BMB
Reports

BMB Rep. 2013; 46(12): 611-616
www.bmbreports.org

Molecular mechanisms of luteolin-7-O-glucoside-induced growth inhibition on human liver cancer cells: G2/M cell cycle arrest and caspase-independent apoptotic signaling pathways

Yu-Jin Hwang[1,2], Eun-Ju Lee[1], Haeng-Ran Kim[1] & Kyung-A Hwang[1,]*

[1]Department of Agrofood Resources, National Academy of Agricultural Science, RDA, Suwon 441-853, [2]Department of Biotechnology & Bioengineering, Sungkyunkwan University, Suwon 440-746, Korea

Luteolin-7-O-glucoside (LUT7G), a flavone subclass of flavonoids, has been found to increase anti-oxidant and anti-inflammatory activity, as well as cytotoxic effects. However, the mechanism of how LUT7G induces apoptosis and regulates cell cycles remains poorly understood. In this study, we examined the effects of LUT7G on the growth inhibition of tumors, cell cycle arrest, induction of ROS generation, and the involved signaling pathway in human hepatocarcinoma HepG2 cells. The proliferation of HepG2 cells was decreased by LUT7G in a dose-dependent manner. The growth inhibition was due primarily to the G2/M phase arrest and ROS generation. Moreover, the phosphorylation of JNK was increased by LUT7G. These results suggest that the anti-proliferative effect of LUT7G on HepG2 is associated with G2/M phase cell cycle arrest by JNK activation. [BMB Reports 2013; 46(12): 611-616]

INTRODUCTION

Apoptosis or programmed cell death is a normal component of the development and health of multicellular organisms. Cells die in response to a variety of stimuli, and in apoptosis, they do so in a controlled, regulated fashion. This makes apoptosis distinct from necrosis, another form of cell death, in which uncontrolled cell death leads to the lysis of cells, inflammatory responses, and potentially, to serious health problems. Apoptosis, in contrast, is a process in which cells play an active role in their own death. Apoptosis plays an important role in embryogenesis, metamorphosis, cellular homeostasis, tissue atrophy, and tumor regression. It is defined by morphological changes that include cell shrinkage, chromatin condensation, nuclear fragmentation, membrane blebbing, and apoptotic body formation (1-3). Oxidative stress and cell-cycle regulation are two essential elements in the apoptosis process. Apoptosis may be triggered by oxidative insults (4). Reactive oxygen species (ROS) are important chemical messengers in normal cells. They keep the balance with antioxidants in healthy cells (5). The accumulation of ROS results in oxidative stress, which mostly results in cell apoptosis (6). In addition, cell cycle arrest and apoptosis are closely linked to cell proliferation in mammalian cells (7-9). Because cancer involves deregulated cell proliferation and survival, inducing cell-cycle arrest is a feasible treatment to forestall continued tumor proliferation (10). A variety of natural and chemical compounds has been reported to interfere with the cell cycle, promote or inhibit apoptosis, produce important effects on their signal transduction and development progress, and even result in the death of tumor cells (11-13).

Recent scientific efforts have focused on the potential roles of extracts of traditional herbs as alternative and complementary medications for cancer treatment. Flavonoids, a kind of polyphenol, have three phenolic subcomponents and are also commonly referred to as bioflavonoids (14). Phytochemicals in the flavonoid family have noted bioactivities to suppress the ROS, inflammation, and growth of tumors (15, 16).

Luteolin-7-O-glucoside (LUT7G), a flavone subclass of flavonoids, can be found in wild edible vegetables such as *Ailanthus altissima*. LUT7G possesses potential antibacterial, antifungal (17), antioxidant (18), and anti-inflammation effects (19). However, there has been no report related to the regulation of cell cycle and apoptosis on hepatocarcinoma cells. We investigated the anti-proliferation of LUT7G on tumor cells and the cellular mechanism of the cytotoxicity of LUT7G in HepG2 cells.

