Chelidonium majus L. extract induces apoptosis through caspase activity via MAPK-independent NF-κB signaling in human epidermoid carcinoma A431 cells

SEUNG-WON PARK[1], SEONG RYUL KIM[2], YOUNGCHUL KIM[3], JANG-HOON LEE[3], HONG-JUNG WOO[3], YEO-KWANG YOON[3] and YOUNG IL KIM[4,5]

[1]Department of Biotechnology, Catholic University of Daegu, Daegu 712-702; [2]Department of Agricultural Biology, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-701; [3]Department of Internal Medicine, College of Korean Medicine, Kyung Hee University, Seoul 130-872; [4]Medical Science Research Institute, Kyung Hee University Medical Center, Seoul 130-872; [5]East-West Medical Research Institute, Kyung Hee University, Seoul 130-872, Republic of Korea

Received June 18, 2014; Accepted September 26, 2014

DOI: 10.3892/or.2014.3566

Abstract. *Chelidonium majus* L. *(C. majus* L.*)* is known to possess certain biological properties such as anti-inflammatory, antimicrobial, antiviral and antitumor activities. We investigated the effects of *C. majus* L. extract on human epidermoid carcinoma A431 cells through multiple mechanisms, including induction of cell cycle arrest, activation of the caspase-dependent pathway, blocking of nuclear factor-κB (NF-κB) activation and involvement in the mitogen-activated protein kinase (MAPK) pathway. *C. majus* L. inhibited the proliferation of A431 cells in a dose- and time-dependent manner, increased the percentage of apoptotic cells, significantly decreased the mRNA levels of cyclin D1, Bcl-2, Mcl-1 and survivin and increased p21 and Bax expression. Exposure of A431 cells to *C. majus* L. extract enhanced the activities of caspase-3 and caspase-9, while co-treatment with *C. majus* L., the pan-caspase inhibitor Z-VAD-FMK and the caspase-3 inhibitor Z-DEVE-FMK increased the proliferation of A431 cells. *C. majus* L. extract not only inhibited NF-κB activation, but it also activated p38 MAPK and MEK/ERK signaling. Taken together, these results demonstrate that *C. majus* L. extract inhibits the proliferation of human epidermoid carcinoma A431 cells by inducing apoptosis through caspase activation and NF-κB inhibition via MAPK-independent pathway.

Introduction

Chelidonium majus is commonly known as greater celandine. It belongs to the family Papaveraceae and is widely distributed in Europe and western Asia. Extracts of *Chelidonium majus* L. (*C. majus* L.) have been shown to exhibit a variety of biological effects including anti-inflammatory, antimicrobial, antiviral and antitumor activities (1,2). The antitumor, antigenotoxic and hepatoprotective effects of *C. majus* L. extract suggest that it may be potentially useful as an anticancer therapeutic agent (3).

Apoptosis is mediated through at least 3 major pathways, which are regulated by death receptors, mitochondria and the endoplasmic reticulum. Activation of the apoptosis pathway is a key mechanism by which cytotoxic drugs kill tumor cells. Defects in apoptosis signaling contribute to the drug resistance of tumor cells (4,5). The Bcl-2 family consists of important apoptotic regulators of programmed cell death. This family of proteins includes both anti-apoptotic molecules such as Bcl-2 and pro-apoptotic molecules such as Bax (6). Bcl-2 and Bcl-X_L are members of the Bcl-2 family and are regulated by nuclear factor-(NF)-κB. These proteins can prevent release of cytochrome *c* and activation of caspases (7).

Mitogen-activated protein kinase (MAPK) cascades are involved in the signaling pathways that regulate various cellular responses such as inflammation, proliferation and cell death (8). Three major mammalian MAPK subfamilies have been described: extracellular signal-regulated kinases (ERKs), c-Jun *N*-terminal kinases and p38 kinases. Each MAPK is activated through a specific phosphorylation cascade (9,10). The ERK cascade is activated through receptor-mediated signaling stimuli and is associated with cell proliferation, differentiation and survival (11). However, in some cases, ERK activation contributes to cell death (12).

In the present study, we examined how the effects of *C. majus* L. extract on human epidermoid carcinoma A431 cells are mediated through multiple mechanisms, including activation of cell cycle arrest and the caspase-dependent pathway, blocking of NF-κB activation and mediation of the MAPK pathway in human epidermoid carcinoma A431 cells.

Materials and methods

Preparation of C. majus L. extract. Five hundred grams of *C. majus* L. and 3 l of distilled water were placed into a round-

Correspondence to: Dr Young Il Kim, Medical Science Research Institute, Kyung Hee University Medical Center, 23 Kyungheedae-ro, Dongdaemun-gu, Seoul 130-872, Republic of Korea
E-mail: ewlabkim@khmc.or.kr

Key words: A431 cells, apoptosis, caspase, *Chelidonium majus* L., mitogen-activated protein kinase, nuclear factor-κB

bottomed flask fitted with a condenser and a heating mantle. The contents of the flask were then boiled for 2 h. The resulting water extract was filtered through a Whatman no. 1 filter paper and its volume was reduced using a rotary evaporator (Buchi, Flawil, Switzerland). The concentrated extract was freeze-dried (EYELA, Tokyo, Japan) and stored at 4˚C in a vacuum container until it was used. The final weight of the extract was 100 g, of which 20% was natural product.

