# Benzyl isothiocyanate (BITC) inhibits migration and invasion of human gastric cancer AGS cells via suppressing ERK signal pathways

Chin-Chin Ho, Kuang-Chi Lai, Shu-Chun Hsu, Chao-Lin Kuo, Chia-Yu Ma, Meng-Liang Lin, Jai-Sing Yang and Jing-Gung Chung

*Hum Exp Toxicol* 2011 30: 296 originally published online 24 May 2010
DOI: 10.1177/0960327110371991

HET

Human and Experimental Toxicology
30(4) 296–306

DOI: 10.1177/0960327110371991
het.sagepub.com
SAGE

# Benzyl isothiocyanate (BITC) inhibits migration and invasion of human gastric cancer AGS cells via suppressing ERK signal pathways

**Chin-Chin Ho[1], Kuang-Chi Lai[2,3], Shu-Chun Hsu[4], Chao-Lin Kuo[5], Chia-Yu Ma[6], Meng-Liang Lin[7], Jai-Sing Yang[8] and Jing-Gung Chung[9,10]**

## Abstract

Metastasis suppressors and associated other regulators of cell motility play a critical initial role in tumor invasion and metastases. Benzyl isothiocyanate (BITC) is a hydrolysis compound of glucotropaeolin in dietary cruciferous vegetables. BITC has been found to exhibit prevention of cancers in laboratory animals and might also be chemoprotective in humans. Here, the purpose of this study was to investigate the effects of BITC on cell proliferation, migration, invasion and mitogen-activated protein kinase (MAPK) pathways of AGS human gastric cancer cells. Wound healing and Boyden chamber (migration and invasion) assays demonstrated that BITC exhibited an inhibitory effect on the abilities of migration and invasion in AGS cancer cells. BITC suppressed cell migration and invasion of AGS cells in a dose-dependent manner. Results from Western blotting indicated that BITC exerted an inhibitory effect on the ERK1/2, Ras, GRB2, Rho A, iNOS, COX-2 for causing the inhibitions of MMP-2, -7 and -9 then followed by the inhibitions of invasion and migration of AGS cells in vitro. BITC also promoted MKK7, MEKK3, c-jun, JNK1/2, VEGF, Sos1, phosphoinositide 3-kinase (PI3K), PKC, nuclear factor-kappaB (NF-κB) p65 in AGS cells. Results from real-time polymerized chain reaction (PCR) showed that BITC inhibited the gene expressions of MMP-2,-7 -9, FAK, ROCK1 and RhoA after BITC treatment for 24 and 48 hours in AGS cells. Taken together, the finding may provide new mechanisms and functions of BITC, which inhibit migration and invasion of human gastric cancer AGS cells.

## Keywords

BITC, migration, invasion, MMP-2, MMP-9, human gastric cancer AGS cells

[1] Department of Nursing, Central Taiwan University of Science and Technology, Taichung, Taiwan
[2] Department of Surgery, China Medical University Beigang Hospital, Yunlin, Taiwan
[3] School of Medicine, China Medical University, Taichung, Taiwan
[4] Department of Chemical Engineering, Hsiuping Institute of Technology, Taichung, Taiwan
[5] Department of Chinese Medicine Resources, China Medical University, Taichung, Taiwan
[6] Derpatment of Food and Beverage Management, Technology and Science Institute of Northern Taiwan, Taipei, Taiwan
[7] School of Medical Laboratory Science and Biotechnology, China Medical University, Taichung, Taiwan
[8] Derpatment of Pharmacology, China Medical University, Taichung, Taiwan
[9] Derpatment of Biological Science and Technology, China Medical University, Taichung, Taiwan
[10] Department of Biotechnology, Asia University, Taichung, Taiwan

**Corresponding author:**
Jing-Gung Chung, Department of Biological Science and Technology, China Medical University, No 91, Hsueh-Shih Road, Taichung 40402, Taiwan
E-mail: jgchung@mail.cmu.edu.tw

## Introduction

Currently in the world, gastric cancer is the fourth most common cancer and the second most common cause of cancer death.[1] The reports from the Department of Health, Executive Yuan, ROC (Taiwan), in year 2008 indicated that about 10.0 persons per 100 thousand die annually from gastric cancer. Epidemiological studies indicated that dietary intake of cruciferous vegetables may lower the risk of various types of malignancies.[2]

It is well documented that metastasis of cancer cells are involving various steps and cytophysiological changes including changed adhesion ability between cells and the extracellular matrix (ECM) and damaged intercellular interaction and the ECM degradation that is associated with invasion and migration of tumor cells. Especially, the overexpression of matrix metalloproteinases (MMPs) and urokinase plasminogen activator (u-PA) are shown in the metastasis of cancer cells and MMPs, which are involved in the invasion and metastasis of various tumor cells[3-5] such as MMP-2 and MMP-9, which are capable of degrading most ECM components.[6-8] The inhibition of MMP expressions or enzyme activities can be used as early targets for preventing cancer metastasis.[9-11]

The anticarcinogenic effect of cruciferous vegetables is attributed to organic isothiocyanates (ITCs) and benzyl-ITC (BITC) is one such compound that has attracted a great deal of research interest because of its remarkable anticancer effects.[2,12-14] In rodent models, BITC have been shown to be a potent inhibitor of mammary, lung and liver carcinogenesis induced by environmental and dietary carcinogens.[15-17] BITC can inhibit the proliferation of cancer cells in culture through causing cell-cycle arrest and/or apoptosis induction.[14,18-23] BITC induced cell death in the 1483 human head and neck cancer cell line was regulated by p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase.[20] Despite these evidences, however, the mechanism by which BITC-treatment triggers inhibition of cell metastasis and invasion in gastric cancer cells is not fully understood. For example, the signaling intermediates responsible for the initiation of BITC-mediated migration and invasion remain to be identified. Furthermore, the precise role of MMPs in BITC-mediated cell migration and invasion is unclear. Herein, we demonstrate that BITC treatment inhibits MMP-2 and -9 in gastric cancer cells to affect cell migration and invasion.

