Research Article

Geraniin-mediated apoptosis by cleavage of focal adhesion kinase through up-regulation of Fas ligand expression in human melanoma cells

Jang-Chang Lee[1,2], Chih-Yen Tsai[3], Jung-Yie Kao[4], Ming-Ching Kao[3], Shih-Chang Tsai[3], Chih-Shiang Chang[1], Li-Jiau Huang[1], Sheng-Chu Kuo[1], Jen-Kun Lin[3,5] and Tzong-Der Way[3]

[1] Graduate institute of Pharmaceutical Chemistry, College of Pharmacy, China Medical University, Taichung, Taiwan
[2] School of Pharmacy, College of Pharmacy, China Medical University, Taichung, Taiwan
[3] School of Biological Science and Technology, College of Life Science, China Medical University, Taichung, Taiwan
[4] Institute of Biochemistry, College of Life Science, National Chung Hsing University, Taichung, Taiwan
[5] Institute of Biochemistry and Molecular Biology, College of Medicine, National Taiwan University, Taipei, Taiwan

Geraniin, a form of tannin separated from geranium, causes cell death through induction of apoptosis; however, cell death characteristics for geraniin have not yet been elucidated. Here, we investigated the mechanism of geraniin-induced apoptosis in human melanoma cells and demonstrated that geraniin was able to induce cell apoptosis in a concentration- and time-dependent manner. We also examined the signaling pathway related to geraniin-induced apoptosis. To clarify the relationship between focal adhesion kinase (FAK) and geraniin-induced apoptosis, we treated human melanoma cells with geraniin and found that this resulted dose- and time-dependent degradation in FAK. However, FAK cleavage was significantly inhibited when cells were pretreated with a selective inhibitor of caspase-3 (Ac-Asp-Glu-Val-Asp-CHO). Here, we demonstrated for the first time that geraniin triggered cell death by caspase-3-mediated cleavage of FAK. There were two possible mechanisms for activating caspase-3, mitochondria-mediated and receptor-mediated apoptosis. To confirm the geraniin-relevant signaling pathway, using immunoblot analysis we found that geraniin-induced apoptosis was associated with the up-regulation of Fas ligand expression, the activation of caspase-8, the cleavage of Bid, and the induction of cytochrome c release from mitochondria to the cytosol. Treatment with geraniin caused induction of caspase-3 activity in a dose- and time-dependent manner followed by proteolytic cleavage of poly-(ADP-ribose) polymerase, and DNA fragmentation factor 45. The geraniin-induced apoptosis may provide a pivotal mechanism for its cancer-chemopreventive action.

Keywords: Apoptosis / Caspase-3 / Fas ligand / Focal adhesion kinase / Geraniin

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1 Introduction

Melanoma is a malignant proliferation of melanocytes, previously considered a rare form of skin cancer. The number of new melanomas diagnosed in the United States has been increasing by 2–3% per year over the past decade. Incidence and mortality rate increases are among the largest of all cancers [1]. The prognosis for advanced disease is poor, with a 5-year survival rate of less than 10% [2]. Occasionally patients have prolonged remissions with chemotherapy or biological response modifiers; however, response rates are usually less than 20%. Therefore, novel approaches are needed for the management of melanoma. "Chemoprevention" could be one such approach. Because of the increasing incidence of skin cancer and inadequacies in current treatment, effective chemoprevention strategies need to be developed.

Correspondence: Dr. Tzong-Der Way, School of Biological Science and Technology, College of Medicine, China Medical University, 91 Hsueh-Shih Road, Taichung, Taiwan 40402, R.O.C
E-mail: tdway@mail.cmu.edu.tw
Fax: +886-4-2207-0465

Abbreviations: DFF-45, DNA fragmentation factor 45 ; **FAK**, Focal adhesion kinase; **FasL**, Fas ligand ; **PARP**, poly(ADP-ribose) polymerase ; **PI**, propidium iodide

Many recent studies have indicated that anticancer drugs or cancer-chemopreventive agents act through the induction of apoptosis to prevent tumor promotion, progression, and the occurrence of cellular inflammatory responses other than necrosis [3, 4]. Apoptosis is a gene-directed form of cell death with well-characterized morphological and biochemical features [5]. It causes characteristic morphological changes, including membrane blebbing, cellular shrinkage, and chromatin condensation, that lead to cellular detachment from the substratum in adherent cells or a loss of cell-cell contact in suspension cells [6].

Focal adhesion kinase (FAK), a cytoplasmic tyrosine kinase located in the focal adhesion complex, has been implicated in the integration of signals from integrins and neuropeptides and plays an anti-apoptotic role in anchorage-dependent cells [7]. Overexpression of FAK is a common event in numerous tumor systems, including breast, colon, and thyroid carcinomas, and occurs at early stages of tumorigenesis before a tumor has developed the capacity for invasion and metastasis [8, 9]. Importantly, FAK has been shown to be one of the critical factors protecting cells from apoptosis, but the exact mechanism is unknown [10]. Studies using breast cancer cells demonstrated that FAK-mediated signaling is required for survival of these cells in both cell adhesion-dependent and -independent conditions, and that disruption of FAK functions induces caspase-8-dependent apoptosis, suggesting an important role for FAK in inhibiting death receptor-related apoptosis [11]. LFA-1/FAK/PI3-K/Akt is a survival pathway in multiple myeloma and targeted inhibition of this pathway may provide new therapeutic options [12].

