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Pioglitazone and Rosiglitazone Decrease Prostaglandin E_2 in Non–Small-Cell Lung Cancer Cells by Up-Regulating 15-Hydroxyprostaglandin Dehydrogenase

Saswati Hazra, Raj K. Batra, Hsin H. Tai, Sherven Sharma, Xiaoyan Cui, and Steven M. Dubinett

Department of Medicine (S.H., R.B., S.S., X.C., S.D.), Division of Pulmonary and Critical Care Medicine, Lung Cancer Research Program and Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California; Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, California (R.B., S.S., S.D.); and Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Kentucky (H.T.).

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ABSTRACT

Lung cancer cells elaborate the immunosuppressive and anti-apoptotic mediator prostaglandin E_2 (PGE_2), a product of cyclooxygenase-2 (COX-2) enzyme activity. Because peroxisome proliferator-activated receptor (PPAR)γ ligands, such as thiazolidinediones (TZDs), inhibit lung cancer cell growth, we examined the effect of the TZDs pioglitazone and rosiglitazone on PGE_2 levels in non–small-cell lung cancer (NSCLC) A427 and A549 cells. Both TZDs inhibited PGE_2 production in NSCLC cells via a COX-2 independent pathway. To define the mechanism underlying COX-2 independent suppression of PGE_2 production, we focused on other enzymes responsible for the synthesis and degradation of PGE_2. The expression of none of the three prostaglandin synthases (microsomal PGES1, PGES2 and cystosolic PGES) was down-regulated by the TZDs. It is noteworthy that 15-hydroxyprostaglandin dehydrogenase (15-PGDH), an enzyme that produces biologically inactive 15-keto-prostaglandins from active PGE_2, was induced by TZDs. The TZD-mediated suppression of PGE_2 concentration was significantly inhibited by small interfering RNA to 15-PGDH. Studies using dominant-negative PPARγ overexpression or 2-chloro-5-nitrobenzanilide (GW9662; a PPARγ antagonist) revealed that the suppressive effect of the TZDs on PGE_2 is PPARγ-independent. Together, these findings indicate that it is possible to use a clinically available pharmacological intervention to suppress tumor-derived PGE_2 by enhancing catabolism rather than blocking synthesis.

Lung cancer is the major cause of cancer-related death in the United States. There is an overall 5-year survival of less than 15%, and thus new therapeutic strategies are needed (Parkin, 2001). Recent research has focused on targeted pathways operative in lung cancer pathogenesis.

Increased cyclooxygenase expression (Huang et al., 1998) and elevated PGE_2 production have been implicated in the pathogenesis of several malignancies and are also associated with a poor prognosis in lung cancer (Wolff et al., 1998). Two isoenzymes of cyclooxygenase (COX) have been described: the constitutive enzyme COX-1 and the inducible enzyme COX-2. Elevated expression of COX-2 is found in a variety of malignant tissues, including colon, gastric, esophageal, prostate, breast, and lung carcinomas (Huang et al., 1998; Dannenberg and Zakim, 1999; Molina et al., 1999; Shamma et al., 2000; Shao et al., 2000; Soslow et al., 2000; Williams et al., 2000; Yip-Schneider et al., 2000). The COX enzyme possesses two distinct enzymatic functions: a cyclooxygenase activity that converts arachidonic acids to prostaglandin (PG) G2 and a peroxidase activity, which converts PGG2 to PGH2. PGH2 is then converted to PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2 by their respective synthases (Smith et al., 1991).

PGE_2 may promote malignant growth by stimulating angiogenesis, tumor invasiveness, and apoptosis resistance and

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ABBREVIATIONS: COX, cyclooxygenase; PG, prostaglandin; NSCLC, non–small-cell lung cancer; PGDH, 15-hydroxyprostaglandin dehydrogenase; TZD, thiazolidinedione; PPAR-γ, peroxisome proliferator-activated receptor-γ; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; GW9662, 2-chloro-5-nitrobenzanilide; dn, dominant negative; FBS, fetal bovine serum; siRNA, small interfering RNA; PGES, prostaglandin E synthase; mPGES1, microsomal prostaglandin E synthase; cPGES, cytosolic prostaglandin E synthase; PPRE, peroxisome proliferator-activated receptor response element.

by inhibiting immune surveillance in human non–small-cell lung cancer (NSCLC) (Stolina et al., 2000; Heuze-Vourc'h et al., 2003; Krysan et al., 2004; Põld et al., 2004). One strategy for inhibiting carcinogenesis or treating established disease is to prevent the overproduction of PGE_2 in premalignant or malignant tissues. In fact, in murine models, COX-2 inhibitory drugs or treatment with anti-PGE_2 monoclonal antibody have been demonstrated to reduce tumor growth, leading to prolonged survival (Stolina et al., 2000).