## Materials and methods

### Reagents

Benzyl isothiocyanates (BITC), dimethyl sulfoxide (DMSO) and Tris-HCl were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). RPMI-1640 medium, fetal bovine serum, L-glutamine, penicillin-streptomycin and trypsin-EDTA were obtained from Invitrogen (Carlsbad, California, USA). Primary antibodies used for Western blotting analysis were obtained as follows: antibodies for MMP-2, MMP-7, MMP-9, GRB2, COX-2, ERK1/2, FAK, RhoA, phosphoinositide 3-kinase (PI3K), p-ERK, PKC, Ras, Sos1, iNOS and nuclear factor-kappaB (NF-κB) and second antibody were purchased from Santa Cruz Biotechnology, (Santa Cruz,California) and diluted in phosphate buffered saline (PBS) Tween-20 before use.

Cell cultureThe AGS human gastric cancer cell line was purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were cultured in RPMI-1640 medium supplement with 10% fetal bovine serum, 2 mM L-glutamine adjusted to contain 100 Units/mL penicillin and 100 μg/mL streptomycin (Invitrogen) and were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ and 95% air.

### Wound healing assay

AGS cells ($1 \times 10^5$ cells/well) were placed in six-well plates for 24 hours and at confluence a wound was made with a pipette tip followed by washing with serum-free medium to remove cell debris as described previously.[24] Then, they were photographed under phase-contrast microscope (time = 0), incubated in media with or without BITC (0, 0.25 and 0.5 μM) at 37°C and 5% $CO_2$ and allowed to migrate into the wound area for up to 24 hours and 48 hours. Then they were photographed under phase-contrast microscope. Experiments were performed in triplicate.

### In vitro migration and invasion assays

The examination of AGS cells migration was performed through the chemotactic directional migration by using a 24-well Transwell insert.[25,26] The 8-μm pore filters (Millipore, Massachusetts, USA) were

coated with 30 μg type I collagen (Millipore) for 1 hour and AGS cells ($10^4$ cells/0.4 mL RPMI 1640) were placed in the upper chamber with or without BITC (0.25 or 0.5 μM) then to undergo migration for 24/48 hours. A cotton swab was used to remove the non-migrated cells in the upper chamber, then the filters were stained with 2% crystal violet and the migrated cells adherent to the underside of the filter were counted and photographed under a light microscope at ×200. Each treatment including control was assayed twice and three independent experiments were performed.[27]

The examination of AGS cells invasion was performed by using Matrigel-coated transwell cell culture chambers (8 μm pore size) as described previously.[25,26] AGS cells were cultured for 24 hours in serum-free-RPMI1640 medium, collected and resuspended in serum-free medium, placed in the upper chamber of the transwell insert ($5 \times 10^4$ cells/well) and treated with 0.5% DMSO (as a control) or BITC (0.25 and 0.5 μM). However, the RPMI1640 medium containing 10% fetal bovine serum (FBS) was placed in the lower chamber. All cells in each treatment were incubated for 24 or 48 hours at 37°C in a humidified atmosphere with 95% air and 5% $CO_2$. A cotton swab was used to remove the non-invasive cells maintained in the upper chamber and the invasive cells were fixed with 4% formaldehyde in PBS and stained with 2% crystal violet in 2% ethanol. The invasive cells in the lower surface of the filter which penetrated through the matrigel were counted and photographed under a light microscope at ×200.[27]

### Western blotting analysis

The cell migration and invasion association protein expression was performed previously.[27,28] AGS cells ($1 \times 10^6$ cells/well) were cultured in 6-well plates for 24 hours. BITC was added to cells (each well) at a final concentration of 0.5 μM, while DMSO (solvent) alone was added to control cells. All cells were incubated at 37°C for 0, 6, 12, 24 and 48 hours. Cells were collected and resuspended in ice-cold 50 mM potassium phosphate buffer (pH 7.4) containing 2 mM EDTA and 0.1% Triton X-100 for sonication. The homogenate was centrifuged at 13,000*g* for 10 min at 4°C to remove cell debris. The supernatant was collected and total protein was determined using a Bio-Rad protein assay kit (Hercules, California, USA) with bovine serum albumin (BSA) as the standard. Sodium dodecyl sulfate (SDS) gel electrophoresis and Western blotting were conducted to determine the effects of BITC on protein levels as described previously.[27,28]

### Real-time PCR of MMP-2, -7, and -9, FAK and RhoA

About $1 \times 10^6$ cells/well of AGS cells were cultured in 6-well plates and grown for 24 hours. BITC was added to cells for a final concentration of 2.5 μM for 24 and 48 hours. Cells were collected and total RNA was extracted using the Qiagen RNeasy Mini Kit as described previously.[24,27] RNA samples were reverse-transcribed at 42°C with High Capacity complementary DNA (cDNA) Reverse Transcription Kit for 30 min according to the protocol of the supplier (Applied Biosystems™, Foster City, CA 94404. USA). Quantitative PCR conditions were: 2 min at 50°C, 10 min at 95°C and 40 cycles of 15 sec at 95°C; 1 min at 60°C using 1 μL of the cDNA reverse-transcribed as described above, 2X SYBR Green PCR Master Mix (Applied Biosystems) and 200 nM of forward and reverse primers as shown in Table 1. Applied Biosystems 7300 Real-Time PCR system was used for each assay in triplicate and expression fold–changes were derived using the comparative $C_T$ method.[24,27]

### Statistical analysis

Statistical differences were performed by using the Student's *t* test and considered significant at the $*p < 0.05$. All data are presented as means ± SD of three experiments.

## Results

### BITC inhibited the migration of AGS cells

To investigate BITC affected cell migration, AGS cells were incubated with 0.25 and 0.5 μM of BITC and wound healing assay was induced for physical wounding of the cells plated, which was performed and results are shown in Figure 1A and B. The results indicated that an apparent and gradual increase of cells in the denude zone was observed under a light microscopy. The quantitative data in Figure 1B indicated that a significant inhibition of cell migration was noted and it was also a time-dependent effect.