In 1980, Okuda *et al*. [13] reported that geraniin is the main tannin component in several species of Geranium (Geraniaceae) and Euphorbia (Euphorbiaceae), and that hydrolysis of geraniin produces corilagin. Our study was designed to investigate the chemopreventive potential of geraniin against melanoma. In view of the data that FAK is important for suppressing apoptosis, we attempt to determine whether geraniin-induced apoptosis could lead to disruption of FAK functions.

2 Materials and methods

2.1 Materials

Geraniin was isolated from the leaves of *Macaranga tanarins (L.)* as described previously [14]. The antibodies to Bax and DNA fragmentation factor 45 (DFF-45) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-caspase-3, -8, and -9, anti-Bid, cytochrome *c*, and poly(ADP-ribose) polymerase (PARP) antibodies were from PharMingen (San Diego, CA, USA); anti-Bcl-2, Bcl-XL, Fas, FAK and Fas ligand (FasL) antibodies were purchased from Transduction Laboratory (Lexington, KY, USA); cleavaged caspase-3, and cleavaged caspase-9 were purchased from Cell Signaling Technology (Danvers, MA, USA). Secondary antibodies used were fluorescein isothiocyanate-labeled rabbit anti-mouse IgG (Chemicon), horseradish peroxidase-conjugated anti-mouse, and rabbit IgG. Propidium iodide (PI) and rhodamine 123 were obtained from Sigma (St. Louis, MO, USA). The fluorogenic peptide substrate Ac-Asp-Glu-Val-Asp-AMC (Ac-DEVD-AMC) for general caspase activity was purchased from Bachem (King of Prussia, PA, USA).

2.2 Cell culture

Human melanoma cells A2058 were cultured in DMEM (Gibco) containing 10% fetal bovine serum(Gibco).Cells were maintained at 37°C in a humidified atmosphere at 95% air and 5% CO_2.

2.3 Cell viability

As described previously [15], the effects of geraniin on cell proliferation were examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

2.4 Flow cytometry

Human melanoma cells (1×10^6) were cultured in 100-mm petri dishes and incubated for 24 h. Cells were harvested, washed with PBS, resuspended in 200 μL PBS, and fixed in 800 μL ice-cold 100% ethanol at −20°C. After being left to stand overnight, the cell pellets were collected by centrifugation, resuspended in 1 mL hypotonic buffer(0.5% Triton X-100 in PBS and 0.5 μg/mL RNase) , and incubated at 37°C for 30 min. Then 30 μL PI solution (50 mg/mL) was added, and the mixture was allowed to stand on ice for 30 min. Fluorescence emitted from the PI-DNA complex was quantitated after excitation of the fluorescent dye by FACScan cytometry (Becton Dickinson, San Jose, CA, USA).

2.5 DNA extraction and electrophoresis analysis

A2058 cells (2×10^5 cells/mL) were harvested, washed with PBS, and then lysed with digestion buffer containing 0.5% sarkosyl, 0.5 mg/mL proteinase K, 50 mM Tris (pH 8.0), and 10 mM EDTA at 56°C for 3 h and treated with RNase A (0.5 μg/mL) for another 2 h at 56°C. The DNA was extracted by phenol/chloroform/isoamyl alcohol (25:24:1) before loading and analyzed by 2% agarose gel electrophoresis. The agarose gels were run at 50 V for 120 min in TBE buffer (Tris-borate/EDTA electrophoresis buffer). Approximately 20 μg DNA was loaded in each well and visualized under UV light and photographed.

2.6 Activity of caspase

Cells were collected and washed with PBS and suspended in 25 mM HEPES (pH 7.5), 5 mM $MgCl_2$, 5 mM EDTA, 5 mM

dithiothione, 2 mM phenylmethylsulfonyl fluoride, 10 μg/mL pepstatin A and 10 μg/mL leupeptin after treatment. Cell lysates were clarified by centrifugation at 12 000 rpm for 30 min at 4°C. The supernatants containing 100 μg total protein were incubated with 50 μM MCA-Ac-DEVD at 30°C for 1 h. The fluorescence intensity of MCA release was measured by a Hitachi F-4500 fluorescence spectrophotometer with excitation at 360 nm and emission at 460 nm (Promega's CaspACE™ Assay System, Madison, WI, USA).

2.7 Western blotting

Cells were treated with various agents as indicated in figure legends. After treatment, Western blotting was performed as described previously [15]. Expression levels of actin, Bid, caspase-3, caspase-8, caspase-9, Fas, Bcl-2, FAK, PARP, DFF-45, cleavaged caspase-3, cleavaged caspase-9 and cytochrome c were detected by enhanced chemiluminescence (ECL, Amersham, Arlington Heights, IL, USA).

