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Gallotannin regulates apoptosis and COX-2 expression via Akt and p38kinase pathway in human lung cancer cell line, A549

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Gallotannin (GT) is derived from plant poly phenol and is associated with biological actions in a wide range of cells. In this study, we evaluated the effect of GT on apoptosis and cyclooxygenase-2 (COX-2) expression and attempted to shed light on the mechanism of action in A549 human lung carcinoma cells. We found that GT dramatically induced apoptosis as demonstrated by expression of p53 and active caspase-3 via western blot analysis and fragmented DNA as detected by DNA fragmentation and DAPI staining. We also observed that GT significantly causes COX-2 expression in a dose-dependent manner determined by western blot analysis. Phosphorylation of Akt and p38 was considerably increased by GT in A549 human lung carcinoma cells. Inhibition of Akt and p38kinase with LY294002 or SB203580 suppressed GT-induced apoptosis and COX-2 expression. Furthermore, we have shown that prevention of COX-2 with NS398 or indomethacin does not any effects on apoptosis induced by GT. Taken together, our present results suggest that GT regulates apoptosis and COX-2 expression through Akt and p38kinase pathway in A549, human lung carcinoma cells.

Keywords: gallotannin (GT); A549; apoptosis; cyclooxygenase-2 (COX-2); Akt; p38kinase

Introduction

Lung cancer is the leading cause of cancer-related deaths throughout the world (Petty et al. 2004). In spite of rapid progress in diagnostic and surgery techniques, lung cancer remains one of the most difficult metastatic diseases to treat (Jemal et al. 2010). More effective treatments and better understanding of molecular mechanisms are thus necessary for inhibition of lung tumorigenesis in clinical oncology. Recently, new cancer therapeutic drugs have been developed and some are in clinical trials. However, there is still a need for more effective treatment.

Apoptosis is a representative type of cell death with unrepaired DNA damage and prevents carcinogenesis. It is distinguished by an induction of pro-apoptotic protein such as p53 and activation of caspases (Meier and Vousden 2007). Among various caspases, caspase-3 is the most critical protease in the caspase-dependent apoptosis pathway, which is required for chromatin condensation and fragmentation (Janicke et al. 1998).

Because cyclooxygenase-2 (COX-2), an inflammatory enzyme, is frequently constitutively elevated in cancer cells, including lung cancers (Hosomi et al. 2000), prostate cancers (Kirschenbaum et al. 2001) and breast cancers (Soslow et al. 2000; Subbaramaiah et al. 2003), it has been designated as a critical key target for treatment (Castelao et al. 2003).

It has been established that overexpression of COX-2 promotes resistance to apoptosis in numerous cancer types, and it has been shown to stimulate proliferation, angiogenesis, and invasiveness (Riedl et al. 2004). Thus, high expression of COX-2 is related with aggressive tumor behavior, signifying COX-2 is an independent poor prognosis marker (Brabender et al. 2002).

However, the relationship between COX-2 expression and growth of cancer cells is more complicate than the foregoing results and this is indicated by reports that COX-2 may play pro-apoptotic roles (Zahner et al. 2002). Moreover, COX-2 expression by pharmacological reagents leads to inhibition of colon cancer cell growth (Williams et al. 2003). Also, several studies have reported that resveratrol, a pharmacological inducer of COX-2, is capable of inducing p53-dependent apoptosis by implicating of COX-2 expression in a variety of cell types such as breast, thyroid, head and neck squamous cancer cells. Therefore, COX-2 has both positive and negative functions on a case-by-case basis in cancer cells (Lin et al. 2008). Thus, COX-2 and p53 have an unsteady relationship that can be related to cell growth and apoptosis.

Gallotannin (GT), a plant polyphenol, is widely distributed in various plants, including fruits and foods (Niemetz and Gross 2005), and has shown biological activities including antiinflammatory (Kratz et al. 2008), antioxidant (Grundhofer et al. 2001), antibacterial (Manna et al. 1999), and antiherpetic (Fiuza et al. 2004). GT has been reported anticancer activity in various cancer cells, such as prostate, gastric, colon, breast, and cervical cancer (You et al. 2010).

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Recent reports have indicated that GT exerts anticancer effects by triggering apoptosis in human monocytic cell line U937 and human leukemia cells (Chen et al. 2011). Although these findings demonstrate the anticancer activity, the molecular mechanism by which GT leads to apoptosis of A549 cells is remains unclear. Several signaling pathways are involved in the expression of p53, a tumor-suppressor gene (Hsu et al. 2007). Several studies have found that stimulation of COX-2 also induces p53, which accelerates after post-translation modification in response to DNA damage (de Moraes et al. 2007) and an increase of p53 results in antiproliferative effects, including cell cycle arrest, DNA repair, or apoptosis (Sengupta and Harris 2005).

Previous studies on A549 cells have indicated that Akt and p38kinase pathways mediate apoptosis and inflammation (Jang 2009; Boo et al. 2011). These pathways also play an important role in the regulation of many cellular responses, such as cell differentiation, proliferation, and cell growth. Some pharmacological reagents have been shown to activate Akt and p38kinase, and their activation is implicated in apoptosis and inflammation (Wang et al. 2010).

