Manipulation of Pulmonary Prostacyclin Synthase Expression Prevents Murine Lung Cancer

Robert L. Keith, York E. Miller, Yasushi Hoshikawa, Mark D. Moore, Tracy L. Gesell, Bifeng Gao, Alvin M. Malkinson, Heiko A. Golpon, Raphael A. Nemenoff, and Mark W. Geraci

Division of Pulmonary Sciences and Critical Care Medicine, Department of Medicine, Denver VA Medical Center, Denver, Colorado 80220 [R. L. K., Y. E. M.,] and Department of Medicine, Division of Pulmonary Sciences and Critical Care Medicine [R. L. K., Y. E. M., Y. H., M. D. M., T. L. G., B. G., H. A. G., M. W. G.], Department of Pharmaceutical Sciences [A. M. M.], and Departments of Medicine and Pharmacology [R. A. N.], University of Colorado Health Sciences Center, Denver, Colorado 80262

ABSTRACT

Inhibition of cyclooxygenase (COX) activity decreases eicosanoid production and prevents lung cancer in animal models. Prostaglandin (PG) 12 (PGI2, prostacyclin) is a PGH2 metabolite with anti-inflammatory, anti-proliferative, and antitumorigenic properties. The instability of PGI2 has limited its evaluation in animal models of cancer. We hypothesized that pulmonary overexpression of prostacyclin synthase may prevent the development of murine lung tumors. Transgenic mice with selective pulmonary prostacyclin synthase overexpression were exposed to two distinct carcinogenesis protocols: an initiation/promotion model and a simple carcinogen model. The transgenic mice exhibited significantly reduced lung tumor multiplicity (tumor number) in proportion to transgene expression, a dose-response effect. Moreover, the highest expressing mice demonstrated reduced tumor incidence. To investigate the mechanism for protection, we evaluated PG levels and inflammatory responses. At the time of sacrifice following one carcinogenesis model, the transgenics exhibited only an increase in 6-keto-PGF1α, not a decrease in PGE2. Thus, elevated PGI2 levels and not decreased PGE2 levels appear to be necessary for the chemopreventive effects. When exposed to a single dose of butylated hydroxytoluene, transgenic mice exhibited a survival advantage; however, reduction in alveolar inflammatory response was not observed. These studies demonstrate that manipulation of PG metabolism downstream from COX produces even more profound lung cancer reduction than COX inhibition alone and could be the basis for new approaches to understanding the pathogenesis and prevention of lung cancer.

INTRODUCTION

Lung cancer is the leading cause of cancer death in men and women in North America (1). Although primary prevention of tobacco smoking and smoking cessation are the most effective interventions available, most lung cancers are diagnosed in former smokers (1), underscoring the need for effective chemoprevention strategies. Chemoprotection efforts have focused on dietary factors (particularly vitamins and micronutrients) or COX,5 (PGH2 synthase) inhibition. Multiple epidemiological studies established a significant negative correlation between a diet high in fruits and vegetables and lung cancer incidence (2, 3). In support of this correlation, vitamin A-deficient animals display tracheal and respiratory epithelial metaplasia and keratinization that is reversed on vitamin A supplementation (4, 5), suggesting these compounds are chemopreventive. However, two large studies found that β-carotene supplementation in humans increased lung cancer incidence (6, 7). In murine models of carcinogenesis, vitamin A or β-carotene supplementation increased formation of pulmonary adenomas, underscoring the importance of animal studies before large-scale human trials (8).

COX inhibition has been investigated as a chemopreventive strategy. PGH2, the product of the COX enzymes, is metabolized to a number of eicosanoids, some of which may be procarcinogenic and others, such as PGI2, which may chemopreventive. COX inhibition decreases the levels of PGH2 and all of the downstream PGs and thromboxanes. In a large United States cohort, 32% fewer lung cancers developed in frequent aspirin users (9). Mouse models of lung carcinogenesis display both histological and molecular genetic similarities to adenocarcinoma (10), the most common histological type of human lung cancer. In these models, either nonselective COX-1 and COX-2 inhibition or selective COX-2 inhibition resulted in a 34–52% reduction in lung tumor multiplicity (11, 12). However, whereas the number of tumors (multiplicity) was significantly decreased, all of the animals acquired tumors; thus there was no effect on overall tumor incidence. COX-2 inhibitors have also been associated recently with a potentially unfavorable side-effect profile (13). Chronic administration of lipoxygenase inhibitors that decreased leukotriene formation lowered lung tumor multiplicity by ~30% (14). To date, large-scale interventional trials of COX inhibition in human lung cancer chemoprevention have not been completed.