Considerable evidence suggests that COX-2, an inducible enzyme expressed in response to cytokines, growth factors, and other stimuli, is a potential pharmacologic target for inhibiting or preventing tumor growth (Riedl et al., 2004; Sandler and Dubinett, 2004). Although studies have suggested that COX-2 inhibition may be beneficial in cancer prevention, recent data raise concern regarding cardiovascular toxicities associated with the use of COX-2 inhibition (Schrör et al., 2005). An alternative approach that could potentially avoid this toxicity includes targeting other elements in the prostanoid pathway downstream of COX-2.

The nicotinamide adenine dinucleotide positive-dependent catabolic enzyme 15-PGDH metabolizes PGE_2 to the biologically inactive 15-keto derivatives (Cho and Tai, 2002). 15-PGDH has recently been identified as a tumor suppressor gene (Ding et al., 2005; Wolf et al., 2006). When the expression of 15-PGDH is suppressed, the limited degradation of PGE_2 may lead to increased tumor growth (Ding et al., 2005). Thus, augmentation of 15-PGDH expression and activity could limit PGE_2 without affecting COX-2.

Thiazolidinedione (TZDs), also known as peroxisome proliferator-activated receptor-γ (PPAR-γ) ligands, may modulate cancer progression and have been the subject of extensive investigation. For example, recent studies show that ciglitazone inhibited the growth of lung cancer cells via induction of apoptosis and differentiation (Chang and Szabo, 2000). In this study, we investigated the effect of the TZDs pioglitatzone and rosiglitazone on COX-2 and PGE_2 levels in NSCLC cell lines. We report that both TZDs suppress PGE_2 levels in a COX-2 expression-independent and 15-PGDH-dependent manner.

Methods and Materials

Cell Culture and Reagents. Human A427 (obtained from Dr. J. A. Radosevich, Northwestern University, Evanston, IL) and A549 (American Type Culture Collection, Manassas, VA) are maintained in Dulbecco's modified Eagle's medium (Mediatech, Herndon, VA) supplemented with 4.5 g/liter glucose and 4 mM L-glutamine, 100 units/ml penicillin/streptomycin (Invitrogen, Carsbad, CA), and 10% fetal calf serum (Gemini Bio-Products, Woodland, CA). Cell cultures were grown in 5% CO_2 atmosphere at 37°C. The TZDs used in these studies were pioglitazone (Actos; Eli Lilly, Toronto, ON, Canada) and rosiglitazone (Cayman Chemical, Ann Arbor, MI). DMSO was used as a diluent. The cells were incubated with 1 to 10 μM pioglitazone or rosiglitazone for 24 h for PGE_2 ELISA. Pioglitazone and rosiglitazone were used at a concentration of 10 μM for the rest of the experiments. To assess the role of PPARγ, experiments were performed in the presence of the PPARγ antagonist GW9662 (10 μM) (Sigma-Aldrich Co, St. Louis, MO) or dn.PPARγ.

PGE_2 Enzyme Immunoassay. Cells were plated in six-well plates in RPMI 1640 medium containing 10% FBS and cultured overnight. The next day, cells were treated with pioglitazone and rosiglitazone (10 μM) for 24 h. Arachidonic acid (final concentration, 15 μM) was added to the culture 1 h before collecting the culture medium. Therefore, TZDs and arachidonic acid were both in the medium for only 1 h before collecting the supernatant for PGE_2 assays. PGE_2 levels were determined by PGE_2 enzyme immunoassay kits (Cayman Chemical). Cells were lysed with radioimmunoprecipitation assay buffer and the COX-2 levels were determined by human COX-2 ELISA kits (Assay Designs, Ann Arbor, MI).

COX-2 ELISA. Cells were plated in six-well plates in RPMI 1640 medium containing 10% FBS medium and treated as described above. The lysates were stored at −80°C for protein isolation. COX-2 protein was measured by the Human Cyclooxygenase-2 Enzyme Immunometric Assay Kit (Assay Designs) using 30 μg of each protein sample.

