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ARTICLE

Polyphenol-Rich Extracts from *Solanum nigrum* Attenuated PKC α-Mediated Migration and Invasion of Hepatocellular Carcinoma Cells

Mon-Yuan Yang,[†,#] Li-Sung Hsu,[†,#] Chiung-Huei Peng,[‡] Yuan-Shong Shi,[†,§] Cheng-Hsun Wu,[†,∥] and Chau-Jong Wang*[,†,⊥]

[†]Institute of Biochemistry and Biotechnology, Chung-Shan Medical University, Number 110, Section 1, Chien-Kuo North Road, Taichung 402, Taiwan, [‡]Institute of Biotechnology, Hungkuang University, Number 34, Chung Chie Road, Shalu County, Taichung Shien 433, Taiwan, [§]Section of Pharmaceutical affairs, Changhua Country Public Health Bureau, Number 162, Jhong-shan Road, Changhua City, Changhua County 500, Taiwan, Republic of China, [∥]Department of Anatomy, China Medical University, Number 91, Hsueh-Shih Road, Taichung 404, Taiwan, and [⊥]Department of Medical Research, Chung-Shan Medical University Hospital, Number 110, Section 1, Chien-Kuo North Road, Taichung 402, Taiwan. [#] These authors contributed equally to this work and therefore share the first authorship.

Solanum nigrum L. (SN) has exhibited multiple biological effects such as anti-inflammation and antiproliferation. Protein kinase C (PKC) regulates cellular functions including proliferation, migration, and invasion. In the present investigation, we demonstrated that 12-*o*-tetradecanoylphobor-13-acetate (TPA) and constitutively activated PKC α significantly increased migration and invasion of HepG2 cells, while treatment with water or polyphenol extracts of SN (SNWE or SNPE) attenuated TPA-induced migration and invasion. SNWE and SNPE reduced TPA-elicited PKC α expression in a dose-dependent manner and obviously inhibited TPA-induced phosphorylation of p38 and ERK, respectively. Constitutively activated PKC α (caPKC α) significantly reversed the inhibitory effects of SNWE and SNPE on ERK and p38 activation. However, the antimigration effect of SNWE and SNPE could not be abrogated by caPKC α. Our results revealed the antimigration and anti-invasion effects of both extracts derived from SN, which may act as a promising therapeutic agent for the treatment of hepatocellular carcinoma.

KEYWORDS: *Solanum nigrum*; PKC α; migration; heptocellular carcinoma cells

INTRODUCTION

Solanum nigrum L. (SN) is a native plant abundantly grown in Asia and used as a medical herb in China and India. It was shown that SN exhibited anti-inflammation effects (*1*) and protected hepatic cells from CCl_4-induced damage (*2*). Administration of fruit extracts from SN significantly reduced gastric lesions and promoted the healing of gastric ulcers (*3*). Recently, many reports have demonstrated the anticancer potential of various components derived from SN. Ethanol extracts of SN significantly triggered the apoptosis of human breast cancer cells (*4*). A 150 kDa glycoprotein isolated from SN induced apoptosis of HT-29 and Hep3B cell lines (*5*, *6*). Administration of polysaccharides and alkaloids derived from SN inhibited tumor formation in mice bearing cancer cells (*7*, *8*). Lin et al. had revealed that HepG2 cells underwent autophagy in the presence of SN extracts (*9*).

Hepatocellular carcinoma (HCC) is a highly prevalent disease throughout the world, particularly in the Far East and sub-Saharan Africa, where it is strongly associated with virus infection and exposure to food contaminated with aflatoxin B1 (*10*). Because of its delayed diagnosis and high metastasis rate, HCC is one of the leading causes of cancer-related death in Taiwan. Dysregulation of PKCs led to abnormal cell growth, migration, and invasion which contributed to tumorigenesis and metastasis (*11*). PKC α played the critical role in hepato-tumorigenesis. Perletti et al provided evidence that reduced PKC α expression impaired proliferation of MH1C1 rat hepatoma cells (*12*). Schmitt et al. demonstrated that PKC α was involved in VEGF-induced disruption of the tight junction, which may elicit the spread of hepatoma cells into normal liver parenchyma (*13*). Using antisense oligonucleotide analysis, Wu et al. have demonstrated that TPA-induced migration of hepatoma cells was mainly dependent on PKC α but not PKC βII or PKC ε (*14*). Significantly higher expression of PKC α was associated with the size and stage of tumor, resulting in the poor prognosis of HCC occurring in Taiwan (*15*). Downregulation of PKC α reduced the growth, migration, and invasion of human HCC cells via inactivating p38 (*16*). Disruption of PKC α decreased the migration/invasion-related gene expression and increased the level of p53 and p21, thus inhibiting cell proliferation, invasion, and migration of human HCC cell lines (*17*).

It was demonstrated that SN extracts (SNE) inhibited the proliferation of HCC cells (*9*, *18*). However, little is known about

*To whom correspondence should be addressed. Tel: +866-4-24730022 ext. 11670. Fax: +866-4-23248167. E-mail: wcj@csmu.edu.tw.

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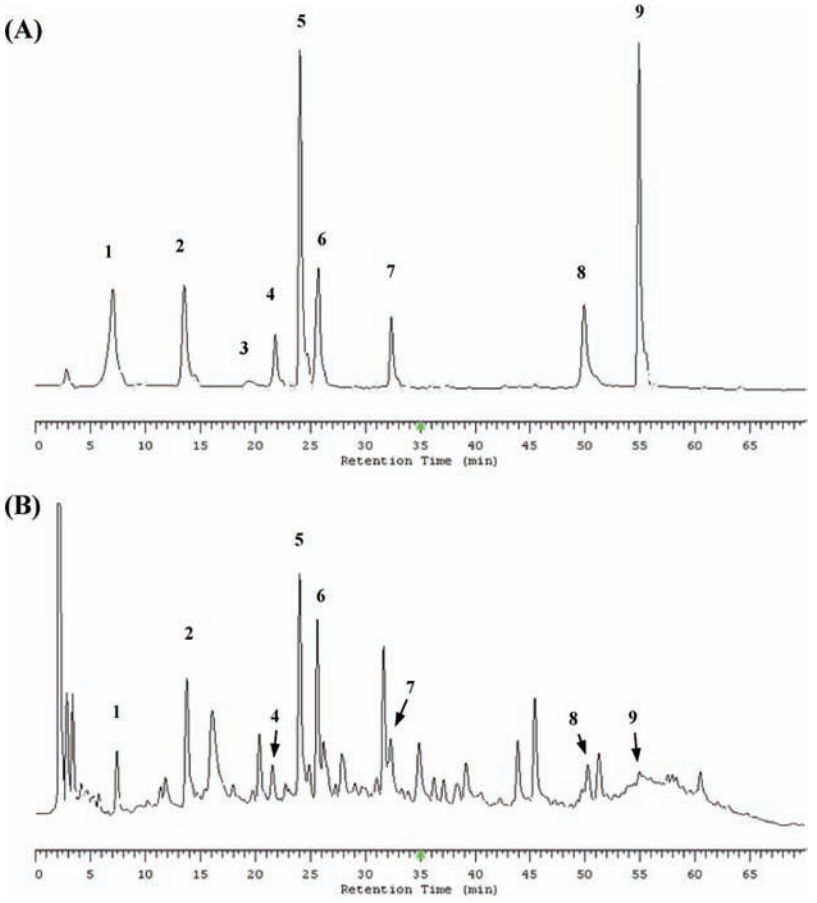