### BITC inhibited the migration and invasion of AGS cells

To further investigate BITC affect cell migration, AGS cells were incubated with 0, 0.25 and 0.5 μM BITC for 24 and 48 hours, AGS cells have an ability

**Table 1.** The DNA sequence was evaluated using the primer expression

| Primer name | Primer sequence |
|---|---|
| Homo MMP-2-F | CCCCAGACAGGTGATCTTGAC |
| Homo MMP-2-R | GCTTGCGAGGGAAGAAGTTG |
| Homo MMP-7-F | GGATGGTAGCAGTCTAGGGATTAACT |
| Homo MMP-7-R | AGGTTGGATACATCACTGCATTAGG |
| Homo FAK-F | TGAATGGAACCTCGCAGTCA |
| Homo FAK-R | TCCGCATGCCTTGCTTTT |
| Homo RhoA-F | TCAAGCCGGAGGTCAACAAC |
| Homo RhoA-R | ACGAGCTGCCCATAGCAGAA |
| Homo ROCK1-F | ATGAGTTTATTCCTACACTCTACCACTTTC |
| Homo ROCK1-R | TAACATGGCATCTTCGACACTCTAG |
| Homo GAPDH-F | ACACCCACTCCTCCACCTTT |
| Homo GAPDH-R | TAGCCAAATTCGTTGTCATACC |

MMP, matrix metalloproteinase; FAK, focal adhesion kinase; RhoA, RAS homologue gene family member A; ROCK1, Rho-associated coiled coil-containing kinase; GAPDH, glyceraldehydes-3-phosphate dehydrogenase. Each assay was conducted at least twice to ensure reproducibility.

to migrate through filters. Results are shown in Figure 2A and B, which indicate that BITC had a significant inhibitory effect on cell migration at concentrations between 0.25 and 0.5 μM and the percent inhibition was at 42%–43% and 37%–44% when cells were incubated with BITC for 24 and 48 hours treatment, respectively. AGS cells have an ability to invade through the filter were coated with Matrigel, and BITC exhibited the significant inhibition of cell invasion at 35%–36% and 33%–50%; this effect in a dose-dependent manner, which is shown in Figure 2C and D. However, the penetration of the Matrigel-coated filter by AGS cells was inhibited in the presence of BITC

### *BITC affected levels of proteins associated with cell migration and invasion in AGS cells*

To determine the effects of BITC on the protein levels associated with migration and invasion in AGS cells, Western blotting was performed and results are shown in Figure 3A–D, which indicate that BITC reduced levels of ERK1/2 (Figure 3A), Ras, GRB2, Rho A (Figure 3B), iNOS, COX-2, uPA (Figure 3C) and MMP-2, MMP-7, MMP-9 (Figure 3D) but increased protein levels of MKK7, MEKK3, c-jun, JNK1/2 (Figure 3A), VEGF, Sos1, PI3K, PKC (Figure 3B), NF-κB p65 (Figure 3C) in examined AGS cells. These effects may lead to the inhibition of migration and invasion of AGS cells.

### *BITC affected MMP-2, MMP-7, MMP-9, FAK, RhoA and ROCK1 mRNA expressions in AGS cells*

To further investigate whether BITC affected migration- and invasion-associated gene expression in AGS cells, cells were treated with BITC (2.5 μM) for 0, 24 and 48 hours. Total RNA was isolated and gene expressions were performed by real-time PCR. The results shown in Figure 4 indicated that the expression levels of MMP-2, MMP-7 and MMP-9 were inhibited after BITC treatment for 24 and 48 hours (Figure 4A). Moreover, FAK, RhoA and ROCK1 mRNA also were decreased at 24 and 48 hours treatment (Figure 4B).

## Discussion

Numerous studies have shown that BITC induced apoptosis in human cancer cells,[2,12-14] but the information regarding the effect of BITC on AGS cell migration and invasion is still not clear. Therefore, in the present study, we offered the effects of BITC on migration and invasion of BITC on human gastric AGS cancer cells and associated protein levels and gene expressions is still unclear.

Our results showed that BITC inhibited the migration and invasion based on the observation such as wound healing assay and migration and invasion assays, and these effects are in dose- and time-dependent manners. Furthermore, we found that BITC decreased the migration- and invasion-associated protein levels such as ERK1/2 (Figure 3A), Ras, GRB2, Rho A (Figure 3B), iNOS, COX-2, uPA (Figure 3C) and MMP-2, MMP-7, MMP-9 (Figure 3D).

It is well documented that cancer cell metastasis is dependent on tumor cell adhesion to the ECM, proteolytic cleavage or destruction of the ECM and cell migration through the resultant defect. MMPs (basement membrane degrading enzymes) are a large family of zinc-dependent endopeptidases and they play an

