PC-SPES down-regulates COX-2 via inhibition of NF-κB and C/EBPβ in non-small cell lung cancer cells

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Abstract. Aberrant expression of COX-2 occurs in many types of malignancies including colon and lung cancers, and is implicated in development and progression of cancer. The molecular mechanisms associated with aberrant expression of COX-2 in lung cancer cells remain to be fully elucidated. In this study, we found that non-small cell lung cancer (NSCLC) NCI-H520 and NCI-H460 cells constitutively expressed COX-2 and produced prostaglandin E2 (PGE2) as measured by Western blotting and enzyme-linked immunosorbent assay (ELISA), respectively. Reporter assays showed that transcriptional regulation of COX-2 was blunted when either the NF-IL6 (C/EBPβ) or nuclear factor-xB (NF-xB) binding site in the COX-2 promoter was mutated, suggesting that C/EBPβ and NF-xB transcription factors have an important role in aberrant expression of COX-2 in these lung cancer cells. In addition, the eight herbal mixture PC-SPES (Lot. 5431219) caused growth arrest and apoptosis of NCI-H520 and NCI-H460 cells in association with blockade of NF-xB and down-regulation of C/EBPβ, resulting in down-regulation of COX-2 and PGE2 in these cells. On the other hand, PC-SPES up-regulated the level of C/EBPβ in these cells. Taken together, C/EBPβ and NF-xB may be promising molecular targets for COX-2 inhibition in lung cancer cells. PC-SPES might be useful in the adjuvant setting for the treatment of individuals with resected NSCLC as well as other types of cancer in which COX-2 is activated.

Introduction

Cyclooxygenase (COX) is the rate-limiting enzyme for the production of prostaglandins and thromboxanes from free arachidonic acid (1,2). Two forms of COX have been described; a constitutively expressed enzyme, COX-1 and an inducible enzyme, COX-2. COX-1 is ubiquitously expressed. COX-2 is an inducible protein which is an essential component of the inflammatory response, as well as involved in the repair of injury (3). Physiological activity of this enzyme provides a benefit to the organism; however, the aberrant or excessive expression of COX-2 has been implicated in a wide variety of pathologic processes including arthritis, carcinoma, as well as septic shock. Abnormal levels of this enzyme have been observed in many types of cancer including those from colon and lung; and studies implicate its involvement in development and progression of cancer (4-6). However, the molecular mechanisms of aberrant expression of COX-2 in cancer cells remain to be fully elucidated.

PC-SPES contains a partially extracted mixture of eight herbs: Dendranthera morfolifolia; Tzvel; Gynodetria Lucidium, Karst; Glycyrrhiza glabra L; Isatis indigotica; Fort; Panax pseudo-ginseng, Wall; Rabdosia rubescens; Scutellaria baicalensis; Georgi and Serenoa repens (7). In previous studies, we and others have shown that PC-SPES mediated an antiproliferative effect on prostate cancer cells in vivo and in vitro (8,9). Recently, we showed that PC-SPES inhibited the proliferation of colon cancer cells and prevented polyp formation in a murine colon cancer model, the Apcmin mouse (10). These mice have a germ-line, non-sense mutation at codon 716 of the adenomatous polyposis coli (APC) gene; and they spontaneously develop multiple polyps in their small and large intestines at the age of 10-12 weeks (11). Therefore, Apcmin mice are considered to be useful for analysis or prevention of human familial adenomatous polyposis (FAP) and sporadic colorectal cancers. The polyp formation was dramatically prevented in Apcmin mice when they were either treated with known COX-2 inhibitors (11,12), or the mice were engineered to have a deletion of the COX-2 gene (11). These results indicated that PC-SPES might act as a COX-2 inhibitor.

In this study, to understand better the molecular mechanisms of aberrant expression of COX-2 in NSCLC
cells, we performed reporter gene assays using a series of mutated COX-2 promoters. Our data indicated that NF-IL6 and NF-xB binding sites of the COX-2 promoter played an important role in the aberrant expression of COX-2 in these cancer cells. Of note, the eight herbal mixture PC-SPES induced the growth arrest and apoptosis of NCI-H520 and NCI-H460 cells. In addition, PC-SPES down-regulated COX-2 transcripts and protein in conjunction with down-regulation of C/EBPβ protein and inhibition of NF-xB activity.

