Magnolol-Induced Apoptosis in HCT-116 Colon Cancer Cells Is Associated with the AMP-Activated Protein Kinase Signaling Pathway

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Colon cancer is the third most common malignancy around the world. Surgery, chemotherapy, and radiotherapy are generally used to treat colon cancer, but no effective therapy for advanced colon carcinoma is available. Therefore, there is a need to identify other therapeutic agents against this disease. Magnolol, a hydroxylated biphenyl compound present in Magnolia officinalis, exerts anticancer potential and low toxicity. Emerging evidence has suggested that activation of AMP-activated protein kinase (AMPK), a potential cancer therapeutic target, is involved in apoptosis in colon cancer cells. However, the effects of magnolol on human colon cancer through activation of AMPK remain unexplored. In this study, we explored whether magnolol exerts an antiproliferative effect and induces apoptosis in HCT-116 human colon cancer cells. Magnolol displayed several apoptotic features, including propidium iodide labeling, DNA fragmentation, and caspase-3 and poly(ADP-ribose) polymerase cleavages. We showed that magnolol induced the phosphorylation of AMPK in dose- and time-dependent manners. The selective AMPK inhibitor compound C abrogated the effect of magnolol on AMPK activation, suppression of proliferation, and caspase-3 cleavage. Magnolol downregulated expression of the antiapoptotic protein Bcl2, upregulated expression of pro-apoptotic protein p53 and Bax, and caused the release of mitochondrial cytochrome c. Magnolol-induced p53 and Bcl2 expression was abolished in the presence of compound C. Magnolol inhibited migration and invasion of HCT-116 cells through AMPK activation. These findings demonstrate that magnolol mediates the anticancer effects of magnolol through apoptosis in HCT-116 cells.

Key words  AMP-activated protein kinase; magnolol; colon cancer

Colon cancer is the third most common malignancy around the world.1) Annually, over 945000 people develop colorectal cancer, and about 492000 patients die from the disease.2) Current treatment for colon cancer mainly includes surgery and chemotherapy along with radiotherapy.3) Despite advances in clinical treatment, the prognosis for patients with colon cancer, particularly those with metastasis, remains unclear. Therefore, there is an urgent need to develop new therapeutic targets and strategies, both of which can be realized through an increasing understanding of the molecular mechanisms governing colon progression.1)

AMP-activated protein kinase (AMPK) is a member of a family of serine-threonine protein kinases that are found in all eukaryotes.4) AMPK is composed of three subunits (α, β, and γ). The AMPK β-subunit is a scaffold for assembly of the trimeric protein, with the α-subunit responsible for kinase activity and the γ-subunit modulating catalytic activity of the α-subunit.5) Activation of AMPK is thought to require phosphorylation of AMPKα Thr172.6) This activated AMPK downregulates ATP-consuming (anabolic) pathways, and up-regulates ATP-generating (catabolic) pathways to maintain energy homeostasis in the cell.7,8) The ability of AMPK to directly sense the energy status of the cell makes it an attractive target molecule for ensuring that cell division proceeds when cells have sufficient metabolic resources to support cell proliferation.9) Several reports have demonstrated that activating AMPK leads to the induction of apoptosis in many human cancer cells including colon cancers.5,10) This activity occurs through various mechanisms, including activation of the p38 mitogen activated protein kinase (MAPK) pathway,1) increased expression of the cell cycle regulatory protein p21WAF1/Cip1,12) inhibition of nuclear factor-κB activity,13) and inhibiting the Akt-mammalian target of rapamycin (mTOR) pathway.14) Additionally, activation of AMPK by phytochemicals, such as quercetin and 24-hydroxyursolic acid, is involved in apoptosis in colon cancer cell lines.15,16) Therefore, AMPK activated by naturally occurring compounds may play a causal role in regulating cell survival and growth for colorectal cancer therapy.

