Induction of apoptosis in human colon cancer HCT-116 cells by anthocyanins through suppression of Akt and activation of p38-MAPK

DONG YEOK SHIN[1], WON SUP LEE[2], JING NAN LU[2], MYUNG HEE KANG[2], CHUNG HO RYU[4], GI YOUNG KIM[5], HO SUNG KANG[6], SUNG CHUL SHIN[3] and YUNG HYUN CHOI[1]

[1]Department of Biochemistry, Dongeui University College of Oriental Medicine and Department of Biomaterial Control (BK21 program), Dongeui University Graduate School, Busan 614-052; [2]Department of Internal Medicine, Institute of Health Sciences and Gyeongnam Regional Cancer Center, Gyeongsang National University School of Medicine, Jinju 660-702; Departments of [3]Chemistry, Research Institute of Natural Science and Research Institute of Life Science and [4]Food Technology and Research Institute of Life Science, Gyeongsang National University, Jinju 660-701; [5]Faculty of Applied Marine Science, Cheju National University, Jeju 690-756; [6]Department of Molecular Biology, Pusan National University, Busan 609-735, Korea

Received July 9, 2009; Accepted September 3, 2009

DOI: 10.3892/ijo_00000469

Abstract. Anthocyanins belong to a class of flavonoids that exhibit important anti-oxidant and anti-inflammatory actions as well as chemotherapeutic effects. However, little is known concerning the molecular mechanisms by which these activities are exerted. In this study, we investigated the anthocyanins isolated from *Vitis coignetiae* Pulliat for their potential anti-proliferative and apoptotic effects on human colon cancer HCT-116 cells. These anthocyanins inhibited cell viability and induce apoptotic cell death of HCT-116 cells in a dose-dependent manner. The apoptotic cell death was caspase-dependent and the anthocyanins regulated anti-apoptotic proteins (IAPs). In addition, apoptosis was associated with activation of p38-MAPK and suppression of Akt. In conclusion, this study suggests that the anthocyanins isolated from *Vitis coignetiae* Pulliat induce apoptosis might at least in part through activating p38-MAPK and suppressing Akt in human colon cancer HCT-116 cells.

Correspondence to: Professor Won Sup Lee, Department of Internal Medicine, Institute of Health Sciences and Gyeongnam Regional Cancer Center, Gyeongsang National University School of Medicine, 90 Chilam-don, Jinju 660-702, Korea
E-mail: lwshmo@hanmail.net; lwshmo@gshp.gsnu.ac.kr

Professor Yung Hyun Choi, Department of Biochemistry, Dongeui University College of Oriental Medicine, 42 San, Yangjung-dong, Busan 614-052, Korea
E-mail: choiyh@deu.ac.kr

Key words: apoptosis, colon cancer, anthocyanins, Akt, p38-MAPK

Introduction

The elderly population has been increasing due to developments in medical science. This elderly population has a high risk for cancer development, and the incidence of cancer and cancer-related mortality is expected to increase. These patients have a higher risk for serious side effects from conventional chemotherapeutic agents. Therefore, drug development is focusing on the less toxic drugs to improve the quality of life. The call for the development of a less toxic drug led us to be interested in the dietary agents because many dietary agents have recently been reported to have anti-cancer activities without notable side effects (1-3). In addition, with the growth of ecological movements, natural products have become more popular for the prevention or treatment of cancer. This has been paralleled by an increase in research focused on natural products.

Vitis coignetiae Pulliat (Meoru in Korea) belongs to the grape family. The fruit has traditionally been used in Korean folk medicine for the treatment of inflammatory disorders and cancer. The intense dark red hue reflects an abundance of anthocyanin pigments the anti-cancer activities of which have been reported *in vitro* and *in vivo* (4-6). Therefore, we postulated that the anthocyanins are major components for the anti-cancer activities of the Meoru. In the previous study (7), the composition of AIMs was falsely stated. The correct composition is: delphinidin-3,5-diglucoside:cyanidin-3,5-diglucoside:petunidin-3,5-diglucoside:delphinidin-3-glucoside:malvidin-3,5-diglucoside:peonidin-3,5-diglucoside:cyanidin-3-glucoside:petunidin-3-glucoside:peonidin-3-glucoside:malvidin-3-glucoside =1.0:0.5:3.4:28.1:6.4:4.2:22.5:4.9:22.5, constituent ratio. Here we tested the anti-cancer activity of AIMs in human solid cancer with special focus on the underlying mechanisms of the anti-cancer activities.