The role of PGI2 in carcinogenesis has been incompletely examined. The potential role of PGI2 in carcinogenesis includes suppression of inflammation (15), platelet inhibition (16), metastasis prevention (17), and reduced growth of established micrometastases (18). PGI2 production in normal lung is well understood. PGI2 is one of the most abundant PGs in normal lung but is produced in very low amounts by human non-small cell lung cancers (19). In contrast, high levels of PGE2 are observed in non-small cell lung cancers containing Ki-ras mutations, because these mutations induce constitutively high expression of cytosolic phospholipase A2 and COX-2 (20). Immunohistochemistry analysis of PGIS has been conducted on human lung tissue. Whereas the enzyme is normally ubiquitously expressed throughout the lung, there is a dramatic reduction of expression in the pleomorphic lesions of primary pulmonary hypertension (21). Notably, these lesions are characterized by the monoclonal proliferation of endothelial cells (22).

To investigate potential chemopreventive properties of PGI2 in murine lung carcinogenesis, we generated transgenic mice expressing rat PGIS under control of the human SP-C promoter (23). PGIS, a 52,000, membrane-associated P450-like enzyme, is the final committed enzymatic step in the production of PGI2, occurring at a branch-point where substrate (PGH2) can be directed toward PGI2, thromboxanes, or PGE2. The human SP-C promoter directs expression of transgenes to alveolar type II and Clara cells (24), the progenitors for human and mouse lung adenocarcinomas.
Our strategy was to examine the effects of selectively elevated pulmonary PGIS activities on mouse lung tumorigenesis as a way of determining the role of PG2 in lung cancer chemoprevention. Two distinct carcinogenesis models, a complete carcinogen model (urethane) and an initiation/promotion model (MCA/BHT), were used to assess the generality of this chemoprevention. Transgenic animals with different degrees of transgene expression were used to ensure that the observed results were not secondary to any effects of random transgene insertion and to evaluate the effect of differing levels of PGIS expression. In both tumor models evaluated, PGIS overexpression significantly reduced both lung tumor multiplicity and incidence in a dose-dependent manner. We conclude that prostacyclin can play a key role in preventing lung carcinogenesis.

MATERIALS AND METHODS

Development and Phenotyping of Transgenic PGIS Overexpressors. Transgenic mice were developed using a construct consisting of a human SP-C promoter and full-length rat PGIS cDNA as described previously (23). The SP-C promoter allows targeted expression to alveolar and airway epithelial cells (24). Transgenic mice were genotyped by performing PCR on genomic DNA isolated from tails as described previously (23). Each line was propagated as heterozygotes. Tg− mice were always bred with wild-type FVB/N (Jackson Laboratory, Bar Harbor, ME) mice to produce the experimental Tg+ mice as well as the Tg− littermates, which were used as controls in all of the experiments. For all of the experiments, F1-generation mice were used.

Determination of PGIS Enzyme Capacity and Activity. Phenotype for the established transgenic lines was determined by measuring total pulmonary levels of 6-keto-PGF1α, the stable metabolite of PGF2α. At the time of sacrifice, the lungs of Tg+ and Tg− were insufflated with 1 × Earles Balanced Salts Solution (Sigma Chemical Co.) containing 0.1% BSA followed by tissue homogenization. To measure PGIS enzyme activity, AA (3 μg/ml) was added to the samples to prevent substrate limitation and then diluted 1:3 with methanol. To measure PGIS enzyme activity, the same procedure was used, except that AA was eliminated from the homogenization. 6-Keto-PGF1α levels were determined as described previously by ELISA (25). For all of the experiments, the tumors represented adenomatous neoplasms, some tumors were paraffin embedded and sectioned before staining with H&E.

