

NAD⁺-Dependent 15-Hydroxyprostaglandin Dehydrogenase Regulates Levels of Bioactive Lipids in Non–Small Cell Lung Cancer

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Abstract

Elevated levels of procarcinogenic prostaglandins (PG) are found in a variety of human malignancies including non–small cell lung cancer (NSCLC). Overexpression of cyclooxygenase-2 and microsomal prostaglandin synthase 1 occurs in tumors and contributes to increased PG synthesis. NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH), the key enzyme responsible for metabolic inactivation of PGs, is down-regulated in various malignancies. The main objective of this study was to elucidate the effect of loss of 15-PGDH on levels of bioactive lipids in NSCLC. We found that levels of cyclooxygenase-2 and microsomal prostaglandin synthase 1 were commonly increased whereas the amount of 15-PGDH was frequently decreased in NSCLC compared with adjacent normal lung. Reduced expression of 15-PGDH occurred in tumor cells and was paralleled by decreased 15-PGDH activity in tumors. Amounts of PGE₁, PGE₂, and PGF_{2α}, known substrates of 15-PGDH, were markedly increased whereas levels of 13,14-dihydro-15-keto-PGE₂, a catabolic product of PGE₂, were markedly reduced in NSCLC compared with normal lung. Complementary *in vitro* and *in vivo* experiments were done to determine whether these changes in PG levels were a consequence of down-regulation of 15-PGDH in NSCLC. Similar to NSCLC, amounts of PGE₁, PGE₂, and PGF_{2α} were markedly increased whereas levels of 13,14-dihydro-15-keto-PGE₂ were decreased in the lungs of 15-PGDH knockout mice compared with wild-type mice or when 15-PGDH was silenced in A549 lung cancer cells. Collectively, these data indicate that 15-PGDH is commonly down-regulated in NSCLC, an effect that contributes to the accumulation of multiple bioactive lipids in NSCLC.

Increased levels of procarcinogenic prostaglandins (PG) have been found in several human tumors including non–small cell lung cancer (NSCLC), which may reflect either increased PG synthesis or decreased catabolism (1–4). The synthesis of PGs from arachidonic acid requires two sequential enzymatic steps. Cyclooxygenase (COX) catalyzes the synthesis of

PGH₂ from arachidonic acid. There are two isoforms of COX: COX-1 is a housekeeping gene that is expressed constitutively in most tissues (5); COX-2 is an immediate-early response gene that is undetectable in most normal tissues but is rapidly induced by oncogenes, growth factors, cytokines, and tumor promoters (6, 7). Specific isomerases [e.g., microsomal prostaglandin synthase 1 (mPGES-1)] then convert PGH₂ to a variety of PGs including PGE₁, PGE₂, and PGF_{2α} (7, 8). Because both COX-2 and mPGES-1 are commonly overexpressed in human malignancies (7, 9–13), enhanced synthesis is likely to explain, at least in part, the increase in PGs found in tumor tissue.

In theory, reduced catabolism of PGs may also contribute to elevated levels of PGs in human malignancies. The key enzyme responsible for metabolic inactivation of PGs is NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH; ref. 14). The human gene for 15-PGDH is located on chromosome 4 and codes for a 29-kDa enzyme that catalyzes the formation of 15-keto-PGs, which possess reduced biological activities compared with PGs (14, 15). 15-Keto-PGE₂ is converted to 13,14-dihydro-15-keto-PGE₂ (14, 16). Reduced amounts of 15-PGDH have been reported in cancers of the colon, breast, lung, and bladder (17–21). This decrease has

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been attributed to reduced gene expression because of either hypermethylation of the promoter or increased binding of repressive transcription factors to E-box elements in the promoter (21, 22). Recent evidence suggests that *15-PGDH* is a tumor suppressor gene. For example, a marked increase in the formation of colon tumors occurred when *15-PGDH* was knocked out in *APC^{Min}* mice (23). Moreover, forced expression of *15-PGDH* suppressed the growth of experimental lung tumors (17). Although *15-PGDH* catabolizes a variety of PGs and lipoxins, its role in regulating the levels of these bioactive lipids in human malignancies is unknown. Given the established link between PGs and carcinogenesis, it is important to determine whether reduced expression of *15-PGDH* contributes to the accumulation of bioactive PGs in tumor tissue.

In the present study, we first determined that the expression of enzymes involved in the synthesis (COX-2, mPGES-1) and catabolism (*15-PGDH*) of PGs was deregulated in NSCLC. Subsequently, levels of PGE₁, PGE₂, and PGF₂α were found to be elevated in NSCLC versus normal lung. Complementary *in vitro* and *in vivo* studies suggested that loss of *15-PGDH* expression in lung cells led to increased amounts of PGE₁, PGE₂, and PGF₂α and reduced levels of 13,14-dihydro-15-keto-PGE₂, a pattern mimicking the profile in NSCLC versus adjacent normal lung. Taken together, these findings suggest that the observed increase in levels of multiple prostanoids in NSCLC reflects reduced catabolism, in addition to increased synthesis.

Materials and Methods

Materials

DMEM, fetal bovine serum, and LipofectAMINE 2000 were from Invitrogen. Rabbit anti-human polyclonal antibodies to mPGES-1 and deuterated eicosanoid standards were acquired from Cayman Chemical. Antibodies to *15-PGDH* were from Novus Biologicals. Goat anti-human COX-2 antibody, antibody to β-actin, Lowry protein assay kits, arachidonate, butylated hydroxytoluene, citric acid, and EDTA were obtained from Sigma Chemical. Western blot analysis detection reagents (enhanced chemiluminescence) were from Amersham Biosciences. Nitrocellulose membranes were from Schleicher and Schuell. All high-performance liquid chromatography-grade solvents were purchased from Fisher Scientific Co. The Bradford protein assay kit was purchased from Bio-Rad.

