Proanthocyanidins Inhibit *In vitro* and *In vivo* Growth of Human Non–Small Cell Lung Cancer Cells by Inhibiting the Prostaglandin E₂ and Prostaglandin E₂ Receptors

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Abstract

Overexpression of cyclooxygenase-2 (COX-2) and prostaglandins (PG) is linked to a wide variety of human cancers. Here, we assessed whether the chemotherapeutic effect of grape seed proanthocyanidins (GSP) on non–small cell lung cancer (NSCLC) cells is mediated through the inhibition of COX-2 and PGE₂/PGE₂ receptor expression. The effects of GSPs on human NSCLC cell lines in terms of proliferation, apoptosis, and expression of COX-2, PGE₂, and PGE₂ receptors were determined using Western blotting, fluorescence-activated cell sorting analysis, and reverse transcription-PCR. *In vitro* treatment of NSCLC cells (A549, H1299, H460, H226, and H157) with GSPs resulted in significant growth inhibition and induction of apoptosis, which were associated with the inhibitory effects of GSPs on the overexpression of COX-2, PGE₂, and PGE₂ receptors (EP1 and EP4) in these cells. Treatment of cells with indomethacin, a pan-COX inhibitor, or transient transfection of cells with COX-2 small interfering RNA, also inhibited cell growth and induced cell death. The effects of a GSP-supplemented AIN76A control diet fed to nude mice bearing tumor xenografts on the expression of COX-2, PGE₂, and PGE₂ receptors in the xenografts were also evaluated. The growth-inhibitory effect of dietary GSPs (0.5%, w/w) on the NSCLC xenograft tumors was associated with the inhibition of COX-2, PGE₂, and PGE₂ receptors (EP1, EP3, and EP4) in tumors. This preclinical study provides evidence that the chemotherapeutic effect of GSPs on lung cancer cells *in vitro* and *in vivo* is mediated, at least in part, through the inhibition of COX-2 expression and subsequently the inhibition of PGE₂ and PGE₂ receptors.

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Introduction

Lung cancer is the leading cause of cancer-related deaths in men and women in the United States and worldwide (1). One of *every* three cancer-related deaths is attributable to lung cancer, and the dismal 5-year survival rate of ~14% has shown no improvement over the past three decades (2, 3). Although a combination of chemotherapy and radiation therapy can improve survival, most patients die of disease progression, which is often associated with acquired or intrinsic resistance to chemotherapeutic drugs (4–6). Cyclooxygenase-2 (COX-2) is frequently constitutively elevated in different human malignancies, including lung cancers (7–10 11) as well as colorectal (12, 13), prostate (14), and breast (15, 16) cancers. Although multiple genetic alterations are necessary for lung cancer development, COX-2 may be a central element in orchestrating this process. Overexpression of COX-2 promotes tumor cell resistance to apoptosis (17, 18) and increases angiogenesis and tumor invasion (19, 20). Cyclooxygenases, which are the rate-limiting enzymes in prostanoid synthesis, convert arachidonic acid into prostaglandin (PG) H₂, a substrate for specific PG synthases (21). Two isoforms of COX, a constitutively expressed form, COX-1, and an inducible form, COX-2, have been identified. COX-2 inhibitors have been shown to inhibit tumor growth and metastasis in several animal models, including models of lung cancer (22). The current treatment strategies for advanced lung cancer include surgical resection, radiation, cytotoxic chemotherapy, and photodynamic therapy (5). In almost two thirds of cases, the cancer has already spread beyond localized disease at the time of diagnosis, limiting therapeutic options (23, 24). Therefore, the exploration and development of more effective chemopreventive/chemotherapeutic agents and therapies that can target the molecules associated with tumor proliferation, angiogenesis, and apoptosis resistance will lead to improved outcomes in patients with lung cancer.