Figure 1. HPLC chromatogram of SNPE. (**A**) HPLC chromatogram of nine kinds of standard polyphenols (1 mg/mL; 10 μL). Peaks: 1, gallic acid; 2, protocatechuic acid; 3, catechin; 4, gallocatechin; 5, caffeic acid; 6, gallocatechin gallate; 7, rutin; 8, quercetin; 9, naringenin. (**B**) HPLC chromatogram of free polyphenols from SNPE (10 mg/mL, 10 μL).

the effects of SNE on the invasion or migration of HCC. In the present investigation, using the in vitro model, we tested the antiprogression ability of both water and polyphenol extracts of SN (SNWE and SNPE, respectively), and the putative role of PKC α in mediating the transduction pathway.

MATERIALS AND METHODS

Materials. Whole plants of SN were collected from a mountain region in Miaoli country, located in central Taiwan. All used chemicals were purchased from Sigma (St. Louis, MO). Antibodies against phospho-p38 and PKC α were obtained from Cell Signaling Technology (Beverly, MA). The Anti-p38, phospho-ERK 1/2, and ERK 1/2 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antiactin and HRP-conjugated secondary antibodies were obtained from Sigma. Constitutively activated PKC α (caPKC α) was a gift from Dr. Jer-Yuh Liu (China Medical University, Taiwan).

Preparation of SNWE and SNPE. The SNWE was obtained as described in previous reports (*9*). Briefly, the SN was collected from the central part of Taiwan. Whole plants were cut into small pieces, dried in the sun, and stored at room temperature for use. Briefly, the dried SN was mixed with water for 30 min and subjected to continuous hot extraction at 100 °C for 40 min. The resulting water extract was filtered and subsequently concentrated in a water bath at 90 °C until it became creamy and was dried in an oven at 70 °C. The analysis of SNWE reveals that it contains 20.4 ± 0.97% total polyphenol (using gallic acid and quercetin as the standard), 14.9 ± 1.3% polysaccharide, and 4.8 ± 0.4% protein. For the preparation of polyphenol extracts of SN (SNPE), we merged 100 g of dried SN powders in 300 mL of ethanol followed by heating at 50 °C for 3 h. The extracts were filtered followed by lyophilization under reduced pressure at room temperature. The powders were resuspended in 500 mL of 50 °C distilled water, followed by extraction with 180 mL of ethyl acetate three times, resolved in 250 mL of distilled water, stored at −70 °C overnight, and lyophilized. The presence and proportion of the main constituents of SNPE have been identified as gallic acid (1.10%), protocatechuic acid (4.56%), gallocatechin (1.38%), caffeic acid (7.18%), gallocatechin gallates (4.74%), rutin (3.00%), quercetin (2.30%), naringenin (4.54%), and unknown components without standards (**Figure 1**). The further identities of the nine components were established from recorded mass spectra. The mass spectra of the phenolic acids are listed in **Table 1**. The polyphenol content of SNPE was estimated about 58.86 ± 0.19% (using gallic acid and quercetin as the standard). The powders were resuspended in distilled water and filtrated by a 0.22 μm filter for use in cell culture.

Cell Culture and Transfection. The human hepatocellular carcinoma cell line HepG2 was cultured with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 100 unit/mL penicillin and 100 μg/mL streptomycin, which were purchased from Gibco/BRL (Gaithersburg, MD, USA) at 37 °C in a humidified atmosphere of 5% CO_2. A vector containing constitutively activated PKC α was transfected into HepG2 cells by Lipofetamine (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendation.

MTT Assay. Cells were seeded in 24-well plates at a density of 2×10^4/mL and treated with the indicated concentration of SNWE or SNPE for 24 h. After the removal of the supernatant, the cells were incubated with medium containing 5.0 g/L 3-(4, 5 dimethylthiazol-2-xl)-2,5 diphenyltetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) and incubated at 37 °C for an additional 4 h. After washing with phosphate buffered saline (PBS), the purple blue formazan was dissolved in 1 mL of isopropanol and the absorbance determined at 563 nm. Cell viability was proportional to the amounts of formazan.

Invasion and Migration Assay. Cells were pretreated with different concentrations of SNWE or SNPE for 24 h, then washed with PBS, and

Table 1. Characterization of Phenolic Compounds of SNPE

peak no.	retention time (min)	assigned identity	recovery (%)	UV λ_{max} (nm)	$[M - H]^-$ (*m*/*z*)	LC/ESI-MS^{2a} (*m*/*z*)
1	8.18	GA	1.100 ± 0.32	270, 225	168.9	125.0
2	14.43	PCA	4.555 ± 1.44	222, 259	153.1	108.9
4	22.07	GC	1.378 ± 0.63	272	305.1	216.6
5	24.52	CA	7.179 ± 1.13	256, 354	178.9	135.0
6	26.08	GCG	4.738 ± 0.93	276	457.1	338.3
7	32.64	R	3.001 ± 0.87	256, 354	609.2	301.1, 343.1
8	50.27	Q	2.304 ± 0.48	266, 368	301.3	151.0, 179.0
9	54.87	N	4.539 ± 1.45	231, 288	271.1	150.9, 177.1

[a] MS^2 run with 30% collision energy. GA, gallic acid; PCA, protocatechuic acid; GC, gallocatechin; CA, caffeic acid; GCG, gallocatechin gallate; R, rutin; Q, quercetin; N, naringenin.