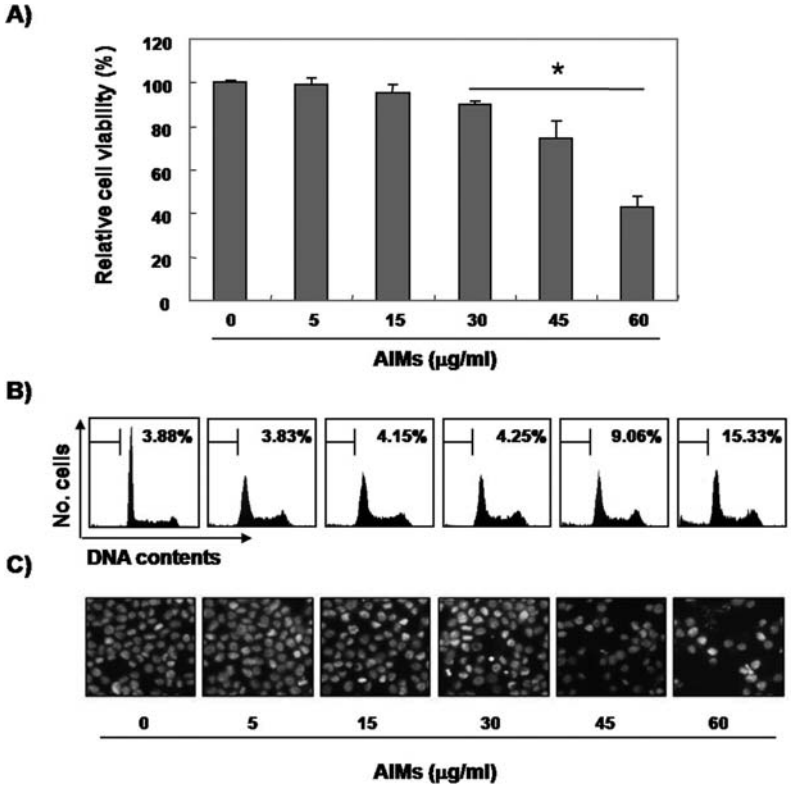

Figure 1. Effects of AIMs on the proliferation of HCT-116 human colon cancer cells. HCT-116 cells were seeded at the density of $5x10^4$ cells per ml. The cells were treated with indicated concentrations of AIMs for 48 h. (A) Cell proliferation was assessed by MTT assay. The data are shown as mean ± SD of three independent experiments. $^*P<0.05$ between the treated and the untreated control group. (B) Effects of AIMs on cell cycle distribution of HCT-116 cells. The cells were treated with indicated concentrations of AIMs for 48 h. The cells with sub-G1 DNA content representing the cells undergoing apoptotic DNA degradation were also analyzed by flow cytometry. (C) Effects of AIMs on the morphology of the nuclei of HCT-116 cells. After fixation, the cells were stained with DAPI solution. Stained nuclei were then observed under fluorescent microscope using a blue filter (magnification x400). The results (B and C) are from a representative experiment of two independent experiments that showed similar patterns.

Materials and methods

Cell culture and chemicals. Human colon cancer cells (HCT116) from the ATCC (Rockville, MD) were cultured in RPMI-1640 medium (Invitrogen Corp., Carlsbad, CA) supplemented with 10% FBS (Gibco BRL, Grand Island, NY), 100 U/ml penicillin, and 100 μg/ml streptomycin in an incubator at 37˚C in a humidified atmosphere of 95% air and 5% CO_2. Molecular mass markers for proteins were obtained from Pharmacia Biotech (Saclay, France). Antibodies against XIAP, cIAP-1, cIAP-2, Bcl-2, Bax, Bcl-XL, Bid, of poly (ADP-ribose) polymerase (PARP), caspase-3, -8 and -9 were purchased form Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody against ß-actin was from Sigma (Beverly, MA). Peroxidase-labeled donkey anti-rabbit and sheep anti-mouse immunoglobulin, and an enhanced chemiluminescence (ECL) kit were purchased from Amersham (Arlington Heights, IL). All other chemicals not specifically cited here were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell viability assays. For the cell viability assay, the cells were seeded onto 24-well plates at a concentration of $5x10^4$ cells/ml, grown to 70% confluence and then treated with the indicated concentration of AIMs for 48 h. Control cells were supplemented with complete media containing 0.1% DMSO (vehicle control). Following treatment, cell number and viability were determined by MTT assays.