Therefore, we investigated the effect of GT on apoptosis and COX-2 expression of A549 human lung cancer cell line focusing on Akt and p38kinase pathways.

Materials and methods

Cell culture and reagents

The A549 cell line was obtained from American Type Culture Collection (Rockville, MD, USA). A549 cells were maintained in RPMI-1640 medium (Invitrogen, Burlington, Canada) containing 10% fetal bovine serum (Invitrogen), 50 μg/ml streptomycin, and 50 units/ml penicillin. Cell cultures were grown at 37°C, in a humidified atmosphere of 5% CO_2 in SANYO incubator. GT, purchased from Sigma-Aldrich (St Louis, MO, USA), and LY294002, SB203580, NS398 and indomethacin obtained from Calbiochem (San Diego, CA, USA) were added to the culture.

Methyl thiazole tetrazolium assay

A549 cells (1×10^4 cells/well) were seeded in a 96-well plate and kept overnight for attachment. The next day the medium was replaced with a fresh medium with various concentrations of GT (25–100 μM), and cells were allowed to grow for 24 h. Four hours before completion of incubation, 10 μl of methyl thiazole tetrazolium (MTT) (10 mg/ml) was added to each well. After completing the incubation, 100 μl of solubilization buffer (10% sodium dodecyl sulfate [SDS] with 0.01 N HCl) was added to each well, and the cells were incubated overnight at 37°C. Color developed after the reaction was measured at 595 nm using a microplate reader.

Flow cytometry

Cells were cultured in 35 mm tissue culture dishes for 24 h. Cells were then treated with GT in the absence or presence of inhibitors (SB203580, LY294002, NS398, and indomethacin) for 24 h. Cells were collected and washed twice with phosphate buffer solution (PBS). After centrifugation (1000 rpm, 10 min), the cells were resuspended in cold 70% ethanol at 4°C for overnight. Ethanol-fixed cells were washed twice with cold PBS, resuspended in PBS containing 50 g/ml RNase A (Sigma-Aldrich, St Louis, MO, USA) at 37°C for 30 min, and stained with 50 μg/ml of propidium iodide (Molecular Probe, Eugene, OR, USA) for 30 min in the dark. Cell populations undergoing apoptosis and that in the subG1 phase were analyzed using a *flow cytometer* (Partec, Munster, Germany). In each sample, 10,000 fluorescent cells were counted.

DNA fragmentation

Cell pellets were resuspended in 750 μl of ice-cold lysis buffer (20 mM Tris–HCl, 10 mM ethylenediaminetetraacetic acid (EDTA), and 0.5% Triton X-100, pH 8.0) for 45 min with occasional shaking. DNA was extracted with phenol and precipitated with alcohol. The pellet was dried and resuspended in 100 μl of 20 mM Tris–HCl, pH 8.0. After digesting RNA with RNase (0.1 mg/ml) at 37°C for 1 h, the samples (5 μg) were electrophoresed through a 1.2% agarose gel in 450 nM Tris–acetate–EDTA buffer, pH 8.0. DNA was photographed under UV light.

Transfection of siRNA

Small interfering RNA for human p38 and Akt-specific and scrambled siRNA (negative control, non-targeting control siRNA) were synthesized by Genolution (Genolution Pharmaceuticals, Inc., Seoul, Korea). A549 cells were cultured and transfected or not, with siRNA or scrambled siRNA at a final concentration of 20 nM using Carrigene reagent (Kinovate, CA, USA) according to the manufacturer's protocol. At 12 h post-transfected cells were stimulated with GT for 24 h.

Western blot analysis

Proteins were isolated in cold RIPA buffer (50 mM Tris–HCl [pH 7.4], 150 mM NaCl, 1% NP-40, and 0.1% SDS, supplemented with protease inhibitors and phosphatase inhibitors) and equal amounts of total cellular proteins were resolved by SDS–PAGE and transferred to

nitrocellulose (NC) membranes (Whatman Schleicher and Schuell, Dachen, Germany). The NC sheet was blocked with 5% non-fat dry milk in Tris-buffered saline. Antibodies to p53 (Santa Cruz, CA, USA), COX-2 (Cayman Chemical, Ann Arbor, MI, USA), pro- and active-caspase-3 (Cell Signaling Technology, Denvers, MA, USA), pp38 (Cell Signaling Technology), p38 (Santa Cruz), pAkt (Cell Signaling Technology), Akt (Santa Cruz), and β-actin (Santa Cruz) were used for probing corresponding NC blots overnight at 4°C. Membranes were then washed three times with Tris-buffered saline/Tween-20 and incubated with horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich, St Louis, MO, USA) for 2 h followed by exposure in an LAS-3000 imager (Fuji Film Co., Tokyo, Japan) according to the manufacturer's instructions.

DAPI (4′,6-diamidino-2-phenylindole) staining

A549 cells were fixed with 3.5% paraformaldehyde in PBS for 15 min at room temperature. The cells were permeabilized in PBS containing 0.1% Triton X-100 for 15 min. The fixed A549 cells were washed with PBS and incubated for 15 min with DAPI (Invitrogen). Next, the cells were washed three times with PBS and observed under a fluorescence microscope.