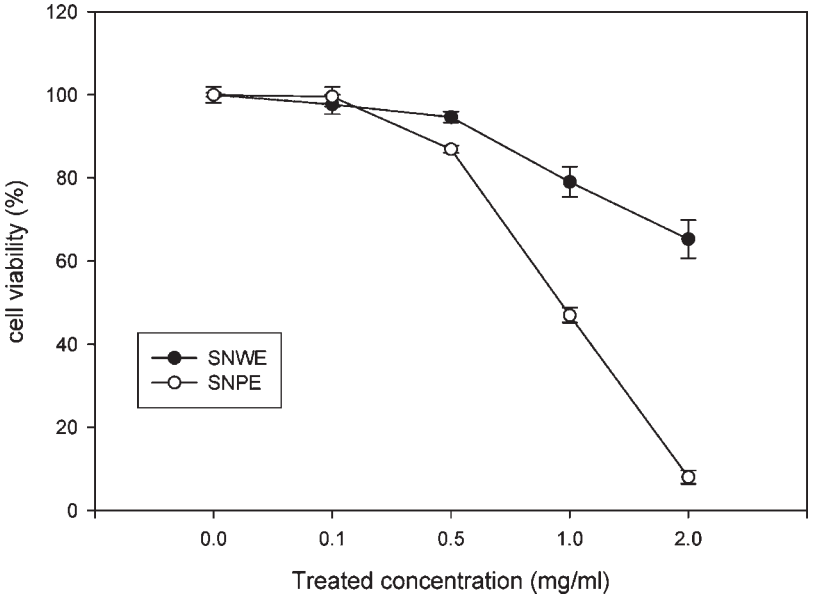

Figure 2. SNWE or SNPE incuced the cytotoxicity of HepG2 cells. HepG2 cells were incubated with various concentrations of SNWE or SNPE (0.1−2 mg/mL) at 37 °C for 24 h. Cell viability was measured by MTT assay. The data were shown as the means ± SD from three replicates per treatment.

trypsinized. After calculating the viability ratio with trypan blue, we seeded alive cells at a density of 5×10^4 in the upper chamber of a 48-well Boyden chamber. For the invasion assay, 10 μL of Matrigel (25 mg/mL, BD. Biosciences, Bedford, MA) was precoated to 8 μm pore size polycarbonate membrane filters. The lower chamber contained 50 nM TPA (Sigma, St. Louis, MO) as a chemo-attractive agent. The chamber was incubated at 37 °C for 6 h. The cells migrating to or invading the lower surface of the membrane were fixed with methanol for 10 min and stained with Giemsa for 1 h. The number of cells was quantified by counting five random microscopic fields (×100 magnification).

Western Blot Analysis. The cell lysates were extracted from SNWE or SNPE treated cells. Protein concentration was measured using a Bradford protein assay kit (Bio-Rad, USA). Fifty micrograms of protein was separated by a 10% polyacrylamide gel and electrotransferred to a nitrocellulose membrane. The membrane was blocked by PBS containing 0.5% nonfat milk for 1 h at room temperature or overnight at 4 °C. After washing with phosphate buffered saline containing 0.1% Tween-20 (PBST), we incubated the membrane with primary antibodies at room temperature for 1 h. The membrane was washed with PBST, followed by reacting with horseradish peroxidase-conjugated goat antimouse IgG antibody (Sigma, St. Louis, MO; 1:5000 dilution). The membrane was extensively washed with PBST, and the reactive signal was detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, UK). The β-actin expression was used as the internal control. Band detection was thereafter revealed by enhanced chemiluminescence using ECL Western blotting detection reagents and exposed in FUJFILM LAS-3000 (Tokyo, Japan). Protein quantity was determined by densitometry using FUJFILM-Multi Gauge V2.2 software.

Statistical Analysis. Data reported are the mean ± standard deviation of three independent experiments and were evaluated by Student's *t* test. Significant differences were established at $p < 0.05$.

RESULTS

SNWE or SNPE Induced the Cytotoxicity of HepG2 Cells. MTT assay was conducted to determine if SNWE and SNPE could exhibit the cytotoxic effect on HCC cells. No cytotoxicity was observed in cells treated with 0.1 mg/mL SNWE or SNPE, whereas significantly reduced viability was found in cells treated with 0.5, 1, and 2 mg/mL SNWE or SNPE. The IC_{50} of SNWE and SNPE was 2.18 and 0.86 mg/mL, respectively (**Figure 2**). Our results indicated that both SNWE and SNPE decreased cell viability in HepG2 cells, while SNPE possessed more cytotoxic potential.

SNWE and SNPE Inhibited TPA-Induced Scattering of HepG2 Cells. It has been demonstrated that the tumor promoter 12-*o*-tetradecanoylphobor-13-acetate (TPA) triggered the scattering of HepG2 cells (*14*). To address if SNWE and SNPE affected TPA-induced scattering, cell morphological changes were observed. In the presence of 50 nM TPA, the morphology of HepG2 was changed from epithelial-like to fibroblast-like shape. Increased concentration of SNWE or SNPE reversed the TPA-induced alteration of cell shape (**Figure 3**).

SNWE and SNPE Inhibited TPA-Induced Migration and Invasion of HepG2 Cells. Boyden chamber analysis was performed to investigate whether SNWE or SNPE could inhibit TPA-induced

HepG2 migration and invasion. Cells (5×10^4) were seeded on a Boyden chamber and added with TPA with or without pretreatment of various concentrations of SNWE or SNPE.

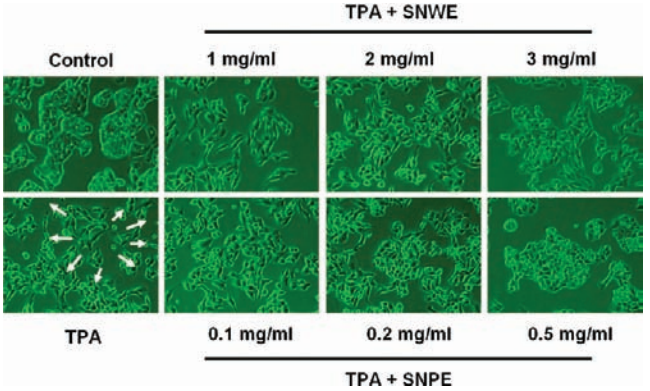

Figure 3. SNWE and SNPE inhibited TPA-induced scattering of HCC cells. HepG2 cells were untreated (control) or treated with 50 nM TPA (TPA) coupled with various doses of SNWE or SNPE for 24 h. EMT-like morphology change of HepG2 cells was observed under a light microscope. The arrow shows the cell scattering induced by TPA. The cells were photographed under ×100 magnification. Data represents one of at least three independent experiments.