Many plant-derived agents have been recognized as potential alternative agents for cancer treatment over the last few years. There is growing interest in phytochemicals with anticancer potential and low toxicity.17) Among them, magnolol (Fig. 1A) is a hydroxylated biphenyl compound isolated from the roots and stem bark of Magnolia officinalis (Magnoliaceae). It is commonly used to treat various ailments due to its muscle relaxant, antioxidative, antiatherosclerosis, antiinflammatory, antiagastic ulcer, antiallergic, antibacterial, antithrombotic, and steroidogenesis-stimulating activities.18) Attention has been paid to its anticancer activity in several cancer cell lines, including colon cancer, hepatoma, leukemia, fibrosarcoma, melanoma, squamous carcinoma, and thyroid carcinoma.18,19) Several researchers have shown that magnolol inhibits proliferation and induces apoptosis in cancer cells by upregulating p21WAF1/Cip1,20) inhibiting DNA synthesis,21) activating the epidermal growth factor receptor (EGFR)/phosphoinositide 3-kinase (PI3K)/Akt signaling pathway,22) as well as Ras/Raf-1/Erk actions.23) However, it remains to be determined whether...
apoptotic cell death is associated with the AMPK signaling pathway in HCT-116 colon cancer cells treated with magnolol. In the present study, we examined the effect of magnolol on HCT-116 colon cancer cell survival and apoptosis, migration, and invasion via AMPK activation.

MATERIALS AND METHODS

Materials RPMI-1640, fetal bovine serum (FBS) and penicillin/streptomycin were obtained from GIBCO/BRL Life Technologies (Grand Island, NY, U.S.A.). Antibodies to caspase-3, poly(ADP-ribose) polymerase (PARP), P-AMPK, AMPK, P-mTOR, mTOR, P-p70S6K, p70S6K, p53 were purchased from Cell Signaling Technology, Inc. (Danvers, MA, U.S.A.), and Bax, Bcl2, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from SantaCruz (Santa Cruz, CA, U.S.A.). Horseradish peroxidase-conjugated anti-mouse and anti-rabbit antibodies were from Transduction Lab (Lexington, KY, U.S.A.). Super Signal® West Pico Chemiluminescent substrate was purchased from PIERCE (Rockford, IL, U.S.A.). Cell Counting Kit-8 was from Dojindo Laboratories (Kumamoto, Japan). PromoKine Mitochondrial Apoptosis Staining Kit was purchased from PromoKine® (Heidelberg, Germany). Magnolol, z-VAD-fmk, compound C (AMPK inhibitor), and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Cell Culture Human colon carcinoma HCT-116 cells were purchased from American Type Culture Collection (ATCC) (Manassas, VA, U.S.A.). HCT-116 cells were grown in RPMI-1640 media supplemented with 10% (v/v) FBS, penicillin (100 U/mL)-streptomycin (100 µg/mL) at 37°C in a humidified CO₂ (5%)-controlled incubator.

Cell Viability Assay Cells were seeded at 5×10^3 cells/mL in 96-well microplates and allowed to attach for 24 h. Magnolol was added to the medium at various concentrations up to 50 µM and different duration. After treatment, cell cytotoxicity and/or proliferation was assessed by Cell Counting Kit-8 (CCK-8). Briefly, highly water-soluble tetrazolium salt, WST-8[2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium, monosodium salt], produced an orange colored water-soluble product, formazan. The amount of formazan dye generated by dehydrogenases in cells was directly proportional to the number of living cells. CCK-8 (10 µL) was added to each well and incubated for 3 h at 37°C, then cell proliferation and cytotoxicity were assessed by measuring the absorbance at 450 nm using microplate reader. Three replicated wells were used for each experimental condition.

Western Blotting Cells were incubated with 10 to 50 µM magnolol for 24 h, and washed twice in cold phosphate buffered saline (PBS). Cells were lysed with lysis buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1% TritonX-100, 0.5% NP-40, 1 mM propidium iodide (PI), 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF)) and placed on ice for 1 h with occasional vortexing. Centrifugation followed at 10000rpm for

Fig. 1. Magnolol Exerts an Antiproliferative Effect and Induces Apoptosis

(A) Chemical structure of magnolol. (B, C) Growth inhibitory activity of magnolol in time-dependent and dose-dependent manners. Cells were treated at the indicated times and concentrations of magnolol. Cell viability (%) was determined by the CCK-8 proliferation assay. Values indicate the mean±standard deviation (S.D.) in triplicate tests. *p<0.05, **p<0.005 compared with untreated cells. (D) Effect of magnolol on apoptosis. Flow cytometric analysis of propidium iodide accumulation after exposing cells to magnolol for 24 and 48 h. Values are expressed as mean±S.D. of three experiments.
10 min and each cell lysates (50 µg) were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane. Blots were blocked with 5% skim milk in PBS containing 0.05% Tween-20 for 1 h at 25°C, then incubated with primary antibodies, followed by incubation with anti-rabbit or anti-mouse horseradish peroxidase-conjugated immunoglobulin G (IgG) and visualized with enhanced chemiluminescence.