RESULTS

Cytotoxic effects of LUT7G on HepG2 cell lines

To determine the cytotoxic effects of LUT7G on HepG2 cells, the cells were exposed to various concentrations of LUT7G (50, 100, and 200 μM) for 24 h. Cells treated with 1% DMSO

*Corresponding author. +82-31-299-0527; Fax: +82-31-299-0504; E-mail: kah366@korea.kr

http://dx.doi.org/10.5483/BMBRep.2013.46.12.133

Received 12 June 2013, Revised 18 June 2013, Accepted 18 June 2013

Keywords: Apoptosis, Cell cycle arrest, Luteolin-7-O-glucoside, MAPK, Reactive oxygen species

ISSN: 1976-670X (electronic edition)

were used as controls. As shown in Fig. 1A, LUT7G decreased cell viability in HepG2 cells in a dose-dependent manner. HepG2 cell proliferation was reduced by 39.8% after exposure to 200 μM LUT7G for 24 h. In addition, there was no cytotoxicity effect of LUT7G on normal cell lines (Huh7 cells) at the concentration tested (Fig. 1B). Microscopic image analysis revealed that LUT7G caused cell shrinkage with a condensed nucleus and a rough plasma membrane, which are indicative of apoptosis (Fig. 1C).

Induction of apoptosis by LUT7G in HepG2 cells

In order to determine whether the anti-proliferative effect of LUT7G was due to apoptosis, HepG2 cells were treated with LUT7G for 24 h, and nuclear Hoechst 33343 staining was performed. As shown in Fig. 2A, nuclei with condensed chromatin and apoptotic bodies, which are typical of apoptosis, were observed in HepG2 cells incubated with LUT7G. The number of apoptotic cells increased as the concentration of LUT7G increased.

Next, we investigated DNA fragmentation in the nucleus using TUNEL staining. As shown in Fig. 2A, LUT7G significantly induced DNA fragmentation in HepG2 cells (yellow-green, TUNEL). Propidium iodide (PI) was used for the counterstain for all nuclei. In agreement with the results in Fig. 2A, LUT7G also increased DNA laddering in HepG2 cells in a dose-dependent manner (Fig. 2B). This suggests that the HepG2 cells may undergo apoptosis after LUT7G treatment, and there is a good correlation between the extent of apoptosis and the inhibition of cell growth.

LUT7G induces apoptosis in HepG2 via a caspase-independent pathway

Because apoptosis can proceed either via caspase-dependent or independent signaling pathways (20, 21), the involvement of

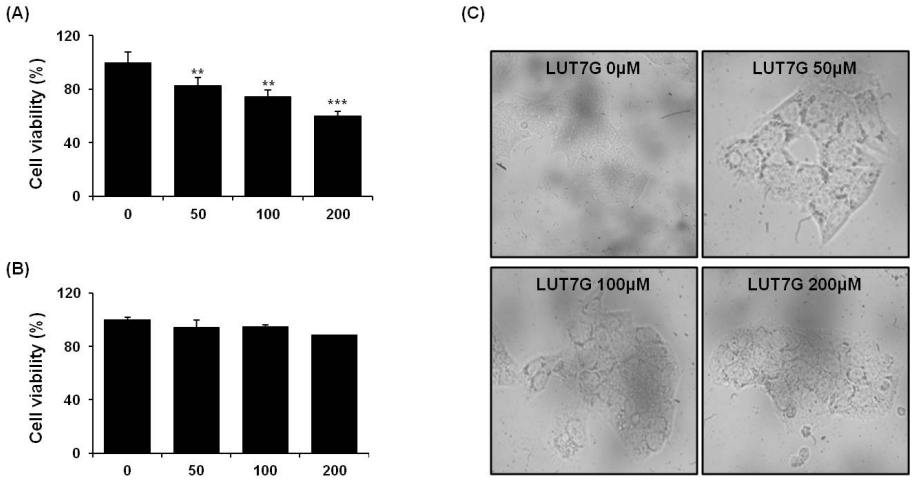

Fig. 1. Inhibitory effect of Luteolin-7-O-glucoside (LUT7G) in HepG2 and Huh7 cells. (A and B) HepG2(A) and Huh7(B) cells were treated with LUT7G at different concentrations (50, 100, and 200 μM). After treatment for 24 h, cell viability was quantified by measuring intracellular ATP. The bars represent the mean ± SEM of 3 experiments performed in triplicate. **P < 0.01 and ***P < 0.001 significantly different from the no treated-group. (C) Light microscopic images after incubation of LUT7G for 24 h in HepG2 (×200).