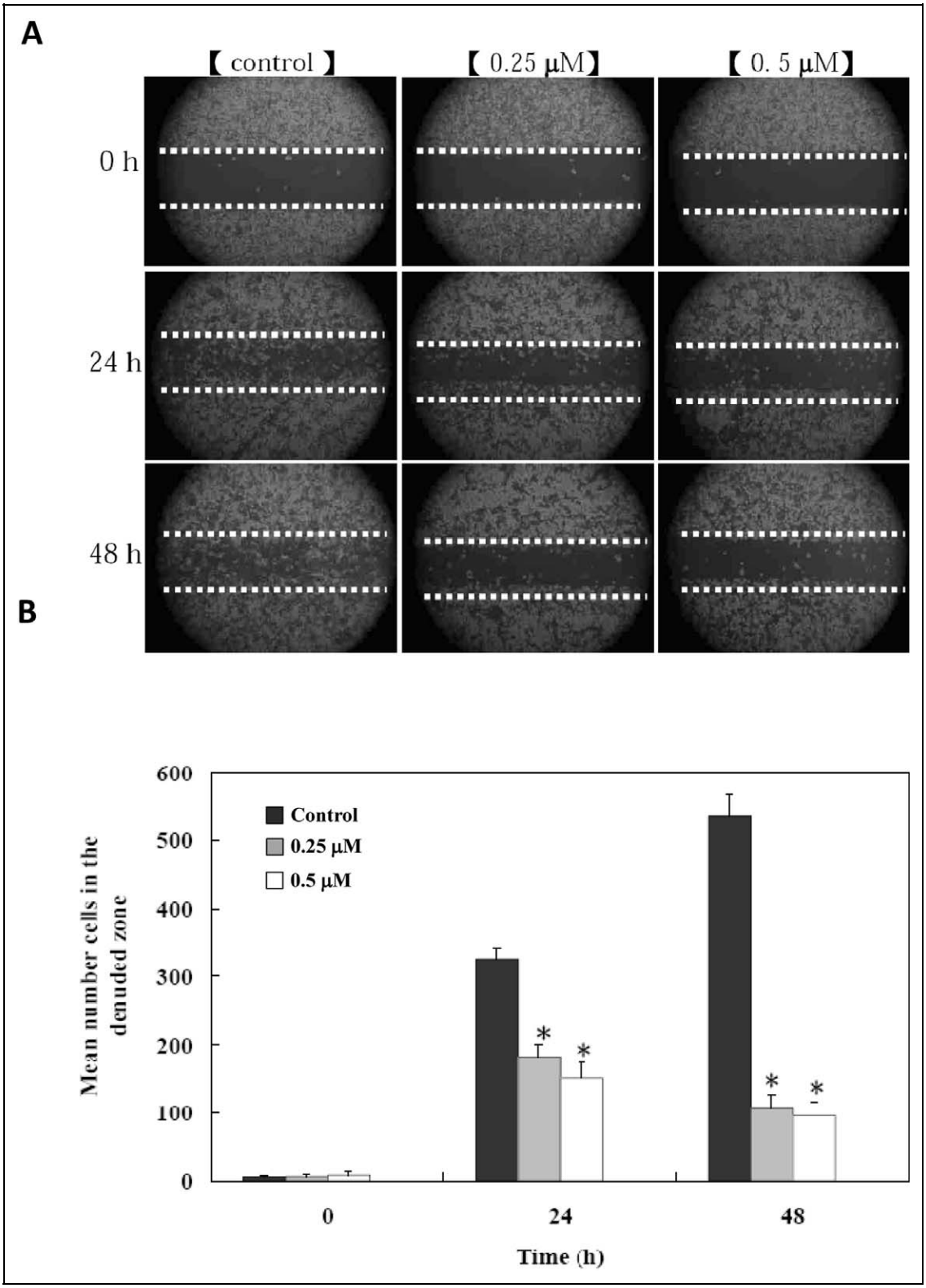


**Figure 1.** Benzyl isothiocyanate (BITC) affected on migration of AGS cells by wound-healing assay. AGS cells were placed on the plates and the injury line was made with a tip. The cells were then incubated with or without 0.25 and 0.5 μM BITC for 0, 24 and 48 hours. (A) Cell migration was examined under microscopy at the indicated time points (×100). (B) The measured width of injury lines was plotted as a percentage of the width at 0 hours. Each bar represents the mean ± SD ($n = 3$).

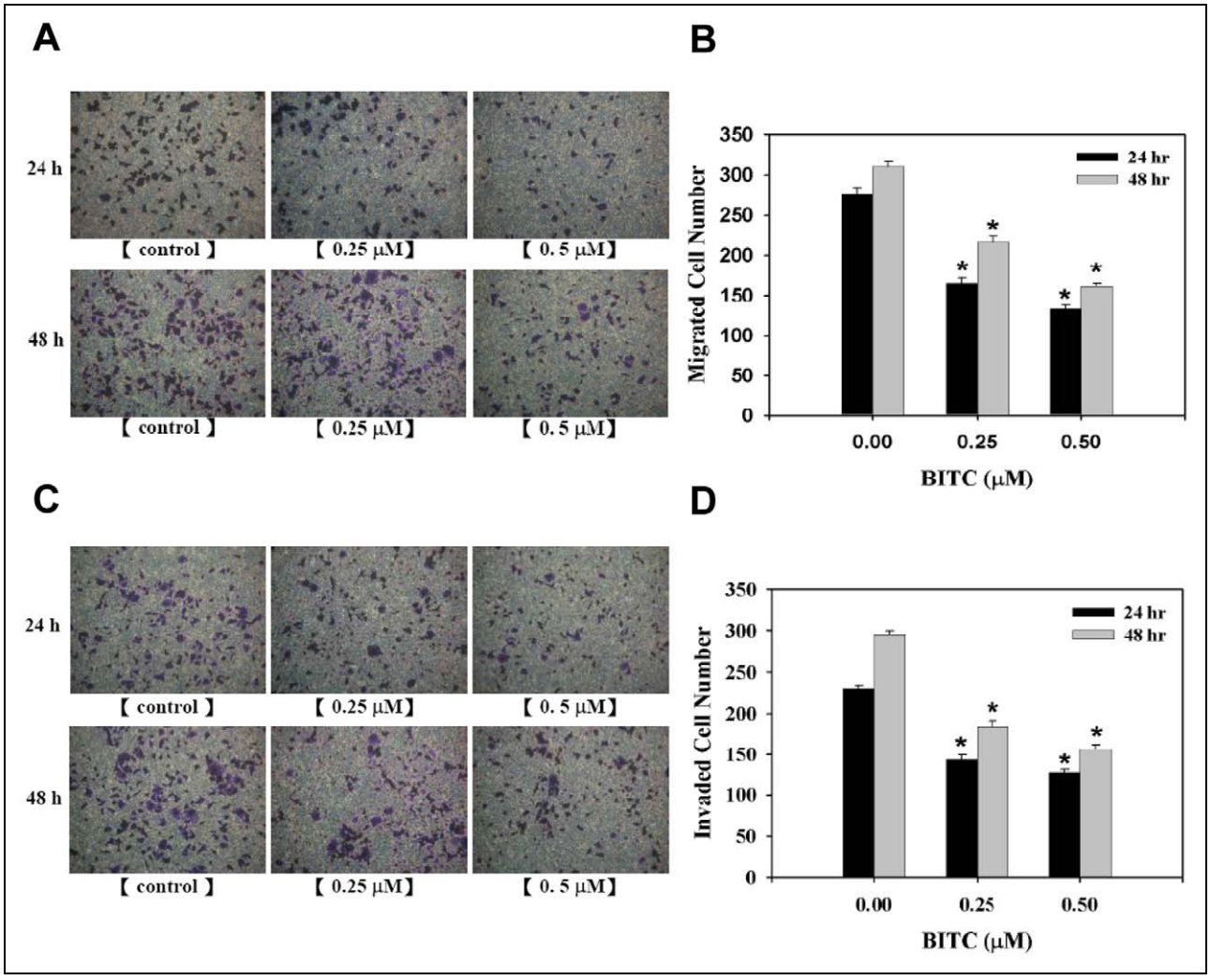


**Figure 2.** Benzyl isothiocyanate (BITC) affected cell migration and invasion of AGS cells. AGS cells were treated with 0, 0.25 and 0.5 μM of BITC for 24 and 48 hours. (A) Cell migration was examined in a Boyden chamber with polycarbonate filters (pore size, 8 μm). (C) Cell invasion was examined in a Boyden chamber; polycarbonate filters (pore size, 8 μm) were precoated with matrigel. Migration (B) and invasion (D) ability of AGS cells were quantified by counting the number of cells that invaded the underside of the porous polycarbonate membrane under microscopy and represent the average of three experiments. *$p < 0.01$ was compared with the untreated control.

important role in tumor angiogenesis, metastasis and growth factor release from the ECM.[29] MMP-2 and MMP-9 have been demonstrated to be associated with the invasive metastatic potential of tumor cells.[9] Overexpression of MMP-2,[30,31] MMP-7[32,33] and MMP-9[34] has been observed in gastric cancer. Our results also showed that BITC suppressed *MMP-2 and MMP-9* gene expression via suppressing the PKCs/MAPK and PI3K/AKT/NF-κB cascades with consequent suppression of migration and invasion by human gastric cancer AGS cells. Our result also showed that BITC inhibited the protein and mRNA levels of MMP-2 in AGS cells. This is in agreement with other reports that showed BITC was also found to inhibit the expression of MT1-MMP mRNA. Because MT1-MMP is responsible for the activation of MMP-2,[35,36] this inhibition by BITC may contribute to the decreased MMP-2 activity at both the protein and mRNA levels.