**Flow Cytometric Analysis** Cells with 1×10⁵ cells/mL were suspended in 300 µL PBS and 700 µL EtOH was added. Cells were incubated at 4°C for 1 h and washed with PBS, and suspended in 250 µL of 1.12% sodium citrate buffer (pH 8.4) together with 12.5 µg of RNase. Incubation was continued at 37°C for 30 min. The cellular DNA was stained by applying 250 µL of propidium iodide (50 µg/mL) for 30 min at room temperature. The stained cells were analyzed by fluorescent activated cell sorting (FACS) on the BD FACSCanto™II flow cytometer using FACSCanto™II System Software for the percentage of apoptotic cells.

**DNA Fragmentation** Cells were plated at 2×10⁵ cells/mL in 12-well plates and added magnolol for 24 h. Both attached and detached cells were collected and lysed in 100 mM Tris (pH 8.0), 20 mM EDTA, 0.8% N-laurylsarcosine sodium salt on ice. The lysates were incubated with 0.2 mg/mL RNase A for 1 h at 37°C, then with 4 mg/mL Proteinase K for 0 h at 50°C. The DNA was precipitated by adding an equal volume of isopropanol, and loaded on to 2% agarose gels and electro-phoresed in 1×TBE buffer for 3 h. Gels were photographed under UV light.

**Wound Migration Assay** Cells were seeded in 6-well plates and incubated for 18 h in serum free RPMI-1640. The cellular monolayer was wounded with a sterile 10 µL pipette tip and washed with serum free RPMI-1640 to remove detached cells from the plates. The cells were incubated in the presence or absence of magnolol for 48 h in RPMI-1640 containing 10% FBS. The medium was replaced with phosphate-buffered saline, and the cells were photographed using a phase-contrast microscope.

**Transwell Invasion Assay** Cells were seeded in 6-well plates and incubated for 18 h in serum free RPMI-1640. The cellular monolayer was wounded with a sterile 10 µL-pipette tip and washed with serum free RPMI-1640 to remove detached cells from the plates. The cells were incubated in the presence or absence of magnolol for 48 h in RPMI-1640 containing 10% FBS. The medium was replaced with phosphate-buffered saline, and the cells were photographed using a phase-contrast microscope.

**RESULTS**

**Magnolol Exerts an Anti-proliferative Effect and Induces Apoptosis in HCT-116 Cells** To test the chemopreventive effects of magnolol on HCT-116 cell proliferation, we treated cells with various concentrations of magnolol (0–50 µM) for 24 and 48 h and analyzed viable cells using the CCK-8 assay. Magnolol induced cell death in dose-dependent (Fig. 1B) and time-dependent manners (Fig. 1C). Treatment with 50 µM magnolol for 24 h resulted in significant decreases in cell viability compared to those in the control group (p<0.005). Treatment with 50 µM magnolol slightly reduced cell viability according to culture time, with 75.3% of the cells surviving after 24 h and 81.7% of the cells surviving after 48 h. To further understand whether magnolol-induced cell death was mediated by apoptosis, we evaluated apoptotic cell death using propidium iodide staining. Magnolol treatment at 50 µM induced apoptosis in 76.1% of the cells after 24 h (Fig. 1D), suggesting that magnolol inhibits cell proliferation and induces apoptosis in HCT-116 colon cancer cells.

**Magnolol Displays Apoptotic Features by Activating Caspase** DNA purified from cells treated with magnolol for 24 h was subjected to agarose gel electrophoresis to assess DNA fragmentation. DNA fragmentation was observed following magnolol (50 µM) treatment (Fig. 2A). To investigate the biological features that lead to apoptosis induced by magnolol, we examined the effect of caspase-3 and poly(ADP-ribose) polymerase (PARP). Cells were treated with magnolol, and Western blotting was performed. Compatible with the DNA fragmentation result, a reduction in the inactive forms of caspase-3 and an increase in the active forms of PARP were observed following magnolol (50 µM) treatment, indicating their activation (Fig. 2B). Pretreatment of cells for 1 h with 100 µM z-VAD-FMK, a pan-caspase inhibitor, completely abrogated PARP cleavage (Fig. 2C). These results indicate that magnolol induces caspase-dependent apoptotic activity.