Phytochemicals, particularly those that can be administered as dietary supplements, offer promising new options for the development of more effective chemopreventive and chemotherapeutic strategies. Grape
seed proanthocyanidins (GSP) have anticarcinogenic properties (25) and seem to exhibit minimal toxicity (26, 27). GSPs are a mixture of polyphenols/flavanols and primarily contain proanthocyanidins (89%), which constitute dimers, trimers, tetramers, and oligomers/polymers of monomeric catechins and/or (−)-epicatechins (25, 27). GSPs are readily available as an extract of grape seeds and this extract, rather than the individual constituents, is used in determining the chemotherapeutic effects of GSPs as it represents a feasible and affordable dietary phytochemical.

Recently, we found that dietary GSPs resulted in the significant inhibition of the growth of human non–small cell lung cancer (NSCLC) cell xenografts in nude mice and that this was associated with the inhibition of lung tumor cell proliferation and the inhibition of angiogenic factors (28). In the present study, we investigated the mechanism responsible for the inhibition of lung cancer cell proliferation using in vitro and in vivo models. We report that treatment of NSCLC cells with GSPs induces apoptotic cell death, and this cell death is mediated through the inhibition of COX-2 expression and the associated inhibition of PGE2 and PGE2 receptors. Our results provide a convincing rationale for the pharmacologic activity of GSPs against NSCLC cells in in vitro and in vivo models.

Materials and Methods

Cell Culture and Cell Lines

The human NSCLC lines (A549, H1299, H226, H460, H1975, H1150, HCC827, and H157) were purchased from the American Type Culture Collection. Normal (nonmalignant) human bronchial epithelial cells (BEAS-2B) from the American Type Culture Collection were used as a control. The lung cancer cell lines were cultured as monolayers in Ham’s F-12 or RPMI 1640 culture media supplemented with 10% heat-inactivated fetal bovine serum (HyClone), 100 μg/mL penicillin, and 100 μg/mL streptomycin and maintained in an incubator with a humidified atmosphere of 95% air and 5% CO2 at 37 °C. The GSPs were dissolved in a small amount of DMSO before addition to the complete media (95% air and 5% CO2 at 37 °C). The GSPs were dissolved in a small amount of DMSO before addition to the complete media (95% air and 5% CO2 at 37 °C). The GSPs were dissolved in a small amount of DMSO before addition to the complete media (95% air and 5% CO2 at 37 °C).

Antibodies, PCR Primers, and Chemicals

Antibodies specific for COX-2, an enzyme immunoassay kit for PGE2 analysis, and an EP4 agonist (PGE1 alcohol) were obtained from Cayman Chemicals. The antibodies specific for EP1, EP2, EP3, and EP4 and their secondary antibodies were obtained from Cell Signaling Technology, Inc. The PCR primers of known sequences of EP1, EP2, EP3, and EP4 were obtained from Invitrogen.

The enhanced chemiluminescence detection reagents for Western blotting were purchased from Amersham Pharmacia Biotech.

Cell Proliferation Assay

The effect of GSPs on the proliferative capacity of the cells was determined using the MTT assay as previously described (28). The effect of GSPs on cell viability was assessed as the percent cell viability compared with vehicle-treated control cells, which were arbitrarily assigned 100% viability.

Analysis of Apoptotic Cell Death by Flow Cytometry

GSP-induced apoptosis of the human NSCLC cells was determined by flow cytometry using the Annexin V–conjugated Alexa Fluor488 (Alexa488) Apoptosis Detection kit following the instructions of the manufacturer, and as described by us (28, 29). Briefly, after overnight serum starvation, cells were treated with varying concentrations of GSPs for 48 h. The cells were then harvested, washed in PBS, and incubated with Alexa488 and propidium iodide in the dark at room temperature. The stained cells were analyzed by fluorescence-activated cell sorting using a FACSCalibur instrument (BD Biosciences) equipped with the CellQuest 3.3 software. The experiments were repeated twice.