Carcinogenesis Protocols. FVB/N mice 8–12 weeks of age were maintained on a standard, antioxidant-free laboratory chow (Lab Diet; PMI Nutrition International, St. Louis, MO) and given food and water ad libitum. They were kept on cedar-free bedding with a 12-h light/dark cycle in a climate-controlled animal facility. Animals were subjected to one of the following experimental protocols:

(a) Urethane carcinogenesis: A single urethane (Sigma Chemical Co., St. Louis, MO) dose (1 mg/g mouse weight) dissolved in normal saline, was administered i.p., and animals were sacrificed 14 weeks later;
(b) MCA/BHT carcinogenesis: A single dose of i.p. MCA (15 μg/g mouse weight) was administered followed by eight weekly i.p. doses of BHT (Sigma Chemical Co.; the first dose was 150 μg/g mouse weight, and subsequent doses were 200 μg/g mouse weight) dissolved in corn oil. Mice were sacrificed 20 weeks after the MCA dose; or
(c) Corn Oil: Four weekly i.p. doses of corn oil delivery vehicle were administered and the animals sacrificed 20 weeks after the first dose.

Tumors were enumerated in fresh lungs inflated at a pressure of 15 cm with 10% buffered formalin under a dissection microscope (×5 magnification). All of the tumors were dissected from the lung parenchyma. To ensure that all of the tumors represented adenomatous neoplasms, some tumors were paraffin embedded and sectioned before staining with H&E.

6-Keto PGF1α and PGE2 Assays to Determine the Balance of PGIS and PGE2 Synthase Activity. To determine the relative production of both 6-keto PGF1α and PGE2 in the lungs of experimental animals, animals had 6-keto PGF1α and PGE2 levels determined at the conclusion of the carcinogenesis protocols. Lung homogenates were prepared as above, both with the addition of AA (3 μg/ml) to measure enzyme capacity and without AA to measure in vivo activity. Determination of pulmonary 6-keto PGF1α and PGE2 levels by ELISA was performed as described previously (25). The assays were performed in a blinded fashion using coded sample tubes.

BHT Induction of Pulmonary Inflammation. To determine whether there existed a difference in the inflammatory response to BHT between Tg− and Tg+, the high-expressing Tg+ mice (with a >2.5-fold increase in lung PGIS activity; Fig. 1) and Tg− were exposed to an inflammation-inducing insult. Tg− and Tg+ littermates, 8–12 weeks of age, underwent an i.p. injection of BHT (either 150 or 200 μg/g mouse weight); controls were injected with the corn oil vehicle alone. Cell counts, differentials, and protein measurement were performed on the lungs which survived after the BHT injections. Surviving mice had bronchoalveolar lavage performed 3 or 5 days after BHT treatment. Tracheal intubation with a 24-gauge Angiocatheter was performed, and three consecutive 1-ml aliquots of normal saline (0.9% NaCl) were instilled into the lungs and then removed. Protein concentrations were determined from the first lavage aliquot (26). The cells were pooled from all of the aliquots to yield final cell counts and differentials.

Statistical Analysis. All of the values were expressed as means ± SE. For tumor multiplicity, cell counts, and PGIS mRNA levels, the data were normally distributed, and unpaired t tests were performed using GraphPad Prism 2.01 for Windows 95 (GraphPad Software for Science Inc., San Diego, CA). For tumor incidence, GraphPad was used to perform Fisher’s exact test. For simultaneous 6-keto PGF1α and PGE2 level determinations, Pearson r correlation coefficients were calculated. Data were considered significant at the P < 0.05 level.

RESULTS

Two Distinct Transgenic Lines with Different PGIS Expression Levels Were Investigated. Five transgenic lines were successfully established using the FVB/N strain (23). The transgene was passed to offspring following Mendelian rules. For our studies two specific lines were used, representing low- and high-expressing lines as determined by pulmonary 6-keto PGF1α levels (as below) and PGIS mRNA on Northern analysis (data and methods published previously in Ref. 23). The low-expressing Tg− line exhibits a 50% increase in pulmonary 6-keto PGF1α levels compared with Tg− littermates [2104 ± 195.7 ng/g tissue (n = 16) versus 1431 ± 182 ng/g tissue (n = 20); *P < 0.05]. The highest expressing line exhibits a >2.5 fold increase in lung 6-keto PGF1α [3107 ± 608 ng/g tissue (n = 10) versus 1159 ± 277 ng/g tissue (n = 11); **P = 0.01; Fig. 1].
A PGIS Capacity Correlates with in Vivo PGIS Activity. Excess AA was added to samples to measure the PGIS enzyme capacity of Tg<sup>+</sup> and Tg<sup>-</sup> littermates. To determine whether this enzyme capacity correlates with the in vivo PGIS activity of Tg<sup>+</sup> and Tg<sup>-</sup>, eicosanoid levels from 5 Tg<sup>+</sup> and 5 Tg<sup>-</sup> were assayed for 6-keto-PGF<sub>1α</sub> levels both with and without the addition of AA (3 μg/ml). Without the addition of AA (a measure of in vivo PGIS activity), Tg<sup>+</sup> demonstrate increased production of lung 6-keto PGF<sub>1α</sub> compared with Tg<sup>-</sup> [2080 ± 204 ng/g tissue (n = 5) versus 731 ± 241 ng/g tissue (n = 5); P < 0.005, data not shown]. The addition of AA showed the increased PGIS capacity of the Tg<sup>+</sup> over the Tg<sup>-</sup> [3930 ± 373 ng/g tissue (n = 5) versus 1222 ± 382 ng/g tissue (n = 5); P = 0.001, data not shown].

