Effects of *Coriandrum sativum* on Migration and Invasion Abilities of Cancer Cells

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Summary *Coriandrum sativum* (coriander) is an annual herb in the Apiaceae family. Its leaves and seeds are used for cooking. Coriander has several beneficial functions such as anti-inflammatory, analgesic and anti-cancer effects. Although anti-carcinogenic potential of coriander has been known well, the effects of coriander on cancer metastasis have not yet been fully elucidated. In the present study, the effects of coriander on migration and invasion were investigated in vitro and in vivo by using human hepatocellular carcinoma cell line (HepG2) and mouse melanoma cell line (B16F10). The migration and invasion abilities of cancer cells had been evaluated by trans-well double chamber and these abilities were significantly impaired by treatment of cancer cells with coriander extract whose concentration did not affect proliferation. The treatment of cancer cells with coriander extract significantly reduced both matrix metalloproteinase 2 (MMP-2) and urokinase-type plasminogen activator (u-PA) activities, which were involved in cell migration and invasion, in their conditioned media. Furthermore, coriander extract suppressed the phosphorylation of Erk 1 or IkB in B16F10 cells, and inhibited the expression of MMP-2 or u-PA mRNA. After injection of B16F10 cells into the tail vein of C57BL/6J mice, the number of metastatic regions in lungs were counted. Mice fed with diet containing coriander possessed a smaller number of metastatic regions than those fed with control diet. It was suggested that coriander extract might have the abilities to suppress cancer cell migration and invasion, indicating that coriander provides the improvement of cancer prognosis.

Key Words coriander, MMP-2, u-PA, metastasis, hepatocellular carcinoma, HepG2, melanoma, B16F10

*Coriandrum sativum* (coriander) is an annual herb in the Apiaceae family which has been wildly used in cooking as a spice worldwide (1). The leaves of coriander are used as decoration or flavor source in Asian and Mexican dishes and used in the process for pickling vegetables outside of Asia (2, 3). On the other hand, the coriander roots, with more intense flavor than the leaves, are used in a variety of Asian cuisines, especially in Thai dishes as soup or curry pastes.

In recent years, it was reported that coriander had several physiological properties, such as anti-inflammatory, analgesic, antioxidant, anti-diabetic, anti-mutagenic and anti-carcinogenic effects (4–6). In progression of malignant tumor, not only proliferation but also metastasis is important and critical. Migration and invasion of cancer cells are involved in cancer metastasis. Although there were several reports about anti-carcinogenic and anti-proliferative effects of coriander (6–8), the effect of coriander on migration and invasion of cancer cells has not yet been fully elucidated. Therefore, the aim of this study was to investigate the effects of coriander on migration and invasion of cancer cells and the underlying molecular mechanisms.

Liver cancer is the fifth most diagnosed cancer in men and the ninth most diagnosed cancer in women. The new liver cancer cases in 2018 were almost 840,000, and it was also a leading cause of cancer deaths worldwide (9). Melanoma is known to be the most dangerous type of skin cancers, with nearly 300,000 new cases worldwide in 2018 (9). In this study, the effects of coriander on migration and invasion abilities of cancer cells were examined by using two different types of cancer cells lines, HepG2, and B16F10. HepG2, liver cancer cell line, was derived from human liver tissue with a well-differentiated hepatocellular carcinoma (10). B16F10 is a murine melanoma cell line from a C57BL/6J mouse (11).

Metastasis plays a critical role in the progression of malignant tumors. The majority of cancer patients...
have metastasis which is associated with an extremely poor prognosis. During metastasis, tumor cells secrete certain proteins, growth factors, and cytokines through activation of specific signaling pathways, allowing them to invade the surrounding tissues \((12)\). The metastatic cascades including migration and invasion are dependent on alteration of cytoskeletal proteins, cell adhesion ability, and degradation of extracellular matrix (ECM). It is well known that matrix metalloproteinases (MMPs) \((13, 14)\) and/or urokinase-type plasminogen activator (u-PA), are overexpressed in metastatic process \((15)\). MMPs directly destroy ECM \((16)\) and u-PA induces the degradation of ECM through activation of plasminogen to plasmin. Plasmin does not only destroy ECM, but also activates pro-MMPs into MMPs \((17)\).