2.8 Cytochrome c release

Mitochondrial and cytosolic fractions were prepared by resuspending cells in ice-cold buffer A (250 mM sucrose, 20 mM HEPES, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothione, 17 μg/mL phenylmethylsulfonyl fluoride, 8 μg/mL aprotinin, and 2 μg/mL leupeptin (pH 7.4). Cells were passed through a needle ten times. Unlysed cells and nuclei were pelleted by centrifugation for 10 min at $750 \times g$. The supernatant was then at $100\,000 \times g$ for 15 min. This pellet was resuspended in buffer A and represents the mitochondrial fraction. The supernatant was centrifuged at $100\,000 \times g$ for 1 h. The supernatant from this final centrifugation represents the cytosolic fraction.

3 Results

3.1 Treatment with geraniin causes dose-dependent reduction in cell survival

The structure of geraniin is illustrated in Fig. 1A. First, to test the effect of geraniin on cell viability, A2058 cells were treated with different concentrations of geraniin. After 24 h of treatment, the percentage of living cells was determined and it was shown that geraniin induced a dose-dependent decrease in cell viability (Fig. 1B).

3.2 Induction of apoptosis by geraniin

Geraniin-treated A2058 cells underwent apoptosis in a dose- and time-dependent manner as measured by flow cytometry using PI staining [11]. As seen in Fig. 2A, the percentage of apoptotic human melanoma cells was 3.06%, 3.52%, 5.6% and 14.71% after 24-h incubation with 0, 10,

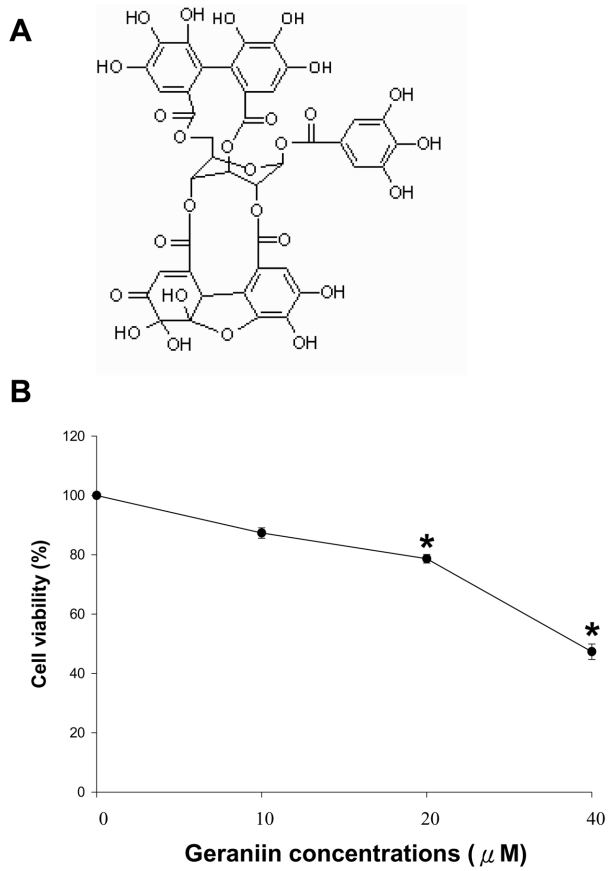

Figure 1. Effect of geraniin on the viability of human melanoma cells. (A) Structure of geraniin. (B) A2058 cells were treated with DMSO (Con) or various concentrations (10–40 μM) of geraniin for 24 h, and subsequent cell viability was measured by an MTT assay. Results from three separate experiments were averaged and are presented as mean ± SE. The Student's *t*-test was used to determine the significance of inhibition (*$p < 0.01$).

20 and 40 μM geraniin, respectively. The percentage of apoptotic human melanoma cells was 2.31%, 3.31%, 17.65%, 49.5% and 60.3% after incubation with 40 μM geraniin for 0, 12, 24, 36 and 48 h, respectively (Fig. 2B). DNA fragmentation, a hallmark of apoptosis, was demonstrated by incubating A2058 with different concentrations of geraniin (Fig. 2C, right panel) for 24 h. DNA fragmentation became apparent at 20 μg/mL or 40 μg/mL of geraniin treatment, and these DNA fragmentation responses were dose dependent. When cells were treated with 40 μg/mL geraniin, DNA ladders were just visible as early as 12 h after treatment, and gradually increasing DNA fragmentation was observed from 12 to 36 h (Fig. 2C, left panel).

3.3 Cleavage of FAK by geraniin during apoptosis

FAK is overexpressed in tumor cells. FAK has been shown to play important roles in regulating cell migration, cell