Nuclear staining. After treatment with the indicated concentration of vitamin C, the cells were harvested, washed with phosphate-buffered saline (PBS) and fixed with 3.7% paraformaldehyde in PBS for 10 min at room temperature. Fixed cells were washed with PBS and stained with 2.5 μg/ml 4,6-diamidino-2-phenylindole (DAPI) solution for 10 min at room temperature. The cells were washed two times with PBS and observed under a fluorescent microscope.

Flow cytometry assay. The cells were plated at a concentration of $2x10^5$ cells/well in 6-well plates. Twenty-four hours later, the cells were subsequently treated with the indicated concentration of AIMs for 48 h. The cells were washed twice with cold PBS and then centrifuged. The pellet was fixed in 75% (v/v) ethanol for 1 h at 4˚C. The cells were washed once with PBS and resuspended in cold PI solution (50 μg/ml) containing RNase A (0.1 mg/ml) in PBS (pH 7.4) for 30 min in the dark. Flow cytometry analyses were performed using FACSCalibur (Becton-Dickinson, San Jose, CA). Forward light scatter characteristics were used to exclude the cell debris from the analysis. Approximately 10,000 cells were evaluated for each sample. Two independent experiments were performed.

Western blotting. The concentrations of cell lysate proteins were determined by means of the Bradford protein assay (Bio-Rad Laboratories, Richmond, CA, USA) using bovine

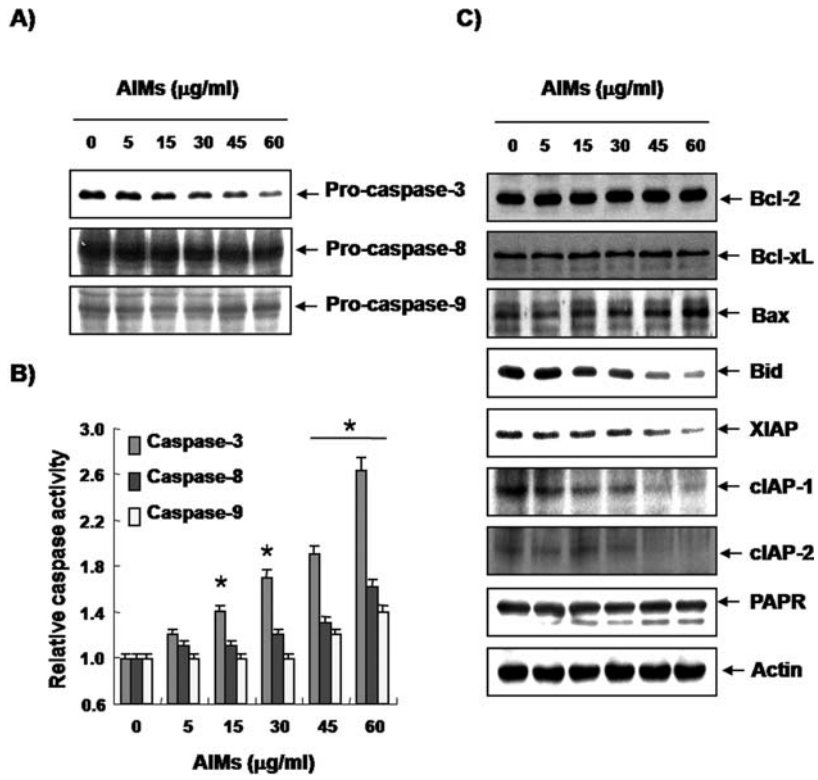

Figure 2. Activation of caspases during the AIM-induced apoptosis in HCT-116 cells. HCT-116 cells were incubated at indicated concentrations of AIMs for 48 h. (A) Equal amounts of cell lysate (30 μg) were resolved by SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the anti-caspase-3, -8, -9 and anti-PARP antibodies. The proteins were visualized using an ECL detection system. ß-actin was used as an internal control. (B) The cell lysates from the cells treated with AIMs for 48 h were assayed for *in vitro* caspase-3, -8 and -9 activity using DEVD-pNA, IETD-pNA and LEHD-pNA respectively, as substrates. The released fluorescent products were measured. Each bar graph represents mean ± SD of three independent experiments. (C) The equal amounts of proteins were then separated by SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were probed with the indicated antibodies and detected by an ECL detection system. *P<0.05 between the treated and the untreated control group.

serum albumin as the standard. The protein was resolved by electrophoresis, electrotransferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA), and then incubated with primary antibodies followed by secondary antibody conjugated to peroxidase. Blots were developed with an ECL detection system.