Measurement of PGE_2 production

A549 cells were grown on 96-well plates and treated with GT in the presence or absence as indicated for each experiment. Prostaglndin E2 (PGE_2) levels in the culture medium were measured by competitive immunoassay using Correlate-EIA Prostaglandin kit (Assay Designs, Ann Arbor, MI, USA).

Statistics

The values given are means ± SEM. The significance of difference between the experimental groups and controls was assessed by a one-way analysis of variance test. The difference is significant if the p-value is <0.05.

Results

GT inhibits cell growth and causes apoptosis of A549 cells

Previous data have shown that GT treatment results in inhibition of growth and induction of apoptosis in a wide variety of cancers (Maurya et al. 2011). To ascertain the inhibitory effect of GT on the growth of A549 cells, cells were treated with 25–100 μM GT for 24 h and proliferation and viability were determined using MTT assay (Figure 1A).

As shown in Figure 1A and B, GT inhibited growth of A549 cells in a concentration-dependent manner (50–100 μM), but low dose of GT (25 μM) did not notably prevent growth of A549 cells as assessed using MTT assay (Figure 1A). In addition, noticeable morphological changes such as rounding and floating of the cells in GT-treated cells at 24 h were examined using a phase contrast microscope. These morphological changes showed that GT might cause apoptosis in A549 cells (data not shown). With the highest concentration (100 μM), up to 40% inhibition of proliferation was observed after 24-h treatment (Figure 1A). GT additionally induced apoptotic cell death, as determined by DNA fragmentation (Figure 1B). Because sodium nitroprusside (SNP), a nitric oxide donor, has been known as an inducer of apoptosis in various cell lines, we used as a positive control for DNA fragmentation in our data (Yoon et al. 2003) (Figure 1B). GT increased the ratio of apoptosis at 100 μM GT (Figure 1B).

Using flow cytometry analysis, we evaluated the percentage of cells in the sub-G1 phase in the presence of GT. GT induced an increase in the percentage of hypoploid cells in a dose-dependent manner starting at 1.1% in the control and reaching 34.2% in the presence of 100 μM of GT (Figure 1C). GT at concentration of 100 μM increased the hypoploid cells in a time-dependent manner (Figure 1D). The hypoploid cells gradually increased from 6 h, following treatment with GT (Figure 1D). However, we did not observe an effect of GT on cell cycle arrest at the G2/M phase.

These results indicated that GT could inhibit the growth of cells via induction of apoptosis without regulating of cell cycle (Figure 1).

Apoptosis can be regulated by signal transduction related with the activation of p53 and caspase-3 (Wyllie et al. 1980). In order to verify the induction of apoptosis, A549 cells were exposed to a series of concentrations of GT for 24 h. The expression of p53 and active caspase-3 was analyzed by western blot analysis (Figure 2A).

We found that GT increased the expression of p53 and active caspase-3 in a dose-dependent manner (Figure 2A, upper panel). Accumulation of p53 was induced in a time-dependent manner (Figure 2A, lower panel). The expression of p53 was strongly induced 4.5-fold after 24 h and was detectable as early as 30 min after exposure to GT, with the highest expression observed at 24 h (data not shown).

These effects also demonstrated that GT causes apoptosis via p53 and caspase-3 activation pathway (Figure 2A).

GT induces the expression of COX-2 in A549 cells.

Because COX-2 expression is involved in cancer cell proliferation, we examined the expression of COX-2. To determine whether GT is able to induce COX-2