Source and Administration of GSPs

We routinely receive GSPs for our research from Kikkoman Corporation, Japan. The GSPs preparation contains ~89% proanthocyanidins, with dimers (6.6%), trimers (2.9%), and oligomers (74.8%) as described earlier (26, 27). GSPs are stable for at least 2 y when refrigerated at 4°C. The experimental AIN76A control diet containing GSPs is prepared in pellet form by TestDiet for our research using the GSPs that we provide for this purpose.

Analysis of PGE2 by Enzyme Immunoassay

Skin or tumor samples were homogenized in 100 mmol/L phosphate buffer (pH 7.4) containing 1 mmol/L ethylenediamine tetraacetic acid and 10 μmol/L indomethacin using a polytron homogenizer (PT3100, Fisher Scientific). The supernatants were collected after centrifugation and the concentration of PGE2 was determined in supernatants using the Cayman PGE2 Enzyme Immunoassay kit following the manufacturer’s protocol.

Preparation of Cell or Tumor Xenograft Lysates and Western Blot Analysis

Following treatment of the NSCLC cells with or without GSPs, the cells were harvested, washed with cold PBS, and lysed with ice-cold lysis buffer supplemented with protease inhibitors, as detailed previously (28, 29). Lysates of tumor xenografts were prepared similarly. For immunoblotting, the proteins were resolved on 10% Tris-glycine gels and transferred onto a nitrocellulose membrane. After blocking the nonspecific binding sites, the membrane was incubated with the primary antibody.
at 4°C overnight. The membrane was then incubated with the appropriate peroxidase-conjugated secondary antibody and the immunoreactive bands were visualized using enhanced chemiluminescence reagents. Each membrane was stripped and reprobed with anti-β-actin antibody to verify equal protein loading.

COX-2-Small Interfering RNA Transfection of A549 and H1299 Cells

Human-specific COX-2 small interfering RNA (siRNA) was transfected into A549 and H1299 cells using the siRNA Transfection Reagent kit (Santa Cruz Biotechnology, Inc.) according to the manufacturer’s protocol. Briefly, 2 × 10⁶ cells per well were seeded in a six-well plate and allowed to grow to 70% confluency. The COX-2 siRNA mix with transfection reagents was overlaid on the cells for ~6 h at 37°C and transferred into 2× growth medium for about 18 to 20 h. At 24 h posttransfection, fresh medium was added to the cells, and the cells incubated for an additional 48 h. Thereafter, the cells were harvested and analyzed for cell death using a trypan blue exclusion assay. The knockdown of COX-2 expression in cells after transfection was confirmed using Western blot analysis.

Reverse Transcription-PCR for EP Receptors

apoptosis (Fig. 1C, left): 0 μg/mL (vehicle control, 6.3%), 20 μg/mL (18.6%, P < 0.05), 40 μg/mL (32%, P < 0.01) and 80 μg/mL (49.3%, P < 0.001; summarized in Fig. 1D). Similar results were obtained on GSP treatment of A549 cells for 72 hours except that the percentages of apoptotic cells were slightly higher than 48 hours (data not shown). Treatment of H1299 cells with GSPs at the various concentrations of GSPs also resulted in a significant
Figure 2. GSPs inhibit the basal levels of COX-2 and PGE2 expression in NSCLC cells. A and B, the basal constitutive expression levels of COX-2 and PGE2 in seven different NSCLC cell lines and normal human bronchial epithelial cells were determined by (A) Western blot analysis of cell lysates to estimate the basal levels of COX-2 and (B) an enzyme-linked immunoassay of PGE2 in samples of cell homogenates. C, treatment of H1299, A549, and H460 cells with GSPs inhibits the basal levels of COX-2 expression dose dependently. Cells were treated with the various concentrations of GSPs for 48 h, then harvested, and cell lysates were subjected to Western blot analysis. D, the amount of PGE2 was determined in the cell homogenates from the same experiment as detailed in C using an enzyme immunoassay. The concentration of PGE2 is expressed in terms of pg/mg protein; columns, mean of three independent experiments; bars, SD. Significant inhibition versus non–GSP-treated controls: *, \( P < 0.05 \); ¶, \( P < 0.01 \); †, \( P < 0.001 \).
Human NSCLC Cells Overexpress COX-2 and Exhibit Enhanced PGE2 Production

