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Research Article

Korean Red Ginseng Suppresses Metastasis of Human Hepatoma SK-Hep1 Cells by Inhibiting Matrix Metalloproteinase-2/-9 and Urokinase Plasminogen Activator

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Korean red ginseng and ginsenosides have been claimed to possess wide spectrum of medicinal effects, of which anticancer effect is one. The present study was undertaken to investigate the antimetastatic effect of Korean red ginseng on human hepatoma as well as possible mechanisms. The inhibitory effect of the water extract of Korean red ginseng (WKRG) on the invasion and motility of SK-Hep1 cells was evaluated by the Boyden chamber assay *in vitro*. Without causing cytotoxicity, WKRG exerted a dose-dependent inhibitory effect on the invasion and motility, but not adhesion, of highly metastatic SK-Hep1 cells. Zymography analyses revealed significant downregulating effects on MMP-2, MMP-9, and uPA activities in SK-Hep1 cells. Western blot analyses also showed that WKRG treatment caused dose-dependent decreases in MMP-2 and MMP-9 protein expressions. Moreover, WKRG increased the levels of TIMP-1, TIMP-2, and PAI-1. The present study not only demonstrated that invasion and motility of cancer cells were inhibited by WKRG, but also indicated that such effects were likely associated with the decrease in MMP-2/-9 and uPA expressions of SK-Hep1 cells.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in Taiwan. Metastasis is characteristic of highly malignant cancers with poor clinical outcomes. Most patients with HCC die within one year after diagnosis largely because of frequent tumor metastasis [1]. One critical characteristic of metastatic cancer cells is the ability to dissolve basement membranes and extracellular matrix (ECM). This degradative process is mediated mainly by matrix metalloproteinases (MMPs), a large family that can degrade all known components of ECM [2]. MMP-2 and MMP-9 are abundantly expressed in various malignant tumors and play critical roles in tumor metastasis. Thus, the inhibition of MMP activity is important in terms of preventing cell metastasis [3]. Interestingly, MMP-9 expression levels are especially high in hepatoma cells, such as SK-Hep1 cells, and the enzyme has been studied in diverse malignant tumors because of its inducible character [4]. MMP-9 inhibitors have been developed and are currently being investigated in clinical trials [5].

Korean red ginseng (*Panax ginseng* C.A. Meyer) has been claimed to possess anticancer effects. The major active components of ginseng are triterpene saponins, known as ginsenosides, which are divided into two groups: protopanaxadiol type (Rb_1 group) and protopanaxatriol type (Rg_1 group). Protopanaxadiol-type ginsenosides are metabolized by human intestinal bacteria to 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (M1, also known as compound K), which is then absorbed from the gastrointestinal tract [6]. The metabolite has a variety of pharmacological activities including antitumor, antidiabetic, anti-inflammatory, and antiallergic effects [7]. We have established an *in vitro* model of cancer metastasis using SK-Hep1 cells, and showed

Table 1: Analytical data received from the Korean Ginseng Corporation on the ginsenoside contents of WKRG and EKRG.

	Ginsenoside (mg/g extracts)						
	Rb_1	Rb_2	Rg_1	Rg_3	Rc	Rd	Re
WKRG	11.79	4.68	6.14	0.44	4.71	1.22	4.71
EKRG	4.03	1.97	0.52	2.89	1.98	1.51	1.18

that the anti-metastatic pathway was associated with the inhibition of MMP-2, MMP-9, and urokinase plasminogen activator (uPA), as well as the elevated levels of tissue inhibitor of metalloproteinases (TIMPs) and plasminogen activator inhibitor (PAI-1). Since Korean red ginseng has been reported to exhibit anticancer effects, we aimed to evaluate the anti-metastatic effects of Korean red ginseng by using experimental models with SK-Hep1 cells.

2. Materials and Methods

2.1. Chemicals. Dulbecco's modified Eagle's medium (DMEM) and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Trypsin-EDTA, fetal bovine serum (FBS), and penicillin/streptomycin were from Gibco Life Technologies, Inc. (Paisley, UK). Other chemicals were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Cell culture supplies were purchased from Costar (Corning, Inc., Cypress, CA, USA). Anti-MMP-2 (SC-373914), anti-MMP-9 (SC-10737), and anti-PAI-1 (SC-6642) mouse monoclonal antibodies were purchased from Santa Cruz Biotechnology Co. (Santa Cruz, CA, USA).

2.2. Korean Red Ginseng Extracts. Water and ethanolic extracts of Korean red ginseng (WKRG and EKRG) were offered by the Korean Society of Ginseng and were stored under light protection before the experiment. Analytical data on the ginsenoside contents of WKRG and EKRG has also been received as an accompaniment, as presented in Table 1. Additionally, it was informed that WKRG was comprised of 61.62% of solids and 36.68% of moisture, and EKRG was comprised of 61.62% of solids and 38.38% of moisture.