PGIS-overexpressing Mice Develop Fewer Lung Tumors. Transgenic overexpression of PGIS significantly decreased tumor multiplicity in both carcinogenesis models. Fig. 2 illustrates the gross (Fig. 2A) and microscopic (Fig. 2B) appearance of the tumors found in the animals at the time of sacrifice. The microscopic appearance (Fig. 2B) is characteristic of the adenomas produced by both the urethane and the MCA/BHT protocols. In urethane-treated mice, PGIS overexpression significantly decreased tumor multiplicity (Fig. 3A). Tg<sup>+</sup> mice expressing low levels of PGIS exhibited a 50% reduction in urethane-induced tumor multiplicity (3.4 ± 0.4 versus 6.8 ± 0.6 tumors/mouse; *P < 0.0001; Fig. 3A) and a 66% reduction in the MCA/BHT model (2.5 ± 0.7 versus 7.5 ± 0.5 tumors/mouse; *P < 0.001; Fig. 3B). Untreated mice (both Tg<sup>-</sup> and Tg<sup>+</sup>), receiving either the corn oil delivery vehicle or normal saline without carcinogen, failed to develop tumors.

Higher Levels of PGIS Expression Afford Greater Chemoprotection in Both Carcinogenesis Models. Tg<sup>+</sup> mice expressing the highest levels of PGIS exhibited even greater chemoprotection than the low expressing line, demonstrating an 85% reduction in urethane-induced tumor multiplicity compared with Tg<sup>-</sup> littermates (0.8 ± 0.14 versus 5.2 ± 0.69 tumors/mouse; **P < 0.0001; Fig. 3A). Similarly, in the MCA/BHT protocol, the highest expressing animals showed a more marked reduction in tumor multiplicity, yielding a 92% reduction in tumor multiplicity compared with transgene negative littermates (0.4 ± 0.4 versus 5.2 ± 1.2 tumors/mouse; **P < 0.01; Fig. 3B).

Transgenic Mice with the Highest PGIS Expression Had a Decreased Incidence of Lung Tumors. Whereas all of the lower expressing Tg<sup>+</sup> animals and their Tg<sup>-</sup> littermates developed tumors with both protocols (incidence of 100%), the highest expressing Tg<sup>+</sup> mice demonstrate a reduction in tumor incidence. For the urethane protocol, lung tumor incidence was greatly decreased in the high-expressing mice, with 44% (8 of 18) of the Tg<sup>+</sup> mice remaining tumor free as compared with the 100% tumor incidence in Tg<sup>-</sup> littermates (P = 0.01; Fisher’s exact test). The individual data are shown in Fig. 4.