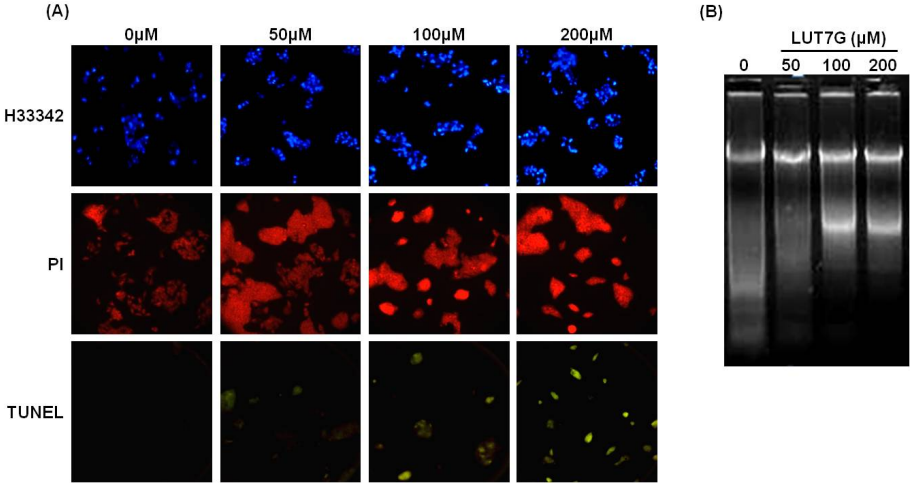

Fig. 2. Luteolin-7-O-glucoside (LUT7G) induces morphological changes of nuclear structure and DNA fragmentation in HepG2 cells. (A) Cells were incubated with LUT7G (50, 100, and 200 μM) for 24 h, followed by detection of apoptotic morphology by staining with a fluorescent DNA-binding dye. Hoechst 33342(H33324) and DNA fragmentation was detected by TUNEL staining. Identical fields were observed for TUNEL-positive for all nuclei. (B) Cells were treated with LUT7G for 24 h, and total genomic DNA was isolated. DNA laddering was run on 1% agarose gels and visualized by ethidium bromide staining.

caspases in LUT7G-induced HepG2 cell apoptosis was assessed. Expressions of the intracellular proteins related to apoptosis, such as PARP and caspase-3, -8, and -9, were investigated to understand the mechanisms by which LUT7G-induces apoptosis in HepG2 cells. As shown in Fig. 3A, the level of the PARP was decreased, and the level of cleaved PARP was increased in LUT7G-treated HepG2 cells. In contrast, the expression of caspase-3, -8 and -9 were not changed. These results suggest that the HepG2 cell apoptosis induced by LUT7G is not dependent on the activation of the caspase family of proteins.

To analyze other possible causes of growth inhibition, we examined the apoptotic effect of LUT7G on ROS generation and cell cycle arrest, which are known to be essential evidence of apoptosis. To investigate the intracellular levels of ROS, cell-permeable probe DCF-DA was utilized. Non-fluorescent DCF-DA, hydrolyzed to DCFH inside the cells, yields highly fluorescent DCF-DA in the presence of intracellular hydrogen peroxide and related peroxides (22). As shown in Fig. 3B, HepG2 cells treated with LUT7G for 24 h revealed ROS generation dose-dependently induced by LUT7G.

Next, we investigate the effect of LUT7G on the cell cycle using flow cytometry. LUT7G treatment arrested HepG2 cells at the G_2/M phase (Fig. 3C). The maximum G_2/M phase percentage of 34.01% occurred with 200 μM LUT7G treatment for 24 h. Therefore, LUT7G induced the G_2/M cycle arrest of HepG2 cells.

