Original Article

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Gigantol-induced apoptosis in lung cancer cell through mitochondrial-dependent pathway

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Abstract

Lung cancer is identified as a major cause of cancer-related death. Death in this cancer associates with the acquisition of apoptosis avoidance mechanism. Apoptosis, a programmed cell death mechanism, plays an important role on normal cell development and tissue homeostasis. The aberrances on cell death mechanism contribute to several pathogenesis including cancers. Several efforts have been paid in investigation of anticancer agents from natural sources. Gigantol (GG), an agent isolated from Thai orchid, *Dendrobium draconis*, possesses various pharmacological activities; however, its effect on cancer apoptosis was not investigated before. This study demonstrates for the first time that GG induced lung cancer cells apoptosis which was prevented by the addition of Z-VAD-FMK, caspase 3 inhibitor. Western blot analysis reveals that anti-apoptotic Bcl-2 and Mcl-1 were dramatically downregulated, in concomitance with an upregulation of proapoptotic Bax protein in response to GG treatment. Anti-apoptosis FLIP was not significantly changed, suggesting that GG-mediated apoptosis was through mitochondrial-dependent mechanism. Interestingly, the fact that GG showed no cytotoxic effect on normal keratinocyte Hacat cells, supports its selectivity on cancer cells. This finding sheds light on an anticancer activity of GG, providing an evidence for development of this agent for cancer therapy.

Key Words: Apoptosis, Gigantol, Mitochondrial pathway, Lung cancer

Introduction

Lung cancer becomes the major cause of high lethal rate of cancer patients [1]. It appears to have defect in cell death machinery that lead to uncontrolled cell growth and potentiated malignancy [2]. Apoptosis, a programmed cell death mechanism, is classified into mitochondrial and death receptor pathways [3]. In mitochondrial apoptotic pathway, apoptotic stimulations trigger the release of cytochrome c from mitochondria, leading to caspase-9 activation and consequent apoptotic signaling initiation [4]. In case of death receptor pathway, the assembly of death ligands such as Fas ligand (FasL), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and tumor necrosis factor (TNF) to their receptors recruit

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death-inducing signaling complex (DISC), activate caspase-8 and trigger following death signaling cascades [5]. Apoptotic response to death receptor pathway in type 2 cells was dependent on mitochondrial pathway through Bid cleavage [6].

Several apoptotic-related proteins were identified such as anti- and pro-apoptotic members in Bcl-2 family and cellular FLICE-like inhibitory protein (cFLIP) [7]. Most of cancers exhibit the imbalance expressions of these apoptotic regulatory proteins [8-10], which lead to the deregulated tumor growth and the resistance to chemotherapy [11-13]. Recently, it has showed that lung cancer cells resist to cisplatin-induced cell death through upregulation of antiapoptosis Bcl-2 protein [14]. The increase of endogenous level of Mcl-1 and FLIP was also shown to associate with the avoidance to TRAIL-induced apoptosis [15,16]. The suppression of anti-apoptotic proteins or upregulation of proapoptotic protein would therefore be therapeutic approach to attenuate uncontrolled cell growth and restore apoptosis sensitivities in cancer.

Currently, cancer research emphasizes the discovery of new drug to improve this aberrant apoptotic behavior in cancers. Natural product is an important source for new drug development [17], where several therapies at present such as paclitaxel [18] and vinblastin [19] were firstly originated from. Recently, the compounds isolated from the family of *Orchidaceae* have been shown their cytotoxicity in various cancers including lung, liver, stomach and colon cancers [20-22]. Gigantol (GG), a bibenzyl compound (Figure 1), was found in *Dendrobium* species such as *Dendrobium draconis* [23], *Dendrobium nobile* [24] and *Dendrobium densiflorum* [25]. This compound was gained increase interest due to its promising pharmacological activities including anti-inflammation [26], anti-oxidation [27] and anti-proliferation [28]. However, the effect of GG on cancer apoptosis was not clarified before. This study demonstrated that GG was able to induce apoptotic cell death through suppression of anti-apoptotic Bcl-2 and Mcl-1, together with an upregulation of pro-apoptotic Bax. The result of study provides information for development of GG as a promising anticancer agent.