Determination of caspase activity. Caspase activities were determined by colorimetric assays using caspase-3, -8, and -9 activation kits according to the manufacturer's instructions. The kits utilize synthetic tetrapeptides labeled with p-nitroanilide. Briefly, the cells were lysed in the supplied lysis buffer. The supernatants were collected and incubated with the supplied reaction buffer containing dithiothreitol and substrates at 37˚C. The caspase activity was determined by measuring changes in absorbance at 405 nm using the microplate reader.

Statistical analyses. Each experiment was performed in triplicate. The results were expressed as mean ± SD. Significant differences were determined using the Student's t-test. Statistical significance was defined as P<0.05.

Results

AIMs induce apoptosis in HCT-116 cells. To investigate the anti-tumor activity of AIMs, human colon cancer HCT116 cells were treated with various concentrations of AIMs for 48 h. We used a relatively low concentration range in which we previously demonstrated apoptotic activity in the hematologic malignant cells, U937 cells *in vitro* (7). The concentration range was easily attainable *in vivo*. The growth of HCT116 cells were inhibited by AIM treatment in a dose-dependent manner, and IC_{50} for the 48h-AIM treatment was <60 μg/ml in HCT-116 cells (Fig. 1A).

To assess how AIMs affected cell growth, we determined the effects of AIMs on the cell cycle of HCT-116 cells by flow cytometry. A significant accumulation of cells with sub-G1 DNA content was noted in a dose-dependent manner, but no other remarkably detectable cell cycle changes were observed in HCT-116 cells treated with the indicated concentration of AIMs for 48 h (Fig. 1B).

To investigate whether the viability decrease in HCT-116 cells was due to the induction of apoptosis, we assessed the changes in nuclear morphology of AIM-treated cells with DAPI staining. The DAPI staining revealed the condensed and fragmented nuclei at higher concentration. This is usually witnessed in apoptosis (Fig. 1C).

AIMs induce caspase-dependent apoptosis. Caspases are known as important mediators of apoptosis and contribute to leading cells undergoing apoptosis to irreversible cell death. Therefore, we measured the activation of caspase-3,-8 and -9 with both the Western blot and colorimetric activity assay. As shown in Fig. 2A, the treatment of AIMs decreased the

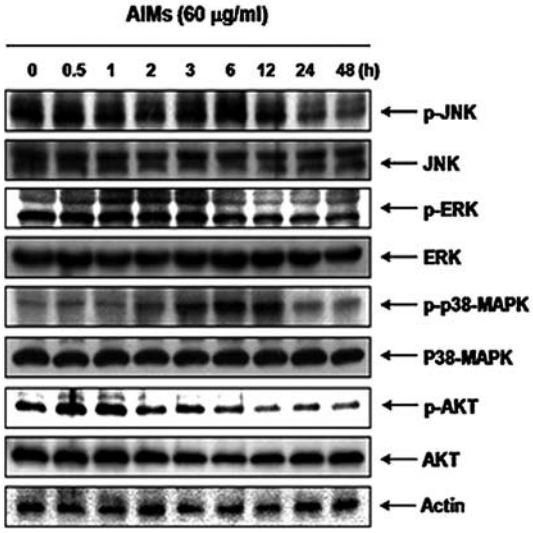

Figure 3. Effects of AIMs on mitogen-activated protein kinase (MAPK) and Akt pathway in HCT-116 cells. The cells were treated with AIMs for the indicated times. Equal amounts of cell lysate (30 μg) were resolved by SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the indicated antibodies and detected by the ECL system. The results are from a representative experiment of at least three independent experiments that showed similar patterns.

expression levels of pro-caspase-3, -8 and -9 in a concentration-dependent manner. To confirm this finding, we assayed proteolytic activity of caspases using colorimetric assay kits. We found that AIMs caused marked increases in the proteolytic activities of caspases (Fig. 2B). Western blot analysis also showed the progressive proteolytic cleavage of PARP protein, which is a downstream target of the activated caspase-3, in a concentration-dependent manner in HCT-116 cells after AIM treatment (Fig. 2C). These findings suggest that AIMs should induce apoptotic death through a caspase-dependent pathway.

AIMs modulate Bcl-2 and IAP family members. To investigate the effects of AIMs on the apoptosis-regulating proteins, we measured the levels of Bcl-2 and IAP family members, which play a crucial role in apoptosis conferring cancer cells drug resistance (8). Western blot analyses revealed that AIMs induced reduction in the expressions of anti-apoptotic proteins (XIAP, cIAP-1 and cIAP-2) whereas the expression of pro-apoptotic Bax remained unchanged at the concentration of ≤45 μg/ml (Fig. 2C). Our results suggested that suppression of the expression of anti-apoptotic proteins might be another mechanism through which AIMs enhance apoptosis in HCT-116 cells.