In the next experiment, we examined the effects of BITC on MAPK pathway including ERKs, p38 kinase and JNK levels in AGS cells. The results from Western blotting demonstrated that BITC inhibited the levels of ERK1/2. These protein kinases are MAPK family enzymes associated with MMP-9 expression in both gene and protein levels. MAPK signalling is involved in the synthesis of MMPs and their activation.[37,38] The results in the present study

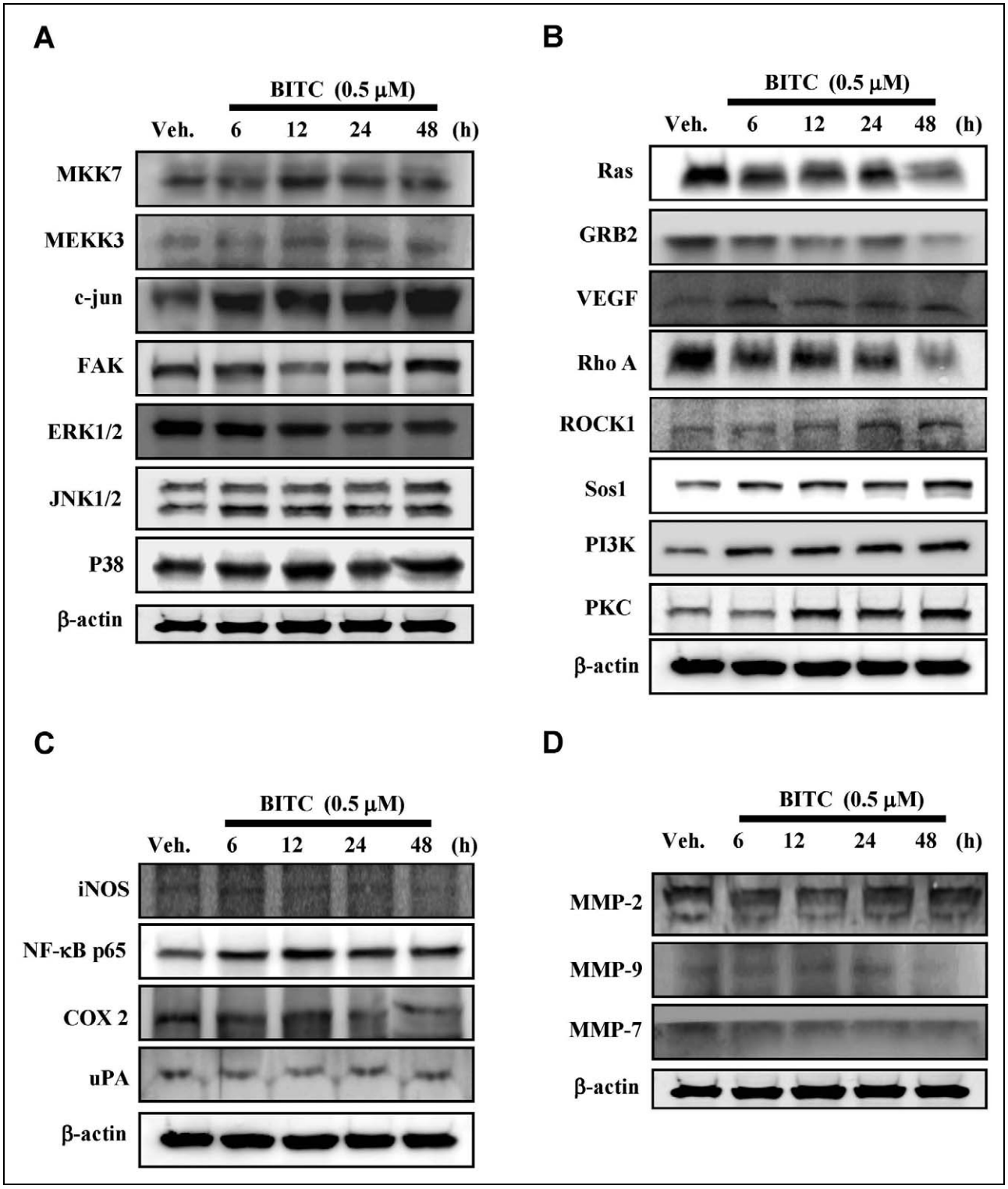


**Figure 3.** Benzyl isothiocyanate (BITC) affected the protein levels associated with migration and invasion in AGS cells. AGS cells were treated with 0.5 μM of BITC for 6, 12, 24 and 48 hours. The total proteins were collected and the protein levels (A): MKK7, MEKK3, c-jun, FAK, ERK1/2, JNK1/2 and P38; (B): Ras, GRB2. VEGF, Rho A, ROCK-1, Sos1, PI3K and PKC; (C): iNOS, NF-κB, COX-2 and u-PA; (D): MMP-2, MMP-7 and MMP-9 were examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting as described in 'Materials and methods.'