Human specimens

Specimens were obtained at the time of surgery from patients with pulmonary adenocarcinoma or squamous cell carcinoma. Tissue samples were obtained from a nonnecrotic area of the tumor and from adjacent normal-appearing lung tissue. Samples were immediately snap frozen in liquid nitrogen and stored at -80°C until analysis. In addition, tissue sections for immunohistochemical staining were fixed in 10% buffered formalin and embedded in paraffin. The study was approved by the Committee on Human Rights in Research at Weill Cornell Medical College.

Animal model

Wild-type and *15-PGDH* knockout mice were bred in the Case Western Reserve Animal Resource Center under a protocol approved by the Institutional Animal Care and Use Committee. Genotyping was carried out as described previously (24). At the time of sacrifice, lungs were snap frozen for future analysis.

Cell culture

The A549 human lung adenocarcinoma cell line was obtained from American Type Culture Collection and maintained in DMEM with

low glucose, supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin.

Western blot analysis

Tissue or cell lysates were prepared by treatment with lysis buffer as described previously (25). Lysates were sonicated for 3 × 10 s on ice and centrifuged at 14,000 × g for 10 min at 4°C to remove the particulate material. The protein concentration of the supernatant was measured using the method of Lowry et al. (26). SDS-PAGE was done under reducing conditions on 12.5% polyacrylamide gels. The resolved proteins were transferred onto nitrocellulose sheets and then probed for COX-2, mPGES-1, *15-PGDH*, and β-actin.

15-PGDH activity assay

Human lung tissue was suspended in 0.05 mol/L Tris buffer (pH 7.5), sonicated for 3 × 10 s on ice, and then centrifuged at 14,000 × g for 10 min at 4°C to remove the particulate material. *15-PGDH* enzyme activity in tissue lysates was assayed by measuring the transfer of tritium from 15(S)-[15-³H]PGE₂ to glutamate by coupling *15-PGDH* with glutamate dehydrogenase as described previously (27).

Transient transfection

15-PGDH siRNA and nonspecific siRNA were obtained from Dharmacon. A549 cells were transfected with 100 pmol of siRNA using DharmaFECT transfection reagent according to the manufacturer's instructions. Following transfection for 24 h, the medium was changed. Twenty-four hours later, the media and cells were collected for analysis.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections from surgical resection specimens containing primary lung squamous cell carcinomas and adenocarcinomas were evaluated for the presence and distribution of *15-PGDH*. Four-micron-thick tissue sections of the tumor and nonneoplastic lung parenchyma from each case were immunohistochemically stained with the *15-PGDH* rabbit polyclonal antibody as described below. Unstained tissue sections were baked, deparaffinized, and rehydrated on the VisionBio Systems BondMax autostainer (VisionBio Systems). Tissue sections were pretreated using the VisionBio Systems heat-induced epitope retrieval solution 1 and incubated with the primary antibody (1:1,625 dilution) for 25 min. The Refine Detection Kit supplied by the manufacturer (VisionBio Systems) was used to block endogenous peroxidase activity and enhance the staining reaction. Two sets of controls were done on unstained tissue sections from each case. The first control included incubation (1 h at room temperature) of the *15-PGDH* antibody with the *15-PGDH* protein (1:1 dilution; Novus Biologicals), dilution with VisionBio Systems diluent (1:800), and application of this solution to the tissue sections according to the methods described above. The second negative control involved replacing the primary antibody with immunoglobulin. Cytoplasmic staining for *15-PGDH* in the lung tumors and nonneoplastic lung tissue was recorded as either absent (negative or weak staining) or present (moderate or strong intensity).

Determination of PG levels

PGs were extracted from cell culture medium using a solid-phase extraction method. Briefly, aliquots (10 μL) of 10% butylated hydroxytoluene and 20 μL of deuterated internal standards (100 ng/mL) were added to 1 mL of cell culture medium. The solution was applied to a Sep-Pak C18 cartridge (Waters Corp.) and PGs were eluted with 1 mL of methanol. The eluate was evaporated under a stream of nitrogen and the residue was reconstituted in 100 μL of a solution of methanol/10 mmol/L ammonium acetate (50:50, v/v). Analysis was carried out using liquid chromatography/tandem mass spectroscopy as detailed below.

PGs were extracted from human and murine lung tissue using a previously described method (28). Briefly, ~50 mg of frozen tissue

were ground to a fine powder and homogenized with an Ultrasonic Processor (Misonix). An aliquot (100 μ L) of homogenate was subjected to extraction with hexane/ethyl acetate (1:1). The upper organic layer was collected and the organic phases from three extractions were pooled and then evaporated to dryness under a stream of nitrogen. All extraction procedures were done at minimum light levels at 4°C. Samples were then reconstituted in 100 μ L methanol/10 mmol/L ammonium acetate buffer, pH 8.5 (50:50, v/v), before liquid chromatography/tandem mass spectroscopic analysis. Protein concentration was determined by the method of Bradford according to the manufacturer's instructions.

Liquid chromatography/tandem mass spectroscopic analyses were done using a QuattroUltima mass spectrometer (Waters) equipped with an Agilent 1100 binary pump high-performance liquid chromatography system (Agilent) using a modified version of the method of Yang et al. (28). PGs of interest were chromatographically separated using a Luna 3- μ m phenyl-hexyl 4.6 \times 100 mm analytic column (Phenomenex). The mobile phase consisted of 10 mmol/L ammonium acetate (pH 8.5) and methanol using a linear methanol gradient that went from 50% to 60% for 10 min and then from 60% to 80% in 4 min. This was then increased to 100% methanol concentration over the next 6 min and kept at this condition for an additional 2 min to achieve chromatographic baseline resolution for the PGs of interest. The flow rate was 0.5 mL/min with a column temperature of 60°C. The mass spectrometer was operated in negative electrospray ionization mode with cone voltage of 40 V. Source temperatures were 125°C with desolvation gas temperature at 350°C. Collision-induced dissociation of the PGs was done using argon gas at a cell pressure of 1.6×10^{-3} Torr. PGs were detected and quantified by multiple reaction mode monitoring of the transitions m/z 351 \rightarrow 271 for PGE₂, m/z 355 \rightarrow 275 for PGE₂-d₄, m/z 353 \rightarrow 317 for PGE₁, m/z 357 \rightarrow 321 for PGE₁-d₄, m/z 353 \rightarrow 309 for PGF_{2 α} , m/z 357 \rightarrow 313 for PGF_{2 α} -d₄, and m/z 351 \rightarrow 333 for 13,14-dihydro-15-keto-PGE₂. The results are expressed as nanograms of PG per milligram of protein.