AIMs induce apoptosis at least in part through the activation of p38-MAPK. Mitogen-activated protein kinase (MAPK) is involved in cell proliferation, differentiation and apoptosis (9-11). To investigate the mechanism responsible for AIM-induced apoptosis, we assessed the changes in MAP kinase activation induced by AIMs. Western blot analysis revealed that 48-h treatment of AIMs activated phosphorylation of p38-MAPK and attenuated phophorylation of JNK which are essential factors in apoptosis signaling (Fig. 3). By contrast, phophorylation of ERK was stimulated by AIMs as early as 30 min and returned to basal status 24 h after AIM treatment. To confirm the involvement of the MAPK in AIM-induced

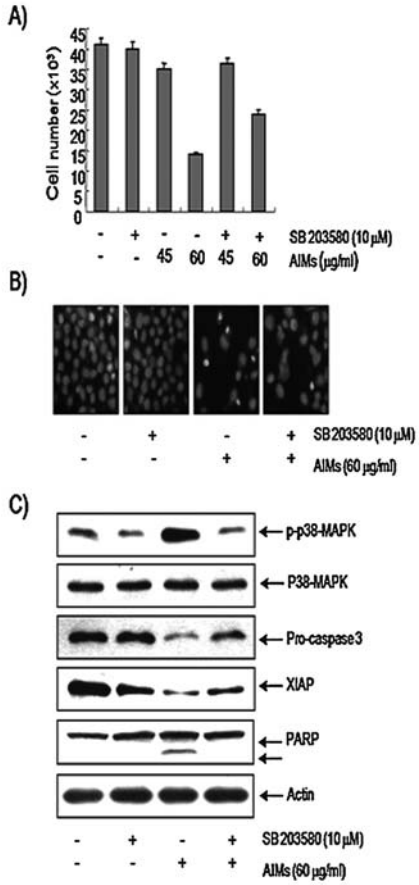

Figure 4. Effects of AIMs on apoptosis by regulating mitogen-activated protein kinase (MAPK) in HCT-116 cells. HCT-116 cells were seeded at the density of $5x10^4$ cells per ml. The cells were treated with SB203580 (10 μM) 1 h before treatment with indicated concentrations of AIMs for 48 h. (A) Cell proliferation was assessed by MTT assay. The data are shown as mean ± SD of three independent experiments. $^*P<0.05$ between the treated and the untreated control group. (B) Nuclei stained with DAPI solution were observed under a fluorescent microscope using a blue filter. (C) Equal amounts of cell lysate (30 μg) were resolved by SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the indicated antibodies and detected by the ECL detection system. The results are from a representative experiment of at least three independent experiments that showed similar patterns.

apoptosis, we performed cell viability assay using p38 inhibitor (SB203580), JNK inhibitor (SP600125), and ERK inhibitor (PD98059). As shown in Fig. 4A and B, p38 inhibitor attenuated cell viability on MMT assay and DAPI staining. P38 inhibitor also attenuated activation of apoptosis-related enzyme (caspase-3) activated by AIMs (Fig. 4C). However, the JNK inhibitor and ERK inhibitor did not show a statistical significance in cell viability assay (data not shown). These findings suggest that AIM-induced apoptosis is mediated at least in part by the activation of p38-MAPK.

AIMs augment apoptosis in part by suppression of XIAP through suppression of phosphorylation of Akt. It has been reported that heat shock protein 90 (Hsp90), which should contribute to the stabilization of p-Akt, is over-expressed in colon cancer (12), and thus Akt/PI3k pathway is activated in colon cancer cells. P-Akt is also an important factor regulating the expression of several pro-apoptotic or anti-apoptotic proteins (13-16). To investigate the possible involvement of PI3k/Akt pathway in AIM-induced apoptosis, we assessed