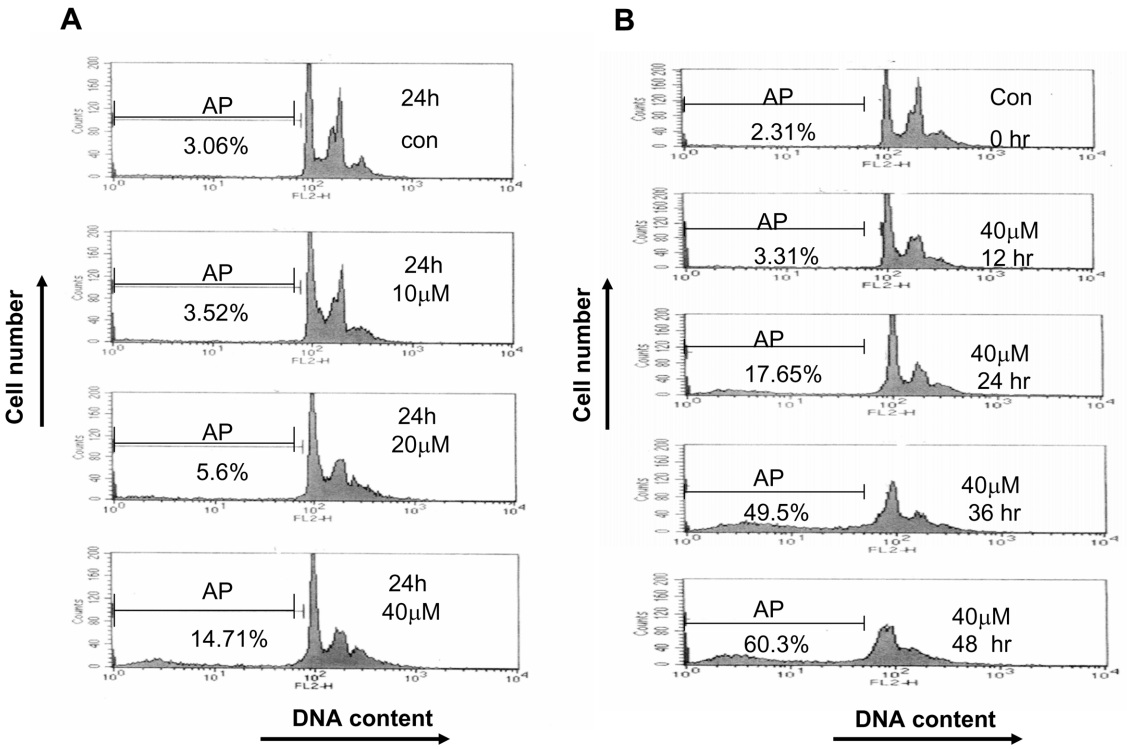

Figure 2. Geraniin induced apoptosis in human melanoma cells. (A) A2058 cells were treated with DMSO (Con) or different concentrations of geraniin for 24 h. (B) A2058 cells were treated with DMSO (Con) or 40 μM geraniin for different time periods. The method for flow cytometry is described in Section 2. Following flow cytometric analysis, the cellular DNA profile was further analyzed by the CellQuest software. Data represent the percentage of cell counts displaying a hypoploid DNA population. (C) DNA ladder formation in A2058 cells incubated with DMSO (Con) or geraniin (40 μM) at 37°C for various times (left column) and various doses for 24 h (right column). Results are from one experiment that is representative of three similar experiments.

cycle progression, and cell survival. Treatment of A2058 cells with 40 μM geraniin caused proteolytic cleavage of FAK at various times (Fig. 3A). Cleavage of FAK was observed during treatment of A2058 cells with different concentrations of geraniin for 24 h (Fig. 3B).

3.4 Induction of apoptosis by geraniin through activation of caspase-3 and cleavage of FAK

Caspases are believed to play a central role in mediating various apoptotic responses. Caspase activities were measured in A2058 cells treated with different concentrations of geraniin for 12 h. As shown in Fig. 4A, the induction of caspase-3 activity by geraniin paralleled the dose-dependent pattern of apoptosis. In addition, we studied the time course caspase-3 activation. Caspase-3 activity was significantly induced, showing a tenfold increase by 12 h after the addition of 40 μM geraniin (Fig. 4B). Moreover, treatment of A2058 cells with geraniin produced an apparent decrease in the expression of procaspase-3, and the active form of caspase-3 was detected at 24 h after treatment with geraniin (Fig. 4C). Cleavage of FAK by caspases during apoptosis has been recently reported [15]. To further explore the possibility that activation of caspase-3 may also induce FAK protein degradation, we tested the effects of tetrapeptide cysteine protease inhibitors on FAK cleavage and cell survival after geraniin treatment on A2058 cells. z-VAD-FMK (100 μM) blocked FAK cleavage and procaspase-3 cleavage (Fig. 4D) in the presence of 40 μM geraniin, significantly increasing cell survival. Moreover, DEVD-CHO (200 mM)

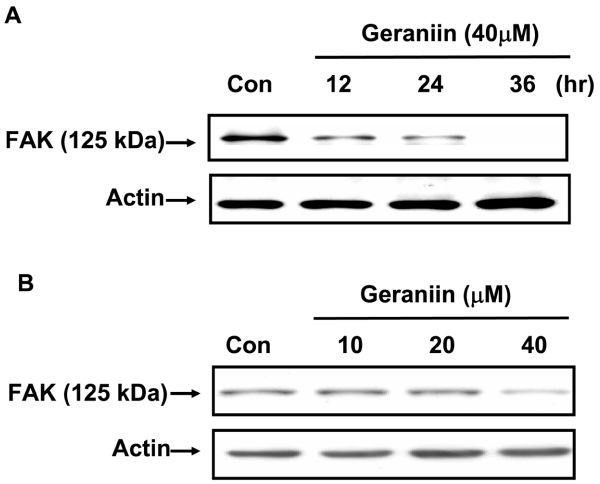

Figure 3. Cleavage of FAK during geraniin-induced apoptosis. A2058 cells were incubated with DMSO (Con) or geraniin (40 μM) at 37°C for various times (A) and for 24 h at various doses (B). Immunoblotting was used to measure levels of full-length FAK and actin. Western blot data presented are representative of those obtained in at least three separate experiments.

suppressed cleavage of FAK. DEVD-CHO preferentially inhibits activation of caspases-3. YVAD-CHO did not suppress FAK cleavage even at a concentration of 200 mM (Fig. 4E). YVAD-CHO preferentially inhibits activation of caspases-1. These data suggest that geraniin mediates cleavage of FAK through activation of caspase-3.