Statistics

Wilcoxon signed rank tests were used to analyze the 15-PGDH activity and PG levels in the paired human samples. Wilcoxon rank sum tests were used to analyze PG levels in the nonpaired samples in cell culture and mice (29). Statistical significance was set at 0.05 (two tailed). Means and SEs were also reported. Analyses were conducted with SAS 9.1 (SAS Institute, Inc.).

Results

Levels of 15-PGDH, COX-2, and mPGES-1 are commonly altered in NSCLC

Western blot analysis was used to evaluate the expression of COX-2, mPGES-1, and 15-PGDH in 19 paired (normal, tumor) samples of NSCLC (Fig. 1A and B). COX-2 (14 of 19, 74%) and mPGES-1 (17 of 19, 89%) were overexpressed in the majority of tumor samples. Conversely, levels of 15-PGDH (15 of 19, 79%) were reduced in most tumors. Consistent with the immunoblot findings, 15-PGDH activity was reduced in the majority of cases of NSCLC regardless of histology (Fig. 1C). Although COX-2 and mPGES-1 were commonly up-regulated and 15-PGDH down-regulated in NSCLC, there was considerable heterogeneity. For example, the magnitude of overexpression of COX-2 and mPGES-1 varied between cases. Moreover, up-regulation of mPGES-1 and down-regulation of 15-PGDH occurred in the absence of COX-2 overexpression in some cases [e.g., case 5 (Fig. 1A) or case 9 (Fig. 1B)]. Collectively, these results imply that the mechanisms controlling the expression of 15-PGDH, COX-2, and mPGES-1 must differ.

Although 15-PGDH activity in lung contributes to the short half-life of PGE₂ in blood (30), little is known about its cellular location. Hence, immunohistochemistry was carried out to localize 15-PGDH in lung tissue. Twenty surgical resection specimens containing primary lung squamous cell carcinomas (9 cases) and adenocarcinomas (11 cases) were evaluated. The nonneoplastic lung parenchyma showed cytoplasmic staining for 15-PGDH in pneumocytes in all 20 cases (Fig. 2A and B). The staining was specific because immunoreactivity was lost when antiserum to 15-PGDH was preadsorbed with 15-PGDH protein (Fig. 2C). All carcinomas showed decreased 15-PGDH staining (Fig. 2E-H), which was limited to <15% of the tumor tissue in all cases, and diminished intensity compared with the adjacent nonneoplastic lung parenchyma.

PG levels are altered in NSCLC

Given the finding that enzymes involved in both the synthesis (COX-2 and mPGES-1) and catabolism (15-PGDH) of PGs are commonly deregulated in NSCLC, we next investigated the potential effect of these changes on PG levels. Twenty paired samples (10 adenocarcinomas and 10 squamous cell carcinomas) of NSCLC were analyzed for PG levels via liquid chromatography/tandem mass spectroscopy. Because the differences observed between tumor and normal were qualitatively similar for adenocarcinomas and squamous cell carcinomas, combined data are shown as NSCLC. The mean concentrations of PGE₁, PGE₂, PGF_{2 α} , and 13,14-dihydro-15-keto-PGE₂ are shown in Fig. 3. Marked increases in amounts of PGE₁ (Fig. 3A), PGE₂ (Fig. 3B), and PGF_{2 α} (Fig. 3C) were found in tumor tissue versus adjacent normal lung. Conversely, the amount of 13,14-dihydro-15-keto-PGE₂ (Fig. 3D), a catabolic product of PGE₂, was significantly reduced from 0.16 to 0.01 ng/mg protein in the same tumor samples. All *P* values were <0.001 from Wilcoxon signed rank tests.

Reduced expression of 15-PGDH contributes to deregulated levels of PGs in NSCLC

PGE₁, PGE₂, and PGF_{2 α} are all known substrates of 15-PGDH. To investigate whether reduced expression of 15-PGDH contributed to the increased levels of each of these PGs in NSCLC, we used both cellular and animal models. First, we used A549 cells, a cellular model that has been used extensively to evaluate the role of PGs in NSCLC (9, 17, 31). In an effort to mimic NSCLC, siRNA was used to knock down 15-PGDH and then PG production was measured. As shown in Fig. 4A, treatment of A549 NSCLC cells with siRNA led to a marked reduction in 15-PGDH protein. Consistent with the PG profile found in NSCLC versus adjacent normal-appearing lung (Fig. 3), silencing of 15-PGDH led to marked increases in the amounts of PGE₁ (Fig. 4B), PGE₂ (Fig. 4C), and PGF_{2 α} (Fig. 4D). These increases were associated with a reciprocal decrease in the production of 13,14-dihydro-15-keto-PGE₂ (Fig. 4E). Hence, siRNA-mediated suppression of 15-PGDH led to increased amounts of PGE₁, PGE₂, and PGF_{2 α} and reduced levels of 13,14-dihydro-15-keto-PGE₂, a pattern mimicking the profile in NSCLC versus adjacent normal-appearing lung (Fig. 3).