**Phosphorylation of AMPK Is Essential for Magnolol-Induced Antiproliferation and Apoptosis** To investigate whether AMPK phosphorylation was responsible for magnolol-induced apoptosis, we treated HCT-116 cells with various concentrations of magnolol (0–50 µM) for 24 h and analyzed apoptosis-related proteins by Western blotting. AMPK phosphorylation at Thr172 increased significantly in dose- and time-dependent manners (Fig. 3A). AMPK activation was associated with decreased mammalian target of rapamycin (mTOR) and 70 kDa ribosomal protein S6 kinase (p70S6K) phosphorylation (Fig. 3A). The selective AMPK inhibitor compound C reversed the magnolol-induced AMPK phosphorylation (Fig. 3B), cell growth inhibition (Fig. 3C), and apoptosis (Fig. 3D) in HCT-116 cells. This result indicates that magnolol-induced antiproliferation and apoptosis are mediated by AMPK activation.

**Magnolol Downregulates the Expression of the Anti-apoptotic Protein Bcl2, Upregulates the Expression of the Pro-apoptotic Proteins p53 and Bax** To evaluate the increase in apoptosis-related proteins in magnolol-induced HCT-116 cells, the expression of the positive mediators of apoptosis, p53 and Bax, as well as a negative regulator, Bcl2, was analyzed by Western blotting. As a result, magnolol induced increases in p53 and Bax levels but decreased Bcl2 levels (Fig. 4A). To investigate the release of mitochondrial protein into
cytosol, the cytosolic and mitochondrial fractions were separated and the translocation of cytochrome c into the cytosol was monitored. Treatment of cells with magnolol resulted in increased translocation of cytochrome c into cytoplasm (Fig. 4B). Next, we examined whether magnolol-induced AMPK activation is associated with the expression of apoptotic proteins. p53 was significantly suppressed in the presence of compound C. In contrast, magnolol-induced decreases in Bcl2 protein expression were reversed by treatment with compound C (Fig. 4C), suggesting that magnolol induces mitochondrial apoptosis via AMPK activation in HCT-116 cells.

Magnolol Inhibits Cell Migration and Invasion
To evaluate the antimetastatic activity of magnolol, we investigated the effect of magnolol on HCT-116 cell migration in a wound migration assay. The migration distance between the leading edge and the wound line was compared between magnolol-treated and untreated cells. Cellular motility was controlled by 50 µM magnolol, and compound C reversed this activity (Fig. 5A). Next, HCT-116 cells were treated with or without magnolol for 48 h to determine whether magnolol also inhibits cell invasion. Treatment of cells with 50 µM magnolol decreased invasive activity. Additionally, the magnolol-induced decrease in Matrigel invasion was reversed by compound C treatment (Fig. 5B). Collectively, these results show that magnolol-induced AMPK activation inhibited the migration and invasion of HCT-116 cells.

Fig. 2. Magnolol Displays Apoptotic Features by Activating Caspase
(A) Fragmentation of genomic DNA by magnolol. Cells were treated for 24 h with the indicated concentrations of magnolol. Fragmented DNA was extracted and analyzed on 2% agarose gel. Camptothecin (10 µM) was used as a positive control. (B) Effect of magnolol-induced apoptosis mediated by caspase activation. Cells were induced with various concentrations of magnolol for 24 h. Cleavage of caspase-3 and poly(ADP-ribose) polymerase (PARP) were measured by Western blot analysis. (C) The pan-caspase inhibitor z-VAD-fmk (100 µM) was pre-treated and added with magnolol (50 µM) in cells, and PARP cleavage was measured by Western blot analysis.

Fig. 3. Phosphorylation of AMP-Activated Protein Kinase (AMPK) Is Essential for Magnolol-Induced Antiproliferation and Apoptosis
(A) Magnolol activated AMPK. After treatment with various concentrations of magnolol (0–50 µM), cells were lysed and the levels of phosphorylated AMPK (Thr172), AMPK, phosphorylated mTOR (Ser2448), mTOR, phospho-p70S6K (Thr389), and p70S6K were determined by Western blot analysis. (B) Magnolol-induced AMPK activation was reversed by compound C (AMPK inhibitor). Cells were treated with magnolol (50 µM) for 24h and then with compound C (10 µM) before being stimulated by magnolol. Phospho-AMPK was determined by Western blot analysis. (C) After pre-exposure of compound C (10 µM), cells were treated for 24 h with magnolol (50 µM), and cell viability was determined by the CCK-8 assay. Data are expressed as percent relative to control. *p<0.05. (D) After pretreatment of compound C (10 µM), cells were treated for 24 h with magnolol (50 µM), and the caspase-3 and PARP cleavage was determined by Western blot analysis.
DISCUSSION