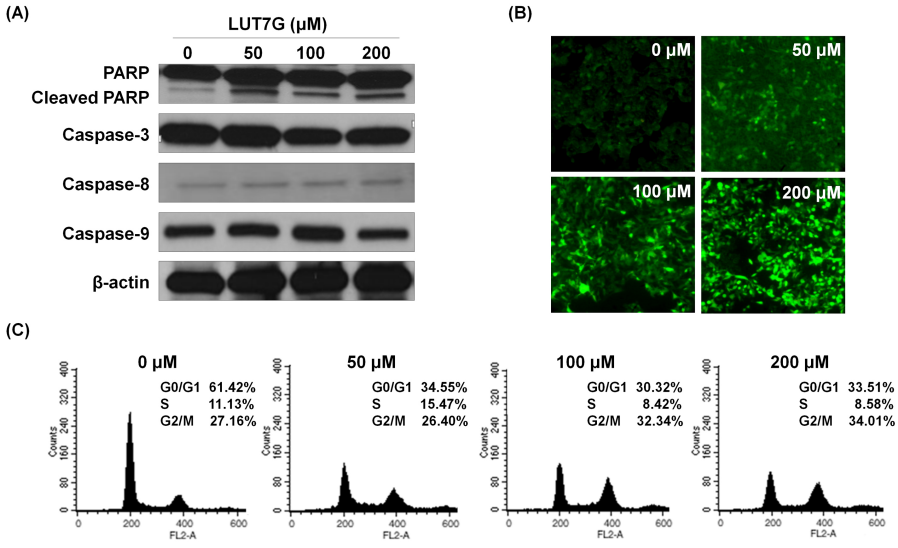

Fig. 3. Luteolin-7-O-glucoside (LUT7G) induces apoptosis in HepG2 via a caspase-independent pathway. (A) Cells were treated with LUT7G at the indicated concentration for 24 h. Whole cell lysates were subjected to western blot analysis of anti-PARP, caspase-3, -8, and -9 antibodies. β-actin was used as an internal control. (B) Cells were treated with LUT7G for 24 h. After staining with DCF-DA for 30 min, the ROS generation was analyzed with microscopy. (C) Cells were treated with LUT7G for 24 h. After fixation and staining with PI for 30 min, the cell cycle profiles were analyzed with flow cytometry. Representative results of the actual cell cycle profile are shown.

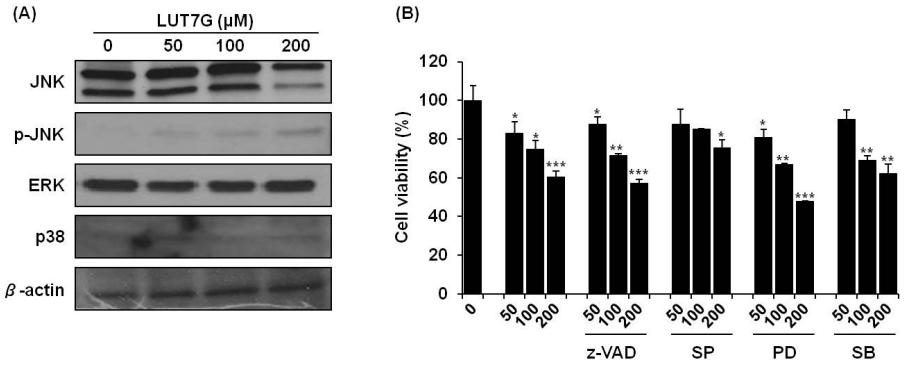

Fig. 4. Inhibition of Luteolin-7-O-glucoside (LUT7G)-induced apoptosis by JNK inhibitor in HepG2 cells. (A) Cells were treated with LUT7G at the indicated concentration for 24 h. Whole cell lysates were subjected to western blot analysis of anti-p38, ERK, JNK, and phospholyrated JNK antibodies. β-actin was used as an internal control. (B) The cells were stimulated with various LUT7G for 24 h and after pre-treatment with 20 μM Z-VAD-FMK (Z-VAD), 20 μM SP600125(SP), 20 μM PD98059(PD) and 20 μM SB203580(SB) for 1 h. Cell viability was quantified by measuring intracellular ATP. The bars represent the mean ± SEM of 3 experiments performed in triplicate. *P < 0.05, **P < 0.01 and ***p < 0.001 significantly different from the no treated-group.

LUT7G induced apoptosis by JNK pathway

MAPKs are activated by various extracellular stimuli, and mediate the signal transduction cascades that play an important role in cell cycle arrest and cell apoptosis (23, 24). Therefore, we next examined the effects of LUT7G on MAPK signaling. The expression of MAPK proteins such as JNK, ERK, and p38 were measured by western blotting. As shown in Fig. 4A, the quantification of band intensity showed that JNK was decreased after LUT7G treatment, but the expression levels of ERK and p38 were not affected. The phosphorylation level of JNK was increased by LUT7G treatment. Next, we investigated the possible roles of MAPKs in LUT7G-induced apoptosis. Cell viability was measured in the presence of specific MAPK inhibitors by intracellular ATP content. The cell viability was similar in the presence of Z-VAD-FMK, PD98059, and SB203580, while it was increased due to SP600125 treatment (Fig. 4B). These results indicate that LUT7G-induced apoptosis may be associated with the upregulation of the JNK pathways.