To further evaluate the role of 15-PGDH as a determinant of PG levels in the lung, we investigated the effects of

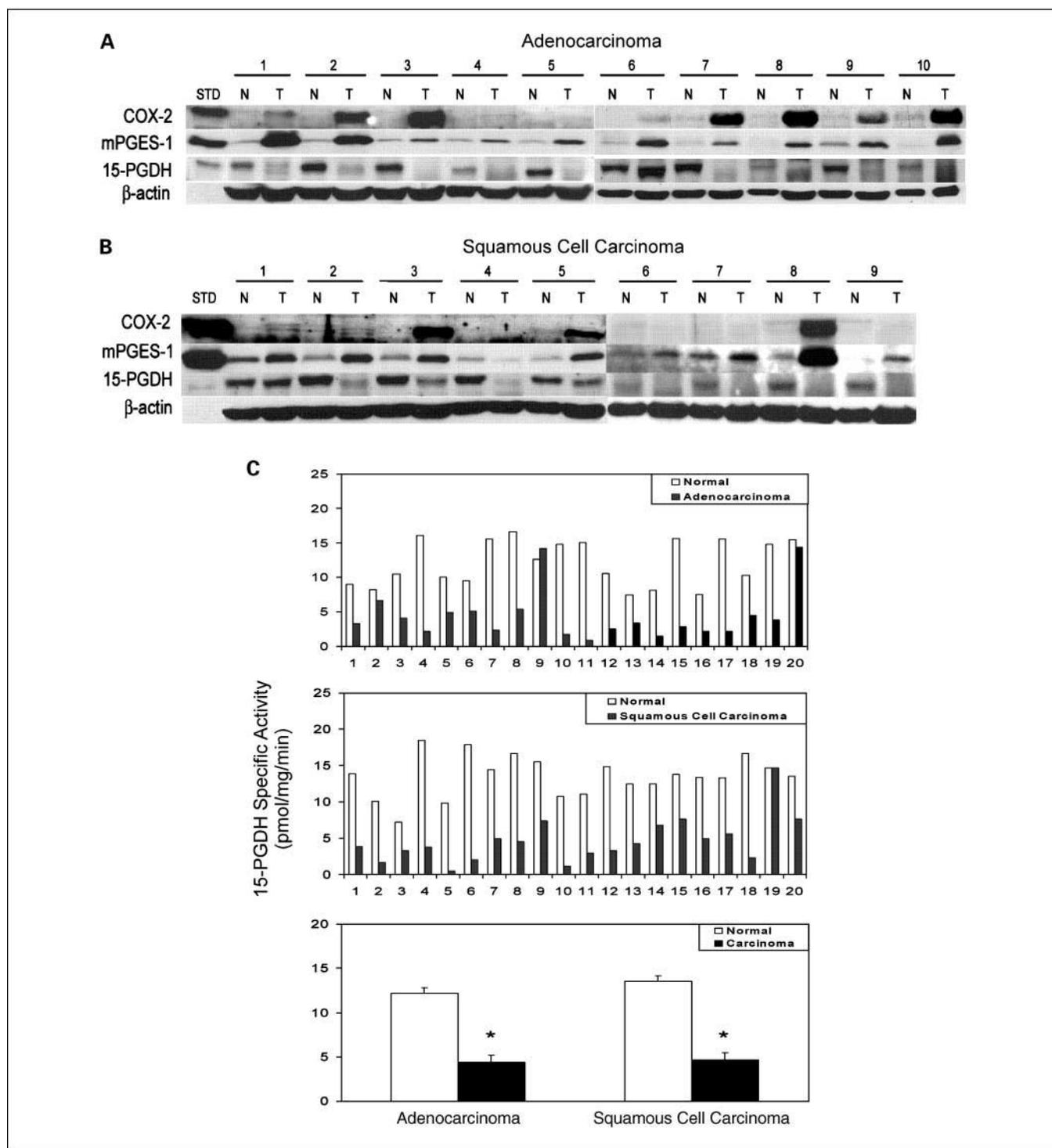


Fig. 1. 15-PGDH is down-regulated whereas COX-2 and mPGES-1 are overexpressed in NSCLC. Levels of COX-2, mPGES-1, and 15-PGDH were measured by immunoblot analysis in 10 paired samples (N, normal; T, tumor) of adenocarcinoma (A) and 9 paired samples of squamous cell carcinoma (B). Tissue lysates (100 µg/lane) were prepared and loaded onto a 12.5% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. Immunoblots were probed as indicated, with β-actin serving as a loading control. C, 15-PGDH activity was measured in 20 paired samples of adenocarcinoma (top) and 20 paired samples of squamous cell carcinoma (middle) of the lung. Cases shown in C are different than those in A and B. C, bottom, summary of the findings in top and middle. Columns, mean (n = 20 per group); bars, SE. *, P < 0.001, compared with adjacent normal lung.

knocking out 15-PGDH on levels of PGs in the murine lung. Consistent with the pattern found in NSCLC versus normal lung, knocking out 15-PGDH led to increased amounts of PGE₁ (Fig. 5A), PGE₂ (Fig. 5B), and PGF_{2α} (Fig. 5C) in

the murine lung. Conversely, amounts of 13,14-dihydro-15-keto-PGE₂ (Fig. 5D) were decreased in lungs of 15-PGDH knockout versus wild-type mice. All differences were statistically significant.

Discussion

In this study, we found that levels of COX-2 and mPGES-1 were commonly increased whereas the amount of 15-PGDH was frequently decreased in NSCLC compared with adjacent normal lung. Reduced expression of 15-PGDH occurred in tu-

mor cells and paralleled decreased 15-PGDH enzyme activity in tumors. Amounts of PGE₁, PGE₂, and PGF_{2α}, known substrates of 15-PGDH, were markedly increased whereas levels of the catabolic metabolite 13,14-dihydro-15-keto-PGE₂ were markedly reduced in NSCLC compared with normal lung.