Western Blot Analysis of Cellular Proteins. NSCLC cells were cultured in a six-well plate for 24 h. Cells were washed with PBS once and lysed with a lysis buffer containing 50 mM Tris-HCl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1× complete protease inhibitor mixture (Roche Diagnostics Corp., Indianapolis, IN), 1 mM Na_3VO_4, and 1 mM NaF. The protein content was measured using Bradford reagent (Bio-Rad Laboratories, Hercules, CA). An equal amount (20 μg) of the whole-cell protein was run and separated by SDS-polyacrylamide gel electrophoresis and transferred on polyvinylidene difluoride membranes (Millipore Corp., Billerica, MA). Proteins were detected by incubating the filter with COX-2 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), Glyceraldehyde-3-phosphate dehydrogenase monoclonal antibody (Advanced Immuno Chemicals Inc., Long Beach, CA) at a concentration of 0.2 to 2 μg/10 ml in Tris-buffered saline (100 mM Tris-HCl and 1.5 M NaCl, pH 7.4) with 5% nonfat milk. For determining the expression of all three prostaglandin E synthases and 15-PGDH after pioglitazone and rosiglitazone treatment, polyclonal antibodies (Cayman Chemical) against prostaglandin E synthase-1 (microso-

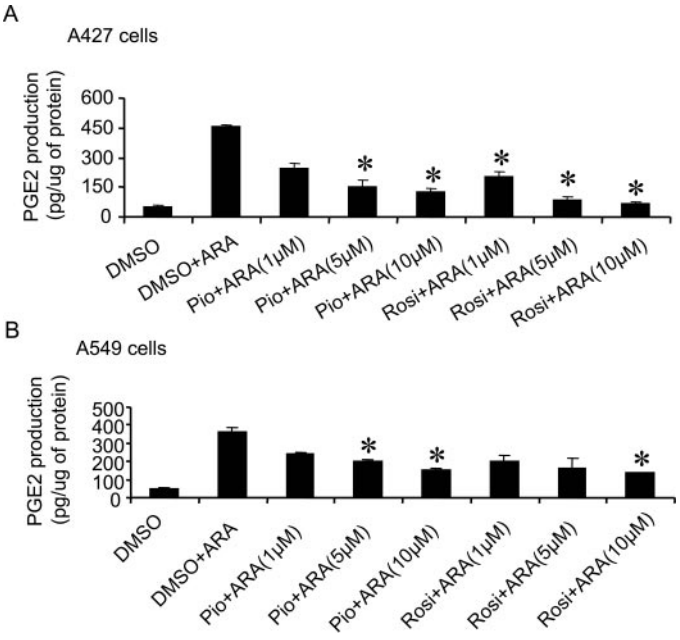

Fig. 1. A and B, pioglitazone and rosiglitazone decrease PGE_2. A427 and A549 cells were treated with 1 to 10 μM concentrations of either pioglitazone or rosiglitazone for 24 h in six-well plates in serum-free medium. Arachidonic acid (15 μM) was applied 1 h before collecting the conditioned medium. PGE_2 concentrations in the medium were then assessed by ELISA. PGE_2 was significantly decreased by pioglitazone and rosiglitazone (10 μM) by approximately 2- and 3-fold, respectively, in the A427 cell line (Fig. 1A) (*, $P < 0.05$, compared with the control "DMSO+ARA"). Pioglitazone and rosiglitazone (10 μM) decreased PGE_2 by approximately 2-fold in A549 cells (B) (*, $P < 0.05$, compared with control "DMSO+ARA"). All data are representative of four independent experiments. ARA, arachidonic acid; Pio, pioglitazone; Rosi, rosiglitazone.

mal), prostaglandin E synthase (cytosolic), prostaglandin E synthase-2 (microsomal) and 15-PGDH were used. The blots were subsequently incubated with an appropriate horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) at 1 μg/10 ml. Detection of proteins on Western blots was achieved using the ECL detection system (Amersham, Piscataway, NJ). Equal protein loading was confirmed by immunodetecting the membranes with anti-GAPDH antibody.