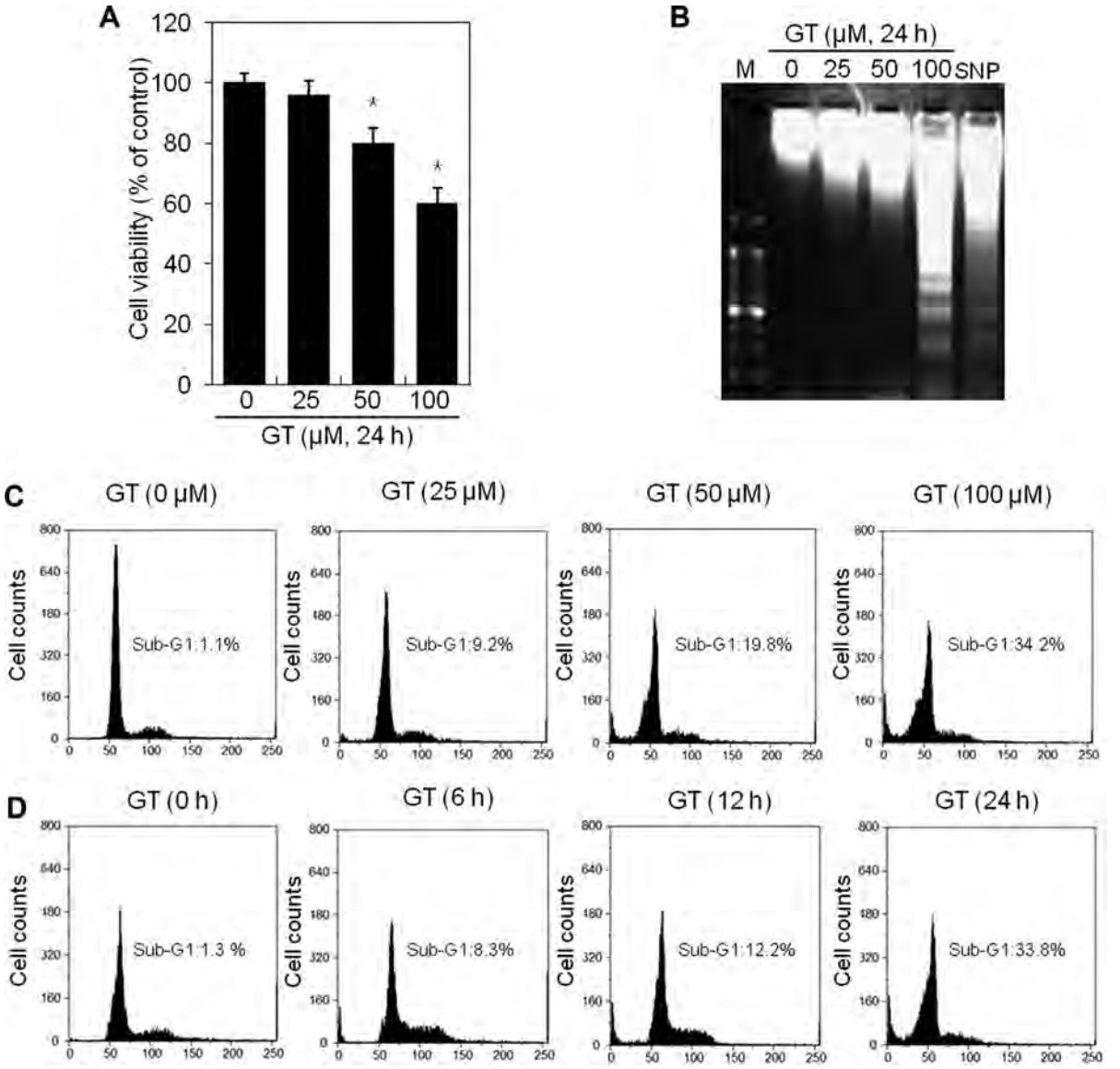

Figure 1. GT inhibits cell growth and induces apoptosis of A549 cells. (A–C) A549 cells were untreated or treated with the indicated concentrations of GT for 24 h. (A) Cell viability was measured by using a methyl thiazole tetrazolium (MTT) proliferation assay kit. (B) Apoptotic cell death was determined by examining DNA fragmentation by electrophoresis. Fragmented DNA was visualized staining with ethidium bromide. Lane M, molecular size markers. Apoptosis cell death from SNP-treated cells was used as a positive control. (C) The percentage of cell in the subG1 phase was determined using FACS analysis. (D) A549 cells were untreated or treated with GT of 100 μM for the specified time period. The percentage of cell in the subG1 phase was detected using FACS analysis. The data in A represent the average values with standard deviation ($n = 4$) and the data in B–D represent the results of a typical experiment conducted three times. *$p < 0.05$ compared with untreated cells.

expression, western blot analysis was performed. Consistent with the expression patterns of p53, we found that GT led to the expression of COX-2 in a dose- and time-dependent manner via western blot analysis (Figure 2B).

These results suggest that GT induces COX-2 expression (Figure 2B). GT regulates apoptosis and inflammation of A549 cells via Akt and p38kinase pathways.

To confirm that the underlying signaling pathway was involved, we further investigated the molecular mechanism of GT on the expression of p53 and COX-2. Because these pathways are related to the induction of apoptosis and inflammation of cancers, we examined whether GT affects the activation of Akt and MAP kinase subtypes, ERK-1/2, p38kinase, and JNK.

Treatment of GT consistently enhanced the activation of Akt and p38kinase in a dose- and