2.3. Cell Culture. Hepatocarcinoma SK-Hep1 cells were purchased from the Bioresources Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were cultured in plastic dishes containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a CO_2 incubator (5% CO_2 in air) at 37°C and subcultured every other day at a dilution of 1 : 5 using 0.05% trypsin-0.02% EDTA in Ca^{2+}-, Mg^{2+}-free phosphate-buffered saline (DPBS).

2.4. Cell Viability. SK-Hep1 cells (2×10^5 cells per well) were seeded in a 96-well plate containing DMEM supplemented with 10% FBS for 1 day to become nearly confluent before being cultured with various concentrations of WKRG and EKRG (0, 100, 200, 400, 800, and 1600 μg/mL) for 24 h. The cells were then washed twice with DPBS and incubated with 100 μL of 0.5 mg/mL MTT for 2 h at 37°C (MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). The medium was discarded and 100 μL of dimethylsulfoxide (DMSO) was added. After 30 min of incubation, the absorbance was read with a microplate reader at 570 nm.

2.5. Determination of MMP-2, MMP-9, and uPA Activities by Zymography. Cells were treated with different concentrations (0, 100, 200, and 400 μg/mL) of WKRG and EKRG for 24 h. The conditioned media were collected, and MMP-2 and MMP-9 activities were assayed using gelatin zymography (7.5% zymogram gelatin gels) according to the method reported by Lai et al. with some modifications which began with electrophoresing (120 V for 90 min) the culture media in a 10% SDS-PAGE gel containing 0.1% gelatin [4]. The gel was then washed at room temperature in a solution containing 2.5% (v/v) Triton X-100, subsequently it was transferred to a reaction buffer containing 1% NaN_3, 10 mM $CaCl_2$, and 40 mM Tris-HCl for enzymatic reaction at 37°C and pH 8.0 before being shaken overnight for 12∼15 h. Next day, the MMP gel was stained with 0.25% (w/v) Coomassie blue in 10% acetic acid (v/v) and 20% methanol (v/v) for 30 min before it was destained in 10% acetic acid (v/v) and 20% methanol (v/v).

Visualization of uPA activity was carried out according to the method reported by Maeda-Yamamoto et al. [8]. Briefly, 2% w/v casein and 20 mg/mL plasminogen were added to an 8% SDS-PAGE gel. Samples each with a total protein of about 20 μg were loaded onto the gel. The uPA activities of cells treated or untreated with WKRG were measured as described in the gelatin zymography assay. The relative MMP-2, MMP-9, and uPA activities were quantified by using Kodak Molecular Imaging Software (Version 4.0.5, Eastman Kodak Company, Rochester, NY) and represented in relative intensities.

2.6. Cell Migration Assay. Tumor cell migration was assayed in transwell chambers (millipore) according to the method reported by Huang et al. with some modifications [9]. Briefly, transwell chambers with 6.5 mm polyvinyl/pyrrolidone-free polycarbonate filters of 8 μm pore size were used. SK-Hep1 cells (5×10^5 mL^{-1}) and WKRG (0–400 μg/mL) were suspended in 100 μL of serum-free DMEM before being placed in the upper transwell chamber; as for the lower chamber, 10% FBS-containing medium was placed in as a chemoattractant. After 24 h of incubation at 37°C, cells on the upper surface of the filter were completely wiped away with a cotton swab, and the lower surface of the filter was fixed in methanol, stained with Giemsa and counted under a microscope at a magnification of 200x. For each replicate, the tumor cells in 10 randomly selected fields were determined, and the counts were averaged.

2.7. Cell Invasion Assay. The invasion of tumor cells was assessed in transwell chambers with 6.5 mm polycarbonate filters of 8 μm pore size, as described in the cell migration assay except that each filter was coated with 100 μL of

Matrigel (1 : 20 dilution in cold DMEM) to form a thin continuous film on the top side of the filter. Cells were adjusted to 5×10^5 mL^{-1}, and 100 μL (containing 5×10^4 cells) in DMEM containing 10% FBS was transferred to each of triplicate wells. After incubating for 24 h, the cells were stained and counted as described previously, and the number of cells invading the lower side was measured [9].

2.8. Cell Adhesion Assay. Each well of a 24-well tissue culture plate was coated with 25 μg of Matrigel and left to air-dry for 40 min. SK-Hep1 cells (5×10^4) and WKRG (0–400 μg/mL) suspended in DMEM containing 0.5% bovine serum albumin were then dispensed into each well. The plate was incubated in 5% CO_2 at 37°C for 1 h and then gently washed thrice with PBS to remove any unattached cells. Attached cells were then stained with hematoxylin and eosin reagent before being counted under a microscope (Eclipse TS100, Nikon, Japan). At least three independent experiments were performed [9].