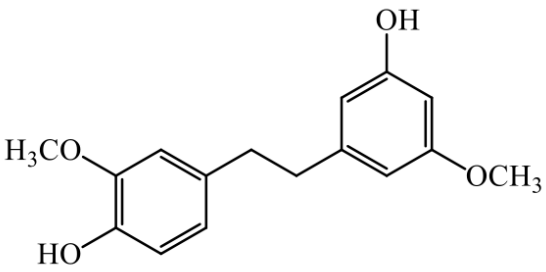

Figure 1 Chemical structure of gigantol

Materials and Methods

Cells and Chemicals. Non-small cell lung cancer H460 cells were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI 1640 containing 10 % fetal bovine serum, 2 mM L-glutamine, and 100 U/ml penicillin/streptomycin in a 5 % CO_2 environment at 37 °C. H460 lung cancer cells in a passage of 50-80 were used in this study. 3-(4,5-dimethyl- thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Sigma Chemical (St. Louis, MO). Gigantol was isolated from *Dendrobium draconis* as previously described [23]. Z-VAD-FMK was from Enzo Life Sciences (Farmingdale, NY). Propidium iodide (PI) and Hoechst 33342 were obtained from Molecular Probes (Eugene, OR). Antibodies for myeloid cell leukemia sequence 1 (Mcl-1), B-cell lymphoma 2 (Bcl-2), Bcl-2, BCL2-associated X protein (Bax), Cellular FLICE-like inhibitory protein (cFLIP), as well as peroxidase-conjugated secondary antibodies were obtained from Cell Signaling (Danvers, MA). The β-actin antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell viability assay. Cell viability was evaluated using the MTT assay. A number of 10,000 cells in 100 µl of RPMI medium were seeded onto each well of 96-well plate and incubated overnight for cell attachment. After that, cells were treated with different concentrations of GG (0, 10, 50, 100, 500 µM) for 24 h. In case of caspase 3 study, cells were pretreated with a caspase-3 inhibitor Z-VAD-FMK (10 µM) for 30 min before gigantol treatment. Medium were then removed, replaced with 500 µg/ml of MTT and incubated at 37 °C for 4 h. An intensity reading of the MTT product was measured at 570 nm using a microplate reader, and the percentage of viable cells was calculated relative to control cells as following equation.

$$\%\text{Cell viability} = \frac{\text{OD570 of treated cells x 100}}{\text{OD570 of untreated control cells}} \quad [1]$$

Apoptosis assay. Apoptosis was determined by Hoechst 33342/PI staining and DNA content analysis. A number of 10,000 cells in 100 µl of RPMI medium were seeded onto each well of 96-well plate and incubated overnight for cell attachment. After that, cells were treated with different concentrations of GG (0, 10, 50, 100, 500 µM) for 24 h. In case of caspase 3 study, cells were pretreated with a caspase 3 inhibitor Z-VAD-FMK (10 µM) for 30 min before gigantol treatment. Cells were washed and incubated with 10 µg/ml Hoechst 33342 and 5 µg/ml PI for 30 min. Nuclei condensation and DNA fragmentation of apoptotic cells and PI-positive necrotic cells were visualized and scored by fluorescence microscopy (Olympus IX51 with DP70). At least three replicates per dose per compound were run in each experiment which 3-5 experiments were performed. Percentage of cells displaying condensed chromatin and/or fragmented apoptotic nuclei and necrotic cell death were determined from 5 replicate photos of each experimental replicate. The percentage of apoptosis or necrosis cells was calculated as following equation.

$$\%\text{Apoptosis or necrosis} = \frac{\left(\text{Number of apoptosis or necrosis cells in treated group}\right) \text{x } 100}{\text{Number of cells per field}} \quad [2]$$

Western blot analysis. Cells were incubated in lysis buffer containing 20 mM Tris·HCl (pH 7.5), 1 % Triton X-100, 150 mM sodium chloride, 10 % glycerol, 1 mM sodium orthovanadate, 50 mM sodium fluoride, 100 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail (Roche Molecular Biochemicals) for 40 min on ice. The cell lysates were collected and the protein content was determined using the Bradford method (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein from each sample (80 µg) were denatured by heating at 95 °C for 5 min with Laemmli loading buffer and subsequently loaded onto a 10 % SDS-PAGE.

After separation, proteins were transferred onto 0.45 µM nitrocellulose membranes (Bio-Rad). The transferred 0.05 % Tween 20) and incubated with the appropriate primary antibodies at 4 °C overnight. Membranes were