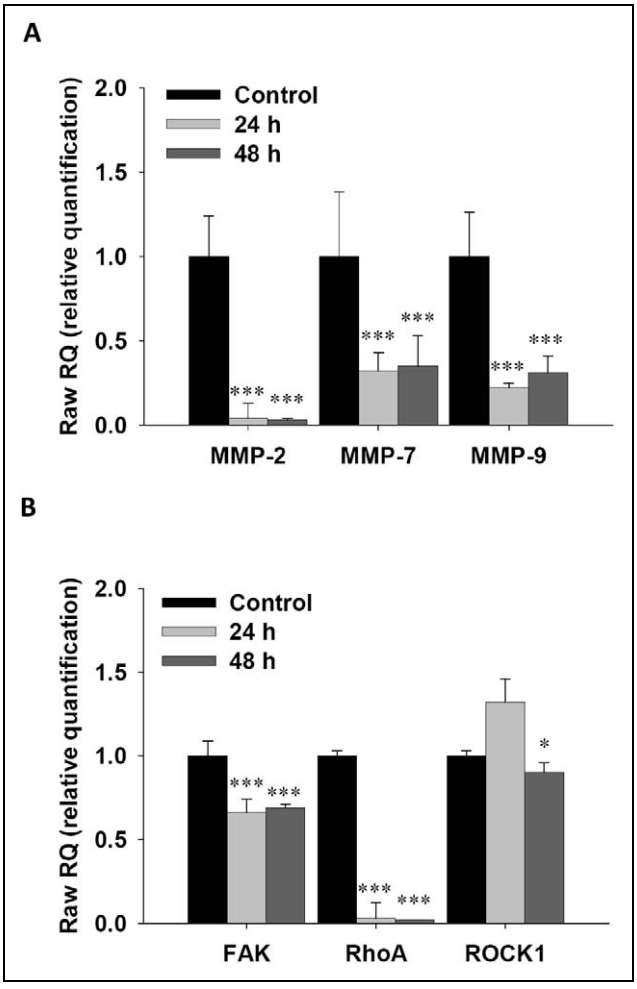


**Figure 4.** Benzyl isothiocyanate (BITC) affected MMP-2, MMP-7, MMP-9, FAK and RhoA mRNA expressions in AGS cells. The total RNA was extracted from BITC (2.5 μM) treatment on AGS cells for 0, 24 and 48 hours, and RNA samples were reverse-transcribed cDNA then for real-time PCR as described in 'Materials and Methods.' The ratios of (A) MMP-2, MMP-7 and MMP-9, (B) FAK, RhoA and ROCK1 mRNA/GAPDH are presented in panel (A) and (B). Data represents mean ± SD of three experiments. *$p < 0.05$ and ***$p < 0.001$ were considered significant.

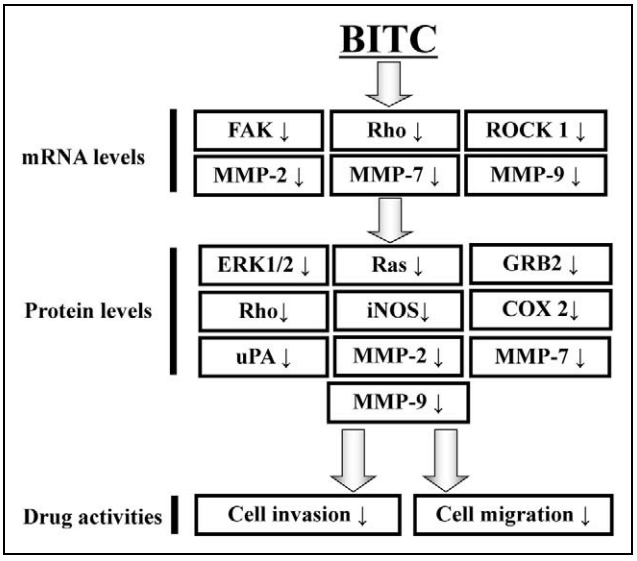


**Figure 5.** The proposed mechanisms of benzyl isothiocyanate (BITC)-inhibited migration and invasion of human gastric cancer AGS cells.

also showed that the regulation of NF-κB, and the upstream of the PI3K/Akt and MAPK (ERK1/2, p38 and JNK) pathways, might be involved in BITC-suppressed MMP-9 expression and invasion in GAS cells. Furthermore, ERK pathways have been found to regulate MMP-2 expression.[39,40] The suppression of ERK activation inhibits MMP-2 promoter activity. The highly metastatic tumors present have MMP-2 overexpressed, and MMP-9 can be stimulated by TNF-α,[41] a growth factor such as VEGF, EGF and TGF-β,[42-45] or Ras oncogene[46-48] through the activation of different intracellular-signaling pathways. The activation of PKC led to translocation of the protein to membranes and for the control of MMP-9 expression via modulating the activation of transcription factors including NF-κB or Sp-1 through MAPK and PI3K signaling pathways.[42-47,49,50] The activation of NF-κB is involved in the induction of the *MMP-9* gene associated with the invasion and metastasis of tumor cells.[41,51] Our results also showed that BITC decreased the levels of uPA, which had been shown to play a major role in the decomposition of basement membranes.[52,53] Several reports indicated that ERK1/2, p38 MAPK and JNK/SAPK play a central role in regulating the expression of MMPs and u-PA.[54-57]

In the present study, BITC also decreased phosphorylation of ERK. Future studies are needed to explore the relationship between the expression of MMPs and phosphorylation of ERK in BITC-treated gastric cancer cells. In summary, the present results may be relevant to the therapeutic targeting of invasion and migration of gastric cancer cells based on the molecular mechanism of BITC through blocking ERK signaling pathways, as well as uPA led to the inhibition of MMP-2 and MMP-9 (Figure 5).

## Funding

This work was supported by grant CMU96-047 from China Medical University, Taichung, Taiwan.