This study examined the effect of magnolol (5,5′-diallyl-2,2′-biphenylidiol, C_{20}H_{30}O_{2}) on the cytotoxic mechanism of HCT-116 colon cancer cells using activation of AMPK, an intracellular energy balancing system. As a potential complementary and alternative medicine, magnolol has been used with other phenolic compounds in Chinese medicine for thousands of years and has a long and safe record of use.18) Because magnolol prevents the promotion of mouse skin tumors in an in vivo two-stage carcinogenesis model,24) the mechanisms responsible for magnolol-induced apoptosis and antiproliferation have been reported for various cancers.18,19) Several reports have demonstrated that magnolol treatment does not affect the viability of normal cells such as human prostate epithelial cells and normal blood cells.18,25) We also observed that magnolol showed no toxicity (<50 µM) in fibroblasts (NIH-3T3) (data not shown). Magnolol is probably a safe molecule for potential clinical use in cancer therapy.

Our results indicate that magnolol induced antiproliferation and apoptosis effects, as determined by the CCK-8 assay, flow cytometry analysis, and Western blotting (Figs. 1, 2). Many researchers have shown the various anticancer activities of magnolol. For example, magnolol suppresses proliferation of cultured human colon cancer cells by inhibiting DNA synthesis21); magnolol-induced apoptotic death is mediated by blocking the EGFR-P13K-Akt pathway and subsequent activation of the Bad-Bax-cytochrome c-caspase pathway in human prostate cancer cells26); magnolol decreases cell number in a cultured human glioblastoma (U373) cancer cell line20); magnolol (100 µM) induces apoptosis in cultured human hepatoma (Hep G2) and colon cancer (COLO 205) cell lines.26) More recently, magnolol-induced apoptosis in human gastric adenocarcinoma (SGC-7901) cells by mitochondrial and P13K-Akt signaling pathways,27) and magnolol has anticarcinogenic effects against UVB-induced skin tumor development in SKH-1 mice and a possible role in apoptosis during skin tumor development has been determined.28) However, some studies claim that magnolol-induced cell death occurs via autophagy not apoptosis,29) and that magnolol increases extracellular-signal-regulated kinase (ERK) activity and prevent cells from entering apoptosis.30) These different views have led us to examine the mechanism of magnolol-induced cell death. In this study, we clearly demonstrate that magnolol induced apoptotic death in HCT-116 cells.

AMPK is an energy-sensing/signaling intracellular protein that is inactive unless it has been phosphorylated by an upstream kinase at a threonine residue (Thr-172) in response to cellular stressors that deplete cellular energy levels and increase the AMP/ATP ratio.31) In the past few years, scientific consensus about the relationship between exercise and cancer has been fundamentally changed. Randomized clinical trials have suggested that the incidence of recurrence of colon and breast cancer is reduced in patients who undergo long-term exercise.32) Although the effect of exercise on reducing the incidence and/or recurrence of such cancers is likely to be complex and involve several factors, such as reducing whole body fat content, it would also be expected that depleting whole-body ATP levels in tissues induced by exercise would raise AMPK activity, which would potentially inhibit cell growth.33) As previously stated, AMPK is involved in cellular homeostasis and it has been recently demonstrated that AMPK is a pivot point between cell survival and apoptosis,34) which may be a potential target for human cancers. For example, 5-amino-1-β-d-ribofuranosyl-imidazole-4-carboxamide (AICAR), one of the pharmacological AMPK activators, has been used widely to suppress cancer cell growth.35) Some natural compounds, such as resveratrol, quercetin, and 24-hydroxyursolic acid induce apoptosis by activating AMPK.36) Our findings demonstrated that magnolol is able to increase AMPK activity (Fig. 3A) and that the AMPK phosphorylation, suppression of cell growth, and apoptosis induced by magnolol was inhibited by compound C (Figs. 3B–D), suggesting that magnolol-induced AMPK activation may be responsible for magnolol-induced apoptosis and that it serves as a positive regulator of apoptosis. AMPK serves as a negative regulator of mTOR via direct phosphorylation of both TSC2 tumor suppressor and Raptor.37) Blocking mTOR by AMPK is vital to determine apoptosis or growth inhibition in cells.38) The p70S6K protein, which is directly downstream of mTOR, is a mitogen activated Ser/Thr protein kinase.39) The mTOR-p70S6K signaling pathway has a major role in cell growth, and aberrant modulation of this pathway can contribute to cancer.40) In this study, mTOR-p70S6K activity was downregulated by magnolol (Fig. 3A). Our observations support that AMPK activation by magnolol negatively regulates mTOR-p70S6K signals for inhibiting growth or inducing apoptosis in colon cancer cells.