In general, the expressions of MMP-2 and u-PA are regulated by signal transduction. In MAPKs signaling pathway, phosphorylation of Erk can upregulate the gene expressions of MMPs and u-PA \((18, 19)\). Similar regulation can be observed when NF-κB is phosphorylated in IKK signaling pathway \((20, 21)\). Therefore, it is suggested that materials, which inhibit the expressions of MMPs and u-PA through signal transduction, may prevent cancer metastasis.

Several findings mentioned above, lead us to investigate the role of coriander in regulation of cancer cell migration and invasion. Therefore, we aimed to clarify the effects of coriander on cancer migration and invasion in vitro and in vivo.

**MATERIALS AND METHODS**

**Extraction of Coriandrum sativum.** Coriandrum sativum used in this study was cultivated in Gunma Prefecture, Japan and purchased from a local market. In order to prepare the extract of coriander, leaves of coriander \((20 \text{ g})\) were lyophilized and dissolved in 10 mL of methanol for 24–48 h. Then, the solution was centrifuged at 3,000 \(×g\) for 10 min to remove the impurities. The supernatant was dried out by evaporator. The extract \((2 \text{ g})\) after evaporation was dissolved in 2 mL of ethanol followed by filtration with Steradisc S25 0.2 \(\mu\text{m}\) (Kurabo, Tokyo, Japan). The extract of coriander was stored at \(-20^\circ\text{C}\) for further use.

**Cell culture.** Human HepG2 cancer cell line was obtained from American Type Culture Collection (ATCC, VA, USA). HepG2 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Nissui Pharmaceutical Co., Tokyo, Japan) with 10% FBS at 37°C in humidified 5% CO\(_2\). Mice B16F10 cancer cell line was obtained from Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University, Tohoku, Japan. B16F10 cells were cultured in Roswell Park Memorial Institute culture medium (RPMI) (Nissui Pharmaceutical Co.) with 10% FBS at 37°C in humidified 5% CO\(_2\).

**Cell proliferation assay.** WST-1 assay (TaKaRa Premix WST-1 cell proliferation assay system, Shiga, Japan) was used to measure the proliferation of cancer cells. All cancer cells were inoculated in 96-well plates \((8,000 \text{ cells/well})\) and incubated for 24 h. After removal of culture medium, cancer cells were incubated with FBS free culture medium containing 0, 3, 5 or 10 mg/mL of coriander extract for 24 h. Then all the culture medium was removed from each well. After washing of each well with PBS, 100 \(\mu\text{L}\) of WST-1 reagent was added into each well and incubated for 15 min at 37°C. And absorbance at 570 nm of wavelength was measured.

**Cell migration assay.** The migration assay was performed by using ThinCertTM 8.0 \(\mu\text{m}\) double chamber (Greiner bio-one. Solingen, German) as described elsewhere \((22)\). Briefly, 500 \(\mu\text{L}\) of culture media containing 0, 3, 5 or 10 mg/mL of coriander extract were added to the lower chamber. And 200 \(\mu\text{L}\) of cell suspensions \((5 \times 10^4 \text{ cells})\) for B16F10 cells or HepG2 cells in FBS free medium were placed in the upper chamber. After incubation for 24 h at 37°C in 5% CO\(_2\), cells on the upper side of the filters were removed with cotton-tipped swap and filters were washed with PBS. Cells on the lower side of the filters were stained with DAPI (AppliChem GmbH, Darmstadt, Germany), which stained nucleus in the cell, for 10 min and the number of cells were counted under a fluorescence microscope (Olympus, Tokyo, Japan). The experiments were carried out in triplicate, and each experiment was repeated for five times.