Cell culture. The human epidermoid carcinoma A431 cell line was purchased from the American Tissue Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA), which contained 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C in a humidified atmosphere of 5% CO_2 and 95% air.

MTT assay. Cell proliferation was evaluated using the CellTiter 96 Aqueous One Solution (Promega, Madison, WI, USA). Cells ($1x10^4$) were incubated with several concentrations of *C. majus* L. extract (0-500 μg/ml) at 37˚C for 24, 48 and 72 h. Cell viability was determined through a colorimetric assay by using PMS/MTS solution. The absorbance was determined at 492 nm with background subtraction at 650 nm.

Cell cycle analysis. Cells ($5x10^5$) were treated with *C. majus* L. extracts for 72 h. At the end of the treatment period, the cells were harvested and washed with PBS. They were then fixed with 70% ethanol for 1 h, treated with RNase A (20 μg/ml) at 37˚C for 1 h and stained with propidium iodide (50 μg/ml). The DNA content at each cell cycle stage was analyzed using a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson, Franklin Lakes, NJ, USA).

Apoptosis assay. The number of apoptotic A431 cells was determined using the Cell Death Detection ELISA^{plus} kit (Roche Molecular Biochemicals, Mannheim, Germany). Cells ($1x10^4$) were incubated with several concentrations of *C. majus* L. extract for 72 h. They were then lysed with the cell lysis buffer (200 μl). The cell lysates were assayed for DNA fragments by using the Cell Death Detection ELISA^{plus} kit according to the manufacturer's protocol. DNA fragmentation was evaluated at 405 nm against an untreated control.

Caspase inhibitor assay. Cells ($1x10^4$) were pre-incubated with several concentrations of the pan-caspase inhibitor Z-VAD-FMK (R&D Systems, Minneapolis, MN, USA) or the caspase-3-specific inhibitor Z-DEVD-FMK for 2 h. Then, 500 μg/ml *C. majus* L. extract was added to the cells and the cells were cultured for a further 72 h. Cell viability was determined through a colorimetric assay using the PMS/MTS solution. The absorbance was determined at 492 nm with background subtraction at 650 nm.

RNA extraction and real-time PCR. Total RNA was purified from cultured cells by using an RNA-Bee solution kit following the manufacturer's protocol (Tel-Test, Friendswood, TX, USA). First-strand cDNA synthesis was performed with 1 μg of total RNA and transcribed into cDNA using a reverse transcription system with random hexamers according to the manufacturer's protocol. The primer sequences used were as follows: cyclin D1 (5'-CCGTCCATGCGGAAGATC-3', 5'-ATGGCCAGCG GGAAGAC-3'; 86 bp), p21 (5'-CAGACCAGCATGACAGAT TTC-3', 5'-TTAGGGCTTCCTCTTGGAGA-3'; 66 bp), Bcl-2 (5'-GATTGATGGGATCGTTGCCTTA-3', 5'-CCTTGGCAT GAGATGCAGGA-3'; 200 bp), Bax (5'-GGATGCGTCCACC AAGAAG-3', 5'-GCCTTGAGCACCAGTTTGC-3'; 216 bp), Mcl-1 (5'-CTCATTTCTTTTGGTGCCTTT-3', 5'-CCAGTCC CGTTTTGTCCTTAC-3'; 117 bp), survivin (5'-GGCCCA GTGTTTCTTCTGCTT-3', 5'-GCAACCGGACGAATGC TTT-3'; 91 bp), β-actin (5'-GCGAGAAGATGACCCAGATC-3', 5'-GGATAGCACAGCCTGGATAG-3'; 77 bp). Real-time PCR was performed using a StepOnePlus Real-Time PCR system with the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster, CA, USA). The PCRs were performed with 1 μl of cDNA in 20 μl reaction mixtures that consisted of 10 μl Power SYBR-Green PCR Master Mix, 2 μl of primers and 7 μl of PCR-grade water. The reactions were performed with a denaturation step at 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec and at 60˚C for 1 min. The crossing point of each target gene with β-actin was calculated by using the formula $2^{-(\text{target gene - β-actin})}$ and the relative amounts of the PCR products were quantified.