DISCUSSION

The proliferation inhibition and apoptotic induction of tumor cells are effective to prevent tumor growth and to eliminate cancers. Although numerous compounds possess antitumor activities, their applications as antitumor agents are greatly restricted by an unknown mechanism (25-27). In the present study, our results demonstrate that LUT7G inhibited growth and induced apoptosis in HepG2 liver carcinoma cells through a caspase-independent pathway, and LUT7G-induced apoptosis appears to be mediated via the induction of G2/M cell cycle arrest and ROS generation. Additionally, this form of cell death requires activation of the JNK pathway.

In the present study, we found that LUT7G was cytotoxic to the human hepatocarcinoma cell line (HepG2). We confirmed the effect on the morphologic features of HepG2 cells. HepG2 cells showed apoptotic body formation and DNA fragmentation with LUT7G treatment, which indicated apoptosis.

One of the main pathways of apoptosis is a caspase-dependent pathway. The caspases are cystein proteases that play key roles in the execution phase of apoptosis. Among the family of caspases, caspase-3 has been reported to be the most frequently activated caspase protease in apoptotic cells, indicating its crucial role in the cell death process (28). In the present study, our data show that LUT7G-induced apoptosis was not inhibited by the broad-spectrum caspase inhibitor Z-VAD-FMK, with no activation of caspase-3, suggesting a caspase-independent signal transduction pathway.

Because increasing evidence has associated apoptosis with ROS generation and cell cycle arrest, we examined the apoptotic effect of LUT7G on these features. ROS generation was effectively enhanced by LUT7G in HepG2 cells. Additionally, HepG2 cells treated with LUT7G were significantly arrested at the G_2/M stage before apoptosis. Therefore, ROS generation and G_2/M cell-cycle arrest probably contribute to LUT7G-induced HepG2 cell apoptosis.

The examination of several signaling molecules may help explain the mechanism of the apoptosis process caused by LUT7G in HepG2 cells. We found that LUT7G triggered phosphorylation of JNK in HepG2 cells.

The mitogen-activated protein kinases (MAPKs) family is one of the signal pathways that has been implicated in oxidative stress-induced cell death cascade (29, 30). Among the MAPKs family, the activation of JNK is commonly linked to promoting cell apoptosis and cell death, and it is thus is also called a stress-activated protein kinase (SAPK) (31, 32). For mammalian cells, researchers have reported that the accumulation of H_2O_2 can activate JNK pathways (33). Also, prolonged JNK activity promotes apoptosis, and can lead to accumulated ROS (34). In addition, JNK may be involved in cell cycle regulation. JNK is associated with the cell cycle with its target molecule c-Jun protein, which was reported to be involved with G_1 phase progression (35). Previous studies reported an association between the JNK signaling pathway and the suppression of cell-cycle progression via the activation of cell cycle inhibitor proteins, including p21 and p27 (36-38).

The results of the present study significantly advance our understanding of the molecular actions of LUT7G. In particular, the novel findings reported here are that JNK activation underlies the anti-proliferative effects of LUT7G, such as G_2/M cell-cycle arrest. Although the present data demonstrate the importance of JNK activation in LUT7G-induced growth inhibition in liver cancer cells, the mechanisms by which JNK regulates apoptotic factors in LUT7G treatment remain to be identified.

In conclusion, we demonstrated that LUT7G could affect the viability of human carcinoma cells. Furthermore, LUT7G induced apoptosis in HepG2 cells in a dose-dependent manner through caspase-independent pathways. With apoptosis, ROS accumulated, and cells were arrested in G_2/M. ROS accumulation and G_2/M cell cycle arrest contributed to the apoptosis process through the JNK pathway. These results provide further insight into LUT7G-induced apoptosis, and a new insight into the molecular mechanisms of LUT7G for cancer intervention.

MATERIALS AND METHODS

Cell culture, antibodies, and chemicals

HepG2 cells were grown in DMEM medium with 10% (v/v) heat-inactivated FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml). The cultures were maintained at 37°C in a 5% CO_2 incubator. Antibodies against PARP, caspase-3, -8, -9, JNK, ERK, p38 MAPK, phosphorylated JNK, and β-actin were purchased from Cell Signaling Technology (Danvers, MA, USA). The ERK1/2 inhibitor PD98059, the p38 inhibitor SB203580, and the JNK inhibitor SP600125 were purchased from Calbiochem (Darmstadt, Germany). The broad-spectrum caspase inhibitor Z-VAD-FMK and PI/RNase A solution were purchased from BD bioscience (San Jose, CA, USA).

