# Prostacyclin Prevents Murine Lung Cancer Independent of the Membrane Receptor by Activation of Peroxisomal Proliferator–Activated Receptor γ

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**Abstract** Overexpression of prostacyclin synthase (PGIS) decreases lung tumor multiplicity in chemical- and cigarette-smoke–induced murine lung cancer models. Prostacyclin signals through a single G-protein–coupled receptor (IP), which signals through cyclic AMP. To determine the role of this receptor in lung cancer chemoprevention by prostacyclin, PGIS-overexpressing mice were crossed to mice that lack the IP receptor [IP(–/–)]. Carcinogen-induced lung tumor incidence was similar in IP(+/+), IP(+/–), and IP(–/–) mice, and overexpression of PGIS gave equal protection in all three groups, indicating that the protective effects of prostacyclin are not mediated through activation of IP. Because prostacyclin can activate members of the peroxisomal proliferator–activated receptor (PPAR) family of nuclear receptors, we examined the role of PPARγ in the protection of prostacyclin against lung tumorigenesis. Iloprost, a stable prostacyclin analogue, activated PPARγ in nontransformed bronchial epithelial cells and in a subset of human non–small-cell lung cancer cell lines. Iloprost-impregnated chow fed to wild-type mice resulted in elevated lung macrophages and decreased lung tumor formation. Transgenic animals with lung-specific PPARγ overexpression also developed fewer lung tumors. This reduction was not enhanced by administration of supplemental iloprost. These studies indicate that PPARγ is a critical target for prostacyclin-mediated lung cancer chemoprevention and may also have therapeutic activity.

In the United States, lung cancer continues to be the leading cause of cancer death in both men and women, and worldwide the lung cancer epidemic will result in millions of cases yearly (1). Whereas tobacco abstinence and smoking cessation are the critical first steps in reducing lung cancer rates, the majority of U.S. lung cancers are diagnosed in former smokers. To date, no effective chemopreventive agents have been discovered. The large at-risk population (current and former smokers) and poor 5-year lung cancer survival rates (2) underscore the need for a better understanding of chemopreventive mechanisms and effective agents.

Prostaglandins play an important role in lung tumorigenesis, and prostaglandin manipulation has been investigated for lung cancer chemoprevention. Cyclooxygenase (COX) inhibition decreases levels of prostaglandins, and large epidemiologic surveys have shown fewer lung cancers in "frequent aspirin users" (3). Human trials evaluating COX inhibition and lung cancer chemoprevention are currently being conducted, but conflicting data in murine studies evaluating the role of nonspecific COX or selective COX-2 inhibition make interpretation of these results difficult. Studies using nonselective COX inhibitors have shown inhibition of lung tumorigenesis in mice (4, 5). However, mice receiving celecoxib (a selective COX-2 inhibitor) showed reduced pulmonary inflammation, but no differences in tumor multiplicity, and an actual increase in tumor size after exposure to an initiator-promoter model of lung tumorigenesis (6). In addition, studies showing excess cardiovascular events associated with chronic COX-2 inhibitor use raise concerns about long-term safety (7, 8). One potential cause for these effects may be lower levels of COX-2–mediated production of prostacyclin, which has been shown to have atheroprotective properties in female mice (9). Additionally, a recent report showed that dysfunctional

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Received 07/18/2008; accepted 07/28/2008.

**Grant support:** NIH grants CA103618, CA108610, and CA58187. R.L. Keith was supported by VA Advanced Career Development and Merit Review Awards from the Department of Veterans Affairs.
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doi:10.1158/1940-6207.CAPR-08-0145

prostacyclin receptor (IP)–mediated signaling (defective adenyl cyclase activation) promoted increased platelet aggregation and a predisposition to atherothrombosis (10).

COX activity generates numerous downstream mediators (including prostaglandins, prostacyclin, and thromboxanes) that can have protumorigenic or antitumorigenic effects (11). Our laboratory has focused on manipulating prostaglandin production distal to the COX enzymes. Prostacyclin ($PGI_2$) is a downstream metabolite of COX activity with anti-inflammatory and platelet inhibitory properties (12). It has also been shown to prevent metastases (13) and inhibit the growth of established micrometastases (14). Previously, we showed that selective pulmonary overexpression of prostacyclin synthase (PGIS) increases pulmonary prostacyclin and prevents lung tumor formation in response to either chemical carcinogenesis or exposure to tobacco smoke in mice (15, 16).