We were interested in determining whether higher levels of COX-2 contribute to the proliferation of lung cancer cells. The levels of COX-2 in lung cancer cells were compared with the levels in normal human bronchial cells. For this purpose, the levels of expression of COX-2 were assessed in cell lysates of various NSCLC cell lines, A549, H1299, H460, H226, H1975, H1650 and HCC827, and BEAS-2B cells, by Western blot analysis. As shown in Fig. 2A, the levels of COX-2 expression were higher in the NSCLC cells than in the normal human bronchial epithelial cells. Among the lung cancer cell lines tested, the A549, H1299, and H460 cell lines expressed higher levels of COX-2. We also determined the levels of PGE2 production in the same cell lines cultured using an identical protocol. Homogenates of equal numbers of cells were analyzed for PGE2 production. As shown in Fig. 2B, the levels of PGE2 were higher in the NSCLC cell lines than the normal human bronchial epithelial cells. As had been observed for the levels of COX-2, the concentrations of PGE2 were higher in the A549, H1299, and H460 cell lines than the other human lung cancer cell lines tested.

GSPs Reduce the Constitutive Overexpression of COX-2 and PGE2 Production in NSCLC Cell Lines

To examine whether GSPs have any effect on the constitutive level of COX-2 in human lung cancer cell lines, we treated the A549, H1299, and 460 cell lines, which have higher levels of COX-2 expression than the other cell lines, with various concentrations of GSPs (0, 20, 40, 60, or 80 μg/mL) for 24 hours. The levels of COX-2 expression in the lysates of the cells was then determined using Western blot analysis. The data revealed that the treatment of lung cancer cells with GSPs resulted in a dose-dependent inhibition of COX-2 expression in all the human NSCLC cell lines tested (Fig. 2C).

As increased expression of COX-2 results in the formation of greater amounts of PG metabolites, we also determined the levels of PG metabolites in these cells with a particular emphasis on PGE2 because PGE2 plays a pivotal role in inflammation-associated diseases, including lung cancer. We found that the treatment of A549, H1299, and H460 cell lines with GSPs for 24 hours resulted in a dose-dependent inhibition of PGE2 production (P < 0.05–0.001) compared with cells which were not treated with GSPs (Fig. 2D).

Treatment of NSCLC Cells with Indomethacin, a Pan-Inhibitor of COX, Inhibits Cell Growth and Increases Cell Death

As we found that the treatment of the various NSCLC cell lines used in this study with GSPs resulted in the inhibition of cell growth/proliferation, and inhibition of cell proliferation is associated with a reduction in the levels of COX-2 expression and PGE2 production, we further sought to determine the effects of indomethacin, a well-known COX inhibitor, on these NSCLC cells. This experiment was done to determine whether the inhibitory effect of GSPs on NSCLC cell growth/proliferation is mediated through its inhibitory effect on COX-2 expression. For this purpose, equal numbers of A549, H1299, H460, and H226 cells were plated in tissue culture plates and treated with various concentrations of indomethacin (0, 20, 40, or 60 μmol/L) for 48 hours. Cell growth and morphology was checked microscopically, and cell viability and cell death were determined using MTT and trypan blue exclusion assays, respectively. As shown in Fig. 3A and B, treatment of the cells with indomethacin resulted in a dose-dependent reduction in the growth of the cells and cell viability compared with nonindomethacin-treated controls (P < 0.01–0.001). The results of the trypan blue exclusion assay revealed that the percentage of dead cells was significantly increased (P < 0.05–0.001) with the increasing doses of indomethacin (Fig. 3C). As overexpression of COX-2 is primarily responsible for rapid cancer cell proliferation and growth, these data suggested that the inhibition of constitutive levels of COX including COX-2 expression in the presence of indomethacin resulted in the induction of NSCLC cell death and inhibition of NSCLC cell proliferation.
Knockdown of COX-2 Leads to the Inhibition of Cell Growth and an Increase in Cell Death in NSCLC Cells

