P < 0.01$ *vs* control, Student's *t* test.

ies have demonstrated roles of MMPs in the migration, invasion and metastasis of HCC[30,31]. The suppression of MMPs, including MMP-2/9, decreases cancer cell invasion and migration. Accordingly, potential inhibitors of MMPs will undoubtedly be valuable in our attempts to treat HCC and other cancers.

DHM is one of the principal pharmacological components of *Ampelopsis grossedentata*, which is a traditional Chinese herb used to treat tinea corporis in south China[11,32]. Recently, a study demonstrated that DHM can inhibit the migration and invasion of human prostate cancer and breast cancer cells and suppress lung metastasis of prostate cancer cells in mice[11]. In this study, we confirmed that DHM can significantly inhibit the migration and invasion of the hepatocellular carcinoma SK-Hep-1 and MHCC97L cell lines. The inhibitory effects of DHM cannot be attributed to a cell growth arrest, because DHM treatment in the tested concentration range (0-100 μmol/L) for 24 h did not affect the viability of SK-Hep-1 and MHCC97L cells. However, previous studies showed that DHM could significantly suppress the proliferation of human HepG2 hepatic cancer cells[22] and LNCap and PC-3 human prostate cancer cells[23]. In this study, we found that DHM could significantly inhibit the adhesion of SK-Hep-1 and MHCC97L cells to fibronectin. Furthermore, we confirmed that DHM inhibited the adhesion of SK-Hep-1 and MHCC97L cells to fibronectin in a concentration-dependent manner. These results are consistent with previous findings that the adhesion ability of B16 mouse melanoma cells treated with DHM was significantly decreased[33].

MMP-9 is an important enzyme in the degradation of the extracellular matrix and basement membrane; MMP-9 therefore has a critical role in tumor invasion and angiogenesis[25,34]. Additionally, MMP-9 is highly expressed in various cancers and cancer cell lines, including hepatocellular carcinoma cells, such as SK-Hep-1 and MHCC97L cells. The suppression of MMP activity, including MMP-9, is an important strategy to prevent cancer cell invasion[35,36]. In our study, we confirmed that DHM could significantly decrease MMP-9 expression in SK-Hep-1 and MHCC97L cells and that this effect is closely associated with inhibitory effects on cell migration, invasion and adhesion. Unexpectedly, the MMP-2 protein remained constant after 24 h of incubation with DHM. Our results demonstrated that DHM suppressed the invasion and migration of SK-Hep-1 and MHCC97L cells directly through the MMP-9 pathway.

The MAPK signal pathways, including JNK, ERK1/2 and p38 MAPK, play an important role in the regulation of cancer invasion and metastasis[37]. MAPK pathways can coordinate the activation of gene expression and regulate cell migration and invasion, exerting a profound effect on cancerous metastasis. It has been shown that MMP expression in cancers involves the activation of MAPK signal pathways[38]. Another study has indicated that JNK, ERK1/2 and p38 are all involved in the regulation of MMP expression in skin fibroblasts[39]. To determine whether the down-regulation of MMP-9 expression is associated with MAPK signal pathways, we assessed the effects of DHM on JNK, ERK1/2, p38 and their phosphorylated forms. Our data demonstrated that DHM treatment could markedly decrease the phosphorylation level of p38 but did not affect the level of total p38 expression in SK-Hep-1 and MHCC97L cells. In addition, phosphorylated ERK1/2 was down-regulated by DHM treatment for 24 h in SK-Hep-1 and MHCC97L cells. The total cellular protein expression of JNK in SK-Hep-1 and MHCC97L cells was down-regulated after DHM treatment for 24 h, and P-JNK was also found to be reduced in a concentration-dependent manner. MAPK signal pathways contain the upstream kinases of MMPs in SK-Hep-1 and MHCC97L cells. Our results suggest that the cooperation of ERK1/2, p38 and JNK plays a crucial role in DHM-mediated cell migration and invasion in SK-Hep-1 and MHCC97L cells. A previous study has demonstrated that the expression of MMP-9 and MMP-2 was down-regulated by nm23-H1 protein, a tumor metastasis suppressor[40,41]. Whether the mechanism of DHM decreasing MMP-9 expression in SK-Hep-1 and MHCC97L cells is associated with the up-regulation of nm23-H1 remains to be clarified in the future. Other

reports have shown that the inhibitory effect of DHM on human prostate cancer PC-3 cell migration and invasion was related to the down-regulation of CXCR4 protein level[11]. We speculate that DHM inhibits cell migration and invasion via different target genes in different cell lines.