## References


1. Ajani JA. Evolving chemotherapy for advanced gastric cancer. *Oncologist* 2005; 3: 49–58.
2. Conaway CC, Yang YM, Chung FL. Isothiocyanates as cancer chemopreventive agents: their biological activities and metabolism in rodents and humans. *Curr Drug Metab* 2002; 3: 233–255.
3. Kilian M, Gregor JI, Heukamp I, et al. Matrix metalloproteinase inhibitor RO 28-2653 decreases liver metastasis by reduction of MMP-2 and MMP-9 concentration in BOP-induced ductal pancreatic cancer in Syrian Hamsters: inhibition of matrix metalloproteinases in pancreatic cancer. *Prostaglandins Leukot Essent Fatty Acids* 2006; 75: 429–434.
4. Mizutani K, Kofuji K, Shirouzu K. The significance of MMP-1 and MMP-2 in peritoneal disseminated metastasis of gastric cancer. *Surg Today* 2000; 30: 614–621.
5. Gullu IH, Kurdoglu M, Akalin I. The relation of gelatinase (MMP-2 and -9) expression with distant site metastasis and tumour aggressiveness in colorectal cancer. *Br J Cancer* 2000; 82: 249.
6. Donadio AC, Remedi MM, Susperreguy S, et al. Extracellular matrix metalloproteinase inducer (EMMPRIN) and matrix metalloproteinases (MMPs) as regulators of tumor-host interaction in a spontaneous metastasis model in rats. *Histochem Cell Biol* 2008; 130: 1155–1164.
7. Cortes-Reynosa P, Robledo T, Macias-Silva M, et al. Src kinase regulates metalloproteinase-9 secretion induced by type IV collagen in MCF-7 human breast cancer cells. *Matrix Biol* 2008; 27: 220–231.
8. Bernhard EJ, Gruber SB, Muschel RJ. Direct evidence linking expression of matrix metalloproteinase 9 (92-kDa gelatinase/collagenase) to the metastatic phenotype in transformed rat embryo cells. *Proc Natl Acad Sci U S A* 1994; 91: 4293–4297.
9. Guruvayoorappan C, Kuttan G. Amentoflavone inhibits experimental tumor metastasis through a regulatory mechanism involving MMP-2, MMP-9, prolyl hydroxylase, lysyl oxidase, VEGF, ERK-1, ERK-2, STAT-1, NM23 and cytokines in lung tissues of C57BL/6 mice. *Immunopharmacol Immunotoxicol* 2008; 30: 711–727.
10. Okada N, Ishida H, Murata N, et al. Matrix metalloproteinase-2 and -9 in bile as a marker of liver metastasis in colorectal cancer. *Biochem Biophys Res Commun* 2001; 288: 212–216.
11. Waas ET, Wobbes T, Lomme RM, et al. Matrix metalloproteinase 2 and 9 activity in patients with colorectal cancer liver metastasis. *Br J Surg* 2003; 90: 1556–1564.
12. Hecht SS. Inhibition of carcinogenesis by isothiocyanates. *Drug Metab Rev* 2000; 32: 395–411.
13. Talalay P, Fahey JW. Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *J Nutr* 2001; 131: 3027S–3033S.
14. Miyoshi N, Uchida K, Osawa T, et al. A link between benzyl isothiocyanate-induced cell cycle arrest and apoptosis: involvement of mitogen-activated protein kinases in the Bcl-2 phosphorylation. *Cancer Res* 2004; 64: 2134–2142.
15. Lin JM, Amin S, Trushin N, et al. Effects of isothiocyanates on tumorigenesis by benzo[a]pyrene in murine tumor models. *Cancer Lett* 1993; 74: 151–159.
16. Sugie S, Okumura A, Tanaka T, et al. Inhibitory effects of benzyl isothiocyanate and benzyl thiocyanate on diethylnitrosamine-induced hepatocarcinogenesis in rats. *Jpn J Cancer Res* 1993; 84: 865–870.
17. Wattenberg LW. Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. *J Natl Cancer Inst* 1977; 58: 395–398.
18. Lui VW, Wentzel AL, Xiao D, et al. Requirement of a carbon spacer in benzyl isothiocyanate-mediated cytotoxicity and MAPK activation in head and neck squamous cell carcinoma. *Carcinogenesis* 2003; 24: 1705–1712.
19. Yang YM, Conaway CC, Chiao JW, et al. Inhibition of benzo(a)pyrene-induced lung tumorigenesis in A/J mice by dietary *N*-acetylcysteine conjugates of benzyl and phenethyl isothiocyanates during the postinitiation phase is associated with activation of mitogen-activated protein kinases and p53 activity and induction of apoptosis. *Cancer Res* 2002; 62: 2–7.
20. Zhang Y, Tang L, Gonzalez V. Selected isothiocyanates rapidly induce growth inhibition of cancer cells. *Mol Cancer Ther* 2003; 2: 1045–1052.
21. Jakubikova J, Sedlak J, Bacon J, et al. Effects of MEK1 and PI3K inhibitors on allyl-, benzyl- and phenylethyl-isothiocyanate-induced G2/M arrest and cell death in Caco-2 cells. *Int J Oncol* 2005; 27: 1449–1458.
22. Srivastava SK, Singh SV. Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. *Carcinogenesis* 2004; 25: 1701–1709.
23. Xiao D, Vogel V, Singh SV. Benzyl isothiocyanate-induced apoptosis in human breast cancer cells is initiated by reactive oxygen species and regulated by Bax and Bak. *Mol Cancer Ther* 2006; 5: 2931–2945.