Cell viability
Cells (1×10^5 cells/well) were added to duplicate 12-well plates and incubated for 4 h, then treated with various concentrations of LUT7G for 24 h. Cell viability was measured with CellTiter Glo (Promega, Madison, WI, USA). Cell viability is presented as the percentage of dead cells in each well.

Nuclear staining with Hoechst 33342
The nuclear morphology of the cells was studied using the cell-permeable DNA-specific dye Hoechst 33342. Approximately 2×10^5 HepG2 cells/well were treated with LUT7G at various concentrations for 24 h. Then, Hoechst 33342 was added to the culture medium at a final concentration of 1 μg/ml, and the plate was incubated for another 10 min at 37°C. The stained cells were then observed under a fluorescence microscope (Carl ZEISS, Oberkochen, Germany) equipped with a SPOT digital camera to examine the degree of nuclear condensation.

TUNEL assay and DNA fragmentation analysis
Cells treated with LUT7G for 24 h were fixed with 4% formaldehyde for 20 min. Cells with fragmented nuclear DNA were detected according to the manufacturer's instructions. PI counterstain was performed at room temperature for all nuclei. The cells were analyzed using a fluorescence microscope. DNA laddering detection was performed according to the manufacturer's instructions (QIAGEN, Hilden, Germany).

Cell cycle analysis
Cells treated with LUT7G were harvested and collected by centrifugation at 1,500 rpm for 10 min and washed with ice-cold PBS. The cell pellet was suspended with 70% ethanol at −20°C overnight, washed, and then incubated with PI/RNase A for 30 min staining in the dark at room temperature. Flow cytometry was used for detection (FACSCalibur, BD biosciences).

ROS generation analysis
For the microscopic detection of ROS formation, cells treated with LUT7G for 24 h were incubated with DCF-DA (25 μM) for 30 min at 37°C in the dark. After several washings with PBS, cells were observed with a fluorescence microscope.

Western blot analysis
Cells were lysed in RIPA buffer (150 mM Sodium Chloride, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 7.5, and 2 mM EDTA) on ice for 30 min. After centrifugation at 4°C for 20 min (12,000 × *g*), the supernatant was collected. Protein concentrations were determined by BCA assay (GenDEPOT, Barker, TX, USA). Equal amounts of cell extracts were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). The membranes were blotted with antibody, and detection was performed with an ECL system (Pierce, Rockford, IL, USA) according to the manufacturer's instructions.

Statistical analysis
Statistical analyses were performed with SPSS statistical software (version 12.0). The data represent the means ± SEM from 3 independent experiments, except where indicated. Statistical analyses were performed by student's *t*-test at a significance level of $P < 0.05$.

Acknowledgements
This study was carried out with the support of the "Research Program for Agricultural Science & Technology Development: (Project No. PJ008462) of the National Academy of Agricultural Science, Rural Development Administration, Korea.