Inhibition of 15-PGDH Expression by Small Interfering RNA. Cells were plated in 24-well plates at 6×10^4 cells per well and grown overnight in RPMI 1640 medium + 10% fetal bovine serum. Cells were transfected with 15-PGDH or negative control (composed of a 19-base-pair scrambled sequence) Silencer small interfering RNA (siRNA) (Ambion, Inc., Austin, TX) using TransMessenger transfection reagent (QIAGEN, Valencia, CA) at different RNA/transfection reagent ratios. In all conditions, we observed a significant suppression of 15-PGDH expression (50–90% inhibition) by using 15-PGDH polyclonal antibody (Cayman Chemical) in Western blotting. For additional experiments, transfection was performed in serum-free RPMI 1640 medium using 1.6 μg of 15-PGDH or control siRNA, 3.2 μl of Enhancer R, and 8 μl of TransMessenger reagent for 3 h followed by the replacement of transfection medium with fresh RPMI 1640 medium supplemented with 10% fetal bovine serum and incubation for an additional 24 h. After 24 h of incubation, the cells were treated with TZD for another 24 h. Arachidonic acid (15 μM) was added 1 h before collecting the medium for PGE_2 ELISA.

Transient Transfection/PGE_2 Assay. The dn.PPARγ construct was a generous gift of Dr. V. K. Chatterjee (Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK) (Gurnell et al., 2000). Cells were seeded at a density of 3×10^6 A549 cells/well in a six-well plate in RPMI 1640 medium containing 10% fetal bovine serum and cultured overnight. For each well, 1 μg of the PCDNA3 control vector or 1 μg of dn.PPARγ vector was transfected with the Effectene transfection reagent (QIAGEN) according to the manufacturer's protocol. The TZDs (10 μM) were added 24 h after the transfection in a serum-free medium for another 24 h. Arachidonic acid (15 μM) was added 1 h before harvesting the medium. Culture medium was collected and PGE_2 levels were determined using PGE_2 EIA kit (Cayman Chemicals, Ann Arbor, MI).

Statistical Analysis*:* Probability values were calculated using two-tailed non paired Student's t test. Tests of statistical significance were significant if $P < 0.05$.

Results

Pioglitazone and Rosiglitazone Inhibit PGE_2 Production in NSCLC Cell Lines. Two NSCLC cell lines, A427 and A549, were used to access the Effect of TZDs on PGE_2 production. These cells were incubated with 1 to 10 μM pioglitazone or rosiglitazone for 24 h. The greatest inhibition of PGE_2 by these TZDs was observed at 10 μM in both A427 (Fig. 1A) and in A549 (Fig. 1B) cells when 1, 5, and 10 μM TZDs were used for the treatment.

Suppression of PGE_2 by Pioglitazone and Rosiglitazone Is Mediated via a COX-2 Expression-Independent Pathway. To determine whether this inhibition of PGE_2 was COX-2-dependent, we tested the capacity of these TZDs to inhibit IL-1β-induced COX-2 expression in NSCLC cells. Neither pioglitazone (Fig. 2A) nor rosiglitazone (Fig. 2A) decreased IL-1β induced COX-2 expression levels, as shown in Western blots. These results were also confirmed by COX-2 ELISA assay as shown in Fig. 2, B and C.

TZDs Do Not Suppress the Expression of PGE Synthases but Up-Regulate 15-PGDH Expression in NSCLC Cells. To understand the mechanism underlying COX-2-independent suppression of PGE_2 by the TZDs in NSCLC cells, the downstream events that regulate PGE_2 production were assessed. For example, an inducible prostaglandin E synthase (PGES) converts COX-derived PGH_2 to PGE_2. Three enzymes possessing PGE synthetic activity have been identified. Microsomal PGES (mPGES1) is an enzyme downstream of COX-2 that affects PGE_2 production, whereas cytosolic PGES (cPGES) is functionally coupled with COX-1 only, and mPGES2 is functionally coupled with both COX-1 and COX-2 (Murakami and Kudo, 2006).

Here, we determined whether these three enzymes (mPGES1, cPGES, mPGES2) were altered by TZDs in A427 and A549 cell lines (Fig. 3, A–D). Western blot analysis of the mPGES1, cPGES, and mPGES2 enzymes revealed no pioglitazone- or rosiglitazone-mediated decrease in expression.