Immunoblot analysis. Cells ($2x10^6$) were treated with various concentrations of *C. majus* L. for 72 h. After treatment, the cells were washed with cold PBS and lysed with the lysis buffer [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4 and 1 μg/ml leupeptin] containing 1 mM PMSF. The protein concentration was determined by means of the BCA protein assay according to the manufacturer's protocol. Thirty micrograms of protein was fractionated by performing SDS-PAGE on a 12% gel and then transferred through electrophoresis onto nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk for 1 h at room temperature and then incubated overnight with anti-NF-κB p65, anti-p38 MAPK, anti-phospho-p38 MAPK, anti-ERK, anti-phospho-ERK, anti-MEK, anti-phospho-MEK (Cell Signaling Technology, Danvers, MA, USA) and anti-β-actin antibodies (Sigma-Aldrich) diluted to 1:1,000 with Tris-buffered saline containing 0.05% Tween-20 (TBS-T). After washing with TBS-T for 1 h, the membranes were treated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibody diluted to 1:2,500 with TBS-T. Then, the membranes were washed with TBS-T for 1 h and the proteins were detected using an enhanced chemiluminescence kit (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Protein expression was analyzed using a Chemiluminescence Imaging System (Davinch-Chemi™, Seoul, Korea).

Statistical analysis. The values are expressed as the mean ± SD. Student's t-test was used to evaluate differences between the control and *C. majus* L. extract-treated samples. The effect of the caspase inhibitors on cell viability was estimated by calculating the differences between the *C. majus* L. extract-treated samples and the *C. majus* L. extract/caspase inhibitor-treated samples. $^{*}p<0.05$ and $^{**}p<0.01$ were considered to indicate statistically significant results.

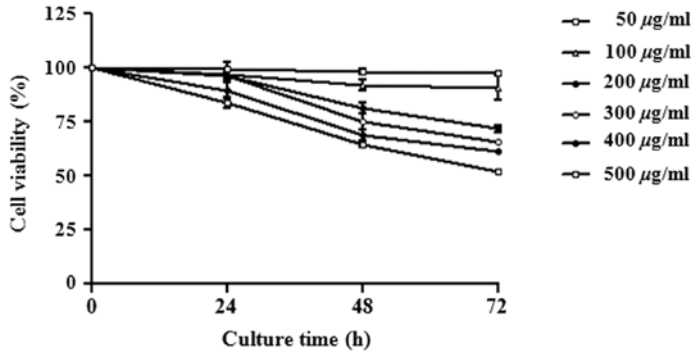

Figure 1. *Chelidonium majus* L. (*C. majus* L.) extract inhibits the proliferation of A431 cells. Cells were treated with various concentrations of *C. majus* L. extract (0-500 μg/ml) for 24, 48, and 72 h. Cell viability was determined using the MTT assay. The data are the mean ± SD of triplicate samples.