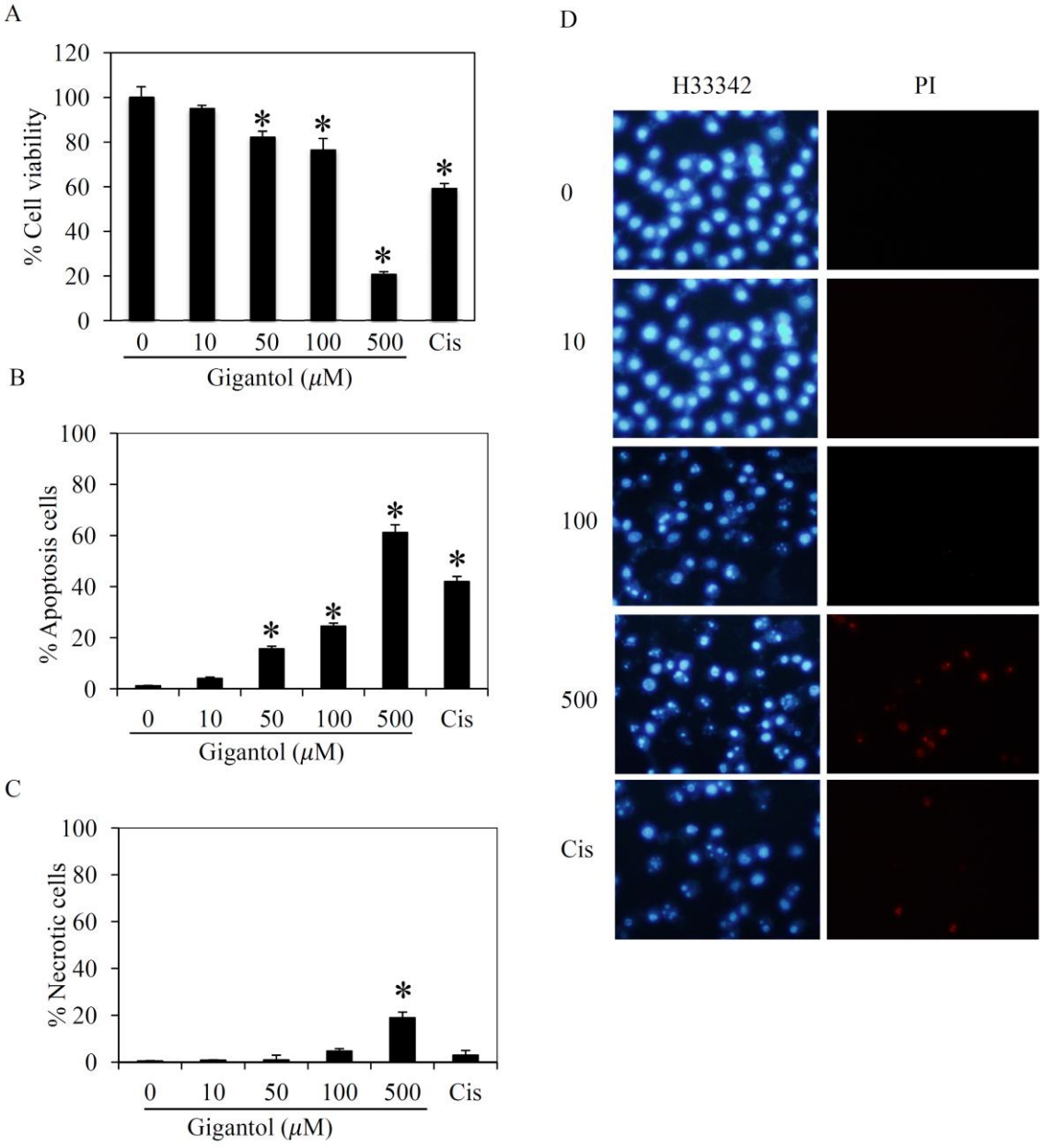

Figure 2 Cytotoxicity and mode of cell death in response to gigantol. **A**: H460 cells were treated with various concentrations of gigantol (0-500 μM) or cisplatin (Cis; 50 μM) for 24 h. Cell survival was determined by a 3-(4,5-dimethly-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The viability of untreated cells was represented as 100 %. **B**: Apoptotic cell death was evaluated using Hoechst 33342 staining, and calculated as a percentage comparing with untreated cells. **C**: Necrotic cell death was evaluated using propidium iodide staining, and calculated as a percentage comparing with untreated cells. **D**: Nuclear staining of apoptosis and necrosis cells were visualized under fluorescent microscope. Data represent the means ± SD (n = 4). *P < 0.05 versus untreated control cells.

blocked for 1 h in 5 % nonfat dry milk in TBS-T (25 mM Tris·HCl pH 7.5, 125 mM NaCl, and washed twice with TBS-T for 10 min and incubated with horseradish peroxidase-coupled isotype-specific antibodies for 1 h at room temperature. The immune complexes were detected by enhancement with a chemiluminescent substrate (Supersignal West Pico; Pierce, Rockfore, IL) and quantified using analyst/PC densitometry software (Bio-Rad).

Statistical analysis. The data are presented as the means ± SD from three or more independent experiments. Statistical differences between the means were determined using an ANOVA and post hoc test at a significance level of $P < 0.05$.