Materials and methods

Cell line. NCI-H520, NCI-H460, and NCI-H1299 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and were grown in RPMI medium (Gibco Life Technologies, Grand Island, NY) with 10% heat-inactivated fetal bovine serum (FCS, Gibco). Murine macrophage RAW264.7 cells were obtained from ATCC and grown in Dulbecco's minimal essential medium (Gibco) with 10% heat-inactivated fetal bovine serum (FCS, Gibco) containing no detectable LPS (<0.006 ng/ml as determined by the manufacturer).

Chemicals. PC-SPES (Lot. 5431219) was obtained from Botanic Lab, Inc. (Brea, CA). One capsule contains 320 mg of powdered herbal extract. Stock solutions of PC-SPES were prepared by exposing these herbal extracts to 70% ethanol (one capsule/1 ml of 70% ethanol). The final concentration of ethanol was <0.02%, and all experimental conditions were compared with vehicle controls. Warfarin and indomethacin were purchased from Sigma Chemical Co. (St. Louis, MO). Oridonin was purified from PC-SPES as previously described (13).

MTT assays. Cells (5x10^4/ml) were cultured in the presence of various concentrations of PC-SPES (0.25-4.0 μl/ml) for 4 days in 96-well plates (Flow Laboratories, Irvine, CA). After cell culture, cell number and viability were evaluated by measuring the mitochondrial-dependent conversion of the tetrazolium salt, MTT (Sigma), to a colored formazan product. MTT (0.5 mg/ml in PBS) was added to each well and incubated for 4 h at 37°C. The medium was carefully aspirated, and dimethyl sulfoxide (DMSO; Burdick & Jackson, Muskegon, MI) was added to solubilize the colored formazan product. Absorbance was read at 540 nm on a scanningmultiwell spectrophotometer (Bio-Rad) after agitating the plates for 5 min on a shaker.

Assessment of apoptosis. Apoptotic cell death was examined by terminal deoxyribonucleotide transferase-mediated dUTP nick end labeling (TUNEL) method using the In Situ Cell Death Detection kit (Roche Molecular Biochemicals, Germany) according to manufacturer's instruction. For quantification, three different fields were counted under the microscope and at least 300 cells were enumerated in each field. All experiments were performed twice.

Transfections and luciferase assay. Wild-type and a series of mutant COX-2 promoters cloned upstream of the luciferase gene in the pX2 vector were a generous gift from Harvey K. Herschman (University of California, Los Angeles) (14). Cells (10^4/ml) were plated on 24-well plates and on the following day, cells were transfected with the indicated plasmids using the GenePORTER transfection reagent (Gene Therapy Systems, Inc., San Diego, CA). Luciferase activity in cell lysates was measured by Dual Luciferase assay system (Promega, Madison, WI) and this was normalized by Renilla activities. All transfection experiments were carried out in triplicate wells and repeated separately at least three times.

Western blot analyses. To prepare whole cell lysates, cells were suspended in lysis buffer [50 mM Tris (pH 8.0), 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% NP-40, 100 μg/ml phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 1 μg/ml pepstatin, and 10 μg/ml leupeptin] and placed on ice for 30 min. After centrifugation at 15,000 x g for 20 min at 4°C, the supernatant was collected.

To prepare nuclear extracts, cells were suspended in ice-cold extraction buffer containing 20 mM HEPES (pH 7.9), 20% glycerol, 10 mM NaCl, 0.2 M EDTA (pH 8.0), 1.5 mM MgCl₂, 0.1% Triton X-100, 1 mM dithiothreitol, 100 μg/ml phenylmethysulfonyl fluoride, 2 μg/ml aprotinin, 1 μg/ml pepstatin, and 10 μg/ml leupeptin. After 10 min of incubation on ice, nuclei were collected by a short spin in a microcentrifuge. The supernatant was removed, and the nuclei were resuspended in ice-cold extraction buffer. After 30 min of incubation, supernatant was collected by centrifugation at 15,000 x g for 20 min at 4°C. Protein concentrations were quantitated using a Bio-Rad assay (Bio-Rad Laboratories, Hercules, CA). Proteins were resolved by 4-15% SDS polyacrylamide gel, transferred to an immobilized polyvinylidene difluoride membrane (Amersham Corp., Arlington Heights, IL), and probed sequentially with antibodies: anti-p53 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-p21 (Santa Cruz Biotechnology), anti-C/EBP-α, -β, anti-COX-1, -2 (Santa Cruz Biotechnology), and anti-actin antibody (Santa Cruz Biotechnology). The blots were developed using the enhanced chemiluminescence kit (Amersham Corp.).