We next examined whether pioglitazone and rosiglitazone could regulate biological inactivation of PGE_2. As described previously, the first step of metabolism of PGE_2 was catalyzed by the 15-PGDH enzyme, which produces biologically inactive 15-keto-prostaglandins. TZDs (such as ciglitazone) have been suggested to inhibit 15-PGDH (Cho and Tai, 2002). However, Western blot analysis revealed that 24-h treatment with pioglitazone (10 μM) (Fig. 3E) or rosiglitazone (Fig. 3F) up-regulated 15-PGDH protein expression in both NSCLC cell lines.

15-PGDH Mediates the Inhibition of PGE_2 by TZDs in NSCLC Cells. To determine the role of 15-PGDH in the reduction of PGE_2 concentrations by pioglitazone and rosiglitazone, A549 cells were transiently transfected with siRNA targeting the 15-PGDH gene. After transfection with 15-

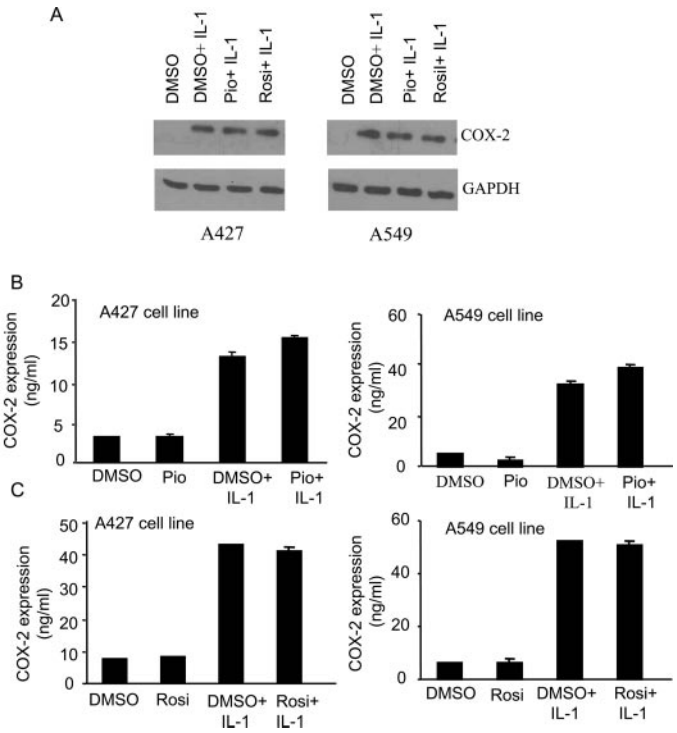

Fig. 2. NSCLC cells were pretreated with IL-β (280 units/ml) for 30 min followed by treatment with 10 μM concentrations of either pioglitazone or rosiglitazone for 24 h in six-well plates in serum-free conditions. Western blot analysis shows that pioglitazone and rosiglitazone did not alter COX-2 protein levels in either A549 or A427 cell line (A). This was further confirmed by COX-2 ELISA assay. Neither pioglitazone (B) nor rosiglitazone (C) affected basal or IL1-induced COX-2 levels in NSCLC cell lines. Data are representative of four independent experiments. Pio, pioglitazone; Rosi, rosiglitazone.

PGDH siRNA, Western blot analysis revealed significant suppression of 15-PGDH protein, whereas it did not affect GAPDH protein expression in A549 cells (Fig. 4A). After 24-h treatment with pioglitazone or rosiglitazone, cell-free culture supernatants were harvested for PGE_2 assays. In the absence of TZDs, 15-PGDH siRNA-treated cells showed a marked increase in PGE_2 concentrations compared with those of control siRNA. (Fig. 4, B and C). Although significant reductions in PGE_2 concentrations (approximately 50% for both pioglitazone and rosiglitazone) were demonstrated in the control siRNA-transfected cells after TZD exposure, these reductions were not evident in 15-PGDH siRNA-transfected cells. This suggests that 15-PGDH induction is critical for the pioglitazone- and rosiglitazone-mediated suppression of PGE_2 levels.