A single receptor for $PGI_2$ has been described (IP/PGIR), which is a member of the G-protein–coupled receptor family, containing seven membrane-spanning domains (17). $PGI_2$ binding to the IP receptor activates $G_s$ and stimulates adenylyl cyclase, leading to increases in intracellular cyclic AMP (cAMP). $PGI_2$ analogues, such as iloprost, have been shown to bind the IP receptor and increase cAMP levels (18). Studies using IP receptor knockout (KO) mice suggest that activation of the receptor mediates inflammation, particularly edema and pain, vascular homeostasis, and thrombosis prevention (for review, see ref. 19). Reports have also shown that $PGI_2$ and its analogues are ligands for peroxisomal proliferator–activated receptors (PPAR), which are members of the nuclear receptor superfamily of regulated transcription factors (20). The purpose of this study was to determine the contribution of IP versus PPARs in mediating the antitumorigenic effects of $PGI_2$. Our experiments show that prostacyclin-mediated lung cancer chemoprevention is independent of the IP receptor and may be secondary to activation of PPARγ. Manipulation of prostaglandin production distal to COX and/or direct activation of PPARγ may represent attractive lung cancer chemopreventive strategies.

## Materials and Methods

### Development of transgenic PGIS overexpressors/IP receptor knockouts

As previously described, PGIS-transgenic mice were developed using a construct consisting of the human surfactant protein-C promoter and full-length rat PGIS cDNA (21). The surfactant protein-C promoter allows targeted expression to alveolar and airway epithelial cells (22). Transgenic mice were genotyped by performing PCR on genomic DNA isolated from tails as previously described (21). IP receptor KO mice were a generous gift of S. Narumiya and were originally developed in the C57BL/6 background (23). The IP KO trait was successfully transferred from the C57BL/6 background to the FVB/N background by completing 10 generations of backcrosses. FVB/N IP KO mice were then bred with PGIS overexpressors to create the experimental animals (PGIS OE/IP KO mice). Animals were bred at the Denver VA Animal Facility. All procedures were done with Institutional Animal Care and Use Committee–approved protocols at the University of Colorado Health Sciences Center and the Denver Veterans Affairs Medical Center.

### Development of PPARγ-overexpressing animals

Animals with lung-specific PPARγ overexpression were created to examine the role of PPARγ in lung tumorigenesis. The 1.5-kb human wild-type (WT) PPARγ1 was ligated into a construct containing the human surfactant protein-C promoter and the SV40 small T intron and poly(A) region (a generous gift of Dr. Jeffrey Whitsett, Department of Pediatrics, University of Cincinnati, Cincinnati, Ohio). Transgenic mice were made at the University of Cincinnati transgenic facility as previously described (21). Individual founders in the FVB strain were propagated and genotyped by PCR using primers for the small T intron to indicate a PPARγ OE transgenic mouse. Animals were bred at the University of Colorado Health Sciences Center animal facility.

### Type II cell isolation and cAMP determination

Murine type II alveolar cells were isolated from IP KO and WT animals to confirm that IP membrane receptor activity had been abolished. Lungs were isolated and perfused with 0.9% saline via the pulmonary artery until thorough blanching had occurred. The type II cells were isolated as previously reported (24). Briefly, the cell suspension was added to antibody plates (CD 45 and CD 32) that had been prewashed twice with DMEM/HEPES and were incubated for 2 h at 37°C in 5% $CO_2$. Following incubation, medium was gently removed from plates and centrifuged (8 min, 130 × *g*). Freshly isolated type II cells from a single mouse lung were resuspended in medium containing 10% FCS with 100 μmol/L I-methyl-3-isobutylxanthine (Sigma Chemical Co.) ± 5 μmol/L iloprost. The cells were incubated at 37°C for 45 min and then washed thrice quickly with cold PBS. The cell pellet was then lysed in cold 0.1 mol/L HCl. Cellular cAMP content was measured using the direct cAMP kit from Assay Designs. Results are reported as picomoles of cAMP per milligram of protein.

### Immunoblotting

Freshly isolated type II were lysed in cold radioimmunoprecipitation assay buffer (10 mmol/L Tris-HCl, 150 mmol/L NaCl, 1% Na-DOC, 1% Triton X-100, 0.1% SDS) containing protease inhibitors, centrifuged at 10,000 × *g* for 5 min, and the supernatant was saved. Protein was quantitated with Bio-Rad Protein Assay reagent (Bio-Rad). Protein samples were run on precast 4% to 20% gradient Tris-HCl gels (Bio-Rad). PPARγ antibody was from Cayman Chemical Co. Blots were visualized by enhanced chemiluminescence, and changes in expression normalized to β-actin expression were determined by densitometry using at least three independent experiments.

### Carcinogenesis protocol

FVB/N mice (PGIS OE/IP KO, PPARγ OE, and WT controls), 8 to 12 weeks of age, were maintained on a standard, antioxidant-free laboratory chow (Lab Diet, PMI Nutrition International) and given food and water *ad libitum*. They were kept on cedar-free bedding with a 12-h light/dark cycle in climate controlled animal facilities. Animals were subjected to a single i.p. injection of ethyl carbamate (Sigma Chemical) at a dose of 1 mg/g of mouse weight dissolved in normal saline. Animals were sacrificed 20 wk later via pentobarbital overdose. Lungs were removed, and tumors were counted and dissected from surrounding tissue and their diameters measured with a dissection microscope (×5 magnification).