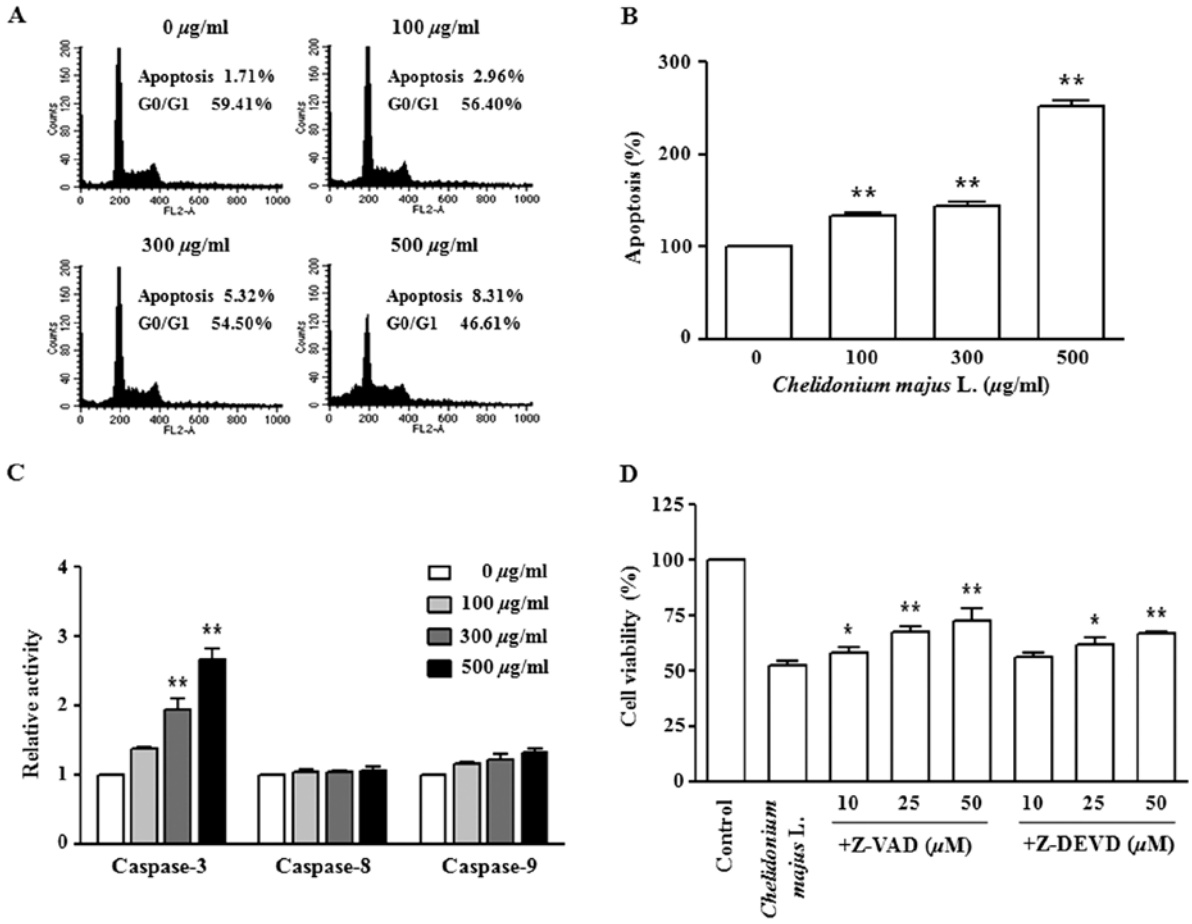

Figure 2. *Chelidonium majus* L. (*C. majus* L.) extract induces apoptosis in A431 cells. Cells were cultured with various concentrations of *C. majus* L. extract for 72 h and stained with propidium iodide. (A) The DNA content was analyzed using flow cytometry. The percentages of cells in the apoptotic and the G0/G1 phases are indicated. (B) Apoptotic cells were detected using a cell death detection ELISA. (C) The enzymatic activities of caspase proteases were measured using a caspase colorimetric assay. The data represent the mean ± SD of 3 independent experiments. $^{*}p<0.05$ and $^{**}p<0.01$ compared to the control. (D) Cells were treated with Z-VAD-FMK (10, 25, and 50 μM) or Z-DEVD-FMK (10, 25, and 50 μM) for 2 h and then incubated with *C. majus* L. (500 μg/ml) for 72 h. Cell viability was measured via the MTT assay. The values are the mean ± SD of three independent experiments. $^{*}p<0.05$ and $^{**}p<0.01$; *C. majus* L. extract-treated cells compared to the *C. majus* L. and caspase inhibitor-treated cells.

Results

C. majus L. extract inhibits cell proliferation. A431 cells were treated with various concentrations of *C. majus* L. extract (0-500 μg/ml) for 24, 48 and 72 h, respectively. The effect of *C. majus* L. extract on cell proliferation was evaluated through an MTT assay. *C. majus* L. extract inhibited proliferation of the cells in a dose- and time-dependent manner (Fig. 1).

C. majus L. extract induces apoptosis. A431 cells were treated with various concentrations of *C. majus* L. extract for 72 h. Flow cytometric analysis showed a dose-dependent increase in the number of cells in the apoptosis phase and a decrease in the number of cells in the G0/G1 phase of the cell cycle (Fig. 2A). Apoptotic cells were then detected using a Cell Death Detection ELISA (Fig. 2B). The number of apoptotic cells increased in a dose-dependent manner following treatment