Results

Cytotoxicity of GG on human lung cancer H460 lung cancer cells. To investigate cell death in response to GG treatment, H460 cells were incubated with various concentrations of GG (0, 10, 50, 100, 500 µM) for 24 h or cisplatin (50 μM) as a positive control and cell viability was examined by MTT assay. Figure 2A shows that the cytotoxicity was first detected in the cells treated with 50, 100, and 500 µM of GG, respectively. Viable cells were decreased to approximately 80, 75 and 20 % in response to 50, 100, and 500 µM of GG; respectively, whereas no significant change was observed in the cells treated with low dose of GG (< 10 µM). This data demonstrated a concentration-dependent effect of GG in H460 lung cancer cells.

Effect of gigantol on human lung cancer H460 cells apoptosis. To investigate cell death mechanism, after treatment with GG (0, 10, 50, 100, 500 µM) or a known apoptosis inducer cisplatin (50 µM), H460 cells were incubated with Hoechst 33342/PI for 30 min and visualized under fluorescent microscope. The results demonstrated that apoptotic nuclei of Hoechst positive cells were remarkable increased to approximately 20–60 % in the cells treated with 50-500 µM of GG compared with non-treated control cells (less than 5 % apoptosis cells; Figures 2B and 2D), and PI-positive necrotic cells were detected only in high doses of GG (500 µM; Figures 2C and 2D). To confirm this finding, H460 cells were treated with 0-500 µM of GG in the presence or absence of a caspase-3 inhibitor, Z-VAD-FMK and cell viability and apoptosis were investigated. Figure 3A shows that GG-induced cell death was suppressed significantly in cells-pretreated with Z-VAD-FMK comparing with GG-treated cells. Importantly, GG-enhanced apoptosis was inhibited by Z-VAD-FMK pretreatment in corresponding with viable cells (Figure 3B).

Together, these data suggest that the cytotoxic effect of 50-100 µM of GG was mainly through apoptotic mechanism, whereas 500 µM of GG caused both apoptotic- and necrotic-cell death. Furthermore neither apoptotic nor necrosis cells were found in cells treated with low dose of GG (<10 µM). The apoptotic doses of GG were further identified their molecular mechanism.

GG induced apoptosis through mitochondrial pathway. To clarify molecular mechanism involved with apoptosis induction, key players in mitochondrial pathway such as Bcl-2, Mcl-1 and Bax, and death receptor pathway such as cFLIP were evaluated. Cells were treated with apoptotic doses of GG (0-100 µM), and western blot analysis reveals that anti-apoptotic Mcl-1 and Bcl-1 were substantial downregulated, together with an increase level of pro-apoptotic Bax in response to GG treatment,

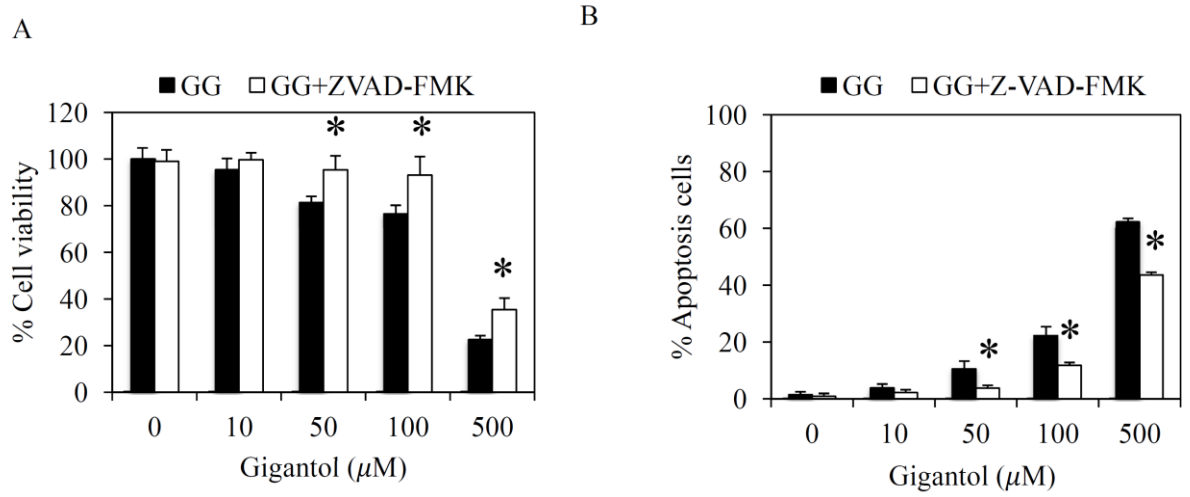

Figure 3 Gigantol induces H460 cell apoptosis through caspase-dependent pathway. H460 cells were treated with 0-500 μM of gigantol (GG) in the present of absence of Z-VAD-FMK, caspase-3 inhibitor (10 μM) for 24 h. **A**: Cell survival was determined by a 3-(4,5-dimethly-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The viability of untreated cells was represented as 100 %. **B**: Apoptotic cell death was evaluated using Hoechst 33342 staining, and calculated as a percentage comparing with untreated cells. Data represent the means ± SD (n = 4). *$P < 0.05$ versus gigantol treated-cells.