No alteration was found in cells treated with low doses of SNWE (below 0.1 mg/mL), whereas at the concentrations of 0.5, 1.0, and 2.0 mg/mL, SNWE significantly inhibited TPA-stimulated migration of HepG2 cells. Similarly, SNPE reduced TPA-elicited migration at concentrations above 0.2 mg/mL (**Figure 4A** and **B**). SNWE and SNPE also significantly abolished the invasion of HepG2 cells (**Figure 5A** and **B**). Taken together, both SNWE and SNPE prevented TPA-induced migration and invasion, while SNPE was more potent.

SNWE and SNPE Reduced PKC α Protein Expression in HepG2 Cells. To investigate if PKC α could mediate the anti-migration and anti-invasion effect of SNWE and SNPE, HepG2 cells were treated with TPA with or without various concentrations of SN. It was demonstrated that TPA enhanced the expression of PKC α, whereas SNWE and SNPE apparently blocked TPA-induced PKC α expression in a dose-dependent manner (**Figure 6**).

SNWE and SNPE Reduced the Phosphorylation of p38 and ERK in HepG2 Cells. The PKC-MAPK pathway has been demonstrated to be involved in the mobility and migration of HCC cells (*19*). Immunoblotting was conducted to analyze if the downstream targets of PKC such as ERK and p38 were involved in the antiprogression effect exerted by SNWE and SNPE. As shown in **Figure 7A**, SNWE inhibited TPA-induced phosphorylation of p38 but not ERK in a dose-dependent manner. However, SNPE

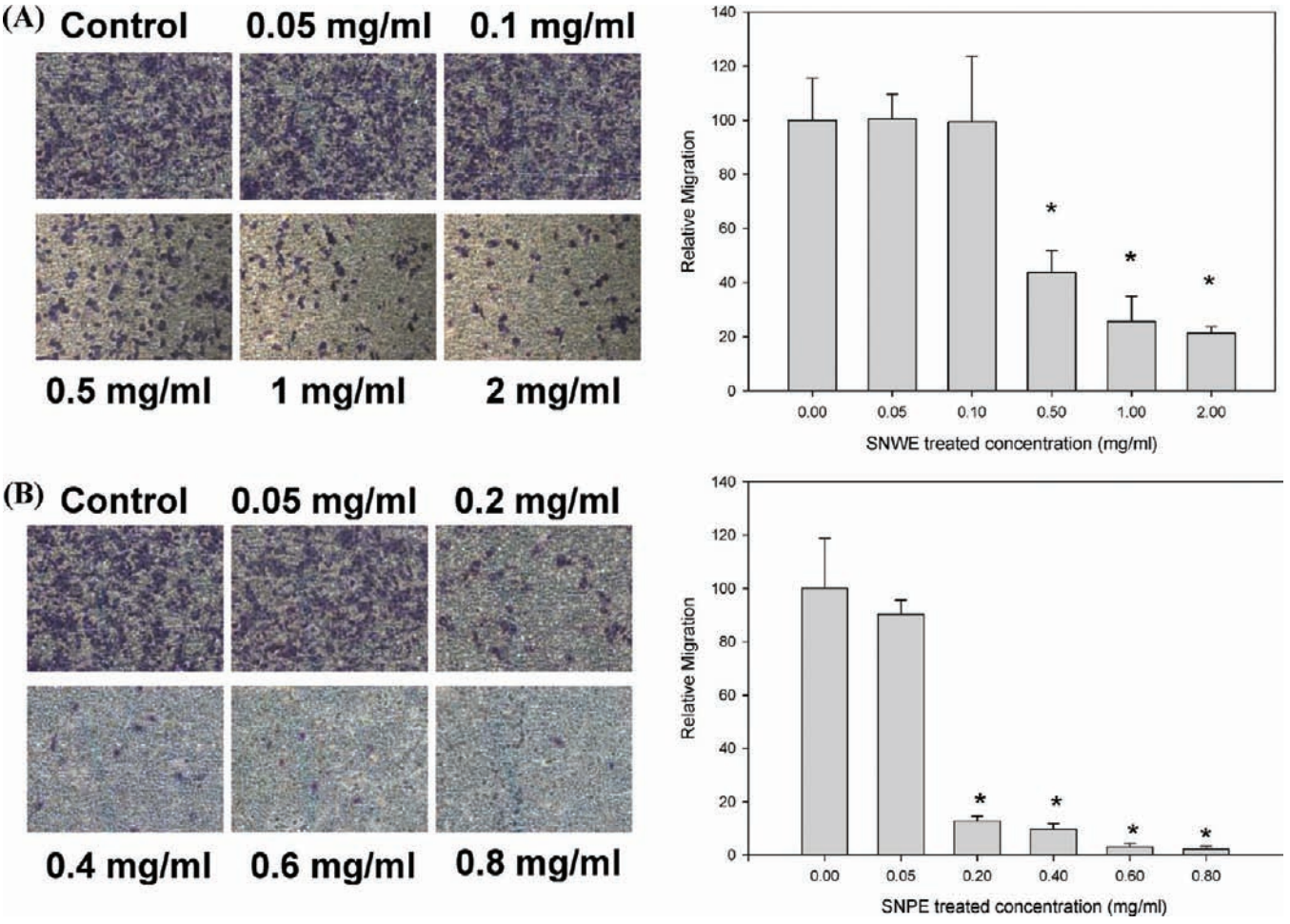

Figure 4. SNWE and SNPE inhibited TPA-induced migration of human HCC cells. HepG2 cells were pretreated with various doses of SNWE (**A**) or SNPE (**B**) for 24 h, then washed with PBS, and trypsinized. After the viability ratio with trypan blue was calculated, we seeded alive cells (5×10^4) in the upper chamber of a 48-well Boyden chamber for 24 h. TPA (50 nM) was applied to the lower chamber as a chemo-attractive agent. Cells that had migrated or invaded the lower surface of the membranes were counted under a light microscope. The migratory effect inhibited by SNWE or SNPE was analyzed by *t* test. The quantitative data was represented as the mean ± SD from three independent experiments. *, $p < 0.01$, compared with the TPA-induced control.