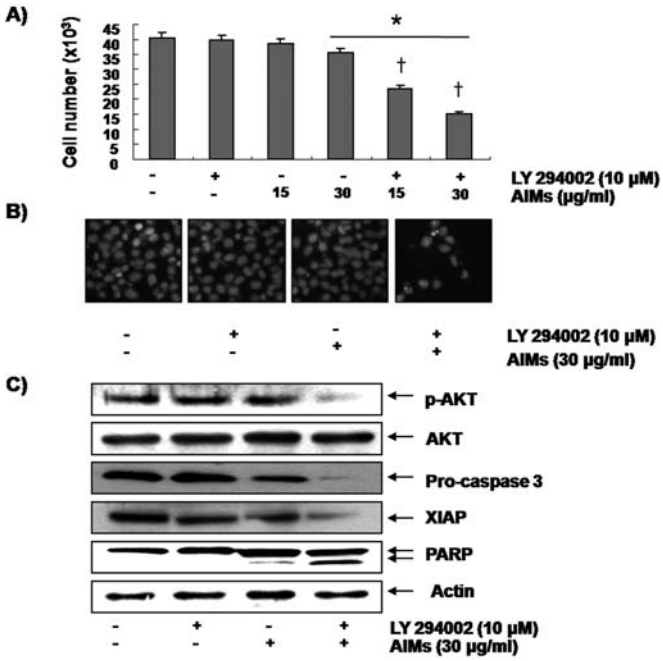

Figure 5. Effects of AIMs on apoptosis by regulating p-Akt in HCT-116 cells. HCT-116 cells were seeded at the density of $5x10^4$ cells per ml. The cells were treated with LY294002 (10 μM) 1 h before treatment with indicated concentrations of AIMs for 48 h. (A) Cell proliferation was assessed by MTT assay. The data are shown as mean ± SD of three independent experiments. *P<0.05 between the treated and the untreated control group. (B) Nuclei stained with DAPI solution were observed under a fluorescent microscope using a blue filter (C). Equal amounts of cell lysate (30 μg) were resolved by SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the indicated antibodies and detected by the ECL detection system. The results are from a representative experiment of at least three independent experiments that showed similar patterns.

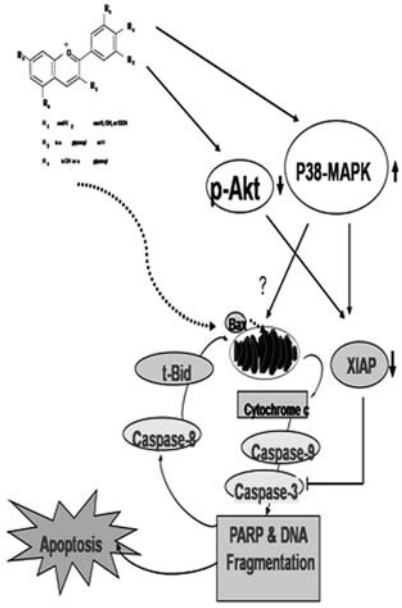

Figure 6. Schematic representation on apoptotic effects of AIMs on HCT-116 human colon cancer cells. AIMs suppress expression of XIAP through inhibition p-Akt, and then this inhibition causes sequential suppression of caspase-3. In addition, AIMs activate p38-MAPK and then this activation causes sequential activation of caspases probably through mitochondrial dysfunction. Taken together, this study suggested that Akt and p38-MAPK are protein kinases that regulate the apoptotic effect of AIMs in human colon cancer HCT-116 cells.

the changes in phophorylation of Akt induced by AIMs. Western blot analysis revealed that Akt was constitutively active in HCT-116 cells and the Akt activation was attenuated by AIMs treatment in time-dependent manner. To confirm the involvement of PI3k/Akt pathway in AIM-induced apoptosis, we performed cell viability assay using LY294002, a representative PI3k/Akt inhibitor. As shown in Fig. 5A and B, LY294002 accentuated cytotoxicity of AIMs on MMT assay and DAPI staining. To confirm this finding at the molecular level, we performed Western blot analysis for apoptosis-related enzyme and p-Akt. As shown in Fig. 5C, the suppression of Akt phosphorylation was related to activation of apoptosis-related enzyme (PARP and caspase-3). XIAP is an important anti-apoptotic protein which is known to be regulated by Akt pathway. We found AIMs inhibited the expression of XIAP in a dose-dependent manner in HCT-116 cells (Fig. 2) and 10 μM LY294002 had synergistic effects with 30 μg/ml or lower concentration of AIMs (Fig. 4). Akt phosphorylation was also positively related to expression of XIAP (Fig. 4C). Taken together, these findings suggest that AIM-induced apoptosis in HCT116 cells could be mediated in part by suppression of XIAP through suppression of PI3K/Akt pathway.

Discussion

This study is designed to determine whether AIMs have anti-cancer activities in human colon cancer cells and further to investigate the underlying mechanisms. In our ongoing efforts to determine the mechanism that mediate the effects of AIMs, we studied the involvement of MAPK and PI3K/AKT pathway. In this present study, we found that AIMs induced apoptosis in human colon cancer HCT-116 cells partly through activation of p38-MAPK and suppression of p-Akt. Our data provide evidence that AIMs may be a good potential anti-cancer agent, modulating p38-MAPK and p-Akt and inducing apoptosis because these are very important molecules in signaling for cancer cell survival and apoptosis. In addition, AIMs augmented the apoptosis by suppression of the anti-apoptotic proteins (XIAP, cIAP-1 and cIAP-2).