**Cell invasion assay.** The invasion assay was performed by using ThinCertTM 8.0 \(\mu\text{m}\) double chamber (Greiner bio-one) as described elsewhere \((23)\). Prior to the invasion assay, the upper side of the filter was coated with Matrigel (Corning® Matrigel® Basement membrane matrix, MA, USA). Briefly, 500 \(\mu\text{L}\) of culture media containing 0, 3, 5 or 10 mg/mL of coriander extract were added to the lower chamber. And 200 \(\mu\text{L}\) of cell suspensions \((5 \times 10^4 \text{ cells})\) for B16F10 cells or HepG2 cells in FBS free medium were placed in the upper chamber. After incubation for 24 h at 37°C in 5% CO\(_2\), cells on the upper side of the filters were removed with cotton-tipped swap and filters were washed with PBS. Cells on the lower side of the filters were stained with DAPI (AppliChem GmbH) for 10 min and the number of cells were counted under a fluorescence microscope (Olympus). The experiments were carried out in triplicate, and each experiment was repeated for five times.

**Zymography assay.** HepG2 and B16F10 cancer cells \((2.0 \times 10^5 \text{ cells/well})\) were cultured in 6-well plates until confluent. After removal of culture medium and washing with PBS, cancer cells were cultured with FBS free medium containing 0, 3, 5 or 10 mg/mL of coriander extract for 24 h. Then the culture medium was collected and stored at \(-80^\circ\text{C}\) until use. Twenty microliters of medium sample was subjected to fibrin zymography or gelatin zymography. The activity of u-PA was measured by fibrin zymography as described elsewhere \((24)\). Briefly, 3.64 mg of bovine plasminogen-rich fibrinogen (Organan Teknika, Dublin, Ireland) was dissolved in the mixture of 2.2 mL of acrylamide \((30 \text{ g/100 mL})\), bisacrylamide \((1 \text{ g/100 mL})\), 2.4 mL of distilled water and 1.7 mL of 1.5 M Tris-HCl buffer (pH 8.8). Then the mixture was added with 36.0 \(\mu\text{L}\) of thrombin \((10 \text{ U/mL})\), 66.5 \(\mu\text{L}\) of 10% SDS, 6.5 \(\mu\text{L}\) of N,N,N,N′-te-


triamethylene-diamine and 66.5 μL of 10% ammonium peroxydisulfate to prepare the fibrin gel. After electrophoresis, the gel was washed with 2.5% Triton X-100 solution twice for 30 min and then incubated in a bath containing 0.1 M glycine-NaOH (pH 8.3) at 37˚C for 24 h. Finally, the fibrin gel was stained with 0.25% Coomassie blue G-250 (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) in 10% acetic acid and 50% methanol, followed by destaining with multiple changes of destaining solution (10% acetic acid and 30% methanol) until lysis bands appeared. The fibrinzymograms were scanned using canon PIXUS MG7130 (Tokyo, Japan) at 400 dpi. The intensities of lysis bands were evaluated by using Fuji Film Multi Gauge (Tokyo, Japan). The activities of MMPs were measured by gelatin zymography as described elsewhere (22). Briefly, the gelatin gel was prepared by mixing with 2 mL of 1.5 M Tris-HCl (pH 8.8), 2 mL of acrylamide (30 g/100 mL), 2 mL of distilled water, 2 mL of gelatin solution (4 mg/mL), 80 μL of 10% SDS, 80 μL of 10% ammonium peroxysulfate and 10 μL of N,N,N,N′-tetramethyl-ethylene-diamine. After electrophoresis, the gel was washed with solution containing 2.5% Triton X-100, 50 mM Tris-HCl, 5 mM CaCl₂, and 1 μM ZnCl₂ twice for 30 min at room temperature. Then the gel was transferred to a reaction buffer, which contained 1% Triton X-100, 50 mM Tris-HCl, 5 mM CaCl₂ and 1 μM ZnCl₂, and was incubated for 24 h. Finally, the gel was stained with 0.25% Coomassie blue G-250 in 10% acetic acid and 50% methanol, followed by destaining with multiple changes of destaining solution until lysis bands appeared. The gelatinzymograms were scanned and the intensities of lysis bands were evaluated as well as fibrinzymograms.