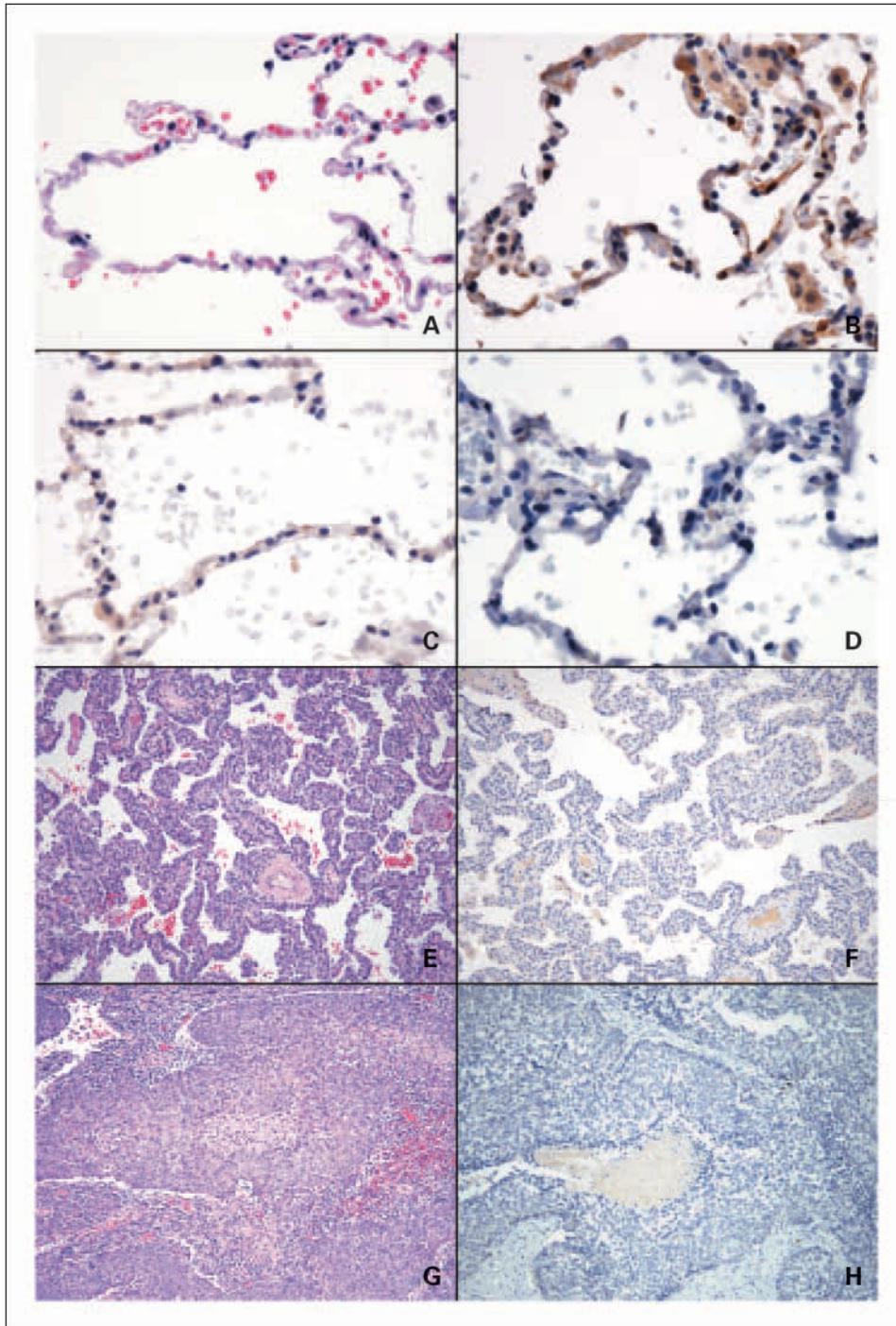


Fig. 2. Immunohistochemical localization of 15-PGDH. Tissue sections from routinely processed lung resection specimens (A; H&E, $\times 200$ magnification) showed strong cytoplasmic staining for 15-PGDH within nonneoplastic pneumocytes when incubated with the anti-15-PGDH antibody (B; hematoxylin counterstain, $\times 200$ magnification). This staining reaction was attenuated when the primary antibody was incubated with recombinant 15-PGDH protein before application to the tissue sections, thereby showing specificity of the antibody for its antigen (C; hematoxylin counterstain, $\times 200$ magnification). Tissue sections treated with immunoglobulin were negative when the primary antibody was omitted (D; hematoxylin counterstain, $\times 200$ magnification). All cases of lung adenocarcinoma (E; H&E, $\times 100$ magnification) and squamous cell carcinoma (G; H&E, $\times 100$ magnification) showed decreased staining with the 15-PGDH antibody (F and H; hematoxylin counterstain, $\times 100$ magnification).