PKC contains classical (α, βⅠ, βⅡ and γ) and novel (δ, ε, η and θ) isoforms. It was reported that PKC-δ plays a key role in MMP-9 induction through the Ras/ERK pathway in MCF-7 cells[42]. Additionally, it has been demonstrated that there is a direct interaction between PKC-δ and the cytoskeleton[43]. The inhibition of PKC-δ can diminish the TNF-α-induced secretion of MMP-9 in neutrophils[44] and decrease eosinophil migration, suggesting an important role of PKC-δ in actin reorganization and the regulation of cell motility[45]. In this study, we found that DHM increased PKC-δ expression in SK-Hep-1 and MHCC97L cells in a concentration-dependent manner.

The mechanism by which DHM regulates invasion and migration through the MMP-9 pathway appears to be complicated. Further studies should be carried out to explore the effects of DHM on the enzymatic activities of MMP-9 and the expression of other MMP family members. In addition, a previous report indicates that the NF-κB and activator protein-1 binding sites in the MMP-9 promoter are involved in MMP-9 transcription in cancer cells[46]. Therefore, the transcriptional activity of MMP-9 should also be examined to confirm whether the DHM-mediated down-regulation of MMP-9 is caused by a suppression of the translocation of NF-κB proteins to the nucleus and/or an inhibition of its DNA binding sites to regulate the transcription of MMP-9.

In conclusion, we found that DHM significantly inhibited the migration and invasion of human hepatocellular carcinoma SK-Hep-1 and MHCC97L cells by decreasing MMP-9 protein expression. Moreover, the down-regulation of MMP-9 is associated with an increase in the level PKC-δ protein and the suppression of the phosphorylation of p38, ERK1/2 and JNK in SK-Hep-1 and MHCC97L cells. These results suggest that DHM has a strong anti-metastatic activity due to its ability to restrain the adhesion, migration and invasion of cancer cells.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is a highly malignant type of solid tumor that is associated with frequent metastasis. It is important to develop a novel chemotherapeutic agent for the treatment of HCC.

Research frontiers

Recently, authors' team has demonstrated that dihydromyricetin (DHM) shows several valuable properties, including the induction of apoptosis in hepatoma cells. However, whether DHM inhibits the invasion and migration of hepatoma cells is not yet known.

Innovations and breakthroughs

This is the first study to demonstrate that DHM can inhibit the migration and invasion of hepatoma cells *in vitro*.

Applications

DHM may serve as a potential effective candidate agent for the prevention of the carcinogenesis and metastasis of HCC.

Peer review

In this study, the authors found that DHM suppressed the migration of two HCC cell lines (SK-Hep1 and MHCC97L). DHM also inhibited the production of MMP9 and the activation of MAPK pathways. These findings are novel and of interest.

REFERENCES

1 **Darvesh AS**, Aggarwal BB, Bishayee A. Curcumin and liver cancer: a review. *Curr Pharm Biotechnol* 2012; **13**: 218-228 [PMID: 21466422]

2 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90

3 **Siegel R**, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* 2011; **61**: 212-236 [PMID: 21685461 DOI: 10.3322/caac.20121]

4 **Bravo-Cordero JJ**, Hodgson L, Condeelis J. Directed cell invasion and migration during metastasis. *Curr Opin Cell Biol* 2012; **24**: 277-283 [PMID: 22209238 DOI: 10.1016/j.ceb.2011.12.004]

5 **Sung HJ**, Kim Y, Kang H, Sull JW, Kim YS, Jang SW, Ko J. Inhibitory effect of Trolox on the migration and invasion of human lung and cervical cancer cells. *Int J Mol Med* 2012; **29**: 245-251 [PMID: 22075980 DOI: 10.3892/ijmm.2011.832]

6 **Lai WC**, Zhou M, Shankavaram U, Peng G, Wahl LM. Differential regulation of lipopolysaccharide-induced monocyte matrix metalloproteinase (MMP)-1 and MMP-9 by p38 and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinases. *J Immunol* 2003; **170**: 6244-6249 [PMID: 12794156]

7 **Gialeli C**, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *FEBS J* 2011; **278**: 16-27 [PMID: 21087457 DOI: 10.1111/j.1742-4658.2010.07919.x]