Western blot analysis. The antibodies used in this study are described as below: Anti-(phospho-) p44/42 MAPK (Erk 1/2) (Thr202/Tyr204) rabbit monoclonal antibody (mAb) (Lot #4370), anti-p44/42 MAPK (Erk 1/2) rabbit mAb (Lot #4695), anti-(phospho-)IkBα (Ser32) rabbit mAb (Lot #2859), anti-IkBα antibody (Lot #9242), anti-NFkB p65 rabbit mAb (Lot #8242), anti-(phospho-) NFkB p65 (Ser536) (Lot #3033) rabbit mAb and anti-β-Actin Rabbit mAb (Lot #4097) were purchased from CST, MA, USA. Anti-MMP2 antibody (Lot #QC0350) was purchased from Sigma-Aldrich, MO, USA. Anti-urokinase antibody (Lot #ab133563) and anti-Lamin B1 antibody (Lot #GR3244890-2) were purchased from Abcam, MO, USA. Anti-rabbit IgG, horseradish peroxidase (HRP) linked antibody (CST) was used as secondary antibody. HepG2 or B16F10 cells (2.0×10⁵ cells/well) were cultured in 6-well plates until confluent. After removal of culture medium and washing with PBS, cancer cells were cultured with PBS free medium containing 0, 3, 5 or 10 mg/mL of coriander extract for 24 h. Next, the culture medium was removed, and cancer cells were washed with PBS. Then, cancer cells were collected and resuspended with RIPA buffer (Thermo Scientific, Rockford, IL, USA) to prepare cell lysates. The cell lysates were used as sample for determination of MMP-2 or u-PA antigen. In order to investigate the signaling pathway in B16F10 cells, B16F10 cancer cells were treated with 10 mg/mL of coriander extract for various time intervals. After removal of culture medium and washing with PBS, the cancer cells were collected and resuspended with RIPA buffer (Thermo Scientific) to prepare cell lysates. On the other hand, B16F10 cancer cells were treated with various concentration of coriander extract for 24 h and then the nuclear fractions of cells were prepared by using Compartmental Protein Extraction Kit (Millipore, Temecula, CA, USA). The concentration of protein in each sample was determined by the Thermo Scientific Pierce BCA Protein Assay kit (Thermo Scientific). The samples contained equal amounts of protein were submitted to 10% SDS-PAGE and separated proteins were transferred to nitrocellulose membranes. The membranes were blocked with blocking buffer (20 mM Tris base, 150 mM NaCl, 0.1% Tween 20, pH 7.4 (TBST) containing 5% skim milk powder) for 1 h at room temperature, followed by incubation with primary antibodies (1: 3,000; with 1% TBST) at 4˚C overnight. After incubation with primary antibodies, the primary antibodies were removed, and the membranes were washed with TBST. Then the membranes were further incubated with secondary antibody for 1 h at room temperature. Then the membranes were washed with 1% TBST. The proteins reacted with antibodies were visualized by LAS-4000 (FujiFilm, Tokyo, Japan). The intensities of the protein bands were evaluated by using Fuji Film Multi Gause.

Real-time PCR. B16F10 melanoma cells (2.0×10⁵ cells/well) were cultured in 6 well dish until confluent, and then the medium was replaced with PBS’ free medium containing 0, 3, 5 or 10 mg/mL of coriander extract. After incubation for 24 h, B16F10 melanoma cells were collected. Total RNA was extracted from B16F10 melanoma cells using RNeasy® Mini Kit (QIA-GEN®, Hilden, Germany). Then, 2 μg of RNA was reversely transcribed into cDNA using SuperScript® III First-Strand Synthesis System for RT-PCR (Invitrogen, CA, USA). Two micrograms of synthesized cDNA was mixed with primers and TaqMan® Fast Advanced Master Mix (Applied Biosystems™, Vilnius, Lithuania), according to the manufacturer’s protocol. The primers for GAPDH (Mm9999999_g1, Lot. 1550483), MMP-2 (Mm01253624_m1, Lot. 1477384) and u-PA (Mm00447054_m1, Lot. 1195135) were purchased from Applied Biosystems™, CA, USA. The real-time PCR assay was carrying out by using Applied Biosystems StepOne Plus Real Time PCR System (Applied Biosystems™, CA, USA). The cycling conditions were 20 s at 95˚C for polymerase activation followed by 40 cycles at 95˚C for 1 s and 60˚C for 20 s.