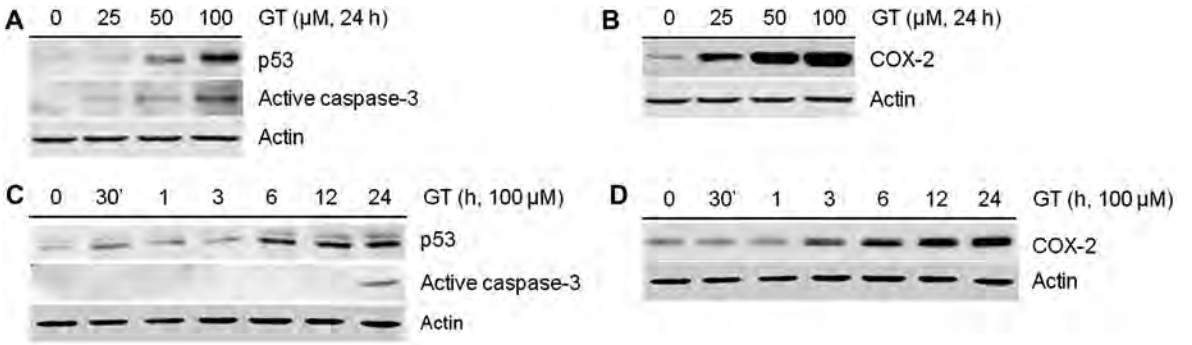

Figure 2. GT causes apoptosis and COX-2 expression of A549 cells. (A and B) A549 cells were untreated or treated with the indicated concentrations of GT for 24 h or 100 μM GT for the specified time periods. (A) Expression of p53, active caspase-3, and actin was determined by western blot analysis. Actin was used as a loading control. (B) A549 cells were untreated or treated with the specified concentrations of GT for 24 h or 100 μM GT for the indicated time periods. Expression of COX-2 and actin was determined by western blot analysis. Actin was used as a loading control. The data represent the results of a typical experiment.

time-dependent manner as determined by phosphorylation of Akt and p38 via western blot analysis (Figure 3). However, the activation of ERK-1/2 and JNK in GT-treated A549 cells was not detected (data not shown). Thus, we next focused on the Akt and p38kinase pathways, which are known important pathway for apoptosis and inflammation (Wang et al. 2011).

Inhibition of GT-induced Akt and p38kinase with LY294002 and SB203580 resulted in a recovery of cell growth (Figure 4A) and blockaded apoptosis (Figure 4B–D). Treatment of LY294002 or SB203580 with GT resulted in a markedly decreased accumulation of subG1 phase cells (Figure 4B).

In addition, treatment of Akt and p38kinase inhibitors in GT-treated cells suppressed the expression of p53, active caspase-3, and COX-2 (Figure 5A and B). Consistent with the effect of COX-2 expression, the production of PGE_2 was decreased by LY294002 and SB203580, detected by PGE_2 assay (Figure 5C). To confirm the importance of p38 and Akt in the regulatory pathway of GT-induced COX-2 and p53 expression, we used siRNA to silence p38 and Akt gene expression. As shown in Figure 5D, expression of p38 and Akt was knocked down with almost 100% efficiency, compared with GT-treated cells. Also, silencing of p38 and Akt with siRNA transfection decreased the expression of COX-2 and p53 (Figure 5D).

These results indicate that Akt and p38kinase play a role in regulating GT-induced apoptosis and inflammation of A549 cells. Taken together, the present results indicate that GT induces not only apoptosis but also inflammation of A549 cells through Akt and p38kinase (Figure 5).

Next, to evaluate the relationship between apoptosis and COX-2 expression, we investigated whether GT-induced apoptosis was regulated by COX-2 expression (Figure 6). A549 cells were treated with GT in the absence or presence of inhibitor of COX-2 or prosta glandins, NS398 or indomethacin, respectively (Figure 6). As shown in Figure 6, GT-caused apoptosis was not reduced by inhibitors of COX-2 determined by MTT assay and western blot analysis via expression of p53 and caspase-3 (Figure 6A).

Taken together, these data represented that expression of COX-2 and p53 induced by GT was any co-relationship. Expression of COX-2 and p53 was modulated by common pathway, Akt and p38kinase (Figure 6).

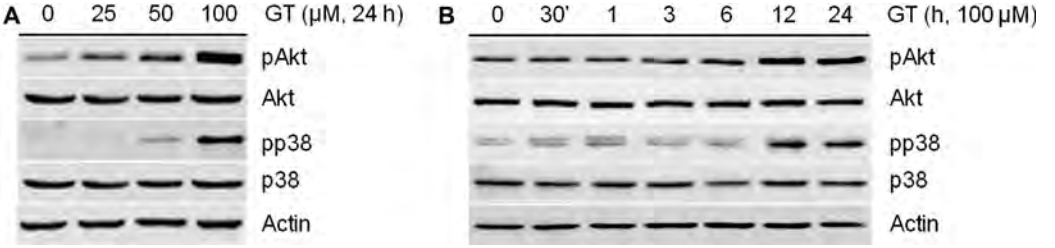

Figure 3. GT results in activation of Akt and p38kinase of A549 cells. (A and B) A549 cells were untreated or treated with the indicated concentrations of GT for 24 h (A) or 100 μM GT for the specified time periods (B). Expression of pAkt, Akt, pp38, p38, and actin was determined by western blot analysis. Actin was used as a loading control. The data represent the results of a typical experiment.