2.9. Wound-Healing Assay. For cell motility determination, SK-Hep1 cells (5×10^4 cells per well) were seeded in a 6-well tissue culture plate and grown to 80∼90% confluence. After aspiration of the medium, the center of the cell monolayer was scraped with a sterile micropipette tip to create a denuded zone (gap) of constant width. Subsequently, cellular debris was washed with PBS, and SK-Hep1 cells were exposed to various concentrations of WKRG (0–400 μg/mL). Wound closure was monitored and photographed at 0, 12, and 24 h with a Nikon inverted microscope. To quantify the migrated cells, pictures of the initial wounded monolayers were compared with the corresponding pictures of cells at the end of incubation. Artificial lines fitting the cutting edges were drawn on pictures of the original wounds and overlaid on the pictures of cultures after incubation. Cells that had migrated across the white lines were counted in six random fields for each triplicate treatment [3].

2.10. Preparation of Whole-Cell Lysates. SK-Hep1 cells (1×10^5 cells) were plated in 100 mm tissue culture flasks and treated with various concentrations of WKRG. SK-Hep1 cells were washed twice with PBS and were scraped into microcentrifuge tubes. The cells were centrifuged at 1,250 g for 5 min, and the pellets were lysed with iced-cold RIPA buffer (1% NP-40, 50 mM Tris-base, 0.1% SDS, 0.5% deoxycholic acid, 150 mM NaCl, pH 7.5), to which was added freshly prepared phenylmethylsulfonyl fluoride (10 mg/mL), leupeptin (17 mg/mL), and sodium orthovanadate (10 mg/mL). After incubating for 5 min on ice, the samples were centrifuged at 10,000 g for 10 min, and the supernatants were collected as whole-cell lysates. The protein content was determined with Bio-Rad protein assay reagent using BSA as the standard. Finally, whole-cell lysate proteins (30–50 μg of purified protein) were boiled for 10 min in electrophoresis sample buffer.

2.11. Western Blotting Analysis. Total cell lysates of equal protein contents from the control and WKRG treated SK-Hep1 cells were resolved on 10∼12% SDS-PAGE gels. Proteins were then transferred onto nitrocellulose membranes (Millipore, Bedford, MA) by electroblotting (Bio-Rad). Nonspecific binding of the membranes was blocked with Tris-buffered saline (TBS) containing 1% (w/v) nonfat dry milk and 0.1% (v/v) Tween-20 (TBST) for more than 2 h. Membranes were washed with TBST for 10 min three times and then incubated with an appropriate dilution of specific primary antibodies in TBST overnight at 4°C. The membranes were washed with TBST before being incubated with an appropriate set of secondary antibody (horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG) for 1 h. After washing the membrane for 10 min three times in TBST, the bands were visualized using ECL reagent (Millipore, Billerica, MA). Band intensity on scanned films was quantified using Kodak Molecular imaging (MI) software and expressed as relative intensity compared with the control.

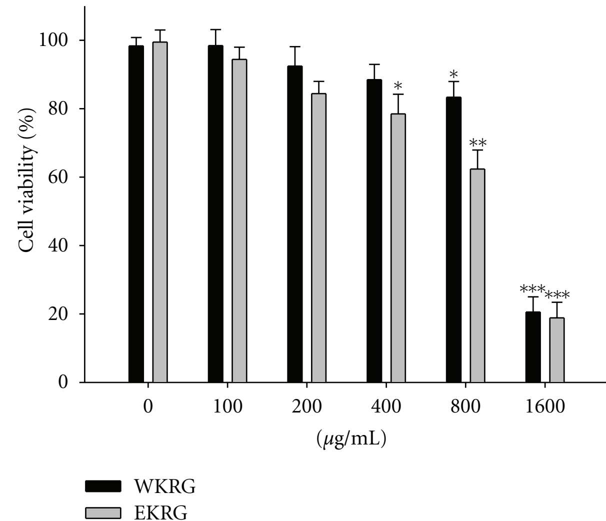


Figure 1: Viability of SK-Hep1 cells incubated with WKRG and EKRG (0, 100, 200, 400, 800, and 1600 μg/mL) for 24 h. Cell viability was measured using MTT assay and is expressed as % of cell survival relative to the control. Values are expressed as means ± SD of three independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ as compared with the untreated control.

2.12. Statistical Analysis. Values were expressed as means ± SD and analyzed using one-way ANOVA followed by Tukey's Test for comparison of group means. All statistical analyses were performed using SPSS for Windows, version 10 (SPSS, Inc.). P values < 0.05 were considered statistically significant.