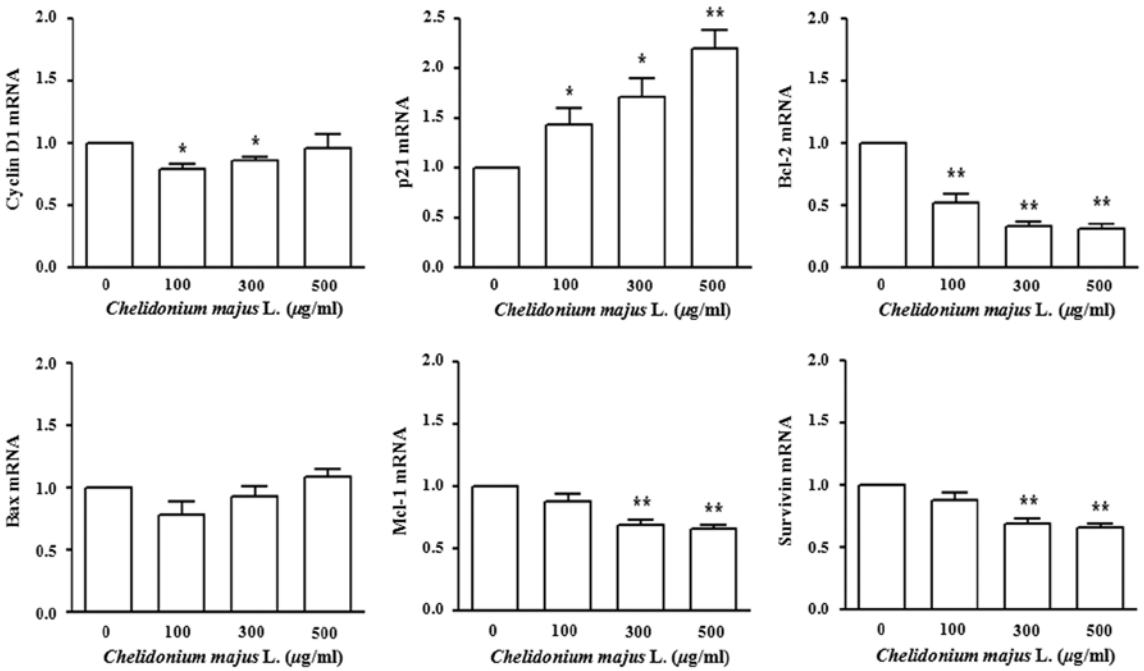

Figure 3. *Chelidonium majus* L. (*C. majus* L.) regulates the mRNA expression of cell cycle- and apoptosis-related genes in A431 cells. Cells were cultured with different concentrations of *C. majus* L. extract for 72 h. mRNA levels were determined using real-time PCR. The crossing points of target genes with β-actin were applied to the formula, $2^{-(\text{target gene - β-actin})}$ and relative amounts were quantified. The data represent the mean ± SD of three independent samples. $^{*}p<0.05$ and $^{**}p<0.01$ compared to the control.

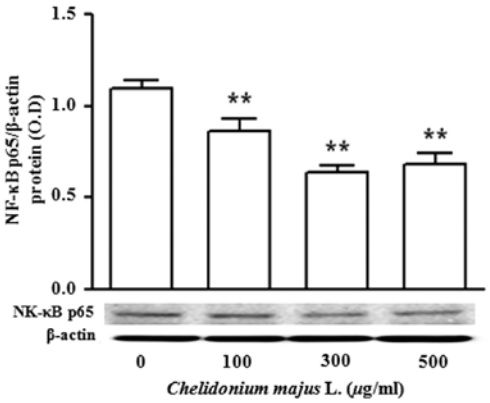

Figure 4. *Chelidonium majus* L. (*C. majus* L.) extract inhibits nuclear factor-κB (NF-κB) p65 activity in A431 cells. Cells were cultured with different concentrations of *C. majus* L. extract for 72 h, and the expression of NF-κB p65 protein was examined by immunoblotting. Densitometric analyses are presented as the relative ratios of NF-κB p65 to β-actin. The data represent the means ± SD of three independent samples. $^{**}p<0.01$ compared with the control.

with *C. majus* L. extract at various concentrations. Caspase activity was assayed using a colorimetric ELISA. Caspase-3 and caspase-9 activities increased in a dose-dependent manner following treatment with *C. majus* L. extract (Fig. 2C). To confirm whether caspase activation was involved in *C. majus* L. extract-induced apoptosis, MTT assay was performed to determine A431 cell proliferation due to treatment with *C. majus* L. extract. Pretreatment of the cells with the pan-caspase inhibitor Z-VAD-FMK and the caspase-3 inhibitor Z-DEVD-FMK increased the *C. majus* L. extract-induced cell proliferation (Fig. 2D).

C. majus L. extract regulates mRNA expression. A431 cells were treated with various concentrations of *C. majus* L. extract for 72 h. Then, the mRNA levels of the cell cycle-related genes cyclin D and p21 and the apoptosis-related genes Bcl-2, Mcl-1, Bax and survivin were examined using real-time PCR. The mRNA levels of cyclin D1, Bcl-2, Mcl-1 and survivin decreased, while those of p21 and Bax increased in a dose-dependent manner (Fig. 3).

C. majus L. extract inhibits NF-κB and increases the activities of p38 MAPK and MEK/ERK. A431 cells were treated with various concentrations of *C. majus* L. extract for 72 h. Changes in the activation and phosphorylation levels of key proteins in the cells were determined by performing western blot analysis. The levels of NF-κB p65 were significantly inhibited (Fig. 4). However, high concentrations (300 and 500 μg/ml) of *C. majus* L. extract induced activities in the levels of phosphorylated p38 MAPK and MEK/ERK1/2 (Fig. 5).

Discussion

The cytotoxic activity of *C. majus* L. on human and animal tumor cell cultures *in vitro* are of great interest and can be considered promising for use in cancer therapy (13). We investigated the effect of *C. majus* L. extract on the viability of A431 cells. *C. majus* L. extract inhibited A431 cell proliferation and this result was consistent with those of Chor *et al* (14), who reported on the activities of several herbs. Our results showed that treatment with *C. majus* L. extract increased the percentage of cells in the apoptotic phase of the cell cycle and enhanced apoptotic death of A431 cells. Taken together, these data suggest that *C. majus* L. extract not only inhibits A431 cell growth and blocks cell cycle progression at the G1 phase,