3.5 Geraniin-induced cytochrome c release and the cleavage of caspase-9

The process of cell death may involve the release of cytochrome c from the mitochondria, which subsequently causes apoptosis by activation of the caspases. Together, these data suggest a linear and specific activation cascade between caspase-9 and caspase-3 in response to cytochrome c released from the mitochondria. We tested whether geraniin could also induced cytochrome c release. As shown in Fig. 5A, geraniin clearly led to the release of cytochrome c into the cytosol in a time-dependent manner. We next examined the cleavage of caspase-9 during treatment of cells with geraniin by immunoblotting. A time-dependent proteolytic cleavage of procaspase-9 and the active form of caspase-9 was demonstrated after treatment with 40 μM geraniin (Fig. 5B).

3.6 Treatment with geraniin causes degradation of PARP

Activation of caspase-3 leads to the cleavage of a number of proteins, one of which is PARP. Although PARP is not essential for cell death, the cleavage of PARP is another hallmark of apoptosis [16]. Treatment of A2058 cells with 40 μM geraniin caused a time-dependent proteolytic cleavage of PARP. The PARP cleavage occurred approximately 12 h after geraniin treatment (Fig. 5C).

3.7 Treatment with geraniin causes the cleavage of DFF-45

DFF has been identified as a heterodimeric protein that triggers DNA fragmentation during apoptosis, although DFF has no nuclease activity [17]. Caspases activated by apoptotic signals cleave DFF-45 to release caspase-activated deoxyribonuclease [18]. We explored the possibility that activation of caspase-3 may also induce DFF-45 protein degradation. Indeed, treatment of A2058 cells with 40 μM geraniin caused proteolytic cleavage of DFF-45 at 24 h. (Fig. 5D). Caspase-3 cleaves DFF-45 and, once caspase-activated deoxyribonuclease is released, it can enter the nucleus where it degrades chromosomal DNA.

3.8 Geraniin-induced apoptosis involves the activation of caspase-8, cleavage of Bid and down-regulation of Bcl-2 expression

Caspases, a family of aspartate-specific cysteine protease, play a pivotal role in the execution of programmed cell death [19]. To further analyze the geraniin-induced apoptosis pathway, we performed a time-course experiment from 12 to 36 h at 12-h intervals after treating A2058 cells with 40 μM geraniin. We investigated the processing of caspase-8, the most proximal caspase during death receptor-mediated apoptosis [20], and found that procaspase-8 was cleaved by 12 h after geraniin treatment (Fig. 6). Previous studies have shown that caspase-8 cleaves Bid to form tBid, which, through direct association with anti-apoptotic members of the Bcl-2 family, releases pro-apoptotic Bax or Bak to generate pores in the mitochondrial membrane, leading to activation of the cytochrome c → caspase-9 → caspase-3 → PARP pathway [21, 22]. In addition, tBid may homodimerize as an alternative mechanism for inducing this mitochondrial pathway. Interestingly, we detected Bid cleavage at 12 h after geraniin treatment in the present study (Fig. 6). We therefore examined additional members of the Bcl-2 family that might interact with tBid and modulate its pore-forming activity in the mitochondrial membrane. The prototypical anti-apoptotic protein Bcl-2 was shown to be decreased in a time-dependent manner by geraniin (Fig. 6).

3.9 Geraniin-induced apoptosis may be mediated by up-regulation of FasL expression

Initiator caspases (including 8, 9, 10, and 12) are closely coupled to pro-apoptotic signals. Once activated, these caspases cleave and activate downstream effector caspases (including 3, 6, and 7), which in turn cleave cytoskeletal and nuclear proteins, such as PARP and lamin A, and