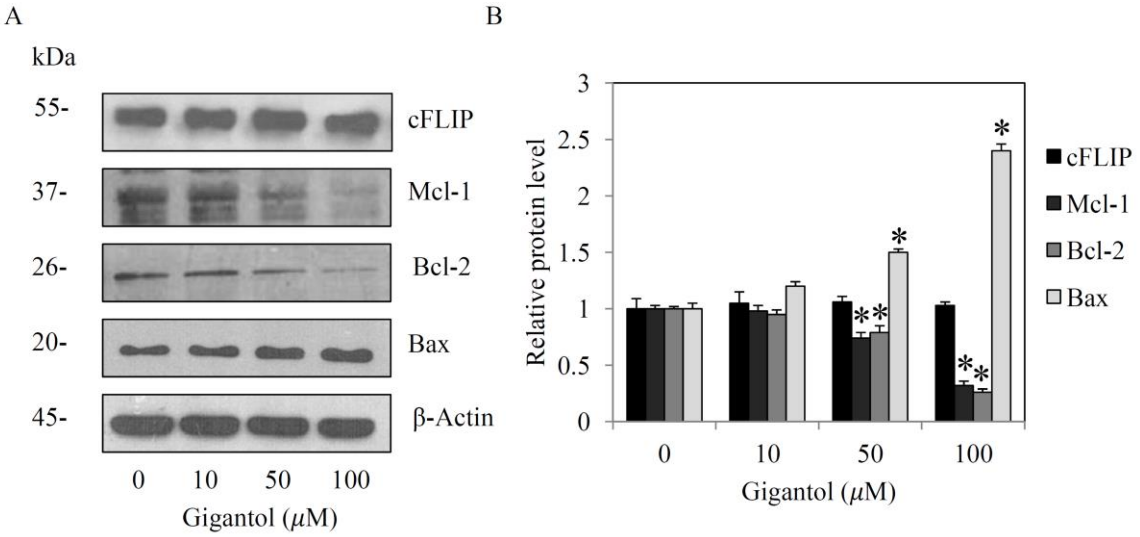

Figure 4 Gigantol mediates lung cancer apoptosis via mitochondrial-dependent mechanism. **A**: H460 cells were treated with gigantol (0-100 μM) for 24 h. The cells were collected and analyzed for myeloid cell leukemia sequence 1 (Mcl-1), B-cell leukaemia/lymphoma-2 (Bcl-2), BCL2-associated X protein (Bax) and Cellular FLICE-like inhibitory protein (cFLIP) expressions by Western blotting. The blots were reprobed with β-actin to confirm equal loading. **B**: The immunoblot signals were quantified by densitometry, and mean data from independent experiments were normalized to the untreated cells. The bars are the means ± SD (n = 4). **P* < 0.05 versus untreated control cells.

whereas the effect on anti-apoptotic cFLIP was not detectable changed (Figure 4). These results indicated that GG mediated lung cancer H460 cell apoptosis via mitochondrial-dependent mechanism.

Cytotoxic effect of GG on normal keratinocyte HaCat cells. The severe side effects of current chemotherapies cause a failure in therapeutic outcome [29]. To investigate whether GG has cytotoxic effect on normal cells, human keratinocyte HaCat cells were treated with GG under the same condition. Interestingly, cell viability assay demonstrated that the effective anticancer dose of GG was not able to mediate HaCat cell death significantly (Figure 5). This result indicated the specificity of GG on cancer.

Discussion

Apoptosis is widely accepted as a programmed cell death that plays an important role in multicellular organisms such as development and maintaining equilibrium of species diversity. Dysregulation of apoptosis regulator was often found in several diseases including cancer [30]. Apoptosis avoidance is one of cancer hallmarks which cancers utilize this characteristic to escape from immune surveillance [31] and chemotherapy [32], which contributes to aggressive disease [33]. Accumulative studies reported that high level of anti-apoptotic Bcl-2 and/or a decreased expression of pro-apoptotic Bax in several cancers were correlated with poor prognosis disease [34,35]. The suppression of anti-apoptotic Bcl-2 and cFLIP were found to attenuate both *in vivo* and *in vitro* tumor growth [12, 13]. In addition, the upregulation of anti-apoptotic Mcl-1 and cFLIP were also shown to against immune surveillance, a critical mechanism inhibiting tumorigenesis [31]. Death ligand of immune cells exhibits the selective killing to tumor cells by which the tumor cells acquires apoptosis resistance through upregulation of these anti-apoptotic proteins [15,36,37]. Aberrant activities of apoptotic regulatory proteins thus represent the potential targets for anticancer drug development.