REFERENCES

1. Curtin, J. F. and Cotter, T. G. (2003) Live and let die: regulatory mechanisms in Fas mediated apoptosis. *Cell Signal.* **15**, 983-992.
2. Lee, E. W., Seo, J. H., Jeong, M. H., Lee, S. S. and Song. J. H. (2012) The roles of FADD in extrinsic apoptosis and necroptosis. *BMB Rep.* **45**, 496-508.
3. Hengartner, M. O. (2000) The biochemistry of apoptosis. *Nature* **407**, 770-776.
4. Lee, D. H., Rhee, J. G. and Lee, Y. J. (2009) Reactive oxygen species up-regulate p53 and Puma; a possible mechanism for apoptosis during combined treatment with TRAIL and wogonin. *Br. J. Pharmacol.* **157**, 1189-1202.
5. Sauer, H., Wartenberg, M. and Hescheler, J. (2001) Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell Physiol. Biochem.* **11**, 173-186.
6. Chandra, J., Samali, A. and Orrenius, S. (2000) Triggering and modulation of apoptosis by oxidative stress. *Free Radic. Biol. Med.* **29**, 323-333.
7. Trivedi, P. P., Roberts, P. C., Wolf, N. A. and Swanborg, R. H. (2005) NK cells inhibit T cell proliferation via p21-mediated cell cycle arrest. *J. Immunol.* **174**, 4590-4597.
8. Okamoto, S., Kang, Y., Brechtel, C., Siviglia, E., Russo, R., Clemente, A., Harrop, A., McKercher, S., Kaul, M. and Lipton, S. (2007) HIV/gp120 decreases adult neural progenitor cell proliferation via checkpoint kinase-mediated cell-cycle withdrawal and G1 arrest. *Cell Stem Cell.* **1**, 230-236.
9. Liang, Q., Xiong, H., Zhao, Z., Jia, D., Li, W., Qin, H., Deng, J., Gao, L., Zhang, H. and Gao, G. (2009) Inhibition of transcription factor STAT5b suppresses proliferation, induces G1 cell cycle arrest and reduces tumor cell invasion in human glioblastoma multiforme cells. *Cancer Lett.* **273**, 164-171.
10. Evan, G. I. and Vousden, K. H. (2001) Proliferation, cell cycle and apoptosis in cancer. *Nature* **411**, 342-348.
11. Choi, J. A., Kim, J. Y., Lee, J. Y., Kang, C. M., Kwon, H. J., Yoo, Y. D., Kim, T. W., Lee, Y. S. and Lee, S. J. (2001) Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *Int. J. Oncol.* **19**, 837-844.
12. Fimognari, C., Nusse, M., Cesari, R., Iori, R., Cantelli-