3. Results

3.1. Cytotoxicity of Korean Red Ginseng Extracts. In the first part of this study, we examined the cytotoxicity of WKRG and EKRG by treating SK-Hep1 cells at various concentrations (0, 100, 200, 400, 800, and 1,600 μg/mL) for 24 h followed by MTT assay. In Figure 1, it is shown that WKRG treatment at the concentration of 0–400 μg/mL and EKRG treatment at the concentration of 0–200 μg/mL exhibited no

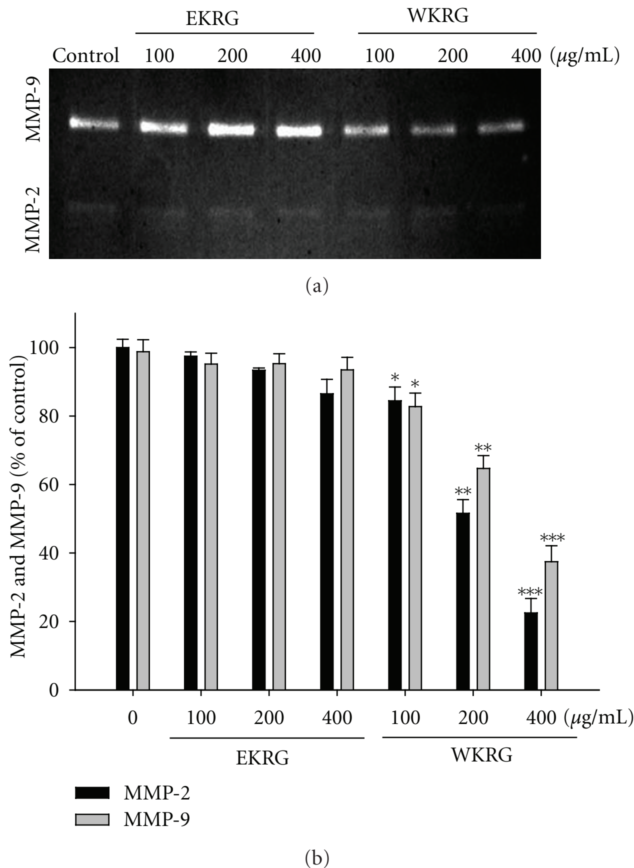


Figure 2: (a): Effects of WKRG and EKRG on MMP-2 and MMP-9 activities of SK-Hep1 cells. Cells were treated with various concentrations (0, 100, 200 and 400 μg/mL) of WKRG and EKRG for 24 h. The conditioned media were collected, and MMP-2 and MMP-9 activities were determined by gelatin zymography. (b) MMP-2 and MMP-9 activities were quantified by densitometric analyses. Values are expressed as means ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ as compared with the untreated control.

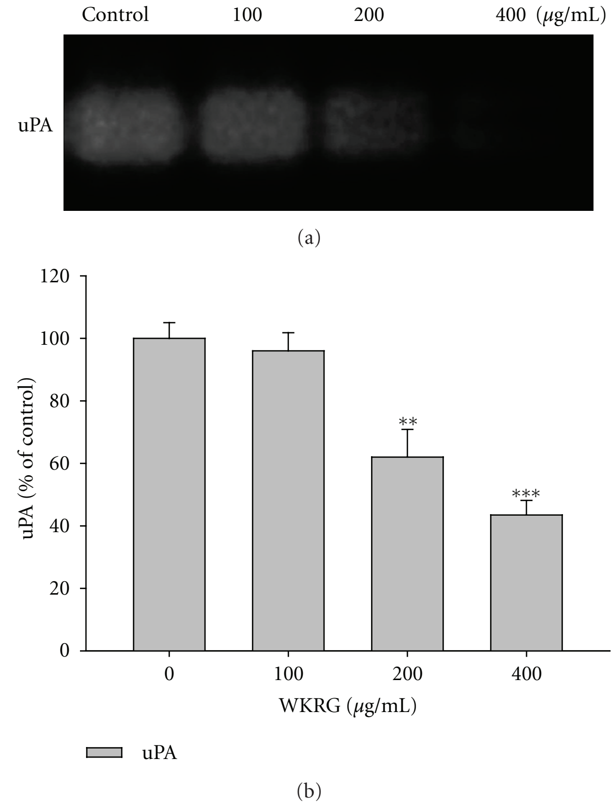


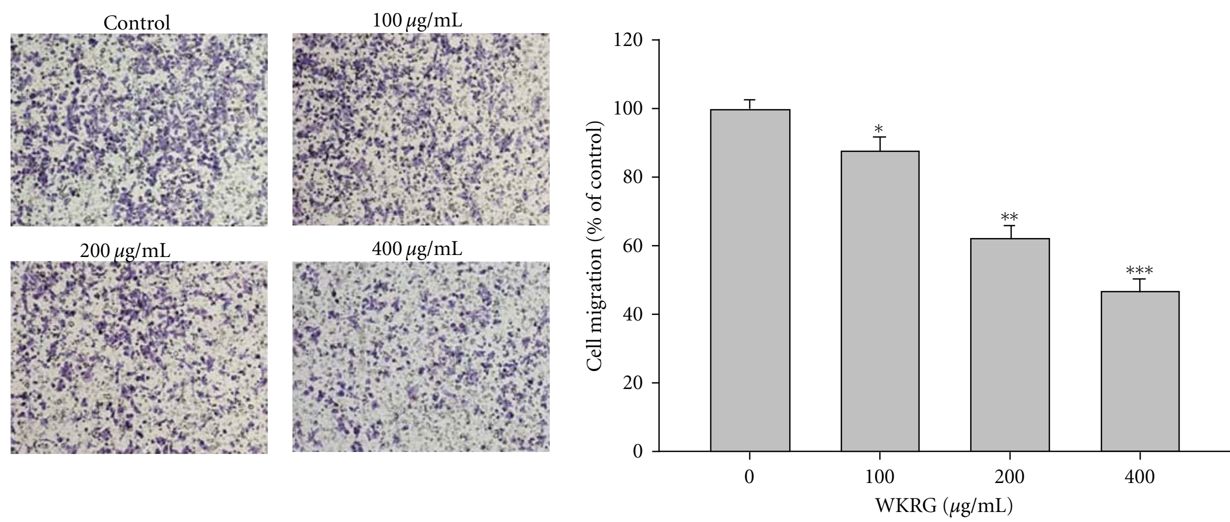
Figure 3: (a): Effect of WKRG on uPA activity of SK-Hep1 cells. Cells were incubated in the presence or absence of WKRG for 24 h. The conditioned media were collected, and uPA activity was determined by casein zymography. (b) uPA activity was quantified by densitometric analysis. Values are expressed as means ± SD of three independent experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared with the untreated control.