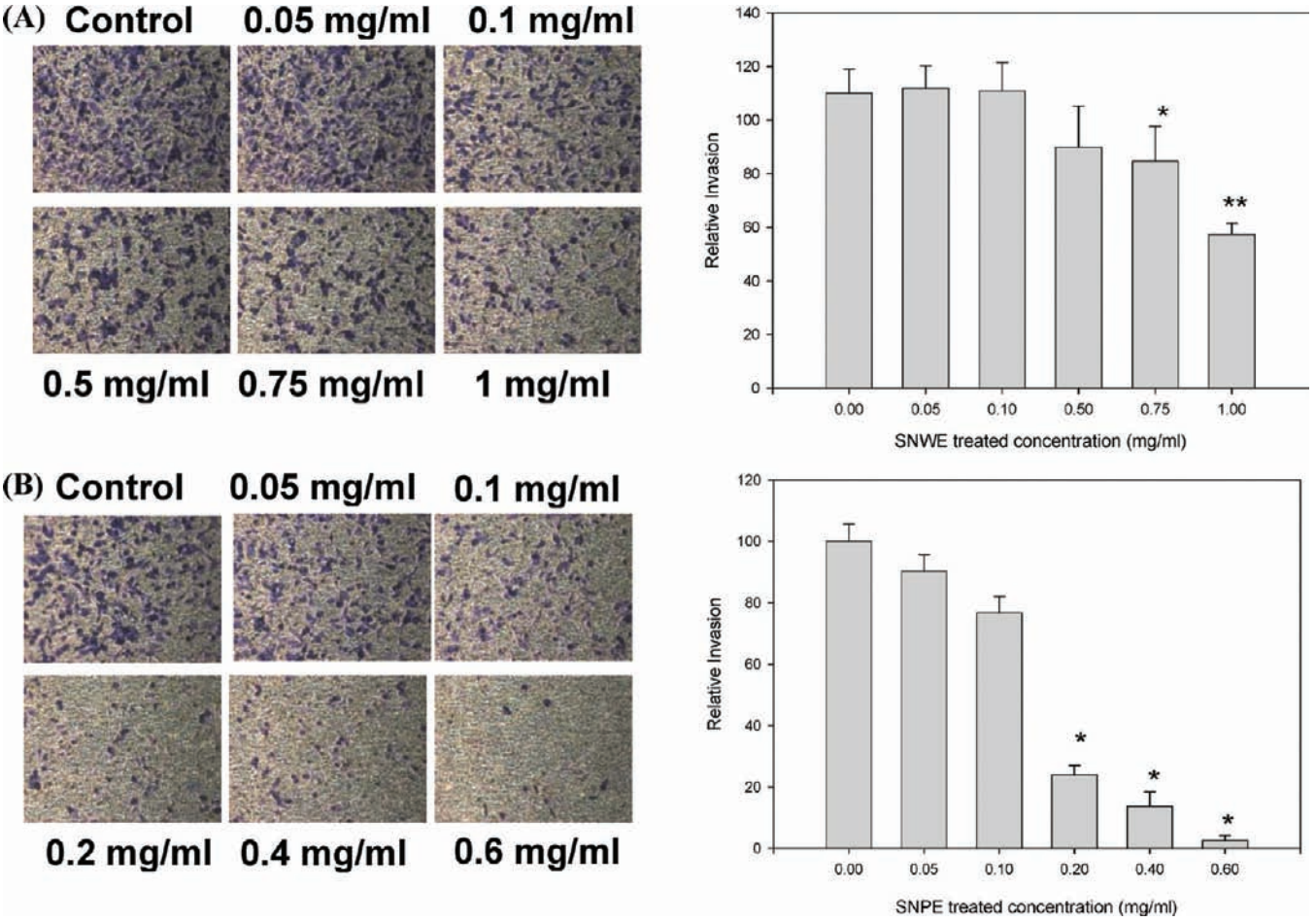

Figure 5. SNWE and SNPE reduced TPA-induced invasion of human HCC cells. HepG2 cells were pretreated with various doses of SNWE (**A**) or SNPE (**B**) for 24 h, then washed with PBS, and trypsinized. After calculating the viability ratio with trypan blue, we seeded alive cells (5×10^4) in the upper chamber of a 48-well Boyden chamber consisting of 8-μm pore-size filters coated with Matrigel basement membrane matrix for 24 h. TPA (50 nM) was applied to the lower chamber as a chemo-attractive agent. Cells that had invaded the lower surface of the membranes were counted under a light microscope. The invasion effect inhibited by SNWE or SNPE was analyzed by *t* test. The quantitative data was represented as the mean ± SD from three independent experiments. *, $p < 0.05$ compared with the TPA-induced control. **, $p < 0.01$ compared with the TPA-induced control.

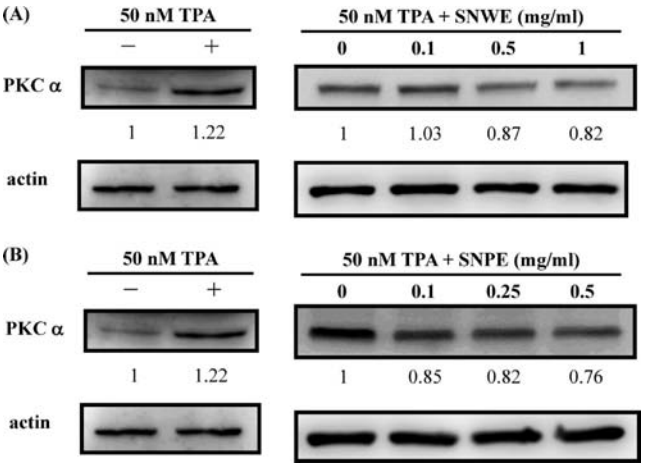

Figure 6. SNWE and SNPE reduced PKC α protein expression in human HCC cells. The HepG2 cells were treated with 50 nM TPA coupled with SNWE or SNPE for 24 h. After the cells were harvested, we did Western blot analyses with anti- PKC α and β-actin antibodies as described in Materials and Methods. The results were represented by using an ECL system and normalized to β-actin. This figure is a representation of three independent experiments with similar results.

significantly reduced TPA-elicited activation of p38 and ERK in a dose-dependent manner (**Figure 7B**). These data indicated that SNWE and SNPE attenuated TPA-induced p38 or p38/ERK activation, respectively.

Constitutively Activated PKC α Reversed the Inhibitory Effects of SNWE and SNPE on Migration of HepG2 Cells. To confirm whether PKC α was involved in the migration of HCC, HepG2 cells were treated with various conditions and then analyzed with migration assay. TPA-stimulated migration was reduced to 60% and 50% in the presence of two PKC α inhibitors, 5 μM Ro-32-0432 and 10 μM Go-6983, respectively (**Figure 8**). On the contrary, constitutively activated PKC α (caPKC α) per se significantly increased the migration of HepG2.

To address whether caPKC α could recover the downstream signals of PKC in the presence of SNWE or SNPE, HepG2 cells were transfected with caPKC α, and the expressions of p38 and ERK were observed. As shown in **Figure 9**, caPKC α increased the phosphorylation of ERK 1/2 and p38 about 1.8- and 2-fold, respectively. Treatment of SNWE or SNPE did not alter the caPKC α-elicited activation of ERK1/2 and p38 (**Figure 9**). However, in the presence of 0.05 and 0.1 mg/mL SNPE, caPKC α-induced migration was decreased 30% and 40%, respectively. Similar results were also obtained in the presence of 0.5 and 1 mg/mL of SNWE (**Figure 10**).