In vivo metastasis model. All animal experimental protocols were approved by the animal committee of Kindai University (Approval number: KAG-29-002) and followed by animal experiment guideline of Kindai University. Male 5 wk old (C57/BL6) mice were purchased from Shimizu Laboratory Supplies Co. Ltd. (Kyoto, Japan). All mice were kept.
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under standard conditions (temperature-controlled by 21 ± 2°C, 12 h light/dark cycle, and free access to chow diet and water). One hundred microliters of PBS containing 1.0 × 10^5 of B16F10 cells was injected into mice tail vein, and mice were separated into four group (n=12 in each group, respectively). One kilogram of coriander leaves was lyophilized into 120 g of powder. Control group was fed with normal diet based on AIN-93G (25), and coriander groups were fed with diets which had been mixed with various concentration of

Fig. 1. Effect of coriander extract on proliferation of B16F10 or HepG2 cancer cell. B16F10 (A) and HepG2 (B) cells were incubated with coriander extract for 24 h. The proliferation abilities were assessed by WST-1 assay. The experiments were carried out in triplicate and each experiment was repeated for five times. The control group (0 mg/mL of coriander extract) was set as 100% and the results were expressed as mean±SD (n=5).

Fig. 2. Effect of coriander extract on migration and invasion of HepG2 and B16F10 cancer cell. B16F10 (A) and HepG2 (B) cells were incubated with coriander extract for 24 h, and the migration ability of each cancer cell was measured by using double chamber. B16F10 (C) and HepG2 (D) cells were incubated with coriander extract for 24 h, and the invasion ability of each cancer cell was measured by using double chamber in which the upper side of the filter was coated with Matrigel®. The number of cells migrated or invaded to lower side of filter was estimated as migration or invasion ability of cells. The experiments were carried out in triplicate, and each experiment was repeated for five times. The results were expressed as mean±SD (n=5). *p<0.05 vs. control (0 mg/mL of coriander extract). **p<0.01 vs. control.
lyophilized coriander (1%, 3%, or 5% w/w). The feeding amounts were recorded every day and the body weights of mice were recorded weekly for 3 wk. After 3 wk, mice were sacrificed and the lungs were resected to count the number of metastatic regions.

Statistical analysis. All data are presented as means±SD. Student’s t-test was performed to analyze the statistical significance of differences between experimental groups. * p value less than 0.05 was considered statistically significant. All experiments in vitro were repeated independently at least three times.

RESULTS
Effect of coriander extract on proliferation of B16F10 or HepG2 cancer cell
When B16F10 cells or HepG2 cells were incubated with 10 mg/mL or less of coriander extract, in comparison to control group no significant differences of proliferation were observed (Fig. 1). The proliferation ability of B16F10 or HepG2 cells was not influenced by treatment with 3, 5 or 10 mg/mL of coriander extract.

Effects of coriander extract on migration and invasion of B16F10 or HepG2 cancer cells
As shown in Fig. 2A, B, the coriander extracts whose concentration did not affect proliferation significantly inhibited the migration ability of B16F10 or HepG2 cells in a dose-dependent manner. Moreover, the coriander extracts also significantly inhibited the invasion ability of B16F10 or HepG2 cells in a dose-dependent manner (Fig. 2C, D).

Fig. 3. Effects of coriander extract on MMP-2 and u-PA activities in the conditioned medium of B16F10 or HepG2 cells. B16F10 (A) or HepG2 (B) cells were incubated with coriander extract for 24 h and MMP-2 activity was measured by using gelatin zymography. The MMP-2 activity in control (0 mg/mL of coriander extract) was estimated as 1.0. B16F10 (C) and HepG2 (D) cells were incubated with coriander extract for 24 h and u-PA activity was measured by using fibrin zymography. The u-PA activity in control (0 mg/mL of coriander extract) was estimated as 1.0. The experiments were carried out in triplicate, and each experiment was repeated for five times. The results are expressed as mean±SD (n=5). * p<0.05 vs. control. ** p<0.01 vs. control.