cytotoxicity against SK-Hep1 cells after 24 h. In all subsequent experiments, only doses below or equal to 400 μg/mL were applied.

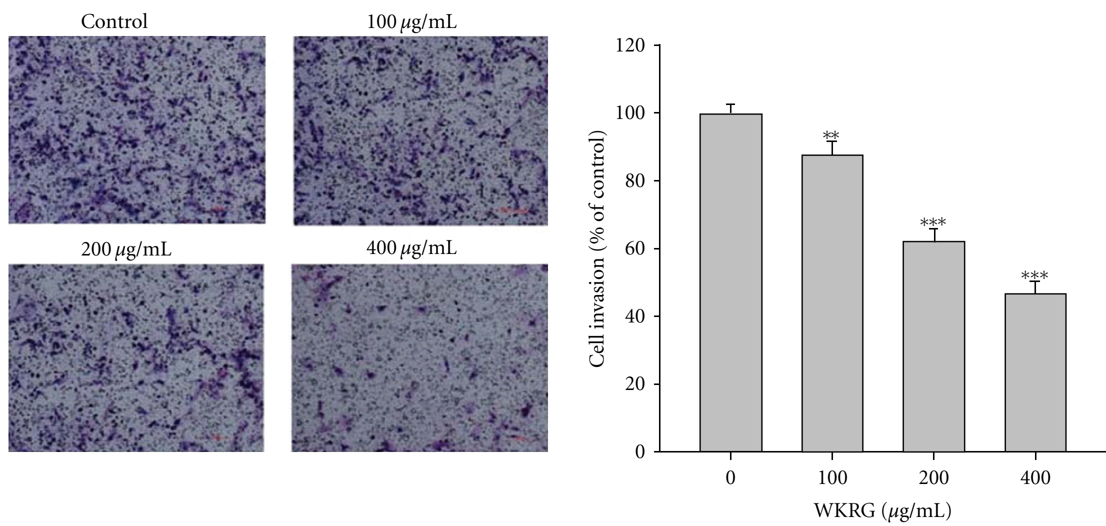
3.2. Inhibition of MMP-2 and MMP-9 Activities by WKRG and EKRG. Metastasis has been found to be accompanied by various physiological alterations involved in the degradation of ECM, including overexpression of proteolytic enzyme activities, such as MMPs or uPA, as well as the migration and invasion of tumor cells into the bloodstream or lymphatic system to spread to other tissues or organs. To investigate whether WKRG and EKRG could inhibit matrix-degrading proteinases, the conditioned media were collected after SK-Hep1 cells had been treated with WKRG and EKRG for 24 h. As shown in Figure 2(a), WKRG inhibited MMP-9 and MMP-2 activities in a concentration-dependent manner, with 62.6% and 81.5% inhibitions at 400 μg/mL after 24 h of incubation (Figure 2(b)), respectively. However, EKRG had no significant inhibition on MMP-9 and MMP-2 activities (Figure 2(a)). Based on the above observation, we used WKRG to do the following experiments in this paper.

3.3. Inhibition of uPA Activity by WKRG. As shown in Figure 3(a), uPA activity was also inhibited in a concentration-dependent manner by WKRG treatment, with 56.6% inhibition at the dosage of 400 μg/mL after incubating for 24 h (Figure 3(b)).