One of the major apoptotic processes in cancer cells involves the mitochondrial pathway. This cell death pathway is...
mediated by the Bcl2 family proteins, a group of antiapoptotic proteins that regulate the passage of small molecules such as cytochrome c, which activate the caspase cascade through the mitochondrial transition pore. Moreover, p53-mediated apoptosis is associated with Bcl2 and Bax, a pro-apoptotic member of the Bcl2 family. To examine the involvement of the mitochondrial pathway in magnolol-induced apoptotic death, we examined the levels of Bcl2, Bax, and p53. Our data indicate that there was an increase in p53 and Bax expression and a decrease in Bcl2 expression, which resulted in a reduction in mitochondrial membrane potential and increased cytochrome c release into cytosol (Figs. 4A, B). One of the main constitu-

tions of the Bcl2 family is the regulation of the mitochondrial transition pore. Moreover, p53-mediated apoptosis is associated with Bcl2 and Bax, a pro-apoptotic member of the Bcl2 family. To examine the involvement of the mitochondrial pathway in magnolol-induced apoptotic death, we examined the levels of Bcl2, Bax, and p53. Our data indicate that there was an increase in p53 and Bax expression and a decrease in Bcl2 expression, which resulted in a reduction in mitochondrial membrane potential and increased cytochrome c release into cytosol (Figs. 4A, B). One of the main constituents, 20(S)-ginsenoside Rg3, found in red ginseng activates apoptosis, and this activity is completely abolished in the presence of compound C or siRNA for AMPK in HT-29 cells. We also observed that the magnolol-induced increase in p53 and cleaved caspase-3 protein expression was significantly decreased by pre-treatment with compound C (Figs. 3D, 4C). In contrast, the magnolol-induced decrease in Bcl2 protein expression was reversed by pre-treatment with compound C (Fig. 4C). Our findings demonstrate that magnolol-induced apoptosis occurred via AMPK activation in HCT-116 cells.

Tumor metastasis, a major cause of morbidity in patients with cancer, is a multi-step process that includes migration, invasion, adhesion, proliferation, and angiogenesis. AMPK activity is involved in cell–cell interaction and inhibits the metastatic potential of several cancers. Magnolol suppresses metastasis by inhibiting invasion, migration, and matrix metalloproteinase activities in PC-3 and HT1080 cells. The antitumor effects of magnolol in an experimental liver and spleen metastasis model using LS174T-YML25 lymphoma and in an experimental and spontaneous lung metastasis model of B16-BL6 melanoma, have been evaluated. In this study, we determined the antitumor effect of magnolol via AMPK activation using wound migration and invasion assays. Our findings demonstrated that magnolol inhibited migration distance that was reversed by pretreatment with compound C (Fig. 5A). Similar to its effects in the migration assay, magnolol inhibited invasion of HCT-116 cells, which was reversed by treatment with compound C (Fig. 5B). Therefore, the antimitastatic action of magnolol is thought to result from AMPK activation that inhibits tumor cell invasion.

In conclusion, we showed that magnolol is a potent cell growth inhibitor and induced apoptosis via AMPK activation in HCT-116 human colon cancer cells. Magnolol strongly induced AMPK activity, and thereby inhibited mTOR/p70S6K signaling pathway. The inhibition of growth and apoptotic protein expression (p53, Bax, Bcl2) was associated with AMPK activation. Magnolol-induced AMPK activation inhibited cancer cell migration and invasion. These findings demonstrate, for the first time, that AMPK mediates the anticancer effects of magnolol through apoptosis in HCT-116 cells.

Acknowledgements This study was financially supported by research fund of Chungnam National University in 2010.

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