Effects of coriander extract on MMP-2 and u-PA activities in the conditioned medium of B16F10 or HepG2 cells
Since MMP-2 and u-PA were thought to be important enzymes in cell migration and invasion, these activities in the conditioned medium were measured by using zymography. The treatments of B16F10 and HepG2 cells with 5 or 10 mg/mL of coriander extract significantly inhibited MMP-2 or u-PA activity in the conditioned medium (Fig. 3). Although the lysis bands derived from MMP-9 activity were only slightly detected, these bands were too weak to compare MMP-9 activity between experimental groups.
Effects of coriander on MMP-2 or u-PA antigen level, and MMP-2 or u-PA mRNA level in B16F10 cells

Since coriander extract inhibited MMP-2 or u-PA activity in B16F10 cells, these antigen levels were examined by using Western blot. The treatment of B16F10 cells with 10 mg/mL of coriander extract significantly reduced MMP-2 or u-PA antigen level in the cell extract of B16F10 cells (Fig. 4A, B). Moreover, it was revealed that the expression of MMP-2 or u-PA mRNA were significantly downregulated by 10 mg/mL of coriander extract (Fig. 4C, D).

Effects of coriander extract on signaling pathway in B16F10 cells

Since 10 mg/mL of coriander extract reduced the expression levels of MMP-2 and u-PA mRNAs, intracellular signaling pathway concerning expression of MMP-2 or u-PA mRNA was investigated. Figure 5A, B, C showed that the treatment of B16F10 cells with 10 mg/mL of coriander extract suppressed the phosphorylation of IkB and Erk 1 in a time dependent manner. Furthermore, the treatment of B16F10 cells with coriander extract did not show remarkable changes of total NF-kB in cytoplasm (Fig. 5D), but decreased localization of phosphorylated NF-kB in nucleus in a dose dependent manner (Fig. 5E, F).

Effect of coriander on metastasis of B16F10 cells in vivo

Anti-metastatic property of coriander was examined by using in vivo metastasis model as described in “Materials and Methods.” In this experimental course, body weight and consumption of diet between control group and coriander groups were no significant differences, respectively (Table 1). Then mice were sacrificed and, lungs were resected to count the number of metastatic regions. The photographs of resected lungs were shown in Fig. 6A. It was observed that the number of metastatic regions were significantly reduced by increasing concentration of lyophilized coriander in their diet (Fig. 6B).

DISCUSSION

Coriander is a well-known spice from Mediterranean region and cultivated in Asia, North Africa and Central Europe (26). Now, coriander is used in the food as a flavor ingredient, rather than spice in traditional dishes. It seems like coriander changes dietary habits all over the world. The report from World Cancer Research Found indicated that saturated fatty acids from cooking oil increased cancer risk. Coriander contains high amount of essential oils which have an ability to decrease saturated fatty acid (27). The extract of coriander root inhibited the proliferation of MCF-7 cells through mitochondrial apoptotic pathways as demonstrated by significant augmentation of caspases-3, -8, and -9 activities (6). Thus, it has been revealed that coriander had anti-carcinogenic and anti-proliferative abilities. However, not only proliferation but also metastasis is critical
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for patients of cancer. The prognosis of cancer depends on metastasis which is induced by migration or invasion of cancer cells. Nevertheless, the effects of coriander on metastasis of cancer cell have not yet been fully elucidated. Since the migration and invasion were involved in metastasis, we investigated the effects of coriander on migration and invasion abilities of cancer cells.

High concentration of coriander extract inhibited the proliferation of cancer cells as demonstrated previously (28). However, in this experimental condition, 10 mg/mL or less of coriander extract did not significantly inhibit proliferation of HepG2 or B16F10 cells (Fig. 1).