Researchers extensively identify the new anticancer agents which natural origin becomes the most interested source. GG is one of attractive agent isolated from Thai orchid. It has recently reported that GG exhibits cytotoxicity to cancer cell lines; for example, it induced human leukemia K562 and HL60 cell death whose IC50 are 19.2 μg/ml and 8.18 μg/ml, respectively [38]; however, the cell death mechanism is remaining unknown. This study demonstrated the first time on anticancer activity of GG in human lung cancer H460 cells. Cytotoxic effect appears in dose range of 50-500 μM of gigantol. A 50-100 μM of gigantol causes apoptosis, a major mode of cell death, whereas high dose (> 500 μM) mediates both apoptotic and necrotic cells (Figure 2). Necrosis is able to trigger neighboring cells damage, suggesting that the optimum doses for anticancer activity are in a range of 50-100 μM. In addition, apoptotic inducing effect of GG was due to, at least in part of, attenuation of anti-apoptotic Bcl-2 and Mcl-1 expression and elevation of pro-apoptotic Bax level (Figure 4). Several evidences indicated that anti-apoptosis cFLIP was raised in various cancer types. The silencing of

such protein was able to restore apoptosis response-triggered by death ligands and chemotherapeutic agents, and inhibited cancer cell growth [12,39,40]. However, in this study, cFLIP is not a target elimination of GG in lung cancer cells (Figure 4). Interestingly, the effective dose of GG on cancer cell death was not affected the viability of normal keratinocyte HaCat cells (Figure 5).

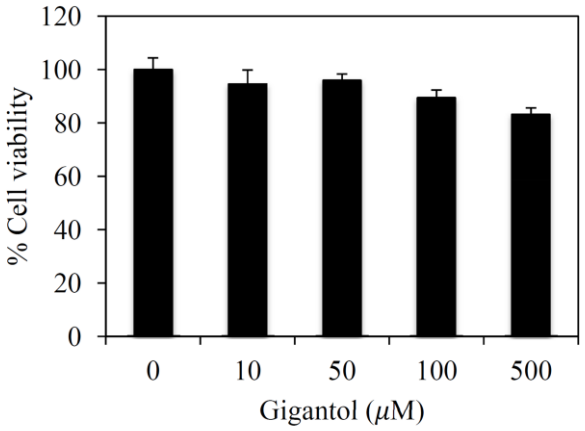

Figure 5 Cytotoxicity of gigantol on human keratinocyte HaCat cells. HaCat cells were treated with various concentrations of gigantol (0-500 μM) for 24 h. Cell survival was determined by a 3-(4,5-dimethly-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The viability of untreated cells was represented as 100 %. Data represent the means ± SD (n = 4). *P < 0.05 versus untreated control cells.

In conclusion, this study shows a concentration-dependent cytotoxic effect of GG on H460 cells. Since several cancers acquire the resistance to apoptosis which lead to advanced stage disease, the result of this study indicates that GG posses a potential apoptotic inducing effect that benefit to the development of this agent for anticancer drug.