Recent evidence suggests that various anthocyanins should exert remarkable cytotoxic effects on malignant cells (3,4,6). These results indicated that apoptosis could be triggered by the activation of a set of caspases and their activation played important roles during apoptosis. In apoptotic processes, caspase-3 has been shown to play a pivotal role in the terminal and execution phase of apoptosis induced by diverse stimuli (17,18). We examined whether the caspase-3 protease is involved in AIM-induced cell death response. Furthermore, this study demonstrated that the activation of apoptosis-related enzyme (caspase-3) by AIMs might be related to the suppression of anti-apoptotic proteins. However, the mechanism of apoptosis triggered by anthocyanins was not fully understood whereas the apoptotic activity of anthocyanin is evident. MAP kinases are important factors in apoptosis signaling. A previous study demonstrated that anthocyanins induced apoptosis in hepatocellular carcinoma cells HepG2 through activation of JNK. In HepG2 cells, anthocyanin induced the production of ROS (4). ROS produced by chemical agents may induce JNK activation and subsequent apoptosis (19). However, AIMs did not induce

JNK activation as well as ROS production even in hepatocellular carcinoma Hep3B cells (data not shown). In addition, the role of JNK is not fully understood in apoptosis in cancer because JNK has pro- or anti-apoptotic functions, depending on cell type, nature of the death stimulus, duration of its activation and the activity of other signaling pathways (20).

In this study, AIMs activated p38-MAPK, which resulted in apoptosis in this study. Previous studies demonstrated that p38-MAPK was important in regulating apoptosis by leading to mitochondrial dysfunction which might be related to BAX phophorylation/activation and subsequent translocation to mitochondria (21,22). We previously demonstrated that AIMs induced mitochondrial dysfunction (7). Consistent with these data, we observed here that AIMs induced activation of p38-MAPK and the inhibitor of p38-MAPK suppressed AIM-induced apoptosis in HCT-116 cells.

p-Akt is an important factor regulating the expression of several pro-apoptotic or anti-apoptotic proteins (13-16). XIAP has been shown to interact with and inhibit caspase-3 activity (23). Recently it has been reported that the expression of p-AKT and XIAP are closely related and Akt is constitutively active in HCT-116 cells (12,14). In this study, we found AIMs could induce caspase-3 activity and the subsequent cleavage of caspase-3 substrate PARP by suppression of XIAP probably through suppression of p-Akt. To our knowledge, this is the first report suggesting that anthocyanin-induced apoptosis is associated with inhibition of XIAP through inhibiting p-Akt.

We found that AIMs inhibited the XIAP, cIAP-1, and cIAP-2 in HCT-116 cells, which are anti-apoptotic proteins and related to drug resistance. These proteins can be regulated by p-Akt through IKK and subsequent activation of NF-κB (12). Therefore, how these anti-apoptotic proteins are regulated by AIMs in HCT-116 warrants further investigation on NF-κB.

Data presented here indicate that AIMs in the concentration of 60 μg/ml has apoptotic activity in human colon cancer HCT-116 cells. Previous studies on the anti-tumor effect of anthocyanins demonstrated that the concentration of AIMs that induce apoptosis *in vitro* may be attainable *in vivo* in xenograft models without potential side effects (3,6).

In conclusion, AIMs induced apoptosis in human colon cancer HCT-116 cells. Akt and p38-MAPK are important protein kinases that regulate the apoptotic effect of AIMs on human colon cancer HCT-116 cells (Fig. 6). AIM-induced apoptosis in HCT116 cells was also mediated at least in part by suppression of XIAP through suppression of p-Akt which had a positive relationship with XIAP. This study provides evidence that AIMs might be a potential anti-cancer agent.

Acknowledgments

This study was supported by a grant from the National R&D Program for Cancer Control, Ministry for Health, Welfare & Family Affairs, Republic of Korea (0820050).