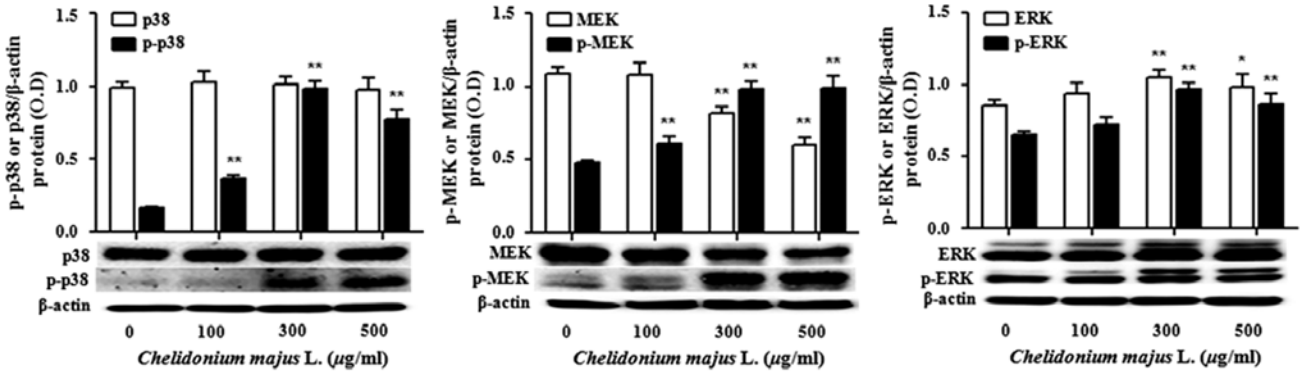

Figure 5. *Chelidonium majus L.* (*C. majus* L.) extract enhances p38 mitogen-activated protein kinase (MAPK) and MEK/ERK phosphorylation in A431 cells. Cells were cultured with different concentrations of *C. majus* L. extract for 72 h, and the expression of p38 MAPK, MEK and ERK protein was examined by immunoblotting. Densitometric analyses are presented as the relative ratios of p38 MAPK, phospho-p38 MAPK, MEK, phospho-MEK, ERK, and phospho-ERK to β-actin. The data represent the means ± SD of three independent samples. $^{*}p<0.05$ and $^{**}p<0.01$ compared with the control.

but also induces apoptosis. A recent study reported a decrease in the number of human hepatic stellate LX2 cells in the G1 phase and accumulation of cells in the sub-G0/G1 phase after incubation with *Orostachys japonicas* (15).

To investigate the molecular mechanism that underlies the apoptosis of A431 cells by *C. majus* L., we assessed the caspase activity of A431 cells during treatment with *C. majus* L. extract. Caspases are central mediators in apoptosis. We found that treatment of A431 cells with *C. majus* L. extract increased their intracellular caspase-3 and caspase-9 activities. This finding was confirmed by experiments using the pan-caspase inhibitor Z-VAD-FMK and the caspase 3-specific inhibitor Z-DEVD-FMK, which enhanced *C. majus* L. extract-mediated cell proliferation. These results suggest that *C. majus* L. extract-induced apoptosis of A431 cells was mediated by caspase activation. The inhibitors of caspase-3 and caspase-9 were previously found to almost completely suppress *Houttuynia cordata* Thunb-induced activities of caspase-3 and caspase-9 in human colon adenocarcinoma HT-29 cells (16). The activities of caspase-3 and caspase-9 and release of mitochondrial cytochrome *c* increased in ovarian cancer cell lines with increasing doses of *Antrodia camphorate* (17).

We investigated the mRNA levels of pro-apoptotic and anti-apoptotic genes in A431 cells that were treated with *C. majus* L. and detected enhanced expression of pro-apoptotic Bax and reduced expression of anti-apoptotic Bcl-2. In addition, the RNA levels of cyclin D1, Mcl-1 and survivin decreased, while that of p21 increased. These results suggest that *C. majus* L. regulated transcription factors of cell cycle-related genes and apoptosis-related genes. Tanshinone IIA was found to exert significant anti-proliferation effects on THP-1 cells through induction of apoptosis. Tanshinone IIA-induced apoptosis of THP-1 cells was found to be mainly related to activation of caspase-3, downregulation of anti-apoptotic proteins Bcl-2 and survivin and upregulation of pro-apoptotic protein Bax (18). Shikonin was previously found to cause cell apoptosis by upregulating p27 and Bax and downregulating Bcl-2 and Bcl-X_L in human colorectal carcinoma COLO 205 cells (19). Wogonin-induced apoptosis was found to be accompanied by significant decreases in Bcl-2 and survivin levels and an increase in Bax levels. In addition, wogonin treatment was found to significantly increase the active apoptotic forms of caspase-3, -8 and -9. Z-DEVD-FMK, which is a specific caspase-3 inhibitor, significantly inhibited wogonin-induced apoptosis of MCF-7 human breast cancer cells (20).