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References

[1] R. Siegel, D. Naishadham, and A. Jemal. Cancer statistics, 2013. *CA. Cancer. J. Clin*. 63(1): 11-30 (2013).
[2] G. I. Evan, and K. H. Vousden. Proliferation, cell cycle and apoptosis in cancer. *Nature.* 411(6835):342-8 (2001).
[3] S. Gupta. Molecular steps of death receptor and mitochondrial pathways of apoptosis. *Life. Sciences.* 69 (25–26): 2957-2964 (2001).
[4] X. Jiang, and X. Wang. Cytochrome C-mediated apoptosis. *Annu. Rev. Biochem.* 73(1): 87-106 (2004).
[5] A. Thorburn. Death receptor-induced cell killing. *Cell. Signal.* 16(2): 139-144 (2004).
[6] N. Özören, and W. S. El-Deiry. Defining characteristics of Types I and II apoptotic cells in response to TRAIL. *Neoplasia.* 4(6): 551-7 (2002).
[7] M. R. Sprick, and H. Walczak. The interplay between the Bcl-2 family and death receptor-mediated apoptosis. *Biophys. Acta.* 1644(2): 125-132 (2004).
[8] T. J. McDonnell, P. Troncoso, S. M. Brisbay, C. Logothetis, L. W. Chung, J. T. Hsieh, and M. L. Campbell. Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. *Cancer. Res.* 52(24): 6940-6944. (1992).
[9] B. Martin, M. Paesmans, T. Berghmans, F. Branle, L. Ghisdal, C. Mascaux, and J. P. Sculier. Role of Bcl-2 as a prognostic factor for survival in lung cancer: a systematic review of the literature with meta-analysis. *Br. J. Cancer*. 89(1): 55-64 (2003).
[10] X. Wang, Y. Wang, J. Zhang, H. P. Kim, S. W. Ryter, and A. M. Choi. FLIP protects against hypoxia/reoxygenation-induced endothelial cell apoptosis by inhibiting Bax activation. *Mol. Cell. Biol*. 25(11): 4742-4751 (2005).
[11] P. A. Ellis, I. E. Smith, S. Detre, S. A. Burton, J. Salter, R. A'hern, and M. Dowsett. Reduced apoptosis and proliferation and increased Bcl-2 in residual breast cancer following preoperative chemotherapy. *Breast. Cancer. Res. Treat.* 48(2): 107-116 (1998).
[12] T. J. Sayers, A. D. Brooks, C. Y. Koh, W. Ma, N. Seki, A. Raziuddin, B. R. Blazar, X. Zhang, P. J. Elliott, and W. J. Murphy. The proteasome inhibitor PS-341 sensitizes neoplastic cells to TRAIL-mediated apoptosis by reducing levels of c-FLIP. *Blood.* 102(1):303-10 (2003).
[13] D. Manka, Z. Spicer, and D. E. Milhorn. Bcl-2/adenovirus E1B 19 kDa interacting protein-3 knockdown enables growth of breast cancer metastases in the lung, liver, and bone. *Cancer. Res.* 65(24): 11689-93 (2005).
[14] L. Wang, P. Chanvorachote, D. Toledo, C. Stehlik, R. R. Mercer, V. Castranova, and Y. Rojanasakul. Peroxide is a key mediator of Bcl-2 down-regulation and apoptosis induction by cisplatin in human lung cancer cells. *Mol. Pharmacol*. 73(1): 119-127 (2008).
[15] P. Chanvorachote, and V. Pongrakhananon. Ouabain downregulates Mcl-1 and sensitizes lung cancer cells to TRAIL-induced apoptosis. *Am. J. Physiol. Cell. Physiol*. 304(3): C263-C272 (2013).
[16] S. J. Lee, H. J. Noh, E. G. Sung, I. H. Song, J. Y. Kim, T. K. Kwon, and T. J. Lee. Berberine sensitizes TRAIL-induced apoptosis through proteasome-mediated downregulation of c-FLIP and Mcl-1 proteins. *Int. J. Oncol*. 38(2): 485-492 (2011).
[17] M. Gordalize. Natural products as leads to anticancer drugs. *Clin. Transl. Oncol.* 9(12): 767-76(2007).
[18] L. W. Chow, B. Xu, S. Gupta, A. Freyman, Y. Zhao, R. Abbas, M. L. Vo Van, and I. Bondarenko. Combination neratinib (HKI-272) and paclitaxel therapy in patients with HER2-positive metastatic breast cancer. *Br. J. Cancer*. 108(10): 1985-1993 (2013).
[19] I. Ospovat, N. Siegelmann-Danieli, T. Grenader, A. Hubert, T. Hamburger, and T. Peretz. Mitomycin C and vinblastine: an active regimen in previously treated breast cancer patients. *Tumori*. 95(6): 683-686 (2009).
[20] C. K. Ho, and C. C. Chen. Moscatilin from the orchid Dendrobrium loddigesii is a potential anticancer agent. *Cancer. Invest*. 21(5): 729-736 (2003).
[21] Y. Chen, J. Xu, H. Yu, C. Qing, Y. Zhang, L. Wang, and J. Wang. Cytotoxic phenolics from Bulbophyllum odoratissimum. *Food. Chem.* 107(1): 169-173 (2008).
[22] A. C. Tsai, S. L. Pan, C. H. Liao, J. H. Guh, S. W. Wang, H. L. Sun, Y. N. Liu, C. C. Chen, C. C. Shen, Y. L. Chang, and C. M. Teng. Moscatilin, a bibenzyl derivative from the India orchid Dendrobrium loddigesii, suppresses tumor angiogenesis and growth in vitro and in vivo. *Cancer. Lett.* 292(2): 163-170 (2010).
[23] B. Sritularak, M. Anuwat, and K. Likhitwitayawuid. A new phenanthrenequinone from Dendrobium draconis. *J. Asian. Nat. Prod. Res.* 13(3): 251-255 (2011).
[24] M. Miyazawa, H. Shimamura, S.-i. Nakamura, and H. Kameoka. Antimutagenic Activity of Gigantol from Dendrobium nobile. *J. Agric. Food. Chem*. 45(8): 2849-2853 (1997).
[25] C. Fan, W. Wang, Y. Wang, G. Qin, and W. Zhao. Chemical constituents from Dendrobium densiflorum. *Phytochem.* 57(8): 1255-1258 (2001).
[26] J. H. Won, J. Y. Kim, K. J. Yun, J. H. Lee, N. I. Back, H. G. Chung, S. A. Chung, T. S. Jeong, M. S. Choi, and K. T. Lee. Gigantol isolated from the whole plants of Cymbidium goeringii inhibits the LPS-induced iNOS and COX-2 expression via NF-kappaB inactivation in RAW 264.7 macrophages cells. *Planta. Med*. 72(13): 1181-1187 (2006).
[27] C. Simmler, C. Antheaume, and A. Lobstein. Antioxidant biomarkers from Vanda coerulea stems reduce irradiated HaCaT PGE-2