Forti, G. and Hrelia, P. (2002) Growth inhibition, cell-cycle arrest and apoptosis in human T-cell leukemia by the isothiocyanate sulforaphane. *Carcinogenesis* **23**, 581-586.
13. Liou, S. F., Lin, H. H., Liang, J. C., Chen, I. J. and Yeh, J. L. (2009) Inhibition of human prostate cancer cells proliferation by a selective alpha1-adrenoceptor antagonist labedipinedilol-A involves cell cycle arrest and apoptosis. *Toxicology* **256**, 13-24.
14. Ko, W. G., Kang, T. H., Lee, S. J., Kim, Y. C. and Lee, B. H. (2000) Effects of luteolin on the inhibition of proliferation and induction of apoptosis in human myeloid leukaemia cells. *Phytother. Res.* **16**, 295-298.
15. Hwang, K. A., Hwang, Y. J., Park, D. S., Kim, J.-H. and Om, A. S. (2011) In vitro investigation of antioxidant and anti-apoptotic activities of Korean wild edible vegetable extracts and their correlation with apoptotic gene expression in HepG2 cells. *Food Chem.* **125**, 483-487.
16. Lee, S. H., Lee, E. and Ko, Y. T. (2012) Anti-inflammatory effects of a methanol extract from *Pulsatilla koreana* in lipopolysaccharide-exposed rats. *BMB Rep.* **45**, 371-376.
17. Kishore, K. C., Arifullah, M., Gayathri, D., Ramagopal, G. and Sathees, C. R. (2007) Phytochemical antimicrobial studies of methy angolensate and luteolin-7-O-glucoside isolated from callus cultures of *Soymida febrifuga*. *Int. J. Biomed. Sci.* **3**, 269-278.
18. Cakir, A., Mavi, A., Kazaz, C., Yildirim, A. and Kufrevioglu, O. I. (2006) Antioxidant activities of the extracts and components of *Teucrium orientale* L. var. orientale. *Turk. J. Chem.* **30**, 483-494.
19. Chung, H. and David, D. K. (2004) Luteolin-7-O-glucoside from dandelion flower suppress iNOS and COX-2 in RAW 264.7 cells. *Mol. Cell Biochem.* **265**, 107-113.
20. Kitanaka, C., Kato, K. and Tanaka, Y. (2007) Ras protein expression and autophagic tumor cell death in neuroblastoma. *Am. J. Surg. Pathol.* **31**, 153-155.
21. Zeuner, A., Eramo, A., Testa, U., Felli, N., Pelosi, E., Mariani, G., Srinivasula, S. M., Alnemri, E. S., Condorelli, G., Peschle, C. and De Maria, R. (2003) Control of erythroid cell production via caspase-mediated cleavage of transcription factor SCL/Tal-1. *Cell Death Differ.* **10**, 905-913.
22. LeBel, C. P., Ischiropoulos, H. and Bondy, S. C. (1992) Evaluation of the probe 2′, 7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chem. Res. Toxicol.* **5**, 227-231.
23. Guay, J., Lambert, H., Gingras, G., Lavoie, J. N., Huot, J. and Landry, J. (1997) Regulation of actinfilament dynamics by p38 map kinase-mediated phosphorylation of heat shockprotein. *J. Cell Sci.* **110**, 357-368.
24. Hedges, J. C., Dechert, M. A., Yamboliev, I. A., Martin, J. L., Hickey, E., Weber, L. A. and Gerthoffer, W. T. (1999) A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration. *J. Biol. Chem.* **274**, 24211-24219.
25. Chan, K. T., Li, K., Liu, S. L., Chu, K. H., Toh, M. and Xie, W. D. (2010) Cucurbitacin B inhibits STAT3 and the Raf/MEK/ERK pathway in leukemia cell line K562. *Cancer Lett.* **289**, 46-52.
26. Rana, S., Gupta, K., Gomez, J., Matsuyama, S., Chakrabarti, A., Agarwal, M. L., Agarwal, A., Agarwal, M. K. and Wald, D. N. (2010) Securinine induces p73-dependent apoptosis preferentially in p53-deficient colon cancer cells. *FASEB J.* **24**, 2126-2134.
27. Wang, Y., Ma, X. Q., Yan, S. S., Shen, S. S., Zhu, H. L., Gu, Y., Wang, H. B., Qin, G. W. and Yu, Q. (2009) 17-Hydroxy-jolkinolide B inhibits signal transducers and activators of transcription 3 signaling by covalently cross-linking Janus kinases and induces apoptosis of human cancer cells. *Cancer Res.* **69**, 7302-7310.
28. Cohen, G. M. (1997) Caspases: the executioners of apoptosis. *J. Biochem.* **326**, 1-6.
29. Fracasso, M. E., Perbellini, L., Soldà, S., Talamini, G. and Franceschetti, P. (2002) Lead induced DNA strand breaks in lymphocytes of exposed workers: role of reactive oxygen species and protein kinase *C. Mutat. Res.* **515**, 159-169.
30. Megan, C., Alice, G. and Patricia, W. (2010) A role for p38 stress-activated protein kinase inregulation of cell growth via TORC1. *Mol. Cell. Biol.* **30**, 481-495.
31. Mansouri, A., Ridgway, L. D., Korapati, A. L., Zhang, Q. X., Tian, L., Wang, Y. B., Siddik, Z. H., Mills, G. B. and Claret, F. X. (2003) Sustained activation of JNK/p38 MAPK pathways in response to cisplatin leads to Fas ligand induction and cell death in ovarian carcinoma cells. *J. Biol. Chem.* **278**, 19245-19256.
32. Bulavin, D., Kovalsky, O., Hollander, M. C. and Fornace, A. (2003) Loss of oncogenic H-ras-induced cell cycle arrest and p38 mitogen-activated protein kinase activation by disruption of GADD45a. *Mol. Cell. Biol.* **23**, 3859-3871.
33. Chang, L. and Karin, M. (2001) Mammalian MAP kinase signaling cascades. *Nature* **410**, 37-40.
34. Luo, J. L., Kamata, H. and Karin, M. (2005) IKK/NF-kappa B signaling: balancing life and death - a new approach to cancer therapy. *J. Clin. Invest.* **115**, 2625-2632.
35. Wisdom, R., Johnson, R. S. and Moore, C. (1999) C-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. *EMBO J.* **18**, 188-197.
36. Davis, R. J. (2000) Signal Transduction by the JNK Group of MAP Kinases. *Cell* **103**, 239-252.
37. Tong, C., Yin, Z., Song, Z., Dockendorff, A., Huang, C., Mariadason, J., Flavell, R. A., Davis, R. J., Augenlicht, L. H. and Yang, W. (2007) c-Jun NH2-Terminal kinase 1 plays a critical role in intestinal homeostasis and tumor suppression. *Am. J. Pathol.* **171**, 297-303.
38. Jee, H., Lee, S. H., Park, J. H., Lee, B. R. Nam, K. T. and Kim, D. Y. (2013) Connexin32 inhibits gastric carcinogenesis through cell cycle arrest and altered expression of $p21^{Cip1}$ and $p27^{Kip1}$. *BMB Rep.* **46**, 25-30.