Evaluation of NF-xB activity by enzyme-linked immunosorbent assay (ELISA). The DNA binding activity of NF-xB in lung cancer cells was quantified by enzyme-linked immunosorbent assay (ELISA) using the Trans-AM NF-xB p65 transcription factor assay kit (Active Motif North America, Carlsbad, CA), according to the instructions of the manufacturer. Briefly, nuclear extracts were prepared as previously described and incubated in 96-well plates coated with immobilized oligonucleotide (5′-AGTTGAGGGGACTTTCC-3′) containing a consensus (5′-GGGACTTTCC-3′) binding site for the p65 subunit of NF-xB. NF-xB binding to the target oligonucleotide was detected by incubation with primary antibody specific for the activated form of p65 (Active Motif North America), visualized by anti-IgG horseradish peroxidase conjugate and developing solution, and quantified at 450 nm with a reference wavelength of 655 nm. Background binding was subtracted from the value obtained for binding to the consensus DNA sequence.

Measurement of prostaglandin E₂. NCI-H520 cells (2x10^5/ml) were plated in 6-wells and cultured with either PC-SPES (2 or 4 μl/ml) or control diluent. After 48 h, culture medium
was collected and the concentration of PGE₂ was measured by ELISA kit (PharMingen, San Diego, CA).

**Statistical analysis.** Statistical differences were determined by two-tailed Student’s t-test.

**Results**

**Molecular mechanisms of constitutive expression of COX-2 in NCI-H520 cells.** Lung cancer cells can express COX-2 (14). For example, we found constitutive expression of COX-2 in NCI-H520 and -H460 cells (Fig. 2A). To begin to investigate how COX-2 transcripts are constitutively regulated in lung cancer cells, NCI-H520 cells were transiently transfected with either the wild-type COX-2 reporter construct, and cultured with either PC-SPES or control diluent. As expected, control diluent cultured NCI-H520 cells secreted 240±80 pg/ml PGE₂/1x10⁵ cells during a 24-h culture. Exposure of these cells to PC-SPES (2 or 4 μl/ml for 24 h) down-regulated the level of PGE₂ by 50% and 60%, respectively (Fig. 2B). Recently, we showed that the eight-herbal mixture PC-SPES inhibited the growth of colon cancer cells in vitro and prevented polyp formation in a murine colon cancer model, the APC<sup>min</sup> mouse in which codon 716 of the APC gene was mutated (10). These results prompted us to hypothesize that PS-SPES could act as a COX-2 inhibitor. Therefore, the effect of PC-SPES on COX-2 was studied at the protein level. Western blot analyses showed that both NCI-H520 and NCI-H460 cells constitutively expressed COX-2 protein; and exposure of these cells to PC-SPES (4 μl/ml, 24 h) down-regulated the level of COX-2 by 50% and 60%, respectively (Fig. 2A).

As an additional evaluation of the effect of PC-SPES on COX-2 activity, we measured the level of PGE₂ in culture medium of NCI-H520 cells treated with PC-SPES (Fig. 2B). Control diluent cultured NCI-H520 cells secreted 240±80 pg/ml PGE₂/1x10⁵ cells during a 24-h culture. Exposure of these cells to PC-SPES (2 or 4 μl/ml for 24 h) decreased the mean level of PGE₂ to 190±60 and 118±37 pg/ml/1x10⁵ cells, respectively, suggesting that PC-SPES blocked COX-2 enzymatic activity.