24. Ho YT, Yang JS, Li TC, et al. Berberine suppresses in vitro migration and invasion of human SCC-4 tongue squamous cancer cells through the inhibitions of FAK, IKK, NF-kappaB, u-PA and MMP-2 and -9. *Cancer Lett* 2009; 279: 155–162.
25. Lu HF, Wang HL, Chuang YY, et al. Danthron induced apoptosis through mitochondria and caspase-3-dependent pathways in human brain glioblastoma multiforms GBM 8401 Cells. *Neurochem Res* 2010; 35: 390–398.
26. Lin SS, Lai KC, Hsu SC, et al Curcumin inhibits the migration and invasion of human A549 lung cancer cells through the inhibition of matrix metalloproteinase-2 and -9 and vascular endothelial growth factor (VEGF). *Cancer Lett* 2009; 285: 127–133.
27. Lin CC, Chen JT, Yang JS, et al. Danthron inhibits the migration and invasion of human brain glioblastoma multiforme cells through the inhibition of mRNA expression of focal adhesion kinase, Rho kinases-1 and metalloproteinase-9. *Oncol Rep* 2009; 22: 1033–1037.
28. Lu HF, Lai KC, Hsu SC, et al. Curcumin induces apoptosis through FAS and FADD, in caspase-3-dependent and -independent pathways in the N18 mouse-rat hybrid retina ganglion cells. *Oncol Rep* 2009; 22: 97–104.
29. Liotta LA, Stetler-Stevenson WG. Tumor invasion and metastasis: an imbalance of positive and negative regulation. *Cancer Res* 1991; 51: 5054s–5059s.
30. Monig SP, Baldus SE, Hennecken JK, et al. Expression of MMP-2 is associated with progression and lymph node metastasis of gastric carcinoma. *Histopathology* 2001; 39: 597–602.
31. Murray GI, Duncan ME, Arbuckle E, et al. Matrix metalloproteinases and their inhibitors in gastric cancer. *Gut* 1998; 43: 791–797.
32. Honda M, Mori M, Ueo H, et al. Matrix metalloproteinase-7 expression in gastric carcinoma. *Gut* 1996; 39: 444–448.
33. Yamashita K, Azumano I, Mai M, et al. Expression and tissue localization of matrix metalloproteinase 7 (matrilysin) in human gastric carcinomas. Implications for vessel invasion and metastasis. *Int J Cancer* 1998; 79: 187–194.
34. Kabashima A, Maehara Y, Kakeji Y, et al. Clinicopathological features and overexpression of matrix metalloproteinases in intramucosal gastric carcinoma with lymph node metastasis. *Clin Cancer Res* 2000; 6: 3581–3584.
35. Ogata R, Torimura T, Kin M, et al. Increased expression of membrane type 1 matrix metalloproteinase and matrix metalloproteinase-2 with tumor dedifferentiation in hepatocellular carcinomas. *Hum Pathol* 1999; 30: 443–450.
36. Tam EM, Moore TR, Butler GS, et al. Characterization of the distinct collagen binding, helicase and cleavage mechanisms of matrix metalloproteinase 2 and 14 (gelatinase A and MT1-MMP): the differential roles of the MMP hemopexin c domains and the MMP-2 fibronectin type II modules in collagen triple helicase activities. *J Biol Chem* 2004; 279: 43336–43344.
37. Munshi HG, Wu YI, Mukhopadhyay S, et al. Differential regulation of membrane type 1-matrix metalloproteinase activity by ERK 1/2- and p38 MAPK-modulated tissue inhibitor of metalloproteinases 2 expression controls transforming growth factor-beta1-induced pericellular collagenolysis. *J Biol Chem* 2004; 279: 39042–39050.
38. Zhong J, Gencay MM, Bubendorf L, et al. ERK1/2 and p38 MAP kinase control MMP-2, MT1-MMP, and TIMP action and affect cell migration: a comparison between mesothelioma and mesothelial cells. *J Cell Physiol* 2006; 207: 540–552.
39. Mitra A, Chakrabarti J, Banerji A, et al. Culture of human cervical cancer cells, SiHa, in the presence of fibronectin activates MMP-2. *J Cancer Res Clin Oncol* 2006; 132: 505–513.
40. Zhang D, Bar-Eli M, Meloche S, et al. Dual regulation of MMP-2 expression by the type 1 insulin-like growth factor receptor: the phosphatidylinositol 3-kinase/Akt and Raf/ERK pathways transmit opposing signals. *J Biol Chem* 2004; 279: 19683–19690.
41. Nee L, Tuite N, Ryan MP, et al. TNF-alpha and IL-1 beta-mediated regulation of MMP-9 and TIMP-1 in human glomerular mesangial cells. *Nephron Exp Nephrol* 2007; 107: e73–e86.
42. Cowden Dahl KD, Symowicz J, Ning Y, et al. Matrix metalloproteinase 9 is a mediator of epidermal growth factor-dependent e-cadherin loss in ovarian carcinoma cells. *Cancer Res* 2008; 68: 4606–4613.
43. Hollborn M, Stathopoulos C, Steffen A, et al. Positive feedback regulation between MMP-9 and VEGF in human RPE cells. *Invest Ophthalmol Vis Sci* 2007; 48: 4360–4367.
44. Lee SO, Jeong YJ, Kim M, et al. Suppression of PMA-induced tumor cell invasion by capillarisin via the inhibition of NF-kappaB-dependent MMP-9 expression. *Biochem Biophys Res Commun* 2008; 366: 1019–1024.
45. Zeisberg M, Maeshima Y, Mosterman B, et al. Renal fibrosis. Extracellular matrix microenvironment regulates migratory behavior of activated tubular epithelial cells. *Am J Pathol* 2002; 160: 2001–2008.

46. Cho HJ, Kang JH, Kwak JY, et al. Ascofuranone suppresses PMA-mediated matrix metalloproteinase-9 gene activation through the Ras/Raf/MEK/ERK- and Ap1-dependent mechanisms. *Carcinogenesis* 2007; 28: 1104–1110.
47. Zhang S, Li Z, Wu X, et al. Methyl-3-indolylacetate inhibits cancer cell invasion by targeting the MEK1/2-ERK1/2 signaling pathway. *Mol Cancer Ther* 2006; 5: 3285–3293.
48. Chung TW, Lee YC, Kim CH. Hepatitis B viral HBx induces matrix metalloproteinase-9 gene expression through activation of ERK and PI-3K/AKT pathways: involvement of invasive potential. *FASEB J* 2004; 18: 1123–1125.
49. Chakraborti S, Mandal M, Das S, et al. Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem* 2003; 253: 269–285.
50. Simon C, Simon M, Vucelic G, et al. The p38 SAPK pathway regulates the expression of the MMP-9 collagenase via AP-1-dependent promoter activation. *Exp Cell Res* 2001; 271: 344–355.
51. Woo JH, Lim JH, Kim YH, et al. Resveratrol inhibits phorbol myristate acetate-induced matrix metalloproteinase-9 expression by inhibiting JNK and PKC delta signal transduction. *Oncogene* 2004; 23: 1845–1853.
52. Duffy MJ, Maguire TM, McDermott EW, et al. Urokinase plasminogen activator: a prognostic marker in multiple types of cancer. *J Surg Oncol* 1999; 71: 130–135.
53. Kwaan HC. The plasminogen-plasmin system in malignancy. *Cancer Metastasis Rev* 1992; 11: 291–311.
54. Chen PN, Hsieh YS, Chiou HL, et al. Silibinin inhibits cell invasion through inactivation of both PI3K-Akt and MAPK signaling pathways. *Chem Biol Interact* 2005; 156: 141–150.
55. Kwon GT, Cho HJ, Chung WY, et al. Isoliquiritigenin inhibits migration and invasion of prostate cancer cells: possible mediation by decreased JNK/AP-1 signaling. *J Nutr Biochem* 2009; 20: 663–676.
56. Lee SJ, Park SS, Lee US, et al. Signaling pathway for TNF-alpha-induced MMP-9 expression: mediation through p38 MAP kinase, and inhibition by anticancer molecule magnolol in human urinary bladder cancer 5637 cells. *Int Immunopharmacol* 2008; 8: 1821–1826.
57. Sun LC, Luo J, Mackey LV, et al. A conjugate of camptothecin and a somatostatin analog against prostate cancer cell invasion via a possible signaling pathway involving PI3K/Akt, alphaVbeta3/alphaVbeta5 and MMP-2/-9. *Cancer Lett* 2007; 246: 157–166.