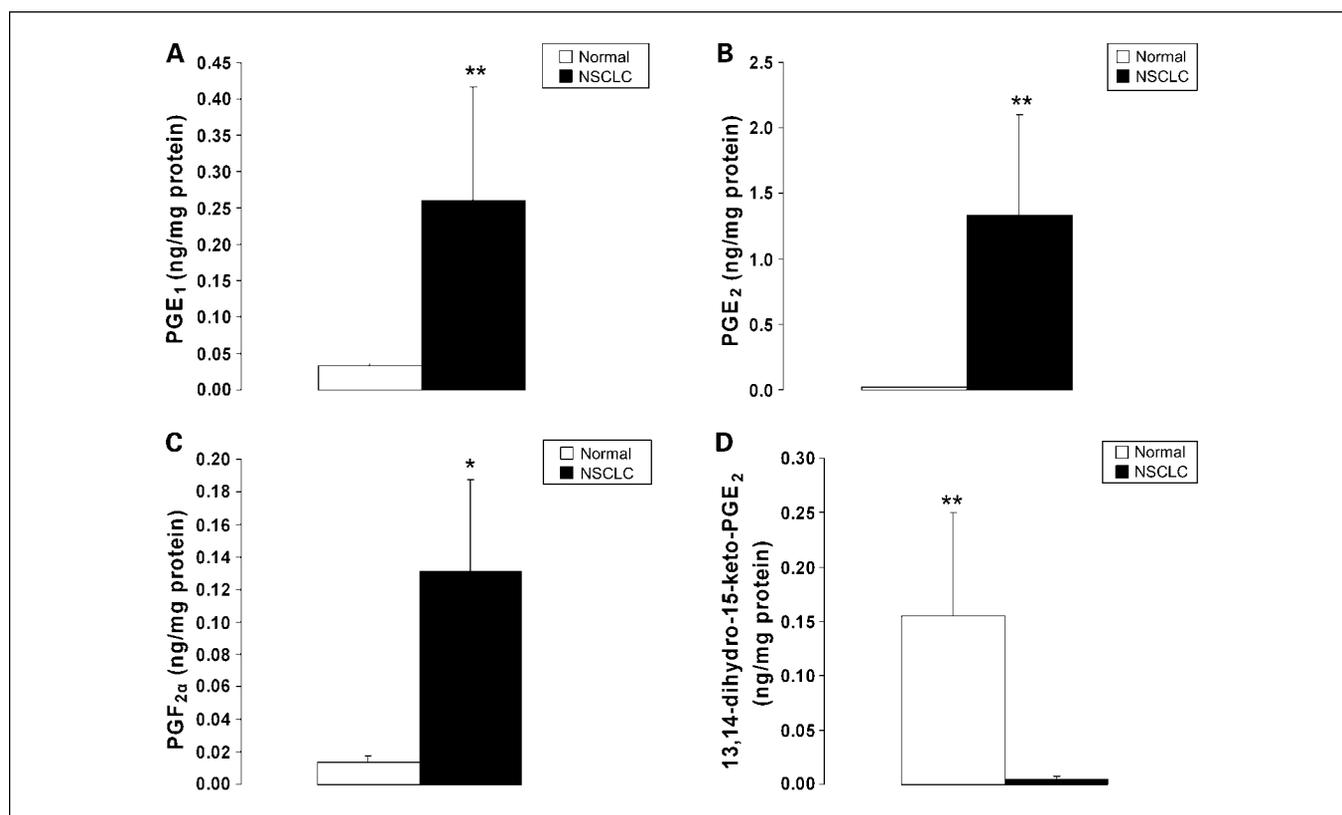


Fig. 3. Levels of PGs are altered in NSCLC compared with adjacent normal-appearing lung. PGE₁ (A), PGE₂ (B), PGF_{2α} (C), and 13,14-dihydro-15-keto-PGE₂ (D) were extracted from normal lung or NSCLC tissue homogenates and quantified, as described in Materials and Methods. Columns, mean ($n = 20$ per group); bars, SE. *, $P < 0.05$; **, $P < 0.01$.

An identical bioactive lipid profile was observed in the lungs of 15-PGDH knockout mice compared with wild-type mice or when 15-PGDH was silenced in A549 lung cancer cells. The fact that levels of PGE₂ increased whereas amounts of 13,14-dihydro-15-keto-PGE₂, a catabolic metabolite, decreased is consistent with reduced expression of 15-PGDH. Taken together, these data strongly suggest that reduced expression of 15-PGDH in NSCLC contributes to the accumulation of multiple procarcinogenic prostanoids in NSCLC. To our knowledge, this is the first evidence that down-regulation of 15-PGDH contributes to the elevation of several procarcinogenic PGs in tumor tissue.

Several lines of evidence, beyond the finding of elevated levels of PGs in tumors, suggest a role for PGs in the development and progression of cancer. PGE₂ can stimulate cell proliferation and motility while inhibiting apoptosis and immune surveillance (7, 9, 13, 32, 33). Importantly, PGE₂ can also induce angiogenesis, at least in part, by enhancing the production of proangiogenic factors including vascular endothelial growth factor (34). Recently, PGE₂ was found to induce an epithelial-to-mesenchymal transition, a process that is believed to be important for tumor metastasis (31). Notably, both PGE₁ and PGF_{2α} also have relevant procarcinogenic effects. Treatment with PGE₁ increased the number of endothelial progenitor cells in the blood and bone marrow of mice and enhanced neoangiogenesis (35). The ability of PGE₁ to induce vascular endothelial growth factor and suppress apoptosis may also contribute to tumor progression (36, 37). PGF_{2α}

also has multiple procarcinogenic effects. For example, PGF_{2α} can stimulate the proliferation, motility, and invasiveness of cancer cells (38, 39). Similar to PGE₁ and PGE₂, PGF_{2α} can induce vascular endothelial growth factor production and angiogenesis while inhibiting apoptosis (40, 41). Thus, down-regulation of 15-PGDH contributes to the accumulation of multiple PGs that are likely, in turn, to enhance tumor progression. In support of this notion, treatment with an anti-PGE₂ monoclonal antibody or a selective COX-2 inhibitor suppressed the growth of transplantable NSCLC (42). Moreover, pharmacologic antagonists or genetic disruption of PG receptors can inhibit the progression of experimental NSCLC (43). In addition to PGE₁, PGE₂, and PGF_{2α}, other eicosanoids including lipoxin A₄, 12(S)-hydroxy-(5Z,8E,10E)-heptadecatrienoic acid, 15-hydroxyeicosatetraenoic acid, 5,15-dihydroxyeicosatetraenoic acid, and 8,15-dihydroxyeicosatetraenoic acid are also excellent substrates for 15-PGDH (44). Based on the current results, it will be of considerable interest to determine whether any of these other known substrates of 15-PGDH accumulate in NSCLC or other tumor types and contribute to tumor progression.