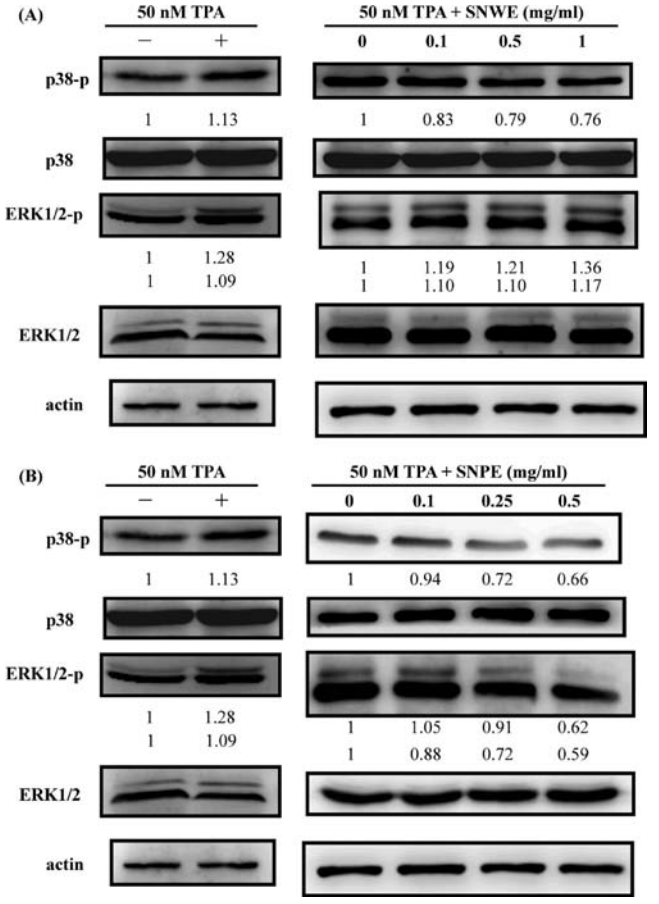

Figure 7. SNWE (**A**) and SNPE (**B**) reduced the protein levels of MAPK family in human HCC cells. The HepG2 cells were treated with 50 nM TPA and various concentrations of SNWE (**A**) or SNPE (**B**) for 24 h. Cell lysates were harvested and subjected into Western blot analysis using primary antibodies as described in Materials and Methods. Results from a representative experiment are shown. The values below the figures represent change in protein expression of the bands normalized to β-actin. This figure is a representation of three independent experiments with similar results.

Effects of SNWE and SNPE Were Not Cell Type-Specific. For understanding if the inhibitory effect of SNWE and SNPE was specific for different cell types, MDA MB-231 (breast cancer cell) and HT-29 (colorectal cell) were used for analysis. It was shown that there existed the inversed correlation between the concentrations of SNWE and SNPE and the viability of tumor cells (**Figure 11A**). Our data also revealed that both SNWE and SNPE attenuated the migration of MDA MB-231 and HT-29 cells (**Figure 11B**). These results indicated that the inhibitory effects of SNWE and SNPE were not cell type-specific.

DISCUSSION

Cell migration triggered many biological changes such as wound healing, invasion, and pathogenesis of tumor metastasis. HCC is one of the poorest prognostic malignancies in Taiwan because of its high prevalence and invasive spread. Hence, it is critical for searching proper agents to prevent the invasion and metastasis of HCC. It has been shown that the natural components isolated from vegetables, fruits, and Chinese herbs exhibited the potency of antimigration and antimetastasis. In the present study, we have demonstrated that the water-soluble and polyphenol extracts isolated from SN also possessed an antiprogressive effects on HCC cells.

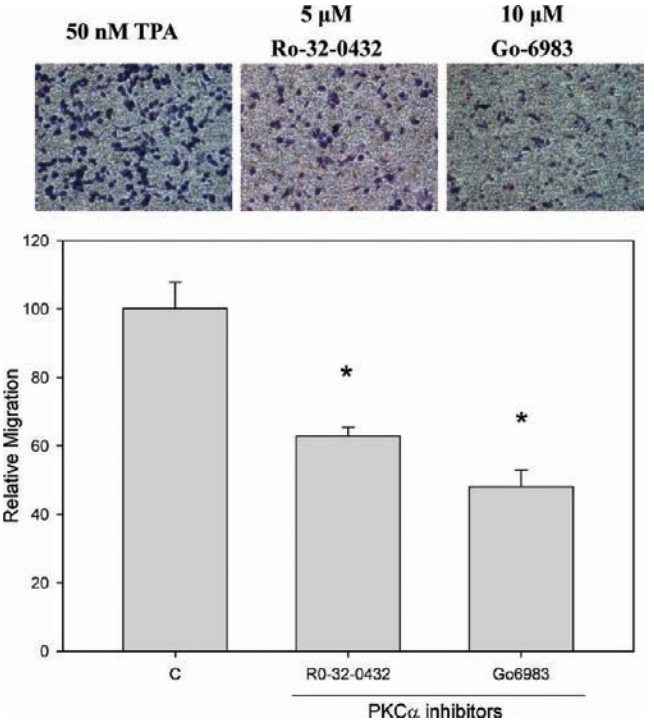

Figure 8. Effects of PKC α inhibitors on TPA-induced migration in human HCC cells. HepG2 cells were treated with 50 nM TPA coupled with PKC inhibitor, 5 μM Ro-32-0432 or 10 μM Go-6983, or vehicle for 24 h. Migration was determined as described in Methods and Materials. The quantitative data was represented as mean $\pm$ SD. * represented significant difference ($p < 0.01$) calculated by *t* test compared to TPA-treated cells.