In the present study, the migration or invasion ability of cancer cells was estimated by counting number of cells in modified double chamber method as described in Materials and Methods. If the proliferation of cells was inhibited by coriander extract, the number of cells would be decreased after incubation for 24 h. Therefore, in the following in vitro experiments, it was decided that cancer cells were treated with 3, 5 or 10 mg/mL of coriander extract which did not affect proliferation.

Migration is one of the most important processes to disseminate cancer cells first. Subsequently, migrated cancer cells invades into another tissues. ECM holds the cells together and provides a porous pathway for the diffusion of nutrients and oxygen to individual cells. The ECM is composed of an interlocking meshwork of heteropolysaccharides and fibrous proteins such as fibrillar collagens, elastin, and fibronectin called as basal lamina. MMPs, especially MMP-2 and MMP-9, play important roles in cancer cell invasion. The activities of MMPs

Fig. 5. Effects of coriander extract on signaling pathway in B16F10 cells. B16F10 cells were incubated with 10 mg/mL of coriander extract for the indicated time, and then the phosphorylation of IκB or Erk was examined by Western blot (A). The intensity of each band was quantified by using densitometer. The phosphorylation levels of IκB (B) and Erk 1 (C) were represented as mean±SD. B16F10 cells were incubated with various concentration of coriander extract for 24 h, then NF-κB in cytoplasm (D), and phosphorylated NF-κB, total NF-κB or Lamin B1 in nucleus (E) were examined by Western blot. The levels of phosphorylated NF-κB in nucleus were corrected by Lamin B1 and represented as mean±SD (F). The experiments were carried out in triplicate, and each experiment was repeated for five times. * p<0.05 vs. control (phosphorylation level of IκB at 0 min). + p<0.05 vs. control (phosphorylation level of Erk 1 at 0 min). ## p<0.01 vs. control (phosphorylated NF-κB in nucleus of B16F10 cells treated without coriander extract).
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575 to degrade type IV collagen, a major component of the ECM, caused metastasis (29). In this study, MMP-9 activity was only slightly detected in zymography. It was considered that MMP-2 rather than MMP-9 more influenced the degradation of ECM in the present study. On the other hand, cell migration was occurred by plasmin generation at focal adhesion sites. Because plasmin catalyzed by u-PA induced detachment of the trailing edge of cell and facilitated the migration of it (30). In addition, it was demonstrated that plasmin activated pro-MMPs to MMPs (17). As shown in Fig. 4, coriander extract inhibited both MMP-2 and u-PA activities. Therefore, it was thought that coriander extract impaired migration and invasion abilities of HepG2 and B16F10 cells through inhibitions of MMP-2 and u-PA activities, suggesting suppression of cancer metastasis.

Several studies showed that Erk 1/2 and NF-kB played an important role in metastasis through their effects on cell migration (31, 32). Indeed, it was reported that MAPKs and IKK signaling pathways upregulated the expression of MMPs (33, 34). Furthermore, it was demonstrated that berberine, one of the main substances of the plant Rhizoma coptidis, greatly reduced the phosphorylation of JNK, p38 and Erk 1/2, and decreased the protein levels of u-PA, MMP-2 and MMP-9 in SCC-4 cell (35). Thus, the Erk 1/2 and NF-kB pathways were thought to play an important role in expression of MMP-2 or u-PA. The present study demonstrated that the treatment of B16F10 cells with coriander extract could not only inhibit the phosphorylation of Erk 1 and IkB, but also downregulate the translocation of phosphorylated NF-kB in nucleus (Fig. 5). Furthermore, coriander extract decreased expression level of MMP-2 or u-PA mRNA and subsequently decreased antigen level of MMP-2 or u-PA in a dose dependent manner (Fig. 4). Based on these findings, it was thought that coriander extract inhibited MMP-2 or u-PA expression through signaling pathways.