### Iloprost chemoprevention

To determine if the chemoprevention observed in the transgenic PGIS overexpressor could be replicated with an oral prostacyclin analogue, animals were fed antioxidant-free chow (AIN-76A, Test Diet) containing 3% ground iloprost tablets (a gift of Schering AG). Iloprost clathrate tablets contain 0.38 mg of iloprost per 65-mg tablet. The iloprost chow was started 1 wk before the urethane treatment and animals were sacrificed 20 wk after urethane exposure. For experiments with the PPARγ OE mice, some animals were also started on iloprost chow 5 wk after urethane administration.

### Iloprost activation of PPAR response element

RL-65 cells, a normal, immortalized rat lung epithelial cell line (25), were obtained from American Type Culture Collection. Lung cancer

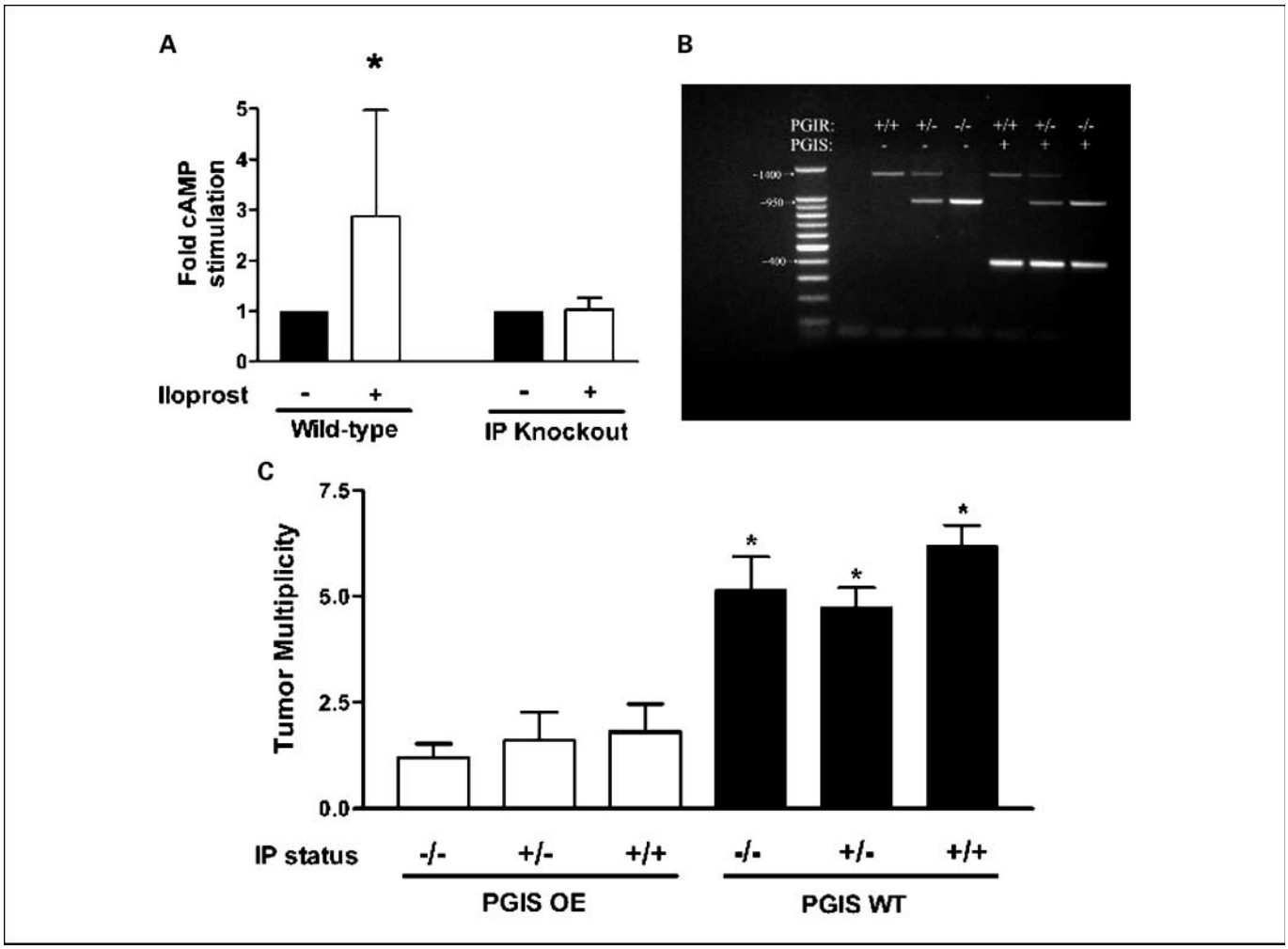


**Fig. 1.** IP receptor status does not effect PGIS-mediated lung tumor prevention. *A*, intracellular cAMP content was measured in type II cells isolated from WT or IP receptor KO mice that were stimulated with iloprost (*white columns*) or vehicle control (*black columns*). cAMP content was only increased in WT mice stimulated with iloprost. *, $P < 0.05$, versus control. *B*, agarose gel electrophoresis of the PCR products for PGIS transgene (400-bp band) and IP receptor KO (PGIR; 1,400-bp WT band and/or 950-bp KO band). *C*, the indicated genetic mice were injected with ethyl carbamate at 1 mg/g of mouse weight. Twenty weeks later, mice were sacrificed and lung tumors counted. PGIS mice developed significantly fewer lung tumors regardless of IP receptor status. *, $P < 0.05$.

cell lines (A549 and H661) were obtained from the University of Colorado Cancer Center. Cells were transiently transfected using Lipofectamine with constructs encoding a PPARγ response element (PPARγ-RE) plasmid or PPARδ response element plasmid (PPARδ-RE; ref. 26), each linked to a luciferase reporter, along with a plasmid encoding β-galactosidase (β-gal) under the control of the cytomegalovirus