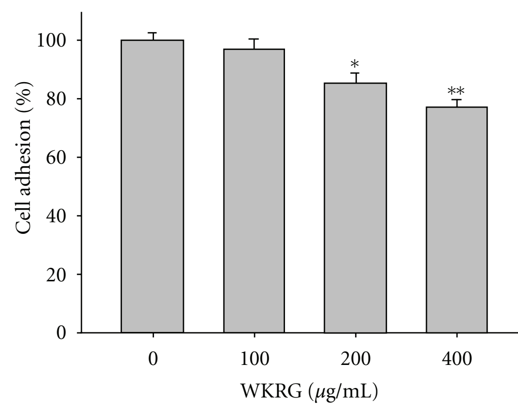
3.4. Inhibition of Migration, Invasion, and Adhesion by WKRG. The inhibitory effects of WKRG on the migration and invasion of SK-Hep1 cells were evaluated by the Boyden chamber assay *in vitro*. We found that WKRG at the concentrations of 0–400 μg/mL obviously decreased both the migration and invasion (Figures 4(a) and 4(b)) of SK-Hep1 cells dose-dependently. IC_{50} values for migration and invasion of WKRG were approximately 282.83 and 135.15 μg/mL, respectively. Since cell-matrix interaction is important for cancer cell invasion, cell-matrix adhesion assay was also performed. The results revealed that WKRG only caused a slight reduction in cell adhesion even at the concentration of 400 μg/mL (Figure 4(c)).



(a)



(b)



(c)

Figure 4: Effect of WKRG on (a) transwell migration, (b) invasion, and (c) adhesion of SK-Hep1 cells. Values are expressed as means ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ as compared with the untreated control.

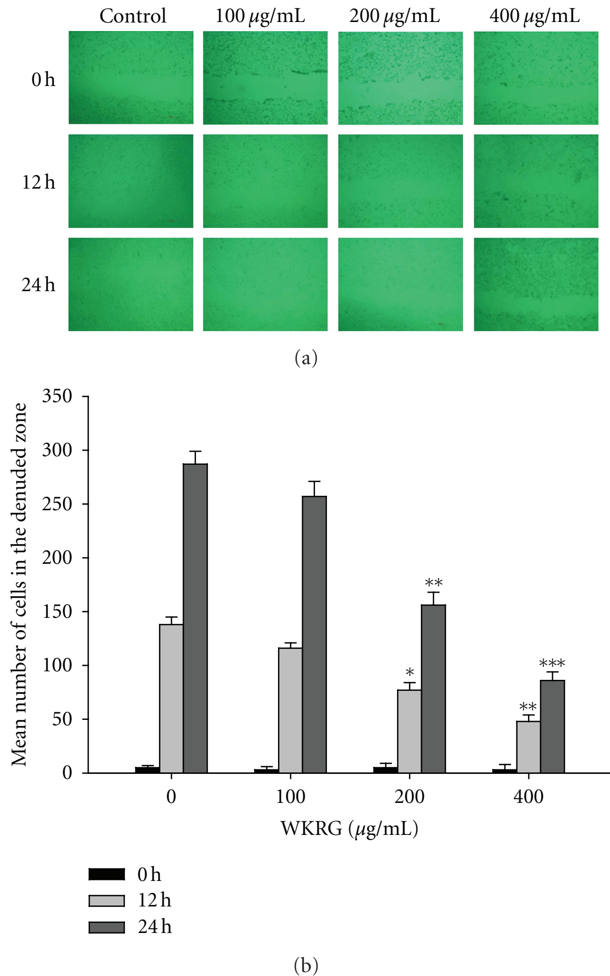


Figure 5: Effect of WKRG on wound healing migration of SK-Hep1 cells. Wound was introduced by scraping the confluent cell monolayer with a pipette tip. (a) Representative photographs of SK-Hep1 cells that received either control or WKRG treatments (0, 100, 200, and 400 μg/mL) for 0, 12 and 24 h. (b) Migrated cells across the black lines were counted in six random fields for each treatment. Quantitative assessment of the number of cells in the denuded zone is expressed as the mean ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ as compared with the untreated control.

3.5. Effect of WKRG on Wound Healing. We further assessed the effect of WKRG on SK-Hep1 cell motility using the wound healing assay, in which the confluent cell monolayer was scraped with a sterile micropipette tip to create a scratch wound. As shown in Figure 5(a), WKRG inhibited the motility of SK-Hep1 cells in a dose-dependent manner, with 65.24% and 70.03% reductions at 400 μg/mL after incubating for 12 and 24 h, respectively (Figure 5(b)).

3.6. Effect of WKRG on MMP-2/-9, TIMP-1/-2, and PAI-1 Protein Expressions. To further explore the mechanisms behind anti-metastatic effect of WKRG, we determined MMP-2, MMP-9, TIMP-1, TIMP-2, and PAI-1 protein levels. As