8 **Lee TK**, Castilho A, Cheung VC, Tang KH, Ma S, Ng IO. CD24(+) liver tumor-initiating cells drive self-renewal and tumor initiation through STAT3-mediated NANOG regulation. *Cell Stem Cell* 2011; **9**: 50-63 [PMID: 21726833]

9 **Ma CY**, Ji WT, Chueh FS, Yang JS, Chen PY, Yu CC, Chung JG. Butein inhibits the migration and invasion of SK-HEP-1 human hepatocarcinoma cells through suppressing the ERK, JNK, p38, and uPA signaling multiple pathways. *J Agric Food Chem* 2011; **59**: 9032-9038 [PMID: 21770460 DOI: 10.1021/jf202027n]

10 **Chen Y**, Lu N, Ling Y, Wang L, You Q, Li Z, Guo Q. LYG-202, a newly synthesized flavonoid, exhibits potent anti-angiogenic activity in vitro and in vivo. *J Pharmacol Sci* 2010; **112**: 37-45 [PMID: 20093787]

11 **Ni F**, Gong Y, Li L, Abdolmaleky HM, Zhou JR. Flavonoid ampelopsin inhibits the growth and metastasis of prostate cancer in vitro and in mice. *PLoS One* 2012; **7**: e38802 [PMID: 22693649 DOI: 10.1371/journal.pone.0038802]

12 **Murakami T**, Miyakoshi M, Araho D, Mizutani K, Kambara T, Ikeda T, Chou WH, Inukai M, Takenaka A, Igarashi K. Hepatoprotective activity of tocha, the stems and leaves of Ampelopsis grossedentata, and ampelopsin. *Biofactors* 2004; **21**: 175-178 [PMID: 15630194]

13 **Shen Y**, Lindemeyer AK, Gonzalez C, Shao XM, Spigelman I, Olsen RW, Liang J. Dihydromyricetin as a novel anti-alcohol intoxication medication. *J Neurosci* 2012; **32**: 390-401 [PMID: 22219299 DOI: 10.1523/JNEUROSCI.4639-11.2012]

14 **He G**, Du F, Yang W, Pei G, Zhu Y. [Effects of tengcha flavonoids on scavenging oxygen free radicals and inhibiting lipid-peroxidation]. *Zhongyaocai* 2003; **26**: 338-340 [PMID: 14535015]

15 **He GX**, Yang WL, Pei G, Zhu YH, Du FL. [Studies on the effect of dihydromyricetin on antilipid-peroxidation]. *Zhongguo Zhongyao Zazhi* 2003; **28**: 1188-1190 [PMID: 15617509]