promoter to normalize for transfection efficiency. Following transfection, cells were incubated overnight and then stimulated with 10 μmol/L iloprost or vehicle control (0.1% DMSO) for an additional 24 h. In a second set of experiments, following transfection, cells were stimulated with 10 μmol/L iloprost, 10 μmol/L of the PPARγ inhibitor T0070907 (Cayman Chemical), or both agents. Cells were harvested, washed, and resuspended in Luciferase Reporter Lysis Buffer (Promega). Cell lysates were centrifuged and analyzed for luciferase and β-gal activities. PPAR-RE activity was normalized to β-gal and presented as relative light units per milliunit of β-gal.

### Macrophage immunohistochemistry

Lungs from iloprost- and control chow–fed animals were evaluated for the presence of macrophages to determine if iloprost altered the number of inflammatory cells. For immunohistochemistry, whole lungs were formalin fixed and paraffin embedded. Paraffin sections were deparaffinized in xylene, rehydrated through a graded ethanol series, and underwent antigen retrieval (antigen unmasking solution; Vector) by heating for 20 min at 125°C in a decloaking chamber (Biocare). Sections were then blocked in 3% goat serum and exposed to rabbit anti-F4/80 (1:100) overnight at 4°C. Antigen/antibody complexes were visualized using kits from Vector Laboratories, and sections lightly counterstained with hematoxylin. Sections were visualized using an Olympus light microscope equipped with SPOT software.

### Statistical analysis

Data are presented as mean ± SE. Differences between groups were identified using the Student unpaired *t* test. One-way ANOVA was used to compare more than two groups, and post hoc Newman-Keul's test was used to identify differences between groups. Under all circumstances, $P < 0.05$ was considered to be significant.

## Results

### Confirmation of PGIS OE/IP KO mouse genotype and phenotype

To examine whether the IP receptor is required for the protective effect of overexpression of PGIS, we created a mouse