production as a result of COX-2 inhibition. *PloS one*. 5(10): e13713 (2010).
[28] C. F. Zhang, M. Wang, L. Wang, M. Iinuma, M. Zhang, L. S. Xu, and Z. T. Wang. Chemical constituents of Dendrobium gratiosissimum and their cytotoxic activities. *Indian. J. Chem.* 47B(6): 952-956 (2008).
[29] A. Rajeswaren, A. Trojan, B. Burnand, and M. Giannelli. Efficacy and side effects of cisplatin- and carboplatin-based doublet chemotherapeutic regimens versus non-platinum-based doublet chemotherapeutic regimens as first line treatment of metastatic non-small cell lung carcinoma: a systematic review of randomized controlled trials. *Lung. Cancer*. 59(1): 1-11 (2008).
[30] U. Fischer, and K. Schulze-Osthoff. New approaches and therapeutics targeting apoptosis in disease. *Pharmacol. Rev*. 57(2): 187-215 (2005).
[31] F. H. Igney, and P. H. Krammer. Immune escape of tumors: apoptosis resistance and tumor counterattack. *J. Leukoc. Biol*. 71(6): 907-920 (2002).
[32] Y. A. Hannun. Apoptosis and the dilemma of cancer chemotherapy. *Blood*. 89(6): 1845-1853 (1997).
[33] B. Favaloro, N. Allocati, V. Graziano, C. Di Ilio, and V. De Laurenzi. Role of apoptosis in disease. *Aging. (Albany NY)*. 4(5): 330 (2012).
[34] G. Gobe, M. Rubin, G. Williams, I. Sawczuk, and R. Buttyan. Apoptosis and expression of Bcl-2, Bcl-XL, and Bax in renal cell carcinomas. *Cancer. Invest*. 20(3): 324-332 (2002).
[35] S. Narayan, J. Chandra, M. Sharma, R. Naithani, and S. Sharma. Expression of apoptosis regulators Bcl-2 and Bax in childhood acute lymphoblastic leukemia. *Hematol*. 12(1): 39-43 (2007).
[36] A. Stephenson-Famy, J. Marks, A. Suresh, S. N. Caritis, H. Simhan, P. Jeyasuria and J. C. Condon. Antiapoptotic signaling via MCL1 confers resistance to caspase-3-mediated apoptotic cell death in the pregnant human uterine myocyte. *Mol. Endocrinol.* 26(2): 320-330 (2012).
[37] C. Xiao, B. F. Yang, N. Asadi, F. Beguinot, and C. Hao. Tumor necrosis factor-related apoptosis-inducing ligand-induced death-inducing signaling complex and its modulation by c-FLIP and PED/PEA-15 in glioma cells. *J. Biol. Chem*. 277(28): 25020-5 (2002).
[38] Y. Chen, J. Xu, H. Yu, C. Qing, Y. Zhang, L. Wang, Y. Liu, and J. Wang. Cytotoxic phenolics from Bulbophyllum odoratissimum. *Food. Chem.* 107(1): 169-173 (2008).
[39] A. R. Safa, and K. E. Pollok. Targeting the anti-apoptotic protein c-FLIP for cancer therapy. *Cancers. (Basel.)* 3(2): 1639-1671 (2011).
[40] A. Apelbaum, G. Yarden, S. Warszawski, D. Harari, and G. Schreiber. Type I interferons induce apoptosis by balancing cFLIP and caspase-8 independent of death ligands. *Mol. Cell. Biol*. 33(4): 800-814 (2013).