Suppression of PGE_2 by Pioglitazone and Rosiglitazone Is PPARγ-Independent. To determine whether the suppressive effects of pioglitazone and rosiglitazone are PPARγ-dependent, a dominant-negative PPARγ plasmid construct (Gurnell et al., 2000) was transfected in A549 cells. As expected, the PPRE activity was increased in the presence of both pioglitazone and rosiglitazone in NSCLC (Fig. 5A). Expression of dn.PPARγ significantly suppressed the induction of basal as well as TZD-mediated PPRE activity in A549 cell lines (Fig. 5A), indicating significant suppression of PPARγ expression by dn.PPARγ. In the presence of overexpression of dn.PPARγ (Fig. 5, B and C), pioglitazone and rosiglitazone maintained the capacity to decrease PGE_2, suggesting that this effect of the TZDs is PPARγ-independent. This observation was further verified by using GW9662, a PPARγ antagonist (Fig. 5, D and E). GW9662 (10 μM) was added 1 h before addition of pioglitazone or rosiglitazone in A427 cells. The experimental results using dn.PPARγ and GW9662 suggest that the suppression of PGE_2 by these TZDs is PPARγ-independent.

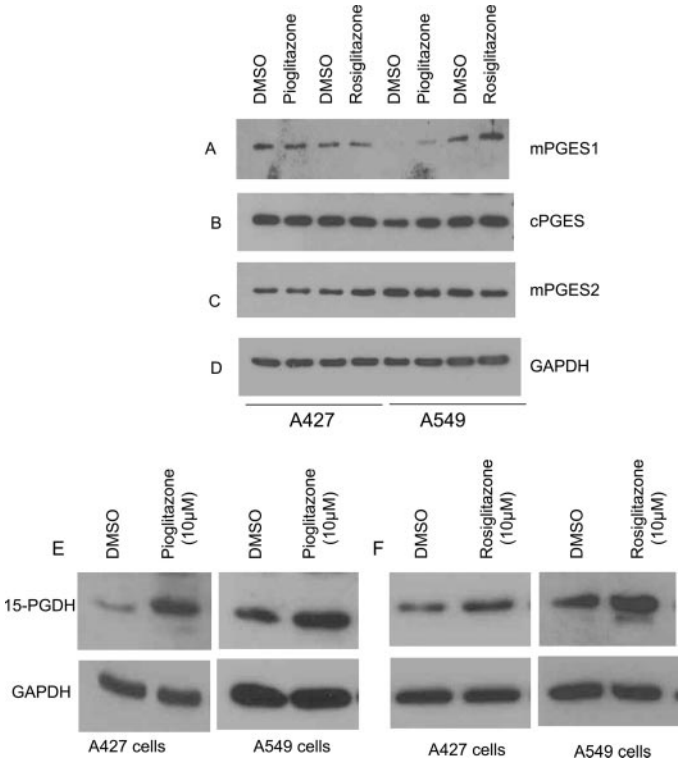

Fig. 3. Neither pioglitazone nor rosiglitazone suppress PGES expression in NSCLC, although they up-regulate 15-PGDH protein expression. The cells were treated with TZDs or DMSO for 24 h in serum-free medium. Both pioglitazone (10 μM) and rosiglitazone (10 μM) failed to inhibit mPGES1 (A), cPGES (B), and mPGES2 (C) expression compared with the DMSO control in both A427 and A549 cells, suggesting that the TZDs suppressed PGE_2 in a PGES-independent manner. The bottom panel of GAPDH expression (D) shows the equal loading of the protein samples. (Data are representative of three independent experiments). Pioglitazone and rosiglitazone increase 15-PGDH protein levels in NSCLC cell lines. Western blots were performed with the whole protein extracts from 24-h TZD-treated cells. Treatment with pioglitazone (10 μM) shows a significant increase in the 15-PGDH protein expression in both A427 and A549 cell lines (E). Rosiglitazone treatment (10 μM, 24 h) up-regulated 15-PGDH protein expression in both A427 and A549 cell lines (F). Blots were stripped and re-probed with anti-GAPDH antibody to verify equal loading (E and F, bottom). (Data are representative of three independent experiments).