Chemoprotection in the PGIS Overexpressors Is Not Solely Attributable to Alterations in PGE<sub>2</sub> Levels. The beneficial effects of PGIS overexpression could be the result of either higher levels of PGL<sub>2</sub> or lower levels of PGE<sub>2</sub> as a result of depletion of the substrate PGH<sub>2</sub>. To investigate whether, at the time of sacrifice, PGIS overexpression shifted the balance of PGH<sub>2</sub> metabolism in favor of PGL<sub>2</sub> and away from PGE<sub>2</sub> (a “steal” phenomenon), we determined the pulmonary levels of these metabolites. Baseline 6-keto-PGF<sub>1α</sub> levels were determined for both transgenic lines (Fig. 1). In addition, at the termination of both carcinogenesis protocols, simultaneous 6-keto-PGF<sub>1α</sub> and PGE<sub>2</sub> levels were determined for both Tg<sup>+</sup> and Tg<sup>-</sup> animals. In both carcinogenesis protocols, the significant elevations in 6-keto-PGF<sub>1α</sub> over baseline persisted at the time of sacrifice, with the same ratios of elevation (i.e., the low-expressing animals had a 50% increase and the high-expressing animals had a >250% increase in 6-keto-PGF<sub>1α</sub>; individual data in Fig. 5, A and B). The MCA/BHT protocol showed no evidence of a steal phenomenon (Fig. 5A). Specifically, in the lower expressing line there were no significant differences between the Tg<sup>+</sup> (n = 8) and Tg<sup>-</sup> (n = 10) when PGE<sub>2</sub> levels were compared (162 versus 131 ng/g lung tissue; P = 0.52). The elevations in 6-keto-PGF<sub>1α</sub> were maintained, as Tg<sup>+</sup> (n = 8) demonstrated higher levels than Tg<sup>-</sup> (n = 10; 1665.6 versus 1325 ng/g lung tissue; P < 0.05). However, in the highest expressing line, animals treated with urethane showed differences between Tg<sup>+</sup> and Tg<sup>-</sup> in regard to PGE<sub>2</sub> levels. Tg<sup>+</sup> (n = 17) demonstrated the anticipated higher 6-keto-PGF<sub>1α</sub> levels than the Tg<sup>-</sup> (n = 11; 6092 versus 2014 ng/g lung tissue; P < 0.0001). The Tg<sup>-</sup> (n = 17) displayed lower PGE<sub>2</sub> levels than their Tg<sup>-</sup> (n = 11) littermates (97 versus 255 ng/g lung tissue; P < 0.0001). The individual data are shown in Fig. 5B. The inverse relationship (negative correlation) in Tg<sup>-</sup> animals between 6-keto-PGF<sub>1α</sub> and PGE<sub>2</sub> for the urethane-treated mice is significant (Pearson test, r = −0.63 at sacrifice; P = 0.037).
after a single exposure to urethane, low-expressing transgenic animals \((n = 22)\) demonstrated a 50% reduction in tumor number compared with transgene negative littermates \((n = 17); 3.4 \text{ versus } 6.8 \text{ tumors/mouse}; **P < 0.0001\). High-expressing transgenic animals \((n = 18)\) showed an 85% reduction in urethane-induced tumor multiplicity compared with transgene negative littermates \((n = 11); 0.8 \text{ versus } 5.2 \text{ tumors/mouse}; ***P < 0.0001\). After an initiation/promotion protocol, low-expressing transgenic mice \((n = 8)\) have a 66% reduction in tumor number compared with Tg\(^{-}\) littermates \((n = 11); 2.35 \text{ versus } 7.5 \text{ tumors/mouse}; *P < 0.0001\). High-expressing transgenic animals \((n = 5)\) demonstrated a 92% reduction in tumor multiplicity compared with Tg\(^{-}\) littermates \((n = 6); 0.4 \text{ versus } 5.2 \text{ tumors/mouse}; **P < 0.01; bars, ±SD\).

**Pulmonary PGIS Overexpressors Exhibit a Survival Advantage after BHT Exposure and Have Significantly Elevated Cell Counts on Day +5 Bronchoalveolar Lavage.** To ascertain whether PGIS overexpression affected the inflammatory response to BHT, we quantitated alveolar cellular composition in the highest expressing transgenic line after two differing doses of BHT. When exposed to i.p. BHT at a dose of 200 mg/kg, all of the high-expressing transgenic animals \((n = 22)\) demonstrated a 50% reduction in tumor number compared with transgene negative littermates \((n = 17); 3.4 \text{ versus } 6.8 \text{ tumors/mouse}; *P < 0.0001\). High-expressing transgenic animals \((n = 18)\) showed an 85% reduction in urethane-induced tumor multiplicity compared with transgene negative littermates \((n = 11); 0.8 \text{ versus } 5.2 \text{ tumors/mouse}; ***P < 0.0001\). After an initiation/promotion protocol, low-expressing transgenic mice \((n = 8)\) have a 66% reduction in tumor number compared with Tg\(^{-}\) littermates \((n = 11); 2.35 \text{ versus } 7.5 \text{ tumors/mouse}; *P < 0.0001\). High-expressing transgenic animals \((n = 5)\) demonstrated a 92% reduction in tumor multiplicity compared with Tg\(^{-}\) littermates \((n = 6); 0.4 \text{ versus } 5.2 \text{ tumors/mouse}; **P < 0.01; bars, ±SD\).