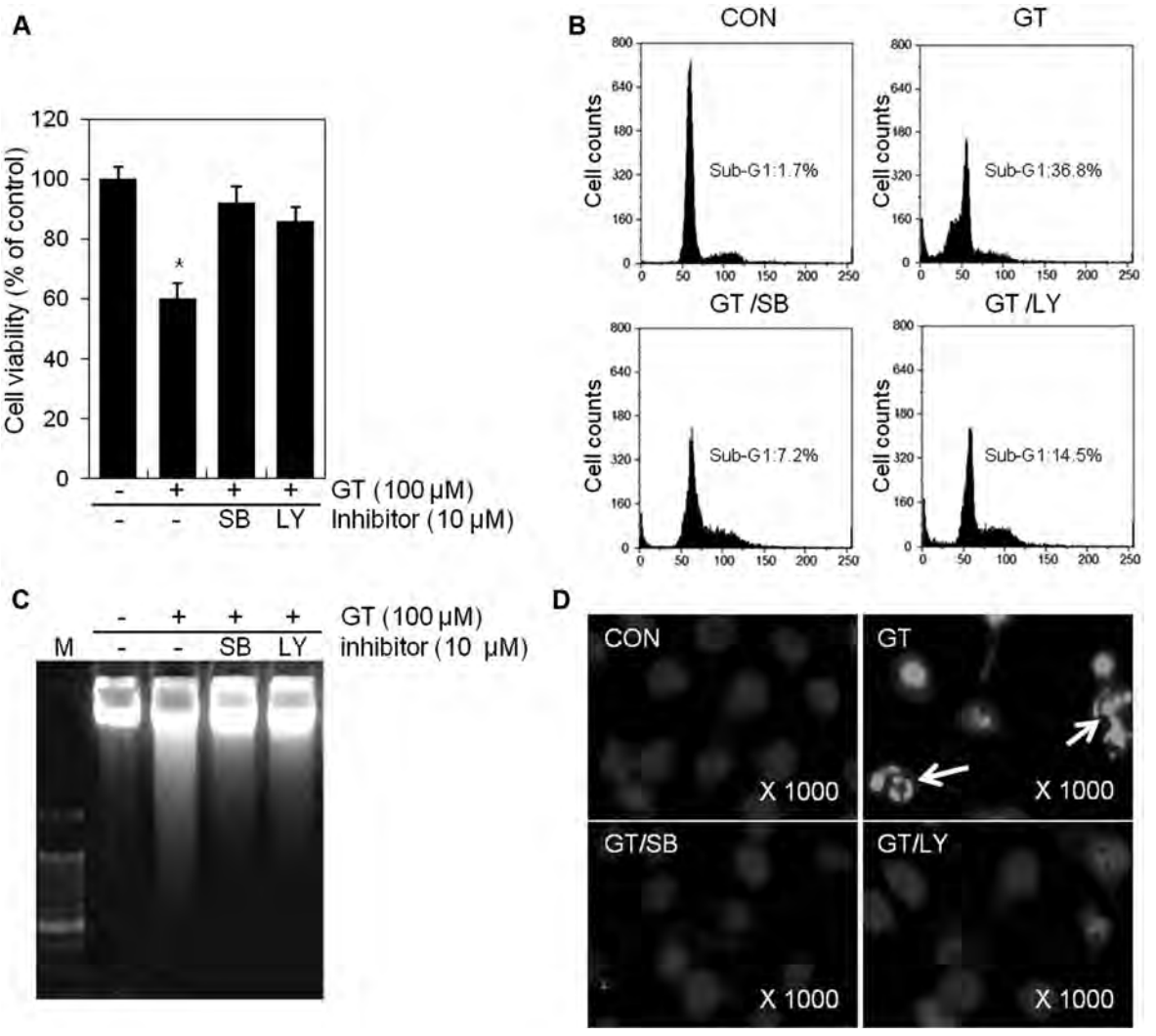

Figure 4. GT induces apoptosis through Akt and p38kinase pathway in A549 cells. (A–D) A549 cells were untreated or treated with 100 μM GT in the absence or presence of 10 μM LY294002 or 10 μM SB203580 for 24 h. (A) Cell viability was measured by using an MTT proliferation assay kit. (B) Apoptosis was analyzed as the subG1 fraction by FACS. (C) Apoptotic cell death was determined by examining DNA fragmentation by electrophoresis. Fragmented DNA was visualized via staining with ethidium bromide. Lane M, molecular size markers. (D) Nucleus of cells was stained with DAPI and observed using fluorescence microscopy. The data in A represent the average values with standard deviation ($n = 4$) and in B–D represent the results of a typical experiment conducted three times. *$p < 0.05$ compared with untreated cells.

Discussion

The molecular basis of various cellular responses including apoptosis and inflammation of lung cancer for therapy must be understood more fully (Breuer et al. 2005). Several previous data have demonstrated that GT has pro-apoptotic and antiinflammatory effects (Kaur et al. 2009). Although many results have shown that GT induces apoptosis in A549 cells, the underlying molecular mechanisms are yet to be properly characterized. In the present study, we focused on the effects of GT on apoptosis and COX-2 expression of lung cancer cells and revealed the signaling pathway mechanism of these pro-apoptotic and pro-inflammatory effects.