In the present study, we found that *C. majus* L. extract inhibited NF-κB activity in A431 cells. *C. majus* L. extract induced cell apoptosis by inhibiting NF-κB via downregulation of the Bcl-2 gene. NF-κB is a common transcription factor that can modulate the expression of apoptosis-related proteins (21). Our results suggest that *C. majus* L. extract functions as an effective apoptosis inducer through an NF-κB-dependent mechanism. Thus, suppression of the NF-κB pathway should be effective at inducing apoptosis of A431 cells. Triptolide was found to induce the apoptosis of human anaplastic thyroid carcinoma cells by downregulating NF-κB expression. It also downregulated the anti-apoptotic proteins Bcl-2 and Bcl-X_L, which are transcriptionally regulated by the NF-κB signaling pathway (22). In the present study, treatment with *C. majus* L. extract led to inhibition of the NF-κB pathway. Thus, suppression of the NF-κB pathway should be effective at inducing the apoptosis of tumor cells.

MAPKs are important regulators of apoptosis, cell proliferation and cell differentiation. Dysregulation of the MAPK pathway is associated with tumor development and progression (23). We demonstrated that high concentrations of *C. majus* L. extract activated p38 MAPK, MEKs and ERKs in A431 cells. Emerging evidence suggests that activation of ERKs contributes to apoptosis. Recent studies showed that kaempferol causes cancer cells to undergo apoptosis through an ERK-dependent pathway (24). Shikonin decreases the levels of phosphorylated EGFR, ERKs and protein tyrosine kinases and increases intracellular levels of apoptosis-related proteins, which leads to apoptosis of epidermoid carcinoma cells (25). The pro-apoptotic effect of wogonin was found to be mediated via activation of ERKs and caspases and is correlated with interruption of the PI3K/Akt/survivin signaling pathways in MCF-7 cells (20). A number of studies have shown the importance of ERK signaling in the regulation of apoptosis (26,27). Although the ERK pathway delivers a survival signal, studies using chemopreventive and chemotherapeutic agents have linked activation of ERKs with induction of apoptosis (28,29).

Scutellaria baicalensis extract was found to prevent hepatic fibrosis by promoting ERK-p53 pathways, which in turn caused G2/M cell cycle arrest and activation of the caspase system resulting in apoptosis. It may also induce Bax expression with concomitant decrease in Bcl-2 and MAPK signaling in HSC-T6 cells (30). Here, we also found that sustained activation of ERKs is involved in the *C. majus* L. extract-induced growth inhibition and apoptosis in A431 cells. In conclusion, the present study demonstrated that A431 cells are susceptible to *C. majus* L. extract-induced inhibition of proliferation and induction of apoptosis, which is mediated in part by caspase activation and NF-κB inhibition via MAPK-independent pathways. These results indicate the need for further research on the *in vivo* efficacy of *C. majus* L. extract as a possible anticancer agent.

Acknowledgements

This research was supported by the Bio-industry Technology Development Program, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (grant no. 311059-4) and partially supported by the Rural Development Administration, Republic of Korea (grant no. PJ008475022012).