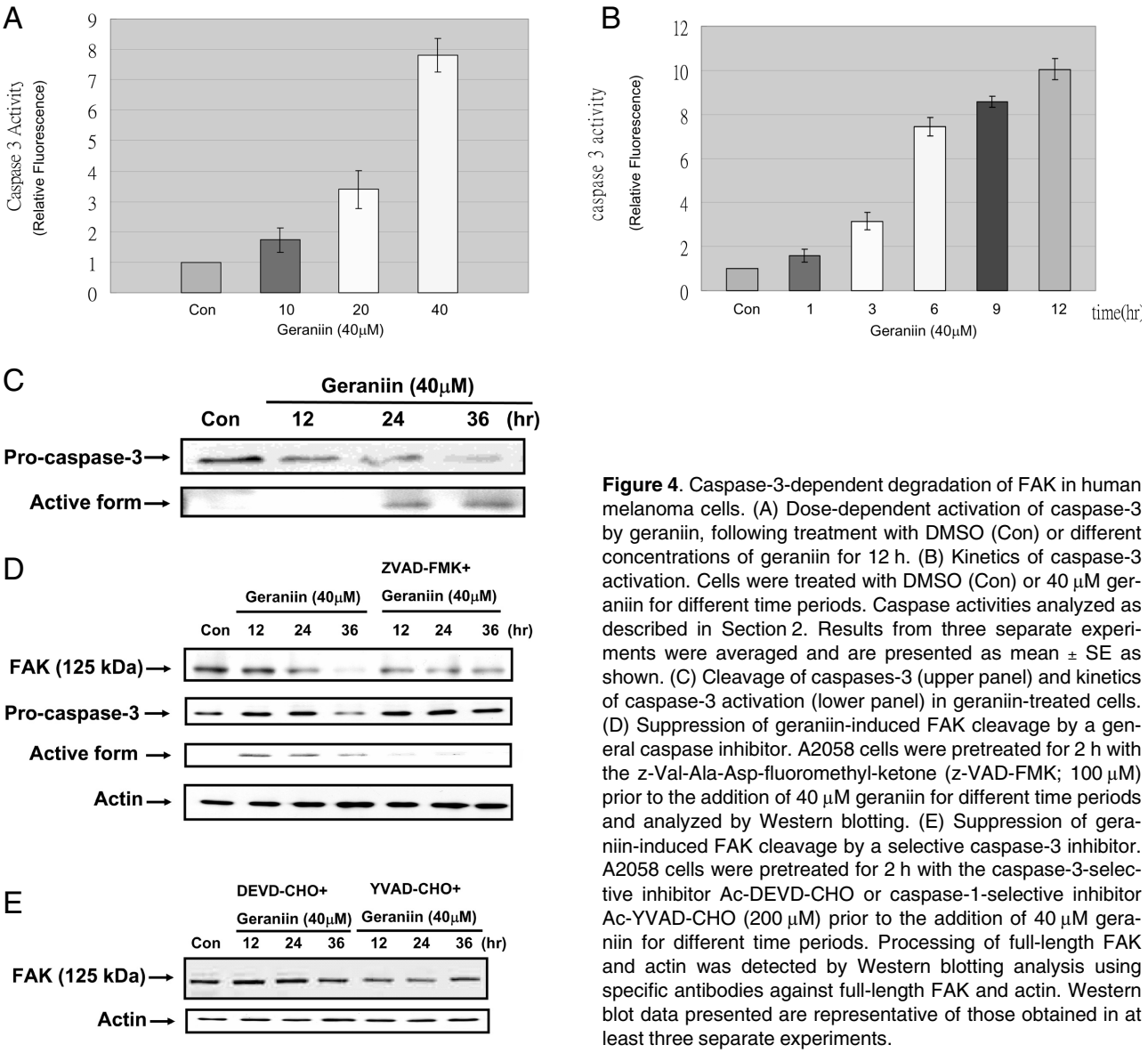

Figure 4. Caspase-3-dependent degradation of FAK in human melanoma cells. (A) Dose-dependent activation of caspase-3 by geraniin, following treatment with DMSO (Con) or different concentrations of geraniin for 12 h. (B) Kinetics of caspase-3 activation. Cells were treated with DMSO (Con) or 40 μM geraniin for different time periods. Caspase activities analyzed as described in Section 2. Results from three separate experiments were averaged and are presented as mean ± SE as shown. (C) Cleavage of caspases-3 (upper panel) and kinetics of caspase-3 activation (lower panel) in geraniin-treated cells. (D) Suppression of geraniin-induced FAK cleavage by a general caspase inhibitor. A2058 cells were pretreated for 2 h with the z-Val-Ala-Asp-fluoromethyl-ketone (z-VAD-FMK; 100 μM) prior to the addition of 40 μM geraniin for different time periods and analyzed by Western blotting. (E) Suppression of geraniin-induced FAK cleavage by a selective caspase-3 inhibitor. A2058 cells were pretreated for 2 h with the caspase-3-selective inhibitor Ac-DEVD-CHO or caspase-1-selective inhibitor Ac-YVAD-CHO (200 μM) prior to the addition of 40 μM geraniin for different time periods. Processing of full-length FAK and actin was detected by Western blotting analysis using specific antibodies against full-length FAK and actin. Western blot data presented are representative of those obtained in at least three separate experiments.

induce apoptosis. Pro-apoptotic stimuli include the FasL, TNF-α, DNA damage and ER stress. Treatment of A2058 cells with 40 μM geraniin for 0, 1, 6, 12, 24 and 36 h induced up-regulated expression of FasL (Fig. 7).

4 Discussion

We have shown that FAK was degraded in an apparently time- and dose-dependent manner in human melanoma cells in the presence of geraniin (Figs. 3A and B), and that DEVD-CHO, a selective caspase-3 inhibitor, could successfully interrupt this event (Fig. 4E). Taken together, our results demonstrate that geraniin induces caspase-3 activity, which leads to cleavage of FAK and cell death. However, whether FAK activity is modulated by dephosphorylation or by caspase-3 cleavage during cell death remains to be determined. To gain an understanding of the downstream mechanism of caspase-3, other substrates such as PARP and DFF-45 were detected (Figs. 5C and D). Interestingly, although geraniin also possesses a tannin structure, no reports about tannin-induced apoptosis through the FAK signaling have so far been reported.