When the dose of BHT was lowered to 150 mg/kg, all of the Tg\(^{-}\) littermates developed tumors \((7.62 \times 10^6; P < 0.05; \text{Fig. } 6)\). Transgenics and Tg\(^{-}\) littermates receiving corn oil alone had no significant differences in cell counts on day +5 \((9.91 \times 10^7 \text{ versus } 1.59 \times 10^8; P = \text{ns}; \text{data not shown})\). Higher cell counts were associated with higher protein levels in all of the animals studied \((\text{Pearson } r = 0.5; P < 0.05)\).

**DISCUSSION**

Pulmonary specific overexpression of PGIS significantly decreases the incidence and multiplicity of murine lung tumors in a dose-dependent fashion. Animals with higher PGIS activity, as evidenced by elevated pulmonary levels of 6-keto PGF\(_{1\alpha}\), were protected from developing tumors in both a single carcinogen model (urethane) and an initiation/promotion model (MCA/BHT). To our knowledge, these are the first studies in transgenic animals to show chemoprevention of lung tumors. These results support the hypothesis that prostacyclin plays a key role in preventing lung carcinogenesis.

Currently, PG\(_I\)\(_2\) can be administered by either continuous i.v. infusion or intermittent inhalation, but the short half-life of PG\(_I\)\(_2\) and difficulties controlling tissue levels have prevented animal studies testing PG\(_I\)\(_2\) as a cancer chemopreventive agent. To overcome drug delivery problems, transgenic mice were developed with pulmonary-specific overexpression of PGIS. To investigate the generality of the PGIS overexpression effect, we used two distinct carcinogenesis protocols. In the first model, urethane (ethyl carbamate), a complete carcinogen that induces pulmonary adenomas \((27)\), was administered. In an initiation/promotion model, MCA, a polycyclic aromatic hydrocarbon found in tobacco smoke that exhibits dose-dependent initiation of murine lung tumors \((28)\), was given followed by multiple weekly treatments with BHT. BHT is a tumor promoter that induces reversible pulmonary damage characterized by alveolar type I cell necrosis, selective pulmonary inflammation, and hyperplasia of alveolar type II cells \((29, 30)\). For both carcinogenesis protocols, we sought to elucidate potential mechanisms for the observed protective effect of PGIS overexpression. Initially, we examined two different lines of transgenic mice with varying levels of PGIS expression to delineate whether higher levels of PGIS expression afforded more chemoprotection. We sought to define alterations in the production of other eicosanoids, namely PGE2, which is known to be pivotal in colon carcinogenesis. We also investigated potential differences in the pulmonary inflammatory response to BHT.

Chemoprotection by PGIS overexpression in distinct carcinogenesis models demonstrates the generality of this prevention. The MCA/BHT protocol is generally accepted as an initiation/promotion model,
PGE\textsubscript{2} levels may be a mechanism for decreased lung cancer incidence after chronic administration of COX inhibitors (33). For the urethane-treated animals, we found a marked steal phenomenon with Tg\textsuperscript{+} mice exhibiting elevated 6-keto PGF\textsubscript{1\alpha} levels and lower PGE\textsubscript{2} levels than Tg\textsuperscript{-} mice. However, we found no difference in the pulmonary PGE\textsubscript{2} levels between Tg\textsuperscript{+} and Tg\textsuperscript{-} mice after the MCA/BHT protocol. The observed chemoprotection despite the lack of differences in the PGE\textsubscript{2} levels in the MCA/BHT model implies that alterations in PGE\textsubscript{2} are not the sole explanation for the chemoprotection. Therefore, lower PGE\textsubscript{2} levels do not represent a necessary condition for protection. This fact, and the observation of greater protection with higher PGI\textsubscript{2} levels, implies that the protective effects are at least partially mediated by elevations of PGI\textsubscript{2}.

The COX enzymes produce substrate for the production of multiple PGs, which we speculate have distinct and perhaps opposing effects on tumorigenesis. COX inhibition can ablate downstream PG production and produce a net antitumorigenic result. Our data suggests that, from a therapeutic standpoint, inhibiting tumorigenic PGs while augmenting antitumorigenic PGs may represent an important strategy for chemoprevention.