It has been shown that the overexpression of COX-2 contributes to cell survival and antiapoptotic effects in cancer cells. We therefore examined whether siRNA knockdown of COX-2 in the lung cancer cells would lead to the inhibition of the growth and induction of cell death of NSCLC cells. The transfection of A549 and H1299 cells with COX-2 siRNA resulted in marked reduction of cell growth and induction of cell death (64–68%, P < 0.001) after 48 hours of transfection compared with control siRNA–transfected A549 and H1299 cells (Fig. 3D).

GSPs Inhibit PGE2-Induced Cell Proliferation of NSCLC Cells

Next, we determined the effect of PGE2 on NSCLC cells and examined whether GSPs inhibit PGE2-induced cell proliferation in human lung cancer cells. For this purpose, A549 and H1299 cells were treated with PGE2 (10 μmol/L) with and without the treatment of GSPs for 48 hours. The cells were then harvested and cell proliferation was estimated using the MTT assay. We found that the treatment of lung cancer cells with PGE2 for 48 hours resulted in a significant increase in proliferation as indicated by the enhanced absorbance at 540 nm compared with the cells that were not treated with PGE2 (Fig. 4A). Pretreatment of A549 and H1299 cells with various concentrations of GSPs (20, 40, or 60 μg/mL) for 48 hours resulted in a dose-dependent inhibition of this PGE2-induced cell proliferation (Fig. 4A). We also examined the effect of GSPs on the proliferation of NSCLC cells that were treated with PGE2 (10 μmol/L) or indomethacin (40 μmol/L) alone or in combination. As shown in Fig. 4B, PGE2 treatment enhanced the proliferation of both A549 and H1299 cells compared with non–PGE2-treated cells. In contrast, indomethacin treatment significantly inhibited (P < 0.01) the proliferation of both cell lines compared with nonindomethacin-treated control cells, as well as PGE2-stimulated cell proliferation (P < 0.05). Under similar conditions, the combined treatment of cells with GSPs + indomethacin synergistically decreased (P < 0.01–0.001) PGE2-stimulated cellular proliferation of both A549 and H1299 cells.

GSPs Block the Upregulation of PGE2 Receptors in Lung Cancer Cells

It is known that PGE2 manifests its biological activity via four known G protein–coupled receptors (i.e., EP1–EP4; ref. 31). Therefore, we determined the effect of GSPs on PGE2 receptors. Analysis of A549 and H1299 cells treated with concentrations of GSPs (0, 10, 20, 40, or 60 μg/mL) for 48 hours by reverse transcription-PCR indicated a dose-dependent decrease in the levels of EP1 and EP4 transcripts (Fig. 5A). The inhibitory effect of GSPs on EP1 was less prominent than EP4. We did not detect any significant changes in the levels of EP2 or EP3 transcripts after GSP treatment of the cells. These results were further verified by Western blot analysis. As shown in Fig. 5B, the levels of EP1 and EP4 were reduced in a dose-dependent manner on treatment with GSPs.