Although it is common for COX-2 and mPGES-1 to be overexpressed and 15-PGDH down-regulated in NSCLC, there is some heterogeneity. This suggests that the mechanisms controlling the expression of these enzymes are not identical. Both COX-2 and mPGES-1 can be induced by oncogenes or proinflammatory cytokines but the detailed control mechanisms are quite different (7, 11, 25). Several

transcription factors, including activator protein 1, regulate the expression of COX-2 whereas early growth response factor 1 seems to be very important for stimulating *mPGES-1* transcription (7, 25). Interestingly, the repressive transcription factors Snail and Slug can bind to conserved E-box elements in the *15-PGDH* promoter and thereby inhibit transcription (22, 45). Alternatively, hypermethylation of the *15-PGDH* promoter has been suggested to contribute to the loss of expression of 15-PGDH in breast cancer (21). Future studies will be needed to evaluate the mechanisms underlying the down-regulation of 15-PGDH expression in NSCLC. It is also important to consider shared regulatory mechanisms. Activation of epidermal growth factor receptor signaling can both induce COX-2 and suppress the expression of 15-PGDH (7, 22, 45). Possibly, activation of this pathway contributes to the up-regulation of COX-2 and down-regulation of 15-PGDH that are found in most cases of NSCLC. It will be worthwhile, therefore, to determine the effects of erlotinib, a selective inhibitor of epidermal growth factor receptor ty-

rosine kinase activity, on the levels of COX-2, 15-PGDH, and bioactive PGs in NSCLC or in intraepithelial neoplasia.

Selective COX-2 inhibitors, prototypic inhibitors of PG synthesis, are active in the treatment and prevention of colorectal adenomas and may have utility in the treatment of NSCLC (46–49). However, chronic use of selective COX-2 inhibitors has been associated with increased risk of cardiovascular complications (50). The mechanisms underlying this toxicity are not fully understood, but inhibition of COX-2 results in the loss of all downstream PGs. It has been suggested that selective COX-2 inhibitors block the production of cardioprotective prostaglandin I₂ by vascular endothelium without inhibiting COX-1-dependent platelet thromboxane A₂ synthesis, supporting a prothrombotic mechanism. To improve the therapeutic index, alternate treatment strategies to suppress the levels or actions of PGs are being explored. Our data indicate that down-regulation of 15-PGDH contributes to the accumulation of procarcinogenic PGs in NSCLC. Hence, agents that induce 15-PGDH should normalize the