References

1. Fik E, Dalgalarrondo M, Haertle T and Gozdzicka-Jozefiak A: Comparative biochemical analysis of lectin and nuclease from *Chelidonium majus* L. Acta Biochim Pol 47: 413-420, 2000.
2. Fik E, Wołun-Cholewa M, Kistowska M, Warchoł JB and Gozdzicka-Jozefiak A: Effect of lectin from *Chelidonium majus* L. on normal and cancer cells in culture. Folia Histochem Cytobiol 39: 215-216, 2001.
3. Biswas SJ, Bhattacharjee N and Khuda-Bukhsh AR: Efficacy of a plant extract *(Chelidonium majus* L.*)* in combating induced hepatocarcinogenesis in mice. Food Chem Toxicol 46: 1474-1487, 2008.
4. Tsuruo T, Naito M, Tomida A, Fujita N, Mashima T, Sakamoto H and Haga N: Molecular targeting therapy of cancer: drug resistance, apoptosis and survival signal. Cancer Sci 94: 15-21, 2003.
5. Debatin KM: Apoptosis pathways in cancer and cancer therapy. Cancer Immunol Immunother 53: 153-159, 2004.
6. Levine B, Sinha S and Kroemer G: Bcl-2 family members: dual regulators of apoptosis and autophagy. Autophagy 4: 600-606, 2008.
7. Liang H, Gu MN, Yang CX, Wang HB, Wen XJ and Zhou QL: Sevoflurane inhibits proliferation, induces apoptosis, and blocks cell cycle progression of lung carcinoma cells. Asian Pac J Cancer Prev 12: 3415-3420, 2011.
8. Dong C, Davis RJ and Flavell RA: MAP kinases in the immune response. Annu Rev Immunol 20: 55-72, 2002.
9. Johnson GL and Vaillancourt RR: Sequential protein kinase reactions controlling cell growth and differentiation. Curr Opin Cell Biol 6: 230-238, 1994.
10. Robinson MJ and Cobb MH: Mitogen-activated protein kinase pathways. Curr Opin Cell Biol 9: 180-186, 1997.
11. Cobb MH: MAP kinase pathways. Prog Biophys Mol Biol 71: 479-500, 1999.
12. Stanciu M, Wang Y, Kentor R, *et al*: Persistent activation of ERK contributes to glutamate-induced oxidative toxicity in a neuronal cell line and primary cortical neuron cultures. J Biol Chem 275: 12200-12206, 2000.
13. Colombo ML and Bosisio E: Pharmacological activities of *Chelidonium majus* L. (Papaveraceae). Pharmacol Res 33: 127-134, 1996.
14. Chor SY, Hui AY, To KF, *et al*: Anti-proliferative and pro-apoptotic effects of herbal medicine on hepatic stellate cell. J Ethnopharmacol 100: 180-186, 2005.
15. Kim YI, Park SW, Choi IH, Lee JH, Woo HJ and Kim Y: Effect of *Orostachys japonicus* on cell growth and apoptosis in human hepatic stellate cell line LX2. Am J Chin Med 39: 601-613, 2011.
16. Tang YJ, Yang JS, Lin CF, *et al*: *Houttuynia cordata* Thunb extract induces apoptosis through mitochondrial-dependent pathway in HT-29 human colon adenocarcinoma cells. Oncol Rep 22: 1051-1056, 2009.
17. Liu FS, Yang PY, Hu DN, Huang YW and Chen MJ: *Antrodia camphorata* induces apoptosis and enhances the cytotoxic effect of paclitaxel in human ovarian cancer cells. Int J Gynecol Cancer 21: 1172-1179, 2011.
18. Liu JJ, Zhang Y, Lin DJ and Xiao RZ: Tanshinone IIA inhibits leukemia THP-1 cell growth by induction of apoptosis. Oncol Rep 21: 1075-1081, 2009.
19. Hsu PC, Huang YT, Tsai ML, Wang YJ, Lin JK and Pan MH: Induction of apoptosis by shikonin through coordinative modulation of the Bcl-2 family, p27, and p53, release of cytochrome c, and sequential activation of caspases in human colorectal carcinoma cells. J Agric Food Chem 52: 6330-6337, 2004.
20. Huang KF, Zhang GD, Huang YQ and Diao Y: Wogonin induces apoptosis and down-regulates survivin in human breast cancer MCF-7 cells by modulating PI3K-AKT pathway. Int Immunopharmacol 12: 334-341, 2012.
21. Reed JC: Apoptosis-targeted therapies for cancer. Cancer Cell 3: 17-22, 2003.
22. Zhu W, Hu H, Qiu P and Yan G: Triptolide induces apoptosis in human anaplastic thyroid carcinoma cells by a p53-independent but NF-κB-related mechanism. Oncol Rep 22: 1397-1401, 2009.
23. Rubinfeld H and Seger R: The ERK cascade: a prototype of MAPK signaling. Mol Biotechnol 31: 151-174, 2005.
24. Jeong JC, Kim MS, Kim TH and Kim YK: Kaempferol induces cell death through ERK and Akt-dependent down-regulation of XIAP and survivin in human glioma cells. Neurochem Res 34: 991-1001, 2009.
25. Singh F, Gao D, Lebwohl MG and Wei H: Shikonin modulates cell proliferation by inhibiting epidermal growth factor receptor signaling in human epidermoid carcinoma cells. Cancer Lett 200: 115-121, 2003.
26. Ma Y, Yu WD, Kong RX, Trump DL and Johnson CS: Role of nongenomic activation of phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase/extracellular signal-regulated kinase kinase/extracellular signal-regulated kinase 1/2 pathways in 1,25D3-mediated apoptosis in squamous cell carcinoma cells. Cancer Res 66: 8131-8138, 2006.
27. Jeon ES, Lee MJ, Sung SM and Kim JH: Sphingosylphosphorylcholine induces apoptosis of endothelial cells through reactive oxygen species-mediated activation of ERK. J Cell Biochem 100: 1536-1547, 2007.
28. Yu W, Liao QY, Hantash FM, Sanders BG and Kline K: Activation of extracellular signal-regulated kinase and c-Jun-NH2-terminal kinase but not p38 mitogen-activated protein kinases is required for RRR-α-tocopheryl succinate-induced apoptosis of human breast cancer cells. Cancer Res 61: 6569-6576, 2001.
29. Xu C, Shen G, Yuan X, *et al*: ERK and JNK signaling pathways are involved in the regulation of activator protein 1 and cell death elicited by three isothiocyanates in human prostate cancer PC-3 cells. Carcinogenesis 27: 437-445, 2006.
30. Pan TL, Wang PW, Leu YL, Wu TH and Wu TS: Inhibitory effects of *Scutellaria baicalensis* extract on hepatic stellate cells through inducing G2/M cell cycle arrest and activating ERK-dependent apoptosis via Bax and caspase pathway. J Ethnopharmacol 139: 829-837, 2012.