16 **Qi S**, Xin Y, Guo Y, Diao Y, Kou X, Luo L, Yin Z. Ampelopsin reduces endotoxic inflammation via repressing ROS-mediated activation of PI3K/Akt/NF-κB signaling pathways. *Int Immunopharmacol* 2012; **12**: 278-287 [PMID: 22193240 DOI: 10.1016/j.intimp.2011.12.001]
17 **Zhong ZX**, Qin JP, Zhou GF, Chen XF. [Experimental studies of hypoglycemic action on total flavone of Ampelopsis grossedentata from Guangxi]. *Zhongguo Zhongyao Zazhi* 2002; **27**: 687-689 [PMID: 12776572]
18 **Zeng S**, Liu D, Ye Y, Wang L, Wang W. [Anti-tumor effects of ampelopsin on human lung cancer GLC-82 implanted in nude mice]. *Zhongyaocai* 2004; **27**: 842-845 [PMID: 15810594]
19 **Zhang B**, Dong S, Cen X, Wang X, Liu X, Zhang H, Zhao X, Wu Y. Ampelopsin sodium exhibits antitumor effects against bladder carcinoma in orthotopic xenograft models. *Anticancer Drugs* 2012; **23**: 590-596 [PMID: 22241170 DOI: 10.1097/CAD.0b013e32835019f9]
20 **Ye J**, Zheng Y, Liu D. [Reversal effect and its mechanism of ampelopsin on multidrug resistance in K562/ADR cells]. *Zhongguo Zhongyao Zazhi* 2009; **34**: 761-765 [PMID: 19624024]
21 **Luo GQ**, Zeng S, Liu DY. [Inhibitory effects of ampelopsin on angiogenesis]. *Zhongyaocai* 2006; **29**: 146-150 [PMID: 16617784]
22 **Gao Q**, Yang X, Ou M. [Effect of serum containing tengcha total flavonoid and dihydromyricetin on proliferation and apoptosis of HepG2 cells]. *Zhongguo Zhongyao Zazhi* 2011; **36**: 500-503 [PMID: 21598552]
23 **Caraballo-Miralles V**, Cardona-Rossinyol A, Garcera A, Villalonga P, Soler RM, Olmos G, Lladó J. SMN deficiency attenuates migration of U87MG astroglioma cells through the activation of RhoA. *Mol Cell Neurosci* 2012; **49**: 282-289 [PMID: 22197680 DOI: 10.1016/j.mcn.2011.12.003]
24 **Doi Y**, Yashiro M, Yamada N, Amano R, Noda S, Hirakawa K. VEGF-A/VEGFR-2 signaling plays an important role for the motility of pancreas cancer cells. *Ann Surg Oncol* 2012; **19**: 2733-2743 [PMID: 22207048 DOI: 10.1245/s10434-011-2181-6]
25 **Kang H**, Jang SW, Ko J. Human leucine zipper protein sLZIP induces migration and invasion of cervical cancer cells via expression of matrix metalloproteinase-9. *J Biol Chem* 2011; **286**: 42072-42081 [PMID: 22009750 DOI: 10.1074/jbc.M111.272302]
26 **Maegdefrau U**, Bosserhoff AK. BMP activated Smad signaling strongly promotes migration and invasion of hepatocellular carcinoma cells. *Exp Mol Pathol* 2012; **92**: 74-81 [PMID: 22024355 DOI: 10.1016/j.yexmp.2011.10.004]
27 **Wu S**, Liu B, Zhang Q, Liu J, Zhou W, Wang C, Li M, Bao S, Zhu R. Dihydromyricetin reduced Bcl-2 expression via p53 in human hepatoma HepG2 cells. *PLoS One* 2013; **8**: e76886 [PMID: 24223706 DOI: 10.1371/journal.pone.0076886]
28 **Zheng H**, Takahashi H, Murai Y, Cui Z, Nomoto K, Niwa H, Tsuneyama K, Takano Y. Expressions of MMP-2, MMP-9 and VEGF are closely linked to growth, invasion, metastasis and angiogenesis of gastric carcinoma. *Anticancer Res* 2006; **26**: 3579-3583 [PMID: 17094486]
29 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576 [PMID: 17570226 DOI: 10.1053/j.gastro.2007.04.061]
30 **Jia YL**, Shi L, Zhou JN, Fu CJ, Chen L, Yuan HF, Wang YF, Yan XL, Xu YC, Zeng Q, Yue W, Pei XT. Epimorphin promotes human hepatocellular carcinoma invasion and metastasis through activation of focal adhesion kinase/extracellular signal-regulated kinase/matrix metalloproteinase-9 axis. *Hepatology* 2011; **54**: 1808-1818 [PMID: 22045676 DOI: 10.1002/hep.24562]
31 **Yu HB**, Zhang HF, Li DY, Zhang X, Xue HZ, Zhao SH. Matrine inhibits matrix metalloproteinase-9 expression and invasion of human hepatocellular carcinoma cells. *J Asian Nat Prod Res* 2011; **13**: 242-250 [PMID: 21409686 DOI: 10.1080/10286020.2010.551641]
32 **Zhou J**, Zeng P, Tu HH, Wang FQ. Development and application of high-performance liquid chromatography for the study of ampelopsin pharmacokinetics in rat plasma using cloud-point extraction. *J Sep Sci* 2011; **34**: 160-168 [PMID: 21246721]