In further experiments, NCI-H520 cells were transiently transfected with the wild-type COX-2 reporter construct, and cultured with either PC-SPES or control diluent. As expected, PC-SPES (2 μl/ml, 24 h) inhibited transcriptional regulation of COX-2 expression by approximately 60% as compared with control diluent treated cells (Fig. 2C). In addition, the effect of PC-SPES on level of COX-2 transcripts was studied using RAW264.7 murine macrophage-like cells; LPS (100 ng/ml, 6 h) increased COX-2 reporter activity by 5.0±2.0-fold and PC-SPES (2 μl/ml, 6 h) blunted this activity by about 50% (Fig. 2D). Recently, we purified oridonin from *Rhabdosia rubescens*, one component of PC-SPES, by high performance liquid chromatography (HPLC) (13). The effect of oridonin on COX-2 reporter activity was investigated. As shown in Fig. 2C, oridonin (5 μg/ml, 24 h) inhibited approximately half the COX-2 reporter gene activity (Fig. 2C), suggesting that oridonin might represent one of the active component(s) of PC-SPES. Recently, Lot. 543129 of PC-SPES which was used in this study, was shown to contain warfarin (483 μg/g) and indomethacin (0.89 mg/g) (16). The final concentration of PC-SPES (2 μl/ml) utilized in our study, would theoretically contain approximately 2x10⁻⁴ M of these reagents. Importantly, even higher doses of warfarin (1x10⁻³ M) and indomethacin (1x10⁻⁴ M) did not inhibit transcriptional regulation of COX-2 expression in NCI-H520 cells (Fig. 2C).

**Effect of PC-SPES on COX-2 promoter activity in NCI-H520 cells.** In further experiments, NCI-H520 cells were transiently transfected with either the wild-type COX-2 promoter or mutated COX-2 promoter constructs (Fig. 1). When both sites were mutated, about 90% reduction of reporter activity occurred. Similarly, when the NF-xB site was mutated, reporter activity was reduced by 60% and combined mutation of the NF-xB and 5’ NF-IL6(2) sites caused approximately 70% reduction. These data indicated that the transcription factors NF-xB and C/EBPβ which bind to NF-xB and NF-IL6 DNA binding site, respectively played an important role in aberrant expression of COX-2 in NCI-H520 NSCLC cells.

**Effect of PC-SPES on NSCLC cells.** NCI-H520 cells were transiently transfected with a NF-xB reporter gene construct, and cultured with either PC-SPES or control diluent. PC-SPES (2 μl/ml, 24 h) inhibited NF-xB reporter activity by approximately 65±8% as compared with control diluent treated cells (Fig. 3A). Oridonin inhibited NF-xB trans-
criptional activity by 37±8% (Fig. 3A). On the other hand, neither warfarin (10-5 mol/l) nor indomethacin (10 -5 mol/l) affected NF-κB activity in NCI-H520 cells (Fig. 3A). Also, PC-SPES inhibited TNF-α-induced NF-κB transcriptional activity in NCI-H520 cells; TNFα increased NF-κB transcriptional activity by about 4.8-fold and PC-SPES (2 μl/ml, 6 h) blunted this activity by 40% (Fig. 3B).

The effect of PC-SPES on NF-κB activity was further confirmed using an ELISA-based assay. Treatment of NCI-H520 cells with PC-SPES (2 μml/ml, 24 h) inhibited the NF-κB binding activity by 60% as compared with untreated control cells (Fig. 3C). As control, 100-fold molar excess of the wild-type NF-κB consensus oligonucleotides was added to the assay with lysate from untreated NCI-H520 cells. Binding was inhibited by at least 80%; in contrast, mutated NF-κB consensus oligonucleotides at the same molar excess were unable to inhibit binding (Fig. 3C), ascertaining the specificity of binding of NF-κB to its consensus binding site. Interestingly,
PC-SPES (2 μl/ml, 24 h) slightly increased AP-1 binding activity (about 1.3-fold) as compared with control diluent treated cells under similar culture conditions (Fig. 3D) (p=0.04). These latter results are consistent with our previous observations showing that PC-SPES activated the JNK/c-Jun/AP-1 signal pathway in human prostate cancer LNCaP cells (17).