Apoptosis is one of the most typical types of cell death (Wyllie et al. 1980). Apoptosis is distinguished by several biological and biochemical changes, such as high mitochondrial membrane permeability, increased caspase activity, and nucleosomal DNA cleavage (Thornberry and Lazebnik 1998). Recently, an increasing body of data suggests that dysregulation of apoptosis according to the level of p53 contributes to carcinogenesis (Williams 1991). p53, a tumor-suppressor protein, defends against cancer through modulation of the cellular response to DNA damage,

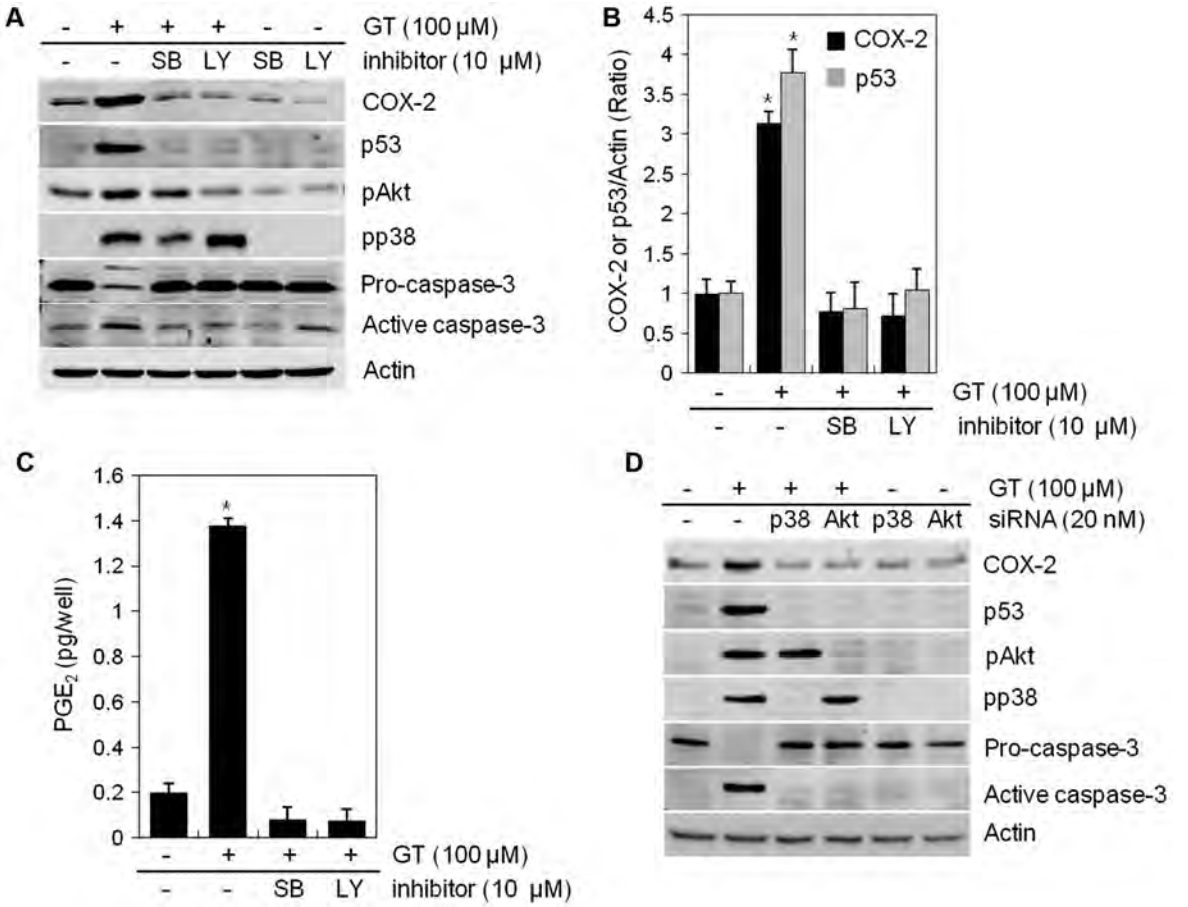

Figure 5. GT regulates COX-2 expression through Akt and p38kinase pathway in A549 cells. (A–C) A549 cells were untreated or treated with 100 μM GT in the absence or presence of 10 μM LY294002 or 10 μM SB203580 for 24 h. (A) Expression of pAkt, pp38, COX-2, p53, pro and active caspase-3, and actin was determined by western blot analysis. Actin was used as a loading control. (B) The relative amounts of p53 or COX-2 were quantified by a densitometric analysis (Image J). (C) PGE_2 production was measured using a PGE_2 assay kit. (D) Cells were cultured and transfected or not, with siRNA or scrambled siRNA at a final concentration of 20 nM. After transfection, the cells were incubated for 12 h and then treated with 100 μM GT for an additional 24 h. Expression of pAkt, pp38, COX-2, p53, pro and active caspase-3, and actin was determined by western blot analysis. Actin was used as a loading control. The data represent the results of a typical experiment. $^*p < 0.05$ compared with untreated cells.

apoptosis, and activation of oncogenes (Williams 1991; Levine 1997).

COX-2 is a key enzyme for synthesis of prostaglandins, which play an important role in the promotion of inflammation, carcinogenesis, invasiveness, and angiogenesis (Huh et al. 2003). Several studies have demonstrated that inhibition of COX-2 with pharmacological agents could be an effective approach for preventing lung cancer (Brown and DuBois 2004). COX-2 has been implicated as a crucial mediator of cancer development. Compared with normal cells, COX-2 is overexpressed in cancer cells. A high level of COX-2 is related with cancer development because it stimulates angiogenesis, which is required for tumor growth. Overexpressed COX-2 in cancer also inhibits apoptosis.