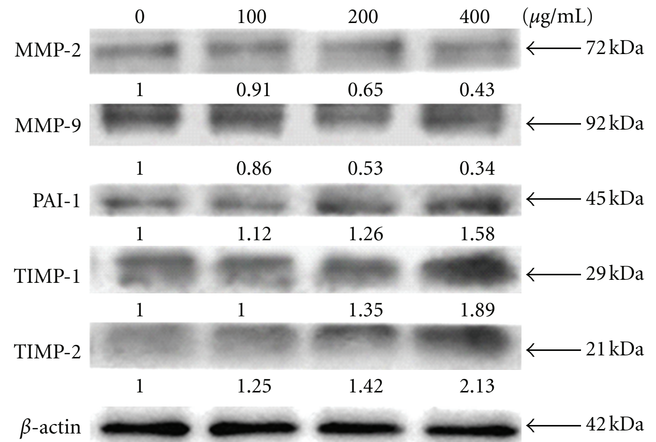


Figure 6: Effect of WKRG on MMP-2, MMP-9, PAI-1, TIMP-1, and TIMP-2 protein levels. SK-Hep1 cells were treated with WKRG (0, 100, 200, and 400 μg/mL) for 24 h, and cell lysates were subjected to SDS-PAGE followed by Western blotting. Levels of these proteins were subsequently quantified by densitometric analyses, with the control set to 100%.

shown in Figure 6, significant reductions in MMP-2 (0.43-fold of control) and MMP-9 (0.34-fold of control) protein levels were observed in SK-Hep1 cells treated with 400 μg/mL of WKRG. Conversely, WKRG strongly increased TIMP-1 and TIMP-2 protein levels concentration dependently, with 1.89- and 2.13-fold increases at 400 μg/mL of treatment, respectively. In addition, WKRG increased the protein level of PAI-1, and the effect was also directly proportional to concentration.

4. Discussion

In this study, we explored the anti-metastatic effect of Korean red ginseng in human hepatoma SK-Hep1 cells. Analytical data received from the Korean Ginseng Corporation showed that there was a great variation in ginsenoside contents between WKRG and EKRG, most likely due to the different extracting processes. Although the difference in the effect of these two extracts on cell viability was not prominent, there was a great variation in their inhibitory effects on MMP-2/-9 activities. WKRG inhibited MMP-2/-9 activities in a concentration-dependent manner, while EKRG had no significant inhibition on MMP-2/-9 activities at all tested concentrations. Based on the above observation, WKRG was selected to do the follow-up experiments in this paper. We found that WKRG also diminished uPA activity of SK-Hep1 cells. Moreover, WKRG significantly inhibited the invasion (assessed using the transwell assay) and migration (examined by both transwell and wound-healing assays) of SK-Hep1 cells. We further demonstrated that WKRG notably inhibited the protein expressions of MMP-2 and MMP-9 and increased the protein levels of TIMP-1, TIMP-2 and PAI-1. These results indicated that antimetastatic effect of WKRG was related to the inhibition of enzymatically degradative processes of tumor metastasis. To our knowledge, this is the first study that attempted to explore biochemical mechanisms

underlying WKRG's inhibitory effect on the metastasis of SK-Hep1 cells.

Panax ginseng is an herb frequently used in traditional oriental medicine for its wide spectrum of medicinal effects such as tonic, immunomodulatory, anticancer, adaptogenic, antiaging, antioxidant, and neuroprotective effects [10]. The major active components of ginseng are ginsenosides. More than forty ginsenosides have, so far, been isolated and each ginsenoside possesses different pharmacological effects [11]. Commercially available Korean ginseng products are classified into fresh ginseng, white ginseng, and red ginseng. White ginseng is fresh ginseng which has been air-dried, while red ginseng is obtained by heating. Although processed differently, both red and white ginseng products are manufactured from 6-year-old fresh ginseng roots; nevertheless Korean red ginseng has higher contents of ginsenosides such as Rh_2, Rg_3, and Rg_5 in comparison to white ginseng [12]. Rh_2 is produced from ginsenoside Rg_3 through bacterial transformation and belongs to the protopanaxadiol family. Rh_2 has attracted considerable attention owing to its potential tumor-inhibitory activity. It constrains cell growth in MCF-7 human breast cancer and SK-Hep1 hepatoma and can induce apoptosis in various cell lines [13]. Ginsenoside Rg_3 has been reported to reduce the gelatinolytic activities of MMP-2 and MMP-9 [14]. Other important ginseng saponins with unique chemopreventive actions include Rb_2 which may partly contribute to the inhibition of lung tumor metastasis by arresting tumor-associated angiogenesis [15], and Rp_1 (a semisynthesized derivative of ginsenoside Rg_5) whose anticancer effect is believed to be achieved by strongly inhibiting tumor cell metastasis and viability, presumably through impeding adhesion and vessel formation [16].

Cell migration is a complex process involving many types of intracellular and extracellular components and is associated with signaling pathways. Since migration is a critical event in cancer progression and especially in metastasis, the inhibitory effect of WKRG on cell migration was evaluated [4]. We found that WKRG significantly inhibited the migration and invasion of SK-Hep1 human hepatoma cells. Invasion of cancer cells through a coated membrane involves not only ECM degradation, but also the formation of adhesive interactions between cells and the matrix. Therefore, the cell adhesion assay was carried out; however we arrived with the result that WKRG only caused a small reduction in cell adhesion. The slight reduction in adhesion may be associated with the significant decreases in migration and invasion by WKRG treatment.