Effect of PC-SPES on levels of C/EBPs in NSCLC cells. C/EBPβ binds to NF-IL6 sites and regulates the expression of numerous genes including COX-2. We therefore explored the effect of PC-SPES on C/EBPs in NSCLC cells. NCI-H520 and NCI-H460 cells were cultured in the presence of either 2 or 4 μl/ml of PC-SPES for 24 h. Protein was extracted and subjected to Western blot analysis. As shown in Fig. 4, both NCI-H520 and NCI-H460 cells constitutively expressed C/EBPβ protein, and PC-SPES down-regulated levels of this transcription factor. For example, PC-SPES (4 μl/ml) reduced the amount of C/EBPβ by 80% and 90% in NCI-H520 and NCI-H460 cells, respectively (Fig. 4). Also, we investigated the level of C/EBPα in the NSCLC cells. As reported previously, the expression of the p42 isoform of C/EBPα was barely detectable in lung cancer cells (18). PC-SPES (4 μl/ml) increased the p42 isoform by 3- or 4-fold in NCI-H520 and NCI-H460 cells, respectively (Fig. 4). On the other hand, the level of the dominant negative p30 isoform of C/EBPα was not significantly modulated.

Previous studies showed that non-steroidal anti-inflammatory drugs (NSAIDs) and acetylsalicylic acid (aspirin) blunted lipopolysaccharide (LPS)-induced expression of COX-2 in murine macrophages via inhibition of the C/EBPβ transcriptional activity (19). Therefore, we explored whether indomethacin down-regulated levels of C/EBPβ in NSCLC cells. Of note, even toxic amounts of indomethacin (10⁻⁴ mol/l) failed to down-regulate C/EBPβ in NCI-H520 cells (Fig. 4B).

Effect of PC-SPES on the proliferation and apoptosis of NSCLC cells. To explore the anti-proliferative and proapoptotic effects of PC-SPES in NSCLC cells, NCI-H520, NCI-H460 and NCI-H1299 cells were cultured in the presence of various concentrations of PC-SPES. PC-SPES effectively inhibited the growth of these cells with an ED₅₀ of approximately
1.8 μl/ml for each of the cell lines as measured by MTT assay on the fourth day of culture (Fig. 5A). The final concentration of PC-SPES (Lot. 5431219) (4 μl/ml) used in our study would theoretically contain approximately 4x10⁻⁶ M of both warfarin and indomethacin. Importantly, even higher doses of both warfarin (1x10⁻⁵ M) and indomethacin (1x10⁻⁵ M) did not inhibit the growth of NCI-H520 cells (Fig. 5B). As shown in Fig. 5C, PC-SPES caused apoptosis of NCI-H520 cells in a dose- and time-dependent manner as measured by TUNEL assay. On the first day of culture, 1 and 4 μl/ml of PC-SPES induced a mean 3±1% and 10±2% of NCI-H520 cells to become apoptotic, respectively. The apoptotic population increased to a mean of 6±0.5% and 15±3%, respectively, on the second day of culture. Less than 1% of control cells were apoptotic on each day of culture (Fig. 5C).

The modulation of expression of the cell cycle ‘checkpoint’ proteins, p21wu1 and p53 were examined by Western blot analysis. NCI-H520 and NCI-H460 cells constitutively expressed wild-type p53 protein. Exposure of these cells to PC-SPES (2 or 4 μl/ml, 24 h) resulted in p53 and p21wu1 increasing 5- to 6-fold compared to untreated, control cells (Fig. 5D).

Discussion

This study demonstrated that NSCLC cells constitutively expressed COX-2 transcripts and protein. Both C/EBPβ and NF-IL6 contributed to aberrant expression of COX-2 in NSCLC cells, and PC-SPES down-regulated levels of C/EBPβ. Accumulating evidence suggests that C/EBPβ can contribute to carcinogenesis and progression of several types of cancer. Forced expression of C/EBPβ could transform normal mammary epithelial cells (20); and aberrant expression of C/EBPβ was found in renal cell carcinoma, but not in the adjacent normal tissues (21). Also, murine C/EBPβ deletional mice were resistant to development of activated ras mediated skin cancers (22). The role of C/EBPβ in human lung cancer is yet to be explored.