Discussion

We report that pioglitazone and rosiglitazone have the capacity to reduce PGE_2 production in NSCLC. To avoid the potential cardiovascular toxicities of COX-2 inhibition, we evaluated pharmacologic agents for their capacity to regulate tumor derived PGE_2 by modulating arachidonic acid pathway elements downstream of COX-2. Because PGE_2 was decreased without changes in COX-2 levels, we concluded that these TZDs reduce PGE_2 in a COX-2 expression-independent manner. We found that PGES protein levels were

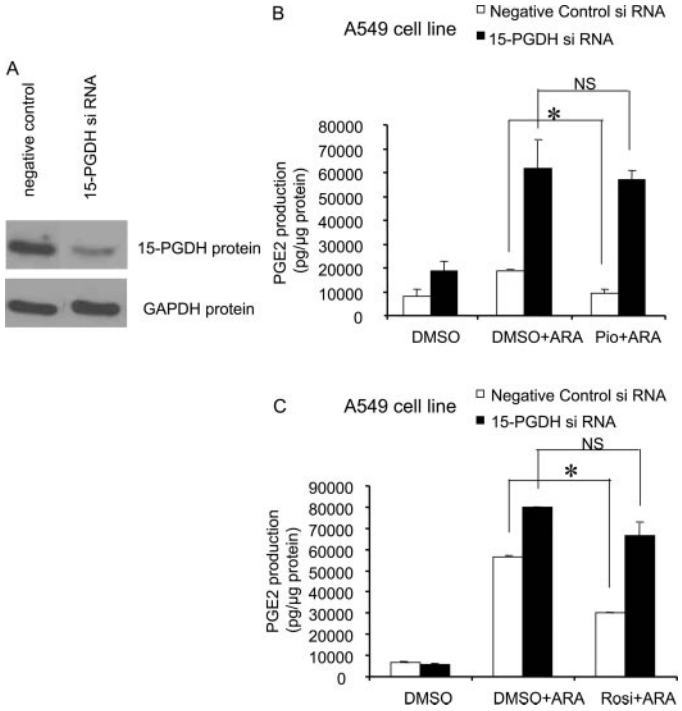

Fig. 4. A–C, 15-PGDH inhibition by siRNA prevents the TZD-mediated suppression of PGE_2 levels in A549 cells. Silencing of 15-PGDH by siRNA transfection shows a significant suppression of 15-PGDH protein, whereas no change in control GAPDH protein expression was demonstrated by Western blot analysis (A). In the control siRNA-transfected cells, PGE_2 levels were increased in the presence of arachidonic acid (15 μM), whereas pioglitazone (B) or rosiglitazone (C) treatment significantly reduced PGE_2 concentrations (*, $P < 0.05$). Inhibition of 15-PGDH by siRNA led to a marked increase in PGE_2 levels compared with the control siRNA transfection in the presence of arachidonic acid (15 μM) (B and C). In the presence of 15-PGDH siRNA, pioglitazone and rosiglitazone did not significantly (NS, nonsignificant) decrease PGE_2 levels (B and C). Data are representative of three independent experiments. ARA, arachidonic acid; Pio, pioglitazone; Rosi, rosiglitazone.

not suppressed by the TZDs in NSCLC cells. In contrast, 15-PGDH expression was up-regulated by TZDs.

Colon cancers have been observed to manifest very limited expression of 15-PGDH; Backlund et al. (2005) also reported that 15-PGDH is down-regulated in colorectal cancer. Furthermore, levels of 15-PGDH are reduced in several other malignancies, including NSCLC; Ding et al. (2005) found that, compared with normal epithelial cells, 15-PGDH expression was diminished in human lung tumors. This led to the suggestion that 15-PGDH, by suppressing the level of PGE_2, may promote susceptibility to apoptosis and thus function as a tumor suppressor gene (Ding et al., 2005). Thus, the development of certain malignancies may require a combination of up-regulated COX-2 expression and a concomitant down-regulation of an opposing and putative tumor suppressor gene, 15-PGDH (Yan et al., 2004). Consistent with these previous investigations, we found that 15-PGDH inhibition by siRNA increases PGE_2 production in A549 cells. We report here that the capacity for pioglitazone and rosiglitazone to decrease PGE_2 was significantly decreased after 15-PGDH si-RNA transfection.

TZDs are currently used for the treatment of type 2 diabetes mellitus (Durbin, 2004) and have been shown to have a broad array of biological activities. Some of these TZDs have been shown to exert anti-inflammatory (Consoli and Devangelio, 2005), antiproliferative (Schmidt et al., 2004), and antiangiogenic effects (Keshamouni et al., 2005). TZD-mediated PPARγ activation has been shown to regulate COX-2 expression in several malignancies including cervical (Han et al., 2003), colon, and liver cancers (Li et al., 2003). It has been reported that TZDs inhibit tumor formation in a variety of animal models, including colon (Yoshizumi et al., 2004) and lung cancers (Keshamouni et al., 2004). PPARγ is expressed in many NSCLC cell lines (Chang and Szabo, 2000), and troglitazone and pioglitazone significantly inhibit angiogenesis in NSCLC (Keshamouni et al., 2005). These two TZDs were also shown to inhibit tumor-associated angiogenesis by blocking the expression of ELR+CXC chemokines.