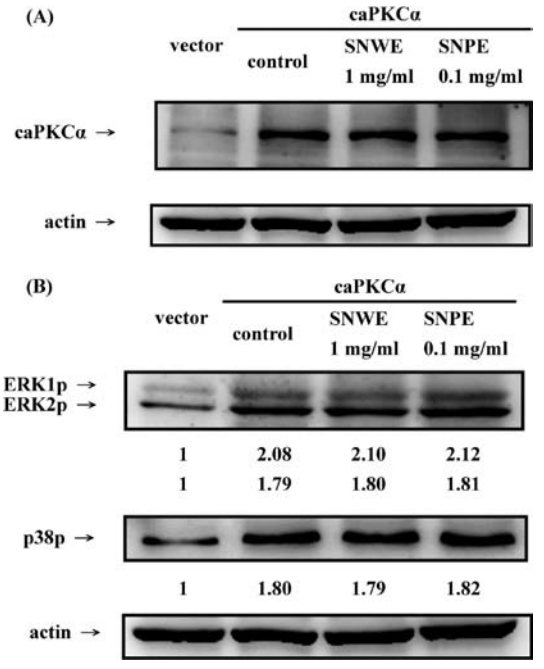

Figure 9. Effects of constitutively activated PKC α on MAPK signal in SNWE and SNPE treated human HCC cells. HepG2 cells were transfected with 8 μg of vector (denoted as vector) or constitutively activated PKC α plasmid (denoted as caPKC α). Twenty-four hours after transfection, cells were treated with 1 mg/mL SNWE or 0.1 mg/mL SNPE for additional 24 h. Cell lysates from each treatment were subjected into Western blot analysis to detect the expression level of caPKC α (**A**) or phosphor-ERK and phosphor-p38 (**B**). Results from a representative experiment are shown. The values below the figures represent change in protein expression of the bands normalized to β-actin.

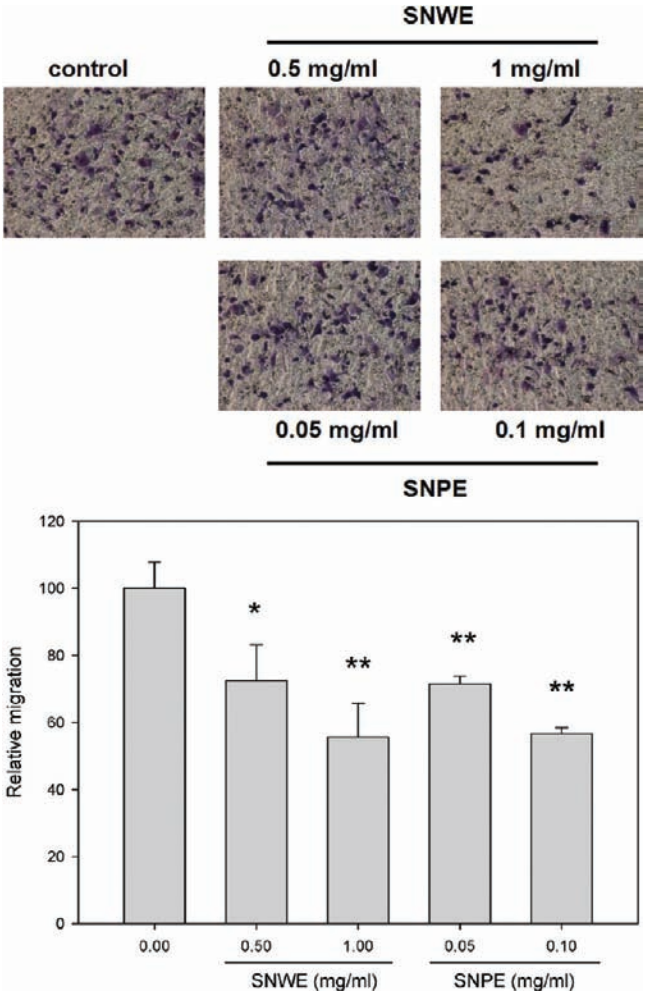

Figure 10. Effects of constitutively activated PKC α on the migration of SNWE and SNPE treated HCC cells. HepG2 cells were transfected with constitutively activated PKC α. Transfected cells were pretreated with SNWE or SNPE, or vehicle for 24 h. Cells (5×10^4) were seeded onto the upper chamber for 24 h. Migrated cells were counted under a light microscope. Each result was obtained from the average of at least three separate experiments. The migratory effect inhibited by SNWE or SNPE was analyzed by *t* test. The quantitative data was represented as mean ± SD. *, $p < 0.05$, compared with the TPA-induced control. **, $p < 0.01$, compared with overexpressed, constitutively activated PKC α.

Recently, much attention has been paid to the PKC family for its regulation of cell migration. Increased expression or activation of PKC was shown to be associated with higher invasive ability in breast cancer and colorectal carcinoma (*20*, *21*). Stable expression of PKC α enhanced the migration of endothelial cells (*22*). Using antisense oligonucleotides to downregulate the expression of PKC α, the human HCC cell lines, HA22T/VGH and SK-Hep-1, were shown to undergo apoptosis and reduce the migration capability (*17*). Our data indicated that either PKC activator TPA or caPKC α significantly induced the migration and invasion of HepG2 cells, whereas PKC α inhibitors decreased TPA-elicited migration. These results suggested that PKC α did play a pivotal role in HCC migration and invasion.

We demonstrated that SNWE and SNPE significantly reduced TPA-induced migration and invasion of HepG2 cells (**Figure 4** and **5**) and other human cancer cell lines (**Figure 11**), indicating that the inhibitory effect of SNWE or SNPE was noncell type-specific. In comparison with their potency, 0.12 mg/mL SNPE exerted an effect similar to that of 0.5 mg/mL SNWE, which inhibited 50% of cell migration of HepG2, suggesting that almost 4-fold of SNWE was needed to exhibit the same effect as SNPE. Alternatively, the content of polyphenol of SNPE (58.8%) was estimated about 3-fold compared to that of SNWE (20.4%) and was supposed to be the most effective component of SN. As observed in the present investigation, SNPE possesses a better effect for regulating cancer cell migration and invasion compared with those of SNWE. The compositions of SN polyphenol such as naringenin, quercetin, and gallic acid have been shown to abrogate migration and invasion of cancer cell lines. Naringenin inhibited hepatocyte growth factor (HGF)-induced migration and invasion in MDA-MB-231 human breast cancer cells (*23*). Quercetin abolished HGF-mediated migration of medulloblastoma cells (*24*) and reduced the invasion of B16-BL6 melanoma (*25*) and osteoblastic cells (*28*). In the present study, SNWE and SNPE decreased TPA-induced PKC α expression in a dose-dependent manner. Recently, quercetin was shown to inhibit the expression of PKC α but not PKC β nor PKC δ in B16-BL6 melanoma cells (*26*). Our results were in accordance with the literature which suggested that the components of SN inhibited tumor cell migration through reducing PKC expression or activation.