Growth of A549 cells dose-dependently decreased and apoptotic cells dose-dependently increased with 25–100 μM GT at 24 h (Figure 1A). Our study provides results demonstrating GT induces COX-2 expression in a dose- and time-dependent manner (Figure 2B). These results suggest that GT facilitates growth of A549 cells, but it conversely inhibits cell growth and induces apoptosis (Figures 1B and 2A).

Many findings have contributed to establishing the relationship between the expression of apoptosis and COX-2 (Benoit et al. 2006; de Moraes et al. 2007).

Recently, Duarte et al. (2009) presented evidence that induction of COX-2 by paclitaxel in A549 cells was independent or dependent on the expression of p53. These results indicated that an increase of COX-2 is not associated with induction of apoptosis in our investigation and it is each regulated independently. We treated with GT in the absence or presence of inhibitors of COX-2 and then confirm that whether treatment of

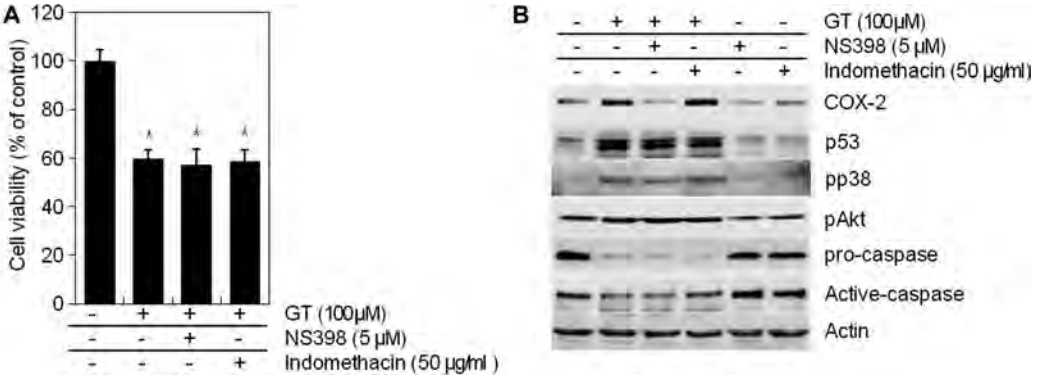

Figure 6. COX-2 expression and apoptosis induced by GT are independently regulated by Akt and p38kinase pathway in A549 cells. (A and B) A549 cells were untreated or treated with 100 μM GT for 24 h in the absence or presence of 5 μM NS398 or 50 μg/ml indomethacin. (A) Cell viability was measured by using MTT proliferation assay kit. (B) Expression of pAkt, pp38, COX-2, p53, pro- and active caspase-3, and actin was determined by western blot analysis. Actin was used as a loading control. The data are the typical results from four independent experiments with similar results. *$p < 0.05$ compared with untreated cells.

COX-2 inhibitors affects apoptosis determined by the expression of p53 and MTT assay (Figure 6).

In addition, previous studies by other investigator have shown that GT induces apoptosis by the activation of caspase-3 in A549 cells (Maurya et al. 2011). Some results have shown that GT causes apoptosis via inhibition of glutathione (GSH) production and synthesis of reactive oxygen species (Inoue et al. 2000). Also, GT decreased the gene expression and production of phorbol 12–myristate 13–acetate-stimulated pro-inflammatory cytokines such as TNF-alpha and IL-6 in human mast cells (Kim et al. 2006). Regulation of apoptosis by GT in HCT-116 was associated with a p53 status. GT did not induce apoptosis in p53 (+/+) cells, a significant induction of apoptosis was observed in p53 (−/−) cells (Al-Ayyoubi and Gali-Muhtasib 2007). Also, in our previous results suggested that GT regulates apoptosis and inflammation via PI3-kinase and p38kinase pathway in MDA-MB-231 human breast cancer cells (Yu and Kim 2011).

Activation of the PI3K and p38kinase is a pivotal factors involved in essential cellular functions in several types of cancer cells including apoptosis, survival, migration, inflammation, and metabolism (Dickinson et al. 2011).

To elucidate the signaling pathway leading to apoptosis and COX-2 expression in GT-treated A549 cells, we focused on Akt and p38kinase, based on previous reports that these intracellular signaling molecules may play a central role in the induction of apoptosis- and inflammation-related genes (Cao et al. 2011).

Our data indicated that GT markedly activated Akt and p38 (Figure 3). By using specific inhibitors of Akt and p38kinase, we established that Akt and p38kinase pathways are crucial mediators in the regulation of GT-induced apoptosis and COX-2 expression (Figures 4 and 5). Moreover, we observed that knockdown of endogenous p38 and Akt by its siRNA greatly suppressed activation of these kinases, and COX-2 and p53 expression in the cells (Figure 5D).

In conclusion, these results provide indication that GT induces apoptosis and inflammation through Akt and p38kinase. The apoptosis-inducing ability of GT could make it a potentially helpful therapeutic agent against lung cancer. However, additional studies are needed to establish the role of GT in inflammation for cancer.

Acknowledgements

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