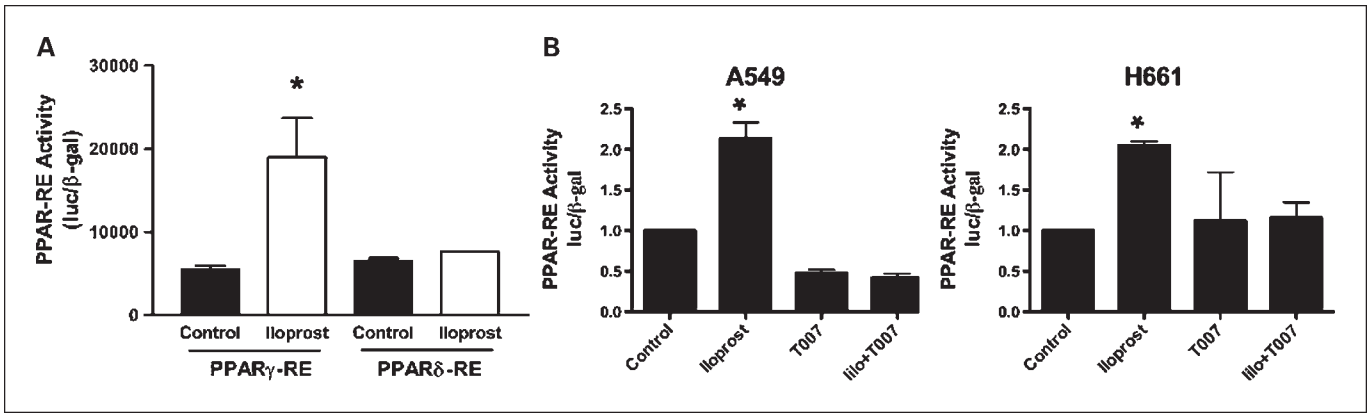


**Fig. 2.** Iloprost treatment drives PPARγ activity *in vitro. A,* RL-65 cells were transiently transfected with a PPARγ-RE/luciferase or PPARδ-RE/luciferase construct along with a plasmid encoding β-gal under the control of the cytomegalovirus promoter to normalize for transfection efficiency. Following transfection, cells were incubated overnight and then stimulated with 10 μmol/L iloprost or vehicle (*Control*) for an additional 24 h. Cells were harvested and PPAR-RE activity normalized to β-gal was determined. *, $P < 0.05$, versus control. *B,* A549 and H661 cells were transiently transfected with the PPARγ-RE reporter construct along with a plasmid encoding β-gal. After an overnight incubation, cells were stimulated with 10 μmol/L iloprost (*Ilo*), 10 μmol/L of the PPARγ inhibitor T0070907 (*T007*), or both agents. Cells were harvested after 24 h and PPARγ-RE normalized to β-gal was determined. *, $P < 0.05$, versus control and T0070907-treated cells.

that overexpressed PGIS and lacked the IP receptor in a congenic FVB/N strain. To transfer the IP KO strain from the C57BL/6 to the FVB/N background, 10 generations of backcrosses were completed. To confirm that the IP KO animals lacked functional cell membrane IP activity, we isolated type II cells from IP KO and WT mice. These type II cells were then exposed to exogenous iloprost, and cell lysates were examined for changes in cAMP levels (Fig. 1A). Type II cells from WT mice showed a 3-fold increase in cAMP levels in response to iloprost, whereas IP KO mice failed to show any significant increase, consistent with a lack of a functional IP receptor.

### PGIS overexpression–mediated prevention of lung tumor formation is independent of membrane IP receptor

PGIS OE/IP KO mice were generated by breeding heterozygote PGIS animals with IP KO animals. PCR-based genotyping of the resultant animals is illustrated in Fig. 1B with the

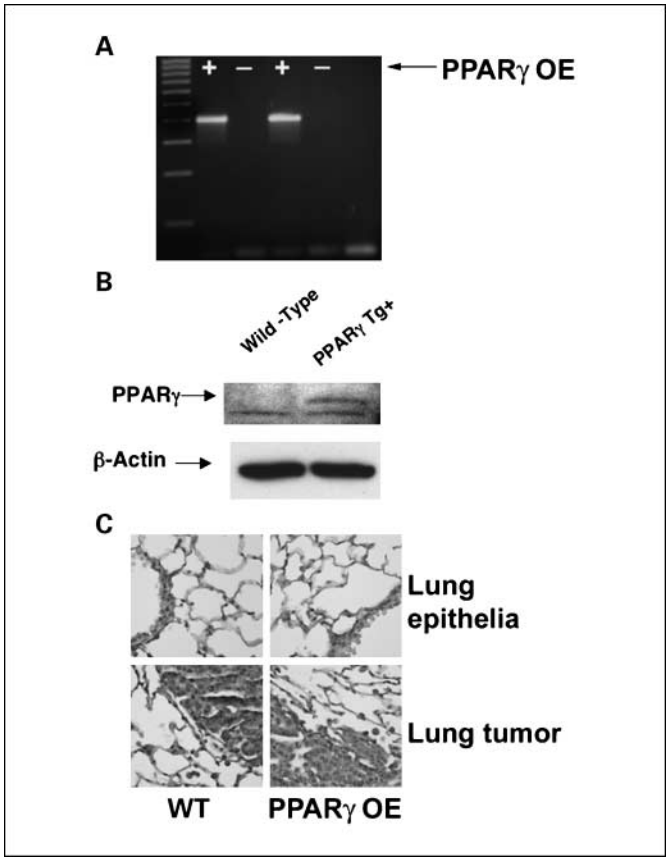


**Fig. 3.** Characterization of PPARγ OE transgenic mice. *A,* agarose gel electrophoresis of the PCR product for the PPARγ transgene (400-bp band) indicating transgene-positive mice. *B,* Western blot of isolated type II cells from PPARγ OE transgenic mice (*PPARγ Tg+*) and WT littermates confirms increased expression of PPARγ (50 kDa) in the overexpressing mice. *C,* representative hematoxylin-stained lung tissue samples from WT and PPARγ transgenic positive (PPARγ OE) mice. Specimens show that PPARγ OE mouse lungs display no anatomic differences and the tumors that develop are similar to those in WT mice (magnification, ×40). Tumors from the animals exposed to urethane also show no differences.