An EP4 Agonist Enhances the Proliferation of Lung Cancer Cells and GSPs Inhibit the EP4 Agonist-Induced Cellular Proliferation of Cells

To further examine the role of PGE2 receptor (e.g., EP4) on the proliferation of NSCLC cells and the therapeutic effect of GSPs, A549 and H1299 cells were treated with an EP4 agonist (PGE1 alcohol) for 24 hours with or without the addition of GSPs. As shown in Fig. 5C, treatment of A549 and H1299 cells with the EP4 agonist resulted in the significant enhancement of cellular proliferation (P < 0.01). Treatment of cells with various concentrations of GSPs significantly inhibited (P < 0.01–0.001) EP4 agonist–induced proliferation in a dose-dependent manner. These data suggest that the stimulation of PGE2 receptor in lung cancer cells has a role in cell proliferation, and that GSPs inhibit the NSCLC cell proliferation, at least in part, by inhibiting the levels of PGE2 receptor.
We have shown earlier that dietary GSPs inhibit the growth of A549 and H1299 NSCLC cells grown as xenografts in athymic nude mice (28). The inhibitory effect of dietary GSPs on the growth of tumor xenografts are shown in Fig. 6A (28). To examine the effect of dietary GSPs in vivo on the levels of COX-2, PGE2, and the receptors of PGE2, we used tumor xenograft samples from our previous experiment (28). As the dietary GSPs at the concentration of 0.5% (w/w) supplemented with AIN76A control diet resulted in a significant inhibitory effect on tumor xenograft growth, we compared the data obtained from the analysis of lysates of A549 and H1299 tumor xenografts obtained from mice fed the control diet without GSPs (group 1) and mice fed the control diet supplemented with GSPs (0.5%; group 2). Western blot analysis revealed that the levels of COX-2 were lower in the tumor xenograft samples of A549 and H1299 cells in mice that were fed the diet supplemented with GSPs than in tumor xenografts from those mice that were not given GSPs in the diet (Fig. 6B). The levels of PGE2 were also significantly lowered ($P < 0.001$) in the tumor xenograft samples of A549 (62%) and H1299 (50%) cells in mice that were fed the diet supplemented with GSPs. Similarly, the levels of the PGE2 receptors EP1, EP3, and EP4 were lower in the tumor xenografts from mice that were fed the GSP-supplemented diet than in the tumor xenografts from control mice that were not given dietary GSPs (Fig. 6B).

**Discussion**

COX-2 is frequently overexpressed in a variety of human malignancies and is linked to all stages of tumorigenesis. Elevated tumor COX-2 expression is associated with
increased angiogenesis, tumor invasion, and suppression of host immunity and promotes tumor cell resistance to apoptosis (31). Because of its important role in tumor progression, COX-2 is a promising target for cancer therapy (31). Although multiple genetic alterations are necessary for lung cancer development, COX-2 enzymatic products may be central to orchestrating this process. Most of the protumorigenic effects of COX-2 have been attributed to its metabolic product, PGE2, an important mediator of tumor growth. A high concentration of PGE2 due to COX-2 overexpression in neoplastic cells shifts the balance of these tumorigenic processes, creating a permissive microenvironment for tumor growth. Therefore, the search of potential COX-2 inhibitors for the prevention or treatment of lung cancer may prove to be an important strategy.

Phytochemicals offer promising options for more effective treatment strategies for lung cancer. GSPs represent one such phytochemical agent that has been shown to have anticarcinogenic activity (25), including activity against lung cancer cells (28). As we had found that...
Proanthocyanidins Target COX-2 and PGE2 receptors

Proanthocyanidins Target COX-2 and PGE2 receptors. A significant finding of the present study was the inhibition of tumor xenograft growth in athymic nude mice fed a diet supplemented with GSPs (0.5%, w/w) was associated with the inhibition of COX-2 and PGE2 expression and a reduction in the levels of PGE2 receptors, EP1, EP3, and EP4. These data suggest that the protective effects of the dietary GSPs on the growth of NSCLC cells in vitro are also mediated through the inhibition of PGE2 and PGE2 receptors.