To gain insight into the mechanism activating the caspase-3, we examined several components of the known apoptosis pathways. Apoptosis is governed by a complex network of anti-apoptotic and pro-apoptotic molecules. The Bcl-2 family proteins play critical role in regulating apoptosis by controlling the mitochondria membrane permeability [23 – 25]. Normally, the Bcl-2 protein functions as a sup-

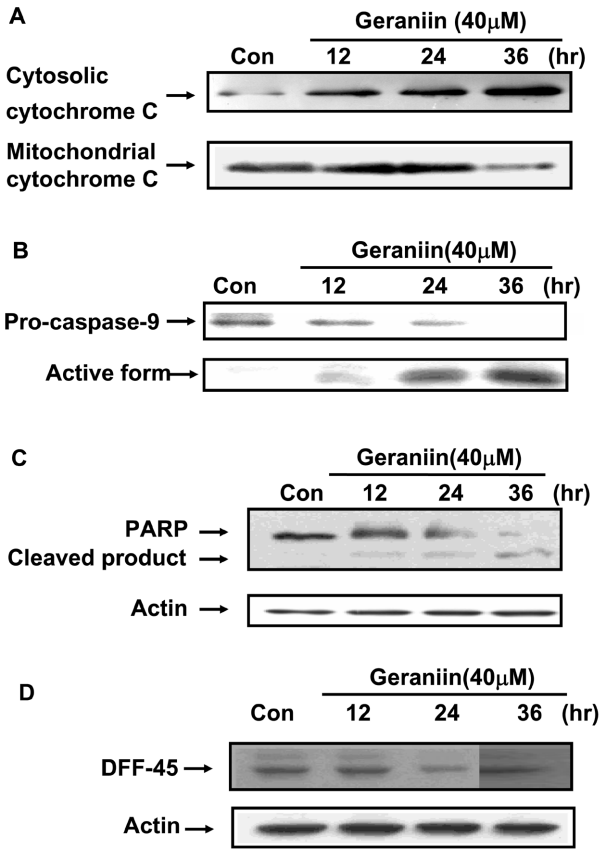

Figure 5. Induction of cytochrome c release and caspase-9 processing in human melanoma cells by geraniin. (A) A2058 cells were treated with DMSO (Con) or 40 μM geraniin for the indicated time periods. Subcellular fractions were prepared as described in Section 2, and cytochrome c was detected using a cytochrome c antibody. (B) Total cell lysates were prepared from A2058 cells treated with DMSO (Con) or 40 μM geraniin for different time periods. Levels of procaspase-9 and cleaved caspase-9 were analyzed by immunoblotting. (C) Cleavage of death substrates during geraniin-induced apoptosis. Kinetics of PARP cleavage by 40 μM geraniin was followed for different time periods and analyzed by Western blotting. (D) Kinetics of DFF-45 cleavage by 40 μM geraniin was followed for different time periods and analyzed by Western blotting. Western blot data presented are representative of those obtained in at least three separate experiments.

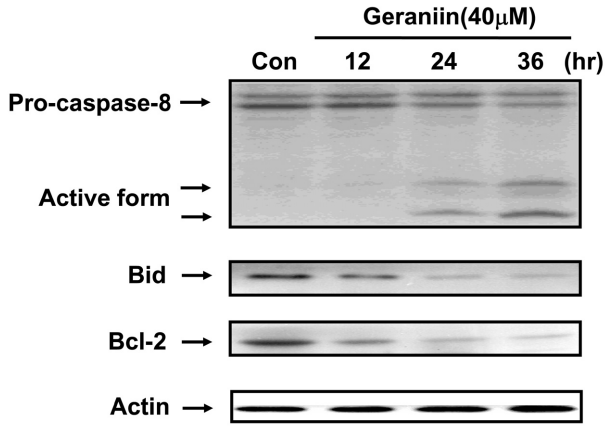

Figure 6. Geraniin-induced apoptosis in human melanoma cells involves the activation of procaspase-8, with cleavage of Bid and reduced expression of Bcl-2. A2058 cells were incubated with DMSO (Con) or 40 μM geraniin for different time periods. Expression of caspase-8, Bid, Bcl-2 and actin was detected by Western blotting analysis using their specific antibodies. Western blot data presented are representative of those obtained in at least three separate experiments.