Previously, other investigators showed that CRE and NF-IL6 sites played a critical role in aberrant expression of COX-2 in colon cancer cells (23). However, the importance of the NF-κB binding site of this gene was unexplored. We found that mutation in the NF-κB site of the COX-2 promoter decreased reporter activity by 60% and mutation of both C/EBP (NF-IL6) binding sites decreased reporter activity by 90%. In addition, our study showed that the COX-2 reporter gene activity decreased only 23±14% when the CRE site of the COX-2 promoter was mutated (Fig. 3). Recently, another study investigating the molecular mechanism of COX-2 expression in murine lung tumor cell lines suggested the importance of the C/EBP but not the CRE site (14), which is consistent with our results. However, their study did not show the importance of NF-κB in the aberrant expression of COX-2. In their study, they employed deletional constructs of the promoter. On the other hand, we used promoter constructs that contained specific mutations of their NF-κB, C/EBP (NF-IL6), CRE and E-box sites. This may explain the discrepancy between our results and theirs. Another possible
explanation for this discrepancy could relate to the type of cells studied. They explored regulation of COX-2 in murine lung cancer cells and we used human lung cancer cells. In our previous study, PC-SPES inhibited growth of COX-2 expressing and non-expressing colon cancer cells (15). Therefore, down-regulation of COX-2 can not totally explain the mechanism by which PC-SPES inhibits the growth of cancer cells. Recently, we explored the effect of PC-SPES on growth inhibition and induction of apoptosis of human prostate cancer LNCaP cells. We found that PC-SPES activated the JNK/c-Jun/AP-1 signal pathway (17). When this signal pathway was blocked by forced-expression of JNK interacting protein-1 (JIP), LNCaP cells became resistant to the growth inhibiting effects of PC-SPES. In the present study, PC-SPES increased DNA binding activity of AP-1 in NCI-H520 cells, suggesting that probably the JNK/c-Jun/AP-1 signal pathway also contributes to the growth inhibition and apoptosis of the cells cultured with PC-SPES.

Another possible mechanism by which PC-SPES inhibited the growth of many types of cancer cells could relate to its ability to down-regulate the level of tubulins in cancer cells; we and others showed that PC-SPES lowered the level of tubulins in colon cancer (10) as well as LNCaP cells (24-26) as these cells underwent apoptosis. Also, Bonham and colleagues found that PC-SPES could inhibit the polymerization of microtubules in LNCaP cells (26). Further studies are required to identify the active component(s) of PC-SPES that affect tubulins.

C/EBPβ was shown to contribute to terminal differentiation of myeloid cells, hepatocytes, as well as adipocytes (27). Targeted inactivation of C/EBPβ in mice demonstrated hyperproliferation of type II pneumocytes and abnormal alveolar structure, and histopathology of the liver displayed a structure resembling regenerative changes or hepatocellular carcinoma (28). We and others have identified mutations in the C/EBPβ gene in individuals with acute myeloid leukemia, myelodysplastic syndrome and NSCLC; and these mutations disrupted normal function of C/EBPβ (29,30). Recently, expression of C/EBPβ was shown to be down-regulated in NSCLC cells compared to normal lung tissues, and forced
expression of the p42 isoform of C/EBPβ reduced proliferation of NSCLC (18). Taken together, these findings reinforce the importance of C/EBPβ in the regulation of cell growth and suggest that it may act as a tumor suppressor. We found that PC-SPES slowed cell growth of NSCLC and up-regulated level of C/EBPβ in these cells. Further studies will explore the molecular mechanism by which the active component(s) of PC-SPES up-regulates C/EBPβ.

We have purified baicalin and oridonin from PC-SPES, and have found that both can inhibit the growth of a wide variety of cancer cells including those from prostate, breast, and lung (13,31). Oridonin, but not baicalin, blunted the LPS-stimulated activity of NF-κB and expression of COX-2 in murine macrophage cells (unpublished data). Oridonin appears to be one of the active component(s) of PC-SPES.

In summary, this study provides the evidence that PC-SPES inhibited the proliferation and induced apoptosis of NSCLC cells. Also, PC-SPES down-regulated levels of COX-2 transcripts and protein in conjunction with inhibition of NF-κB transcriptional activity. It also decreased levels of C/EBPβ and increased level of the p42 isoform of C/EBPα transcriptional activity. It also decreased levels of C/EBPβ in murine macrophage cells (unpublished data). Oridonin appears to be one of the active component(s) of PC-SPES.

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References