With regard to cancer metastasis, animal models in which melanoma cells were injected into tail vein of mice were commonly used (36, 37). In those models, the metastatic regions of melanoma cells could be easily observed in lungs. Therefore, we used B16F10 cells to perform in vivo experiment. Results from in vivo experiment revealed that oral consumption of lyophilized coriander dramatically reduced the number of metastatic regions by means of increasing amount of coriander. This in vivo data is reasonable for the in vitro data in which coriander inhibited migration and invasion abilities of B16F10 cells. As far as we know, this study is the first presentation to demonstrate the effect of oral consumption of coriander on metastasis of B16F10 cells. Namely, it was confirmed for the first time that the

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**Table 1. Time course of dietary intake and mice body weight in metastatic model.**

| Lyophilized coriander in diet (% w/w) | Dietary intake (g/mouse/wk) | Body weight (g/mouse) |
|--------------------------------------|----------------------------|----------------------|
|                                      | 1st week | 2nd week | 3rd week | 1st week | 2nd week | 3rd week |
| 0%                                   | 18.15±0.80 | 23.15±1.06 | 24.53±1.33 | 13.96±3.96 | 15.85±4.36 | 14.96±4.81 |
| 1%                                   | 18.00±0.57 | 23.00±1.27 | 24.83±1.64 | 13.17±4.61 | 14.07±5.21 | 13.21±4.24 |
| 3%                                   | 17.75±2.01 | 23.16±0.93 | 25.00±0.85 | 13.00±4.33 | 15.75±4.62 | 14.75±3.14 |
| 5%                                   | 17.69±0.85 | 23.46±1.71 | 25.15±1.51 | 12.96±5.35 | 14.21±5.20 | 13.21±5.37 |

The values are shown as mean±SD (n=12). Dietary intake and mice body weight were no significant differences between control group (0% of lyophilized coriander in diet) and coriander groups (1, 3, 5% of lyophilized coriander in diet).
administration of coriander suppressed migration and invasion abilities of B16F10 cells and caused inhibition of metastasis.

The limitation of this study is that exactly components of coriander extract which suppress migration and invasion of cancer cells are unclear and effective ingredients are unknown. However, phenolic compounds are major components of spice and aromatic plants. They are one of the largest groups of secondary metabolites and ubiquitous in the plant kingdom (38). Recent studies have shown that coriander was rich in flavonoids, especially quercetin and rutin (6). It was reported that quercetin and rutin were detected in both methanolic and ethanolic extract in coriander (3). Quercetin has been reported to reduce migration and invasion of gastric cancer cells by suppressing phosphorylation of NF-kB, PKC, Erk 1/2 and AMPK, and decreasing u-PA protein level (39). Moreover, quercetin reduced the protein levels of MMP-2 and MMP-9 by suppressing phosphorylation of PI3K and MAPKs (Erk 1/2, p38 and JNK) pathway (40, 41). Also, anti-migration potentials of quercetin and rutin were confirmed by reducing MMP-2 expression in human glioblastoma cells (42). Thus, it was demonstrated that quercetin and rutin could inhibit cell migration and invasion of cancer cells by modulation of MAPKs and IKK signaling pathway. These reports are consistent with our experimental data, suggesting that quercetin and rutin may be the main compounds by which the coriander extract can restrict the invasion and migration abilities of cancer cells. However, it is possible that the substances other than quercetin and rutin in coriander may possess suppression abilities of migration and invasion. In order to identify these components, further study will be needed.

In conclusion, it was revealed that the downregulation of MAPKs and IKK signaling pathway by coriander caused suppression of MMP-2 and u-PA mRNAs expressions in B16F10 cells. Since coriander inhibited the secretion of MMP-2 and u-PA from cancer cells, it was thought that coriander could suppress the invasion and migration abilities of cancer cells. Furthermore, the present study showed for the first time that oral consumption of coriander suppressed metastasis of B16F10 cells in vivo. Based on these findings, it may be possible that coriander provides the improvement of cancer prognosis.

Authorship
Research conception and design: HH and SU; experiments: HH, TN and TY; statistical analysis of the data: HH and SU; interpretation of the data: HH and SU; writing the manuscript: HH and SU.

Disclosure of state of COI
No conflicts of interest to be declared.

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