Furthermore, SNWE and SNPE inhibited the activation of p38 and ERK. It was well accepted that p38 and ERK played an essential role in cell migration (*19*). Recent reports also demonstrated that p38 and/or ERK functioned as downstream targets of PKCs. The PKC α-mediated p38 activation was involved in lipopolysaccharide-stimulated TNF-α production in microglia (*27*). Knockdown of PKC α led to the decrease of phosphorylation of p38 but not ERK nor JNK, which in turn, attenuated the migration and invasion of HCC (*16*). Matsuoka et al. have indicated that tamoxifen decreased the migration of B16-Bl6 melanoma cells through suppression of the PKC α-MEK-ERK pathway (*28*). It has also been shown that downregulation or inhibition of PKC β led to the abolishment of ERK and p38 activation, and subsequently attenuated the migration of HCC cells (*19*). This evidence implied the critical transduction of PKC/ERK and/or p38 in mediating migration and invasion. Intriguingly, with overexpressed caPKC α, SNWE and SNPE still obviously inhibited the migration of HepG2 cells, although the inhibitory effect on the expression of ERK and p38 could not be reversed. It was demonstrated that quercetin reduced the migration of osteoblast cells through directly inhibiting ERK and p38 protein kinases (*29*). However, quercetin was also shown to inhibit the PI-3 kinase/AKT signal pathway which subsequently reduced migration in TNF-α-induced mouse epidermal cells (*30*) and in HGF-induced medulloblastoma cells (*24*). Our results suggested that, in addition to PKC α, extracts of SN might also inhibit other protein kinases involved in cell proliferation and migration.

In summary, both SNWE and SNPE inhibited TPA-stimulated migration and invasion, and reduced the activation of p38 and ERK in HCC cells. Overexpression of caPKC α recovered SNWE or SNPE-reduced p38 and ERK activation, whereas cell migration was still attenuated in the presence of SNWE or SNPE. Our results suggested that SNWE and SNPE may become promising therapeutic agents for preventing the migration and invasion of human hepatocellular carcinoma.

ABBREVIATIONS USED

caPKC α, constitutively activated PKC α; DMEM, Dulbecco's modified Eagle's medium; ERK, extracellular regulated kinase; HCC, hepatocellular carcinoma; JNK, c-jun amino-terminal

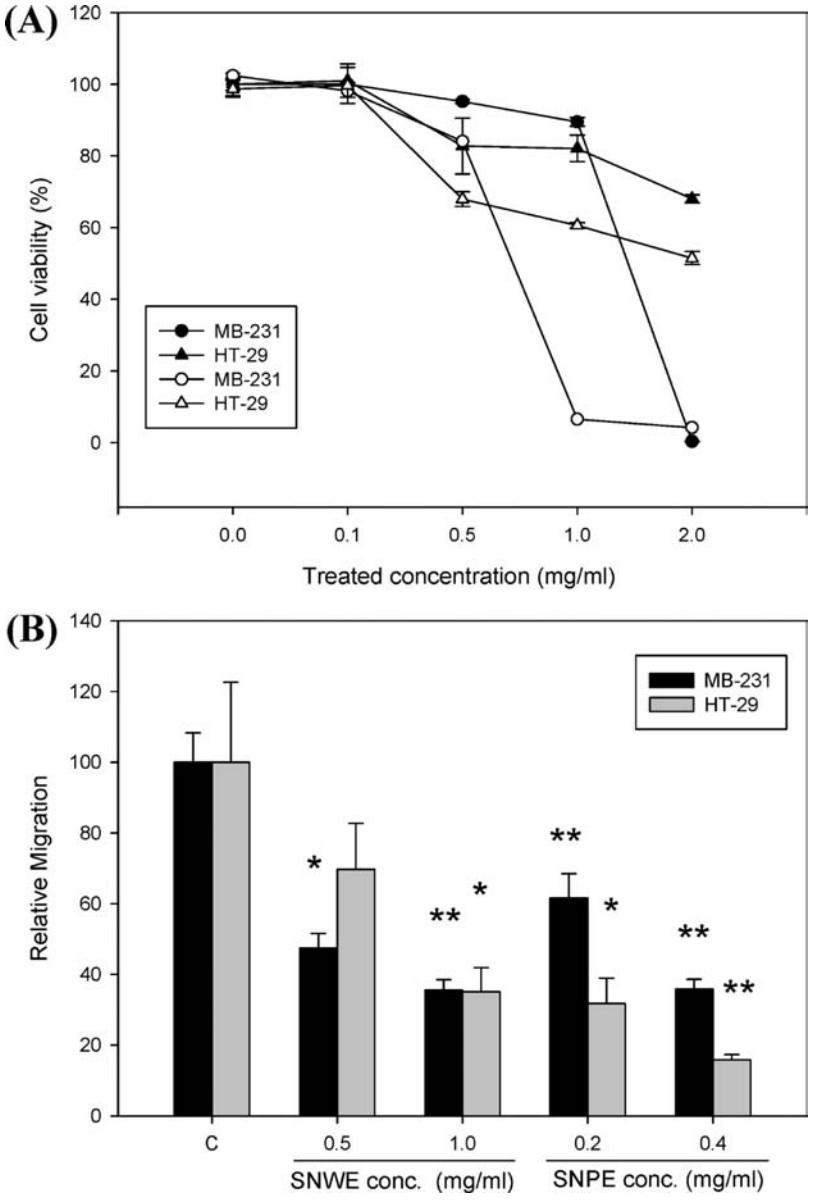

Figure 11. Effects of SNWE and SNPE on cell viability (**A**) and TPA-induced migration (**B**) of different types of cancer cells. The cytotoxicity effects of SNWE (closed symbols) and SNPE (open symbols) on MDA-MB-231 (circle) and HT-29 (triangle) (**A**). Cancer cells were incubated with different concentrations SNWE or SNPE (0.1−2 mg/mL) for 24 h, and the viability rate was determined by MTT assay. Closed circle and triangle denote the presence of SNWE, and open circle and triangle denote the presence of SNPE. SNWE and SNPE inhibited TPA-stimulated migration of MDA-MB-231 and HT-29 (**B**). The migration of MDA-MB-231 (black bar) and HT-29 (gray bar) cancer cells in the presence of TPA (50 nM) is shown. Cells were pretreated with various doses of SNWE or SNPE for 24 h. Migration was induced by 50 nM TPA for 24 h. The quantitative data was represented as mean ± SD. *, $p < 0.05$, compared with the TPA-induced control. **, $p < 0.01$, compared with the TPA-induced control.

kinase; MTT, 3-(4,5 dimethylthiazol-2-xl)-2,5 diphenyl-tetrazolium bromide; PKC, protein kinase C; SN, *Solanum nigrum* L.; SNPE, polyphenol extracts of *Solanum nigrum* L.; SNWE, water extracts of *Solanum nigrum* L.; TPA, 12-*o*-tetradecanoylphobor-13-acetate.

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