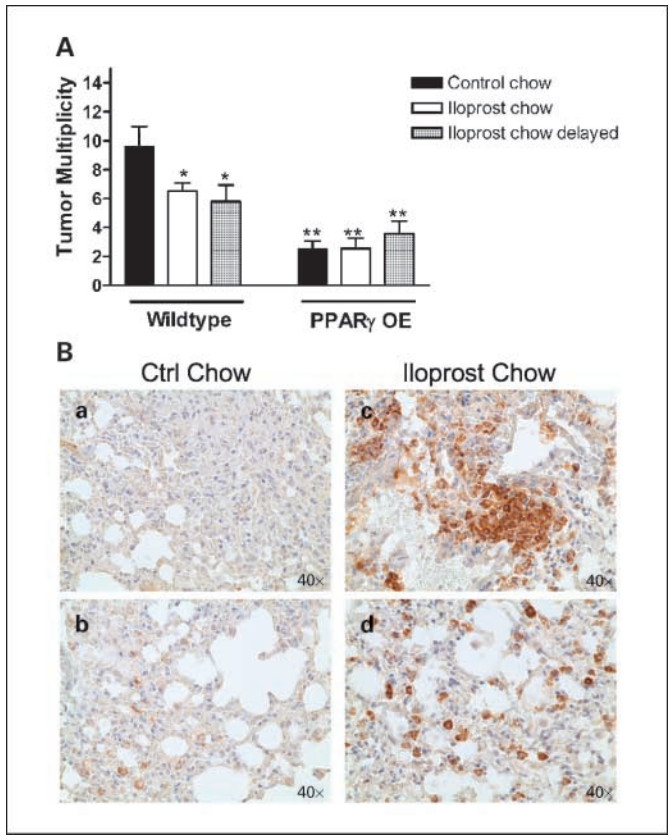


**Fig. 4.** Mice fed iloprost and PPARγ OE transgenic mice develop fewer lung tumors than WT controls. *A,* WT or PPARγ OE mice were injected with ethyl carbamate at 1 mg/g of mouse weight. Twenty weeks later, mice were sacrificed and lung tumors counted. Black columns, mice fed control chow; white columns, mice fed chow containing 3% iloprost from 1 wk before injection through the end of the study; hatched columns, mice fed control chow until 5 wk after the injection and then changed to chow containing 3% iloprost for the remainder of the study. *, $P < 0.05$, versus WT mice fed control chow; **, $P < 0.001$, versus WT mice fed control chow. The difference between WT mice fed iloprost chow and PPARγ mice fed control chow or iloprost chow was also significant ($P < 0.05$). *B,* increased accumulation of macrophages in lungs of iloprost fed mice. WT mice were injected with ethyl carbamate at 1 mg/g of weight. Mice were fed control chow or chow containing 3% iloprost 1 wk before injection through the end of the study. Representative lung tissues from control-fed mice (*a* and *b*) compared with iloprost-fed mice (*c* and *d*) immunohistochemically stained for the macrophage marker F4/80 (*brown reaction color*).

expected genetically modified progeny. The indicated groups of mice were subjected to a single ethyl carbamate injection to initiate lung tumorigenesis. Animals were sacrificed after 20 weeks, and tumor incidence and multiplicity determined. IP-null animals did not show a difference in tumor multiplicity compared with WT (5.1 ± 0.8 versus 6.2 ± 0.48, $P > 0.05$) or IP heterozygotes (5.1 ± 0.8 versus 4.7 ± 0.45, $P > 0.05$; Fig. 1C). Overexpression of PGIS decreased tumor number in WT, IP(−/−), and IP(+/−) mice to the same extent, and these were all significantly less than PGIS WT animals. There were no differences in tumor incidence between the various experimental groups (data not shown). Tumor histologies were reviewed to confirm they represented pulmonary adenomas.

### Prostacyclin analogues activate PPARγ in lung epithelial cells and non–small-cell lung cancer cell lines

Because the chemoprotective effects of $PGI_2$ were independent of IP status, we sought to identify other targets that mediated the antitumorigenic effect seen in PGIS OE mice. Several studies have shown that $PGI_2$ and its analogues can activate members of the PPAR family of nuclear receptors (27, 28). To examine the ability of prostacyclin analogues to engage PPARs, RL65 cells (a nontransformed rat lung epithelial cell line) were transiently transfected with a reporter constructs encoding three copies of a consensus PPARγ-RE or PPARδ-RE (26) linked to firefly luciferase, and then stimulated with iloprost. Iloprost exposure increased PPARγ activity 3-fold ($P < 0.05$), but no concomitant increase in PPARδ-RE activity was observed (Fig. 2A). We further examined the ability of iloprost to activate PPARγ in non–small-cell lung cancer. In two adenocarcinoma cell lines, A549 and H661, iloprost increased PPAR-RE activity by ~2-fold. Furthermore, a specific PPARγ ligand binding antagonist, T0070907, completely blocked this stimulation (Fig. 2B). These data suggest that prostacyclin and iloprost may mediate inhibition of lung tumorigenesis through activation of PPARγ. We next examined the levels of IP expression in the non–small-cell lung cancer cell lines by reverse transcription-PCR. Both cell lines had undetectable levels of IP (data not shown), indicating that the activation of PPARγ was independent of IP expression.