MMPs belong to a family of zinc-dependent endopeptides. They are secreted as inactive proenzymes and are activated by partial proteolytic cleavage. MMP-2, MMP-9 and dominant MMPs are released by most endothelial cells and appear to play important roles in the degradation of type VI collage, a major constituent of basement membrane, in cancer invasion and metastasis [17]. In this study, we observed up to 62.6% and 81.5% downregulations of MMP-2 and MMP-9 activities by WKRG as compared with the control. Although these are not direct inhibitions of enzymatic activity, they seem to be enough to decrease cancer metastasis, based on other studies [18].

The expression of uPA has been suggested to play a critical role in local fibrin deposition/dissolution [17]. Conversion of plasminogen into active plasmin by plasminogen activators, such as uPA, is primary for fibrinolysis to occur. Plasmin degrades fibrin and prevents its extracellular deposition. WKRG caused a reduction in cell surface plasmin activity as evidenced by the uPA activity assay. The importance of fibrinolytic system in wound healing has been demonstrated in plasminogen-deficient mouse models, where healing is weakened primarily due to impaired fibrinolysis, a consequence of insufficient plasmin generation [19]. Furthermore, uPA plays prominent roles in cellular migration and is vital during the initial phases of wound healing [20]. We used the wound healing assay *in vitro* to observe the effect of WKRG on cellular migration. WKRG potently retarded the migration of cells towards the wounded area. The results of this study demonstrated that WKRG inhibited fibrinolysis and cell migration which are vital during the early phase of wound healing.

To further explore the mechanisms underlying the anti-metastatic effect of WKRG, we detected the alteration in levels of several proteins by lysing SK-Hep1 cells after they had been incubated in the presence or absence of WKRG, including MMP-2, MMP-9, TIMP-1, TIMP-2, and PAI-1. There is *in vitro* evidence that MMPs and TIMPs are critical in determining the invasive potential of proliferating tumor cells. MMP-2 and MMP-9 are necessary for the migration of many normal cell types and tumor cells [21]; on the other hand, TIMPs are believed to play important roles in the inhibition of growth and migration, especially in hepatocellular carcinomas [22]. These documented findings strongly suggest anti-metastatic potential of WKRG, as we have demonstrated that WKRG suppressed MMP-2/-9 and enhanced TIMP-1/-2 expressions in terms of protein levels.

PAI-1 is a serine protease inhibitor which inactivates uPA to prevent it from binding to the uPA receptor (uPAR). uPAR is part of the plasminogen activation system which is also involved in the regulation of cell adhesion, migration, and invasion. It can transmit uPA-mediated extracellular signals inside the cell, probably through the association with different types of integrins and ECM components. Thus PAI-1 has the ability to arrest the proteolysis cascade following uPAR activation [23]. Our experiment by Western blot demonstrated that the protein level of PAI-1 was enhanced in a dose-dependent manner by WKRG treatment. In summary of the above results, it is suggested that WKRG's influences on MMP-2, MMP-9, uPA, PAI-1, TIMP-1, and TIMP-2 expressions may have a synergic suppressive effect on the migration and invasion of tumor cells.

In conclusion, we explored the anti-metastatic effects and mechanistic actions of WKRG in human hepatoma SK-Hep1 cells. It was found that WKRG significantly inhibited the invasion and migration of SK-Hep1 cells. Then, we carried forward the study by showing that WKRG notably inhibited the expressions of MMP-2, MMP-9, and uPA and elevated the protein levels of PAI-1, TIMP-1, and TIMP-2. These results not only evidenced anti-metastatic effect of WKRG but also showed that such effect was associated with the inhibition of enzymatically degradative processes of

tumor metastasis. The present study suggests that Korean red ginseng may be developed into a promising agent for cancer therapy. As to which ginsenosides are predominantly responsible for the anti-metastatic effect of Korean red ginseng remain primarily unclear and would require further studies.

Acknowledgments

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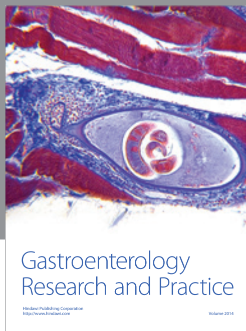

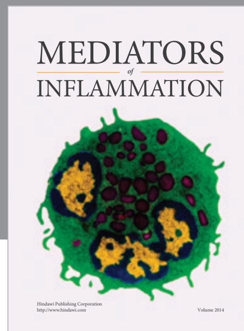

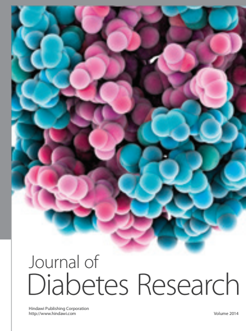

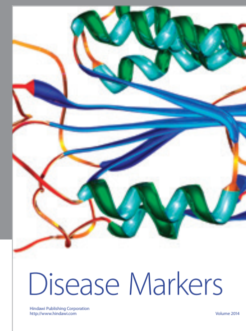

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