33 **Liu DY**, Zheng HQ, Luo GQ. [Effects of ampelopsin on invasion and metastasis of B16 mouse melanoma in vivo and in vitro]. *Zhongguo Zhongyao Zazhi* 2003; **28**: 957-961 [PMID: 15620188]
34 **Nyberg P**, Heikkilä P, Sorsa T, Luostarinen J, Heljasvaara R, Stenman UH, Pihlajaniemi T, Salo T. Endostatin inhibits human tongue carcinoma cell invasion and intravasation and blocks the activation of matrix metalloprotease-2, -9, and -13. *J Biol Chem* 2003; **278**: 22404-22411 [PMID: 12690120 DOI: 10.1074/jbc.M210325200]
35 **Purcell WT**, Rudek MA, Hidalgo M. Development of matrix metalloproteinase inhibitors in cancer therapy. *Hematol Oncol Clin North Am* 2002; **16**: 1189-1227 [PMID: 12512389]
36 **Zhang M**, Altuwaijri S, Yeh S. RRR-alpha-tocopheryl succinate inhibits human prostate cancer cell invasiveness. *Oncogene* 2004; **23**: 3080-3088 [PMID: 15048090 DOI: 10.1038/sj.onc.1207435]
37 **Zhou X**, Yang J, Wang Y, Li W, Li-Ling J, Deng Y, Zhang M. Cucurbitacin B inhibits 12-O-tetradecanoylphorbol 13-acetate-induced invasion and migration of human hepatoma cells through inactivating mitogen-activated protein kinase and PI3K/Akt signal transduction pathways. *Hepatol Res* 2012; **42**: 401-411 [PMID: 22151918 DOI: 10.1111/j.1872-034X.2011.00933.x]
38 **Hwang YP**, Yun HJ, Kim HG, Han EH, Lee GW, Jeong HG. Suppression of PMA-induced tumor cell invasion by dihydroartemisinin via inhibition of PKCalpha/Raf/MAPKs and NF-kappaB/AP-1-dependent mechanisms. *Biochem Pharmacol* 2010; **79**: 1714-1726 [PMID: 20152819 DOI: 10.1016/j.bcp.2010.02.003]
39 **Reunanen N**, Westermarck J, Häkkinen L, Holmström TH, Elo I, Eriksson JE, Kähäri VM. Enhancement of fibroblast collagenase (matrix metalloproteinase-1) gene expression by ceramide is mediated by extracellular signal-regulated and stress-activated protein kinase pathways. *J Biol Chem* 1998; **273**: 5137-5145 [PMID: 9478967]
40 **Kuppers DA**, Lan K, Knight JS, Robertson ES. Regulation of matrix metalloproteinase 9 expression by Epstein-Barr virus nuclear antigen 3C and the suppressor of metastasis Nm23-H1. *J Virol* 2005; **79**: 9714-9724 [PMID: 16014933 DOI: 10.1128/JVI.79.15.9714-9724.2005]
41 **Ohba K**, Miyata Y, Koga S, Kanda S, Kanetake H. Expression of nm23-H1 gene product in sarcomatous cancer cells of renal cell carcinoma: correlation with tumor stage and expression of matrix metalloproteinase-2, matrix metalloproteinase-9, sialyl Lewis X, and c-erbB-2. *Urology* 2005; **65**: 1029-1034 [PMID: 15882758 DOI: 10.1016/j.urology.2004.12.032]
42 **Hsieh HL**, Wu CY, Yang CM. Bradykinin induces matrix metalloproteinase-9 expression and cell migration through a PKC-delta-dependent ERK/Elk-1 pathway in astrocytes. *Glia* 2008; **56**: 619-632 [PMID: 18240315]
43 **Smallwood ND**, Hausman BS, Wang X, Liedtke CM. Involvement of NH2 terminus of PKC-delta in binding to F-actin during activation of Calu-3 airway epithelial NKCC1. *Am J Physiol Cell Physiol* 2005; **288**: C906-C912 [PMID: 15590896 DOI: 10.1152/ajpcell.00484.2004]
44 **Chakrabarti S**, Zee JM, Patel KD. Regulation of matrix metalloproteinase-9 (MMP-9) in TNF-stimulated neutrophils: novel pathways for tertiary granule release. *J Leukoc Biol* 2006; **79**: 214-222 [PMID: 16275891 DOI: 10.1189/jlb.0605353]
45 **Langlois A**, Chouinard F, Flamand N, Ferland C, Rola-

Pleszczynski M, Laviolette M. Crucial implication of protein kinase C (PKC)-delta, PKC-zeta, ERK-1/2, and p38 MAPK in migration of human asthmatic eosinophils. *J Leukoc Biol* 2009; **85**: 656-663 [PMID: 19164129 DOI: 10.1189/jlb.0808492]

46 **Park SK**, Hwang YS, Park KK, Park HJ, Seo JY, Chung WY. Kalopanaxsaponin A inhibits PMA-induced invasion by reducing matrix metalloproteinase-9 via PI3K/Akt- and PKCdelta-mediated signaling in MCF-7 human breast cancer cells. *Carcinogenesis* 2009; **30**: 1225-1233 [PMID: 19420016 DOI: 10.1093/carcin/bgp111]

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