### PPARγ OE mice with selective airway epithelial overexpression developed fewer lung tumors

To directly test the role of PPARγ in chemoprevention of lung tumorigenesis, we created mice with targeted overexpression of PPARγ using the surfactant protein-C promoter (PPARγ OE). Mice were genotyped using PCR (Fig. 3A) and propagated as heterozygotes in the FVB/N strain. Overexpression of PPARγ was verified by immunoblotting isolated type II cells from WT and PPARγ OE mice with anti-PPARγ antibody. PPARγ OE mice showed a marked increase in PPARγ expression (Fig. 3B). PPARγ OE mice did not display any phenotypic abnormalities and had normal lung architecture when H&E stains were reviewed (Fig. 3C).

PPARγ OE mice and WT littermates were injected with 1 mg/g ethyl carbamate and tumors were counted 20 weeks after treatment. PPARγ OE mice developed 2.6 ± 0.53 lung tumors, a 73% reduction ($P < 0.05$) in tumor multiplicity as compared with WT mice (9.6 ± 1.4; Fig. 4), similar to what we have

previously reported (29). To determine if iloprost supplementation could further enhance the chemopreventive effects of targeted PPARγ overexpression, transgenics were given 3% iloprost–impregnated chow 1 week before the ethyl carbamate injection and throughout the remainder of the study, whereas the control animals for these experiments received antioxidant-free control chow. Whereas iloprost decreased tumor number in WT mice (6.5 ± 0.6 versus 9.6 ± 1.4 tumors per mouse, $P < 0.05$), PPARγ OE mice fed iloprost chow showed no further reduction in tumor number when compared with control chow (2.6 ± 0.73 versus 2.6 ± 0.53 tumors per mouse). The lack of additive effects of iloprost and PPARγ overexpression suggest that iloprost and PPARγ may inhibit tumorigenesis through a common pathway, and that the presence of the PPARγ ligand iloprost does not augment the chemoprevention. Tumor burden was similar in all groups, but there was a trend toward smaller tumors in PPARγ overexpressors (data not shown).

To determine whether there is a critical time point for iloprost tumor inhibition, separate groups of both WT and PPARγ OE mice were maintained on control chow for the first 5 weeks after ethyl carbamate administration and were then switched to iloprost-containing chow for the remaining 15 weeks of the experiment. In WT mice, iloprost administration 5 weeks after ethyl carbamate decreased tumor size to the same extent as seen in animals exposed to iloprost throughout the entire 20-week regimen (Fig. 4A). In PPARγ OE mice, iloprost given at 5 weeks after ethyl carbamate had no effect on tumor number, which was still markedly decreased compared with WT controls.

To determine if iloprost treatment altered pulmonary inflammatory cell content, lungs from iloprost- and control chow–fed animals were examined for macrophage presence. Immunohistochemistry with the pan macrophage cell-surface marker F4/80 revealed a marked increase in pulmonary macrophages in iloprost-exposed animals (Fig. 4B).

## Discussion

Pulmonary-specific PGIS overexpression (and resulting elevations in pulmonary $PGI_2$ levels) significantly decreases both tumor incidence and multiplicity in multiple lung tumor models, and we have shown that pharmacologic supplementation with the oral prostacyclin analogue iloprost also decreases tumor multiplicity. The observed chemoprevention is independent of the IP membrane receptor, as IP KO animals that lack PGIS overexpression do not differ from WT mice in lung tumor development. Importantly, the combination of overexpression of PGIS and deletion of the IP receptor is still protective, indicating that $PGI_2$ must act through an alternative pathway or receptor. These findings are in contrast to studies showing that the protective effects of prostacyclin against the development of pulmonary hypertension are in fact mediated through the IP receptor (30). Thus, the beneficial effects of prostacyclin are mediated through distinct pathways in different disease states.

In the studies described above, we provide evidence that the observed chemoprotection is mediated by the nuclear transcription activator PPARγ. Several studies have indicated that $PGI_2$ analogues such as iloprost can activate PPARδ (27). We did not observe this activation in nontransformed lung epithelial cells using a PPARδ-RE. This could be a result of low baseline expression of PPARδ in nonmalignant lung epithelial cells or the absence of critical coactivators. The role of PPARδ in lung cancer chemoprevention remains to be determined. In contrast to PPARδ, prostacyclin analogues like iloprost selectively increase PPARγ activity both in nontransformed epithelial cells and in non–small-cell lung cancer. In human non–small-cell lung cancer cell lines, activation of PPARγ by pharmacologic agents or by molecular overexpression strongly inhibits transformed growth, as assessed by colony formation in soft agar (31, 32). Additionally, non–small-cell lung cancer cells overexpressing PPARγ exhibit significantly less invasiveness and metastasis compared with control cells both *in vitro* and in a rat orthotopic lung xenograft model (31). Similar to PGIS overexpression and oral iloprost, selective pulmonary overexpression of PPARγ decreases tumor multiplicity, and this reduction is not further enhanced by the addition of the PPARγ ligand iloprost (Fig. 4), suggesting that PPARγ is a major effector for the chemopreventive effects of iloprost. In fact, the observed decrease in tumor number was greater in PPARγ OE mice than in mice treated with iloprost. This could be a result of lower PPARγ expression in WT mice, or alternatively, the level of PPARγ activation achieved with systemic iloprost is lower than activation achieved through lung-specific overexpression.