It is also important to consider whether the effect of any chemopreventive agent in an animal model can be translated in human system. For appropriate conversion of chemopreventive/chemotherapeutic agent doses from animal studies to humans, the body surface area normalization method has been recommended (37). In this study, we measured that each mouse (mean weight, 20 g) consumed ~13.5 mg GSPs per day. Based on this information, the human equivalent dose of GSPs was calculated using the following formula:

$$\text{Human equivalent dose (mg/kg)} = \frac{\text{Animal dose (mg/kg) \times Animal K_m factor}}{\text{Human K_m factor}}$$

(K_m factor for mouse = 3; K_m factor for adult human = 37).

If, the normal body weight of a person is considered to be 70 kg, then 3.8 g GSPs will be required for a person per day to produce same level of antilung carcinogenic effects as observed in mice, which seems reasonable, affordable, and attainable.

In summary, the results from this study show for the first time the chemotherapeutic efficacy of GSPs in controlling the proliferation and induction of apoptosis of human NSCLC cells in vitro and tumor xenograft growth in vitro through the inhibition of COX-2 and PGE2 expression, and the role of PGE2 receptors in this process. As the overexpression of COX-2 and subsequently overproduction of PGE2 metabolite play a prominent role in lung cancer risk, the novelty of this study lies in the exploration of a new and more effective chemotherapeutic agent, and that is GSPs. More mechanism-based studies are therefore needed to develop GSPs as a pharmacologic safe agent for the prevention or treatment of lung cancer in humans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

27. Mittal A, Elmets CA, Katiyar SK. Dietary feeding of proanthocyanidins from grape seeds prevents photocarcinogenesis in SKH-1 hairless mice: relationship with decreased fat and lipid peroxidation. Carcino
Correction: Proanthocyanidins Inhibit In Vitro and In Vivo Growth of Human Non–Small Cell Lung Cancer Cells by Inhibiting the Prostaglandin E₂ and Prostaglandin E₂ Receptors

In this article (Mol Cancer Ther 2010;9:569–80), which appeared in the March 2010 issue of Molecular Cancer Therapeutics (1), the blots of EP3 and EP4 along with the blot of β-actin under Fig. 6B (related with PGE₂ receptors part) were incorrectly presented.

Figure 6. A, dietary GSPs inhibit the growth of A549 and H1299 non–small cell lung cancer cells (NSCLC) grown as xenografts in athymic nude mice (29). Tumor xenograft tissues were harvested at the termination of the experiment, and the wet weight of the tumor/mouse in each group is reported in grams as mean ± SD, n = 10. Statistical significance versus non-GSP–treated controls, * P < 0.05; † P < 0.01; ‡ P < 0.001. B, tumor xenograft tissues from control and GSP-treated (0.5%, w/w) mice were used for the analysis of the levels of COX-2 and PGE₂ receptors using Western blotting. Dietary GSPs inhibit the levels of COX-2 and PGE₂ receptors, EP1, EP3 and EP4, in the tumor xenograft tissues grown in athymic nude mice compared with control tumor xenograft tissues. Representative blots from A549 or H1299 xenografts are presented from the independent analysis of tumors from 6 animals per group with identical results. The relative density (arbitrary) of each band after normalization for β-actin is shown under each immunoblot as the fold change compared with non-GSP–treated control, which was assigned an arbitrary unit 1 in each case. PGE₂ was determined in tumor xenograft tissue samples using a PGE₂ immunoassay kit following the manufacturer’s instructions. The concentration of PGE₂ is expressed in terms of pg/mg protein as a mean ± SD, n = 10. Significantly lower versus non-GSP–treated controls. * P < 0.001.
The experiments were repeated in A549 and H1299 cell lines with and without treatment with grape seed proanthocyanidins (GSP) under identical conditions as used before. Cell lysates were subjected to Western blot analysis for the detection of the levels of PGE$_2$ receptors (EP1, EP3, and EP4), and new data were generated to confirm and verify the levels of PGE$_2$ receptors. The equal protein loading on the gel was verified and re-confirmed using antibody against β-actin. The data obtained re-confirm the results originally reported in the article and thus correct our errors. The authors regret this error.

Reference


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