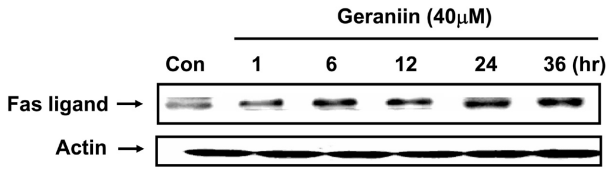

Figure 7. FasL is up-regulated in geraniin-induced apoptosis. A2058 cells were incubated with DMSO (Con) or 40 μM geraniin for different time periods. Expression of FasL and actin was detected by Western blotting analysis using specific antibodies against FasL and actin. Western blot data presented are representative of those obtained in at least three separate experiments.

pressor of apoptosis, whereas Bax is a pro-apoptotic protein in the Bcl-2 family [26, 27]. In response to damage or stress Bax can form transmembrane pores across the mitochondrial membrane and interrupts the normal function of anti-apoptotic Bcl-2 protein [28]. This leads to a loss of membrane potential [28] and an efflux of cytochrome *c* and the apoptosis-inducing factor. Cytochrome *c* together with other components forms a complex with procaspase-9, which finally leads to the activation of caspase-3 [29]. We observed that treatment of human melanoma cells with geraniin decreases the expression of Bcl-2 (Fig. 6, panel 3) and procaspase-9 (Fig. 5B), indicating that the release of cytochrome *c* from mitochondria to cytosol results in the activation of caspase-9 and then caspase-3, with the decreased expression of Bcl-2 protein.

Fas is a major member of the death receptor family, a subgroup of the TNF receptor superfamily, characterized by a cytoplasmic death domain that is responsible for the transmission of apoptotic signaling through interaction with death domain-bearing adaptor molecule [30]. When FasL binds to the Fas death receptor, the adaptor molecule Fas-associated death domain protein (FADD)/Mort-1 becomes recruited to the receptor, allowing binding and proteolysis of procaspase-8 for generation of activated caspase-8 [31]. Bid, a BH3-domain membrane of the Bcl-2 family, is cleaved by caspase-8 following the FasL treatment [32, 33], where the truncated Bid fragment (tBid) translocates to mitochondria and triggers the release of cytochrome *c*. Our results indicated that Bid was cleaved (Fig. 6, panel 2) subsequent to the FasL expression and cleavage of caspase-8 (Fig. 6, panel 1) during geraniin-induced apoptosis in

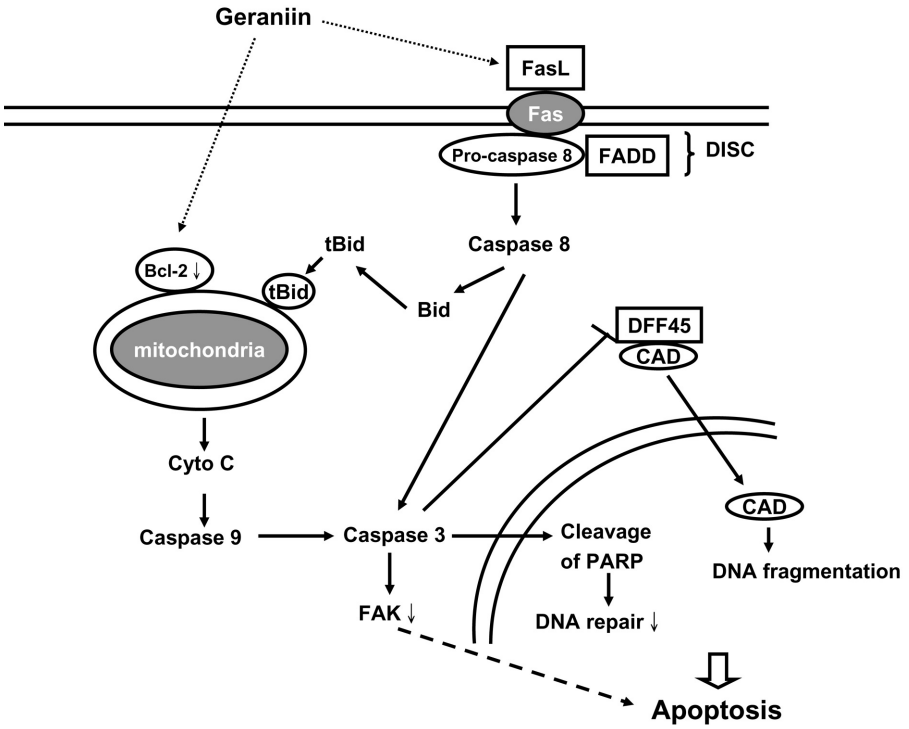

Figure 8. Schematic representation of the molecular mechanism of geraniin-induced apoptosis in human melanoma cells.

human melanoma cells. Cleavage of caspase-8 and Bid may have been efficiently activated by a direct death receptor activation. However, it is unknown how geraniin increases the expression of Fas and FasL. Recently, it was reported that mitochondrial dysfunction is directly related to the enhancement of Fas gene expression [34], suggesting that geraniin may cause damage to mitochondria *via* an unknown pathway, and that a mitochondrial defect might directly or indirectly lead to the overexpression of Fas and FasL proteins.

In summary, the object of our study was to characterize the signaling pathways producing geraniin-mediated apoptosis. Two possible mechanisms are presented (Fig. 8). In the mitochondria-dominated pathway, activation of the caspase cascades responsible for apoptosis occurred following damage to the mitochondrial membrane and release of cytochrome *c*, which were partially initiated by the disturbance of Bcl-2 family proteins. Alternatively, in the receptor-mediated pathway, geraniin might induce the increase of Fas and FasL expression, and resulting in activation of caspase-8, which subsequently promotes proteolytic processing of caspase-3 and Bid. Our results indicate that geraniin may have a potential role in cancer therapeutic and chemopreventive functions.

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The authors have declared no conflict of interest.

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