Chronic inflammation likely plays a critical role in promoting lung carcinogenesis and may account for decreased lung cancer rates associated with anti-inflammatory drug use (9). To determine whether the anti-inflammatory effects of PGI\textsubscript{2} could explain the observed differences in tumor numbers, BHT was administered to Tg\textsuperscript{+} and Tg\textsuperscript{-} mice. BHT is a widely used food additive, which is converted through cytochrome p450 metabolism to an oxidative species inducing pneumotoxicity (34). A significant survival advantage was afforded to the PGIS overexpressors. For the highest PGIS expressors, all of the Tg\textsuperscript{+} survived, and all of the Tg\textsuperscript{-} died. However, the bronchoalveolar lavage yielded unexpected results, with the transgenic animals actually demonstrating augmented inflammatory cell infiltrate and protein leak in response to BHT. Differentials performed on the cells were not statistically different between the Tg\textsuperscript{+} and Tg\textsuperscript{-} animals, showing an overwhelming predominance of alveolar macrophages (>95%), few lymphocytes (1–2%), and rare bronchial epithelial cells (~1%). Therefore, PGIS overexpression results in an augmented acute inflammatory response to BHT, as measured by alveolar macrophage numbers and protein leak, yet it is associated with increased survival. The increased macrophage numbers may also play a critical role in our

relying on the induction of pulmonary inflammation as part of the carcinogenesis process. In distinction to this model, urethane acts as a simple carcinogen and is given in a single dose. Because these two protocols induce tumor production by differing mechanisms, the fact that PGIS overexpression provides protection in both protocols argues that overexpression of PGIS has a more general beneficial effect in preventing lung carcinogenesis.

The highest expressing Tg\textsuperscript{+} mice were protected to a much greater extent than the low-expressing mice. These data show a clear dose-response relationship between the PGIS enzyme activity (50\% versus >250\% increase) and the greater reduction in tumor number in both protocols. Indeed, only in the highest expressing line does true chemoprevention (decrease in tumor incidence) occur. The fact that both lines show a reduction in tumorigenesis argues strongly against these protective effects being solely attributable to mutation at the site of transgene insertion.

Beneficial effects of PGIS overexpression could include either increased PGI\textsubscript{2} levels or decreased PGE\textsubscript{2} levels, which might occur through a steal phenomenon, whereby elevated PGIS preferentially consumes substrate (PGH\textsubscript{2}) and decreases the amount of procarcinogenic eicosanoids such as PGE\textsubscript{2}. PGE\textsubscript{2} plays a critical role in colon carcinogenesis where nonsteroidal anti-inflammatory drugs that decrease PGE\textsubscript{2} levels decrease colorectal polyp burden (size and number) and have induced polyp regression in familial adenomatous polyposis coli syndrome (31). PGE\textsubscript{2} blocks the immune regulation of tumor growth (32), and decreased PGE\textsubscript{2} levels may be a mechanism for decreased lung cancer incidence after chronic administration of COX inhibitors (33).
initiation/promotion tumor model where multiple doses of BHT are administered.

Several lines of evidence suggest that the effects of PG12 are mediated by PG12 activation of the nuclear hormone receptor PPAR, demonstrating the first reported biological function of this receptor signaling pathway (35). The PPARs are ligand-activated transcription factors that are members of the nuclear hormone receptor superfamily. Three distinct isoforms (α, δ, and γ) have been isolated and characterized (36). Recently, Lim et al. (37) presented evidence that PG12 was not signaling through the G-coupled membrane PG12 receptor, PGIR. COX-2-deficient mice demonstrate multiple reproductive failures including a defect in embryo implantation (37). The major PG subtype produced at the implantation site is PG12. High levels of PG12 are produced at the implantation site but no PGIR. Furthermore, administration of PG12 rescues the implantation defect as does intermittent inhaled PG12 (43) to treat pulmonary hypertension, could be coupled with the current safe use of continuous IV (41, 42) or animal models before pursuing large human interventional trials (40).

PG12 mediated PPAR activation reinforces the desirability for preliminary support in chemoprevention. Our findings suggest that manipulation of the AA pathway strategy, our findings suggest that manipulation of the AA pathway intervention/promotion tumor model where multiple doses of BHT are administered.


Manipulation of Pulmonary Prostacyclin Synthase Expression Prevents Murine Lung Cancer

Robert L. Keith, York E. Miller, Yasushi Hoshikawa, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/3/734

Cited articles
This article cites 41 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/3/734.full#ref-list-1

Citing articles
This article has been cited by 24 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/62/3/734.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.