References

1. Liu BL, Zhang X, Zhang W, *et al*: New enlightenment of French Paradox: resveratrol's potential for cancer chemoprevention and anti-cancer therapy. Cancer Biol Ther 6: 1833-1836, 2007.
2. Hatcher H, Planalp R, Cho J, *et al*: Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci 65: 1631-1652, 2008.
3. Ding M, Feng R, Wang SY, *et al*: Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity. J Biol Chem 281: 17359-17368, 2006.
4. Yeh CT and Yen GC: Induction of apoptosis by the anthocyanidins through regulation of Bcl-2 gene and activation of c-Jun N-terminal kinase cascade in hepatoma cells. J Agric Food Chem 53: 1740-1749, 2005.
5. Favot L, Martin S, Keravis T, *et al*: Involvement of cyclin-dependent pathway in the inhibitory effect of delphinidin on angiogenesis. Cardiovasc Res 59: 479-487, 2003.
6. Hafeez BB, Siddiqui IA, Asim M, *et al*: A dietary anthocyanidin delphinidin induces apoptosis of human prostate cancer PC3 cells *in vitro* and *in vivo*: involvement of nuclear factor-kappaB signaling. Cancer Res 68: 8564-8572, 2008.
7. Lee SH, Park SM, Park JH, *et al*: Induction of apoptosis in human leukemia U937 cells by anthocyanins through down-regulation of Bcl-2 and activation of caspases. Int J Oncol 34: 1077-1083, 2009.
8. Debatin KM: Apoptosis pathways in cancer and cancer therapy. Cancer Immunol Immunother 53: 153-159, 2004.
9. Robinson MJ and Cobb MH: Mitogen-activated protein kinase pathways. Curr Opin Cell Biol 9: 180-186, 1997.
10. Nguyen KT, Wang WJ, Chan JL, *et al*: Differential requirements of the MAP kinase and PI3 kinase signaling pathways in Src-versus insulin and IGF-1 receptors-induced growth and transformation of rat intestinal epithelial cells. Oncogene 19: 5385-5397, 2000.
11. Lee JT Jr and McCubrey JA: The Raf/MEK/ERK signal transduction cascade as a target for chemotherapeutic intervention in leukemia. Leukemia 16: 486-507, 2002.
12. Park KA, Byun HS, Won M, *et al*: Sustained activation of protein kinase C downregulates nuclear factor-kappaB signaling by dissociation of IKK-gamma and Hsp90 complex in human colonic epithelial cells. Carcinogenesis 28: 71-80, 2007.
13. Qian J, Zou Y, Rahman JS, *et al*: Synergy between phosphatidylinositol 3-kinase/Akt pathway and Bcl-xL in the control of apoptosis in adenocarcinoma cells of the lung. Mol Cancer Ther 8: 101-109, 2009.
14. Srinivasan S, Koduru S, Kumar R, *et al*: Diosgenin targets Akt-mediated prosurvival signaling in human breast cancer cells. Int J Cancer 125: 961-967, 2009.
15. Wang X, Chen W, Zeng W, *et al*: Akt-mediated eminent expression of c-FLIP and Mcl-1 confers acquired resistance to TRAIL-induced cytotoxicity to lung cancer cells. Mol Cancer Ther 7: 1156-1163, 2008.
16. Oh JH, Lee TJ, Kim SH, *et al*: Induction of apoptosis by withaferin A in human leukemia U937 cells through down-regulation of Akt phosphorylation. Apoptosis 13: 1494-1504, 2008.
17. Ashkenazi A: Targeting death and decoy receptors of the tumour-necrosis factor superfamily. Nat Rev Cancer 2: 420-430, 2002.
18. Thornberry NA and Lazebnik Y: Caspases: enemies within. Science 281: 1312-1316, 1998.
19. Appierto V, Tiberio P, Villani MG, *et al*: PLAB induction in fenretinide-induced apoptosis of ovarian cancer cells occurs via a ROS-dependent mechanism involving ER stress and JNK activation. Carcinogenesis 30: 824-831, 2009.
20. Liu J and Lin A: Role of JNK activation in apoptosis: a double-edged sword. Cell Res 15: 36-42, 2005.
21. Park MT, Kang YH, Park IC, *et al*: Combination treatment with arsenic trioxide and phytosphingosine enhances apoptotic cell death in arsenic trioxide-resistant cancer cells. Mol Cancer Ther 6: 82-92, 2007.
22. Seo SK, Lee HC, Woo SH, *et al*: Sulindac-derived reactive oxygen species induce apoptosis of human multiple myeloma cells via p38 mitogen activated protein kinase-induced mitochondrial dysfunction. Apoptosis 12: 195-209, 2007.
23. Takahashi R, Deveraux Q, Tamm I, *et al*: A single BIR domain of XIAP sufficient for inhibiting caspases. J Biol Chem 273: 7787-7790, 1998.