Our data also indicate that iloprost will have systemic effects, which may be critical for tumor chemoprevention. Iloprost increased the number of macrophages in the lungs of tumor-bearing animals. Macrophages play a complex role in cancer progression (33). Whereas tumor-associated macrophages initially mediate direct cytotoxic effects on tumors, their presence has been implicated in promoting tumor growth and metastasis. In lung cancer, some studies have reported an association of increased tumor-associated macrophages and tumor regression (34). The location of macrophages seems to be important. Increased numbers of macrophages within the tumor itself was associated with a favorable prognosis, whereas increased numbers of macrophages in the stroma surrounding the tumor was associated with an unfavorable prognosis (35). Further studies elucidating the role of macrophages in chemoprevention are required.

Additional support that PPARγ may play an important role in human lung tumorigenesis is found in recent epidemiologic studies of diabetics treated with the thiazolidinediones rosiglitazone and pioglitazone. Lung cancer incidence in diabetics on these PPARγ agonists was decreased by 33% when compared with diabetics on non–PPARγ-modulating treatments (36). Thiazolidinediones have been shown to induce 15-hydroxyprostaglandin dehydrogenase, the enzyme that inactivates the antiapoptotic and immunosuppressive prostaglandin $E_2$ by conversion to 15-keto prostaglandins (37). Therefore, unlike COX inhibition, which would act to decrease prostaglandin $E_2$ and $PGI_2$, thiazolidinediones would be predicted to enhance 15-hydroxyprostaglandin dehydrogenase and selectively lower prostaglandin $E_2$ levels while preserving $PGI_2$. Thiazolidinediones have also been shown to inhibit angiogenesis by blocking ELR + CLC chemokine production in non–small-cell lung cancer (29, 38) and to inhibit primary tumor growth and spontaneous lung metastases in animal models (39). The finding by Govindarajan and colleagues of

decreased lung cancer in diabetics on thiazolidinediones must be tempered against a recent meta-analysis suggesting that diabetics on PPARγ agonists have significantly increased risks of myocardial infarction (40). A more recent interim analysis of clinical events in the Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycaemia in Diabetes (RECORD) study was inconclusive about the effects of rosiglitazone on cardiovascular mortality, and rosiglitazone was associated with an increase in heart failure but not death from cardiovascular causes (41). One critical issue in assessing PPARγ activators will be defining whether the suggested adverse cardiovascular events are mediated through PPARγ or through off-target drug effects. A recent report has shown an IP mutation resulting in defective adenylyl cyclase activation, thereby promoting platelet aggregation and atherothrombosis (10).

Additionally, we show that iloprost given 5 weeks after tumor initiation continues to protect against lung tumorigenesis. Five weeks after urethane administration, microadenomas have formed (42), and the ability of iloprost to block the progression of these microadenomas to visible adenomas suggests that this agent may have chemotherapeutic activity and may be a potential chemopreventive agent in former smokers who have accumulated airway damage. Further studies are in progress to test the ability of iloprost to inhibit non–small-cell lung cancer growth and to define whether this is mediated through PPARγ activation.

Our studies indicate that the protective effect of overexpression of PGIS can be reproduced using a bioavailable prostacyclin analogue, providing a rationale for clinical lung cancer chemoprevention studies in high-risk humans. Potential prevention agents must have excellent safety profiles, and chronic COX-2 administration has been associated with increased cardiovascular mortality (8). Prostacyclin has known atheroprotective properties that may make it a more attractive prevention agent (9). To better investigate this, we have an ongoing clinical chemoprevention trial where patients at high risk for lung cancer are given oral iloprost and assessed for the histologic progression of endobronchial dysplasia. Our data continue to confirm a pivotal role for $PGI_2$ in preventing lung tumorigenesis (the overwhelming majority of which is tobacco smoking associated); PPARγ activation is one mechanism, and PPARγ agonists (as single agents or in combination with other agents) may be ready for chemoprevention and chemotherapeutic trials.

## Disclosure of Potential Conflicts of Interest

R.L. Keith: owns patent on use of prostacyclin analogues for cancer chemoprevention. No other authors disclosed potential conflicts of interest.

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