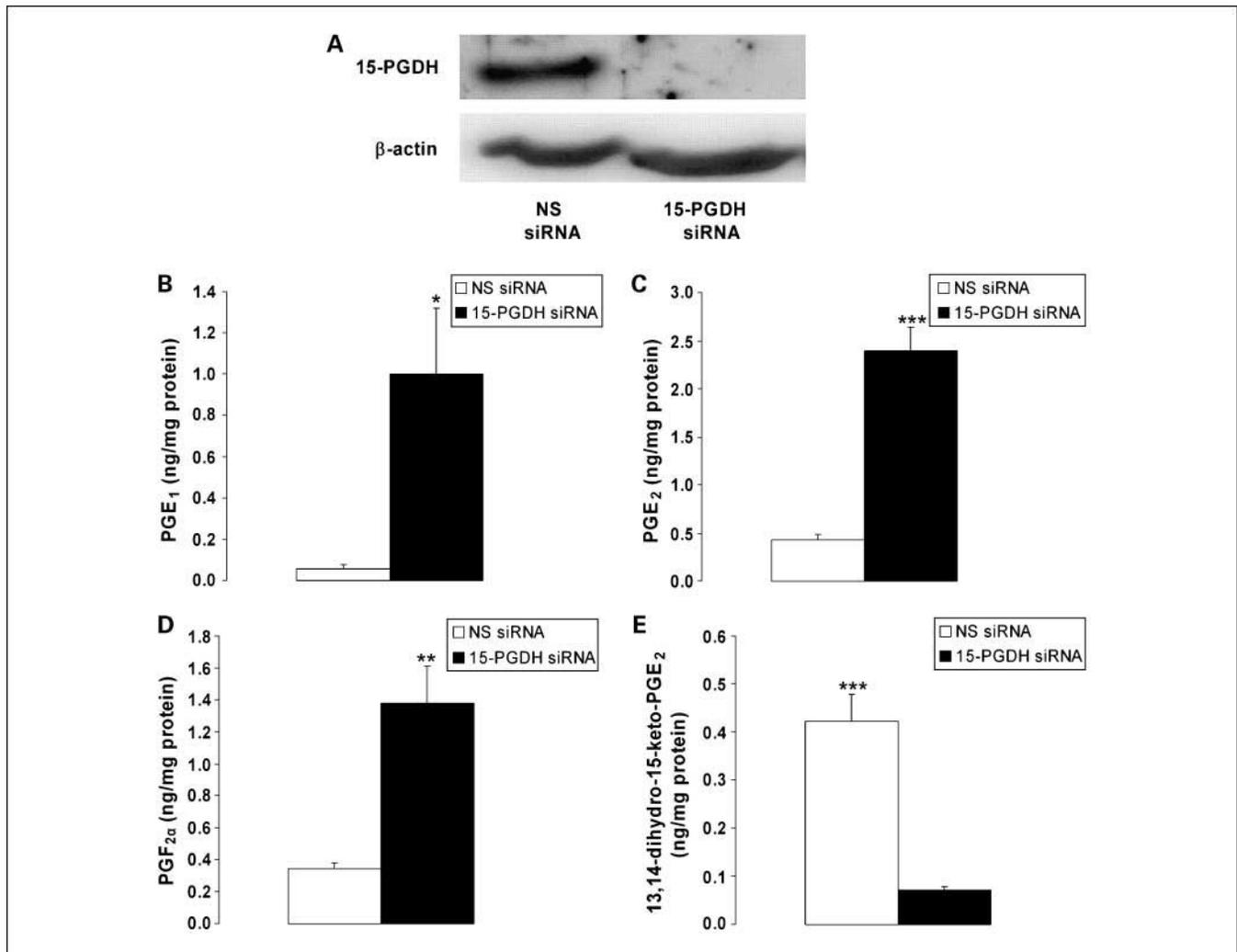


Fig. 4. Silencing of 15-PGDH in A549 cells results in changes in PG production that mimic NSCLC. **A**, A549 cells were grown to 50% confluence before being transfected with either 15-PGDH siRNA or nonspecific (NS) siRNA for 24 h. Subsequently, the medium was changed and cells were allowed to grow for an additional 24 h, at which time both cells and media were collected for analysis. **A**, immunoblot analysis showed reduced levels of 15-PGDH protein in cells transfected with siRNA to 15-PGDH. PGE₁ (**B**), PGE₂ (**C**), PGF_{2 α} (**D**), and 13,14-dihydro-15-keto-PGE₂ (**E**) were isolated from the cell culture media and quantified, as described in Materials and Methods. Columns, mean ($n = 6$); bars, SE. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

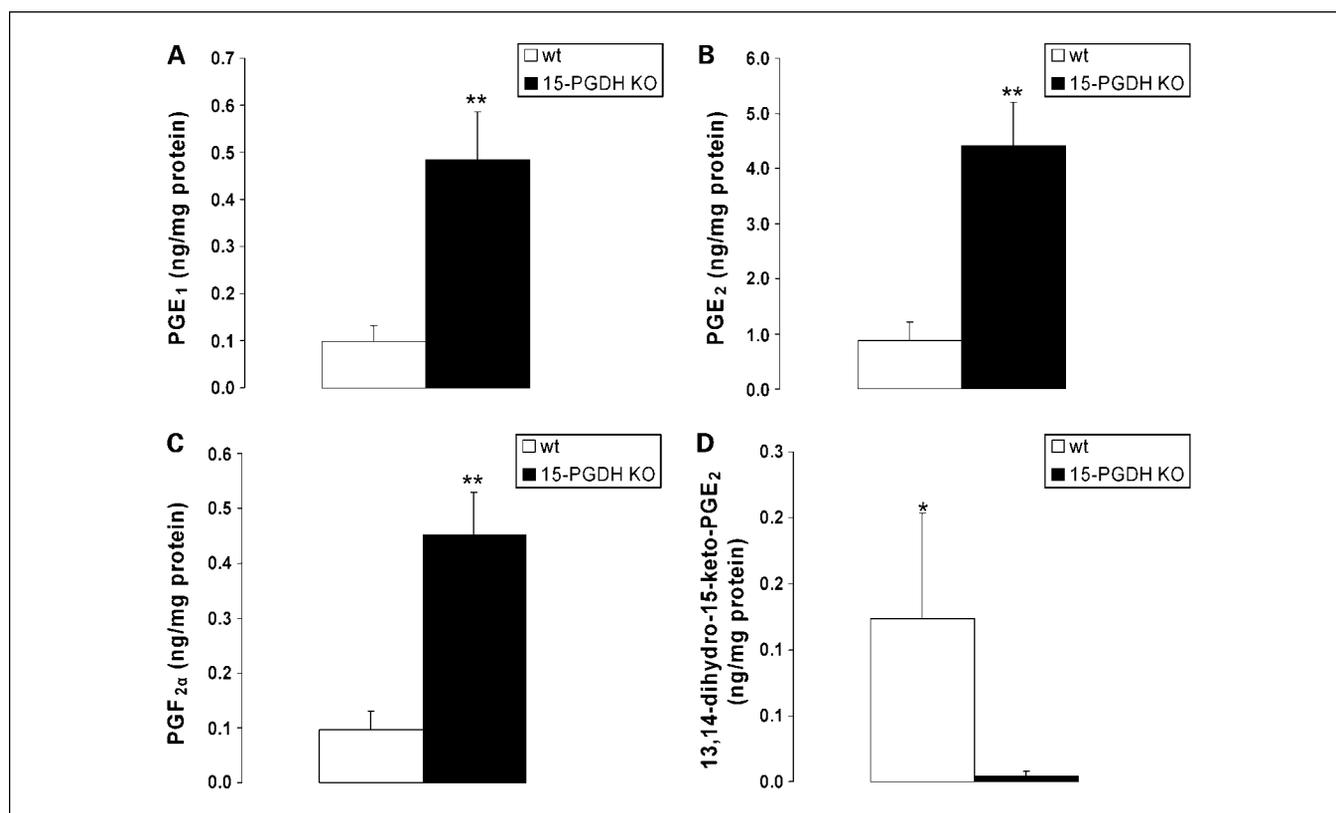


Fig. 5. Knocking out *15-PGDH* results in a PG profile in the murine lung that resembles the pattern found in human NSCLC. PGE₁ (A), PGE₂ (B), PGF_{2α} (C), and 13,14-dihydro-15-keto-PGE₂ (D) were isolated from the lungs of wild-type and *15-PGDH* knockout mice and quantified, as described in Materials and Methods. Columns, mean ($n = 6$ per group); bars, SE. *, $P < 0.05$; **, $P < 0.01$.

levels of PGs in tumor or premalignant tissues. In this context, we note that pioglitazone and rosiglitazone, ligands of the peroxisome proliferator-activated receptor γ , were recently found to up-regulate *15-PGDH* (51). Whether inducers of *15-PGDH* will possess chemopreventive or antitumor activity and an acceptable therapeutic index will need to be evaluated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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