Although TZDs are widely known as ligands for PPARγ, they may mediate receptor-independent effects, as demonstrated here and as previously reported (Chawla et al., 2001; Lennon et al., 2002). For example, by using the embryonic stem cells from PPARγ-null mice, Chawla et al. (2001) found that neither macrophage differentiation nor anti-inflammatory effects of synthetic PPARγ ligands are PPARγ receptor-dependent. To understand whether the suppression of PGE_2 by pioglitazone and rosiglitazone is PPARγ-dependent in NSCLC, we performed experiments using either a dn.PPARγ plasmid vector or a PPARγ inhibitor GW9662. Here, for the first time, we report that pioglitazone and rosiglitazone increase 15-PGDH and thus decrease PGE_2 in a PPARγ-independent manner.

PGE_2 is well-known to play an important role in tumorigenesis. However, the precise role of the 15-PGDH enzyme, which regulates the biological activity via degradation of PGE_2, has not yet been well defined in the pathogenesis of lung cancer.

The potential benefits of inhibiting PGE_2 levels in a COX-2-independent manner include the following. First, promoting 15-PGDH activity could decrease PGE_2 without modify-

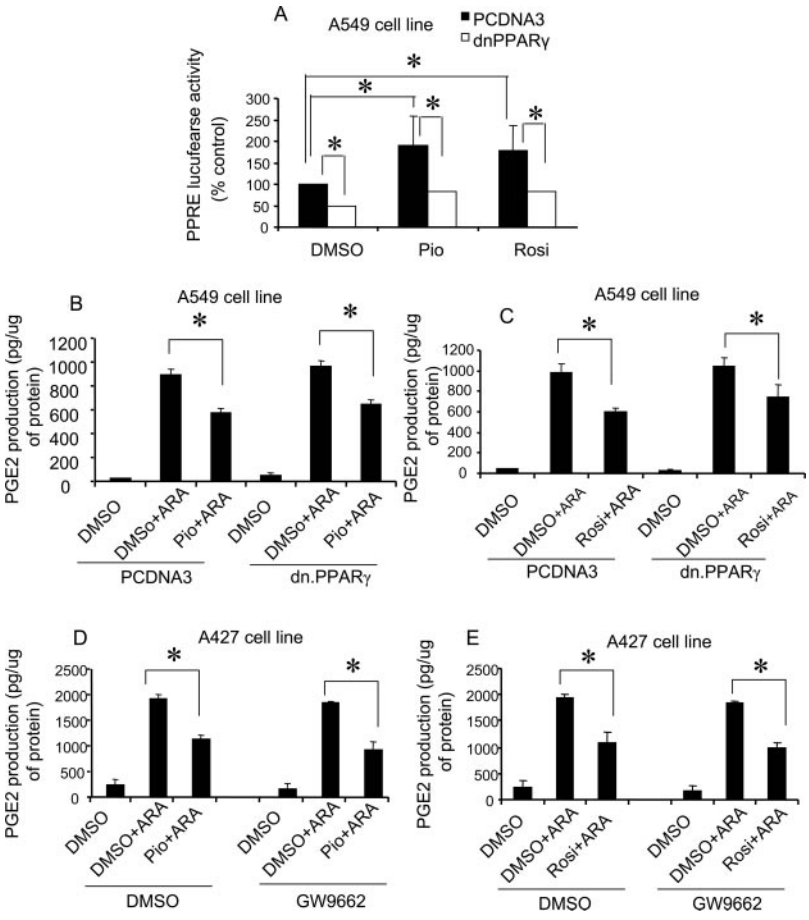

Fig. 5. A–E, PPARγ antagonists do not alter PGE_2 levels in NSCLC cell lines. The cells were first transiently transfected with dn.PPARγ for 24 h followed by incubation with 10 μM pioglitazone and rosiglitazone for another 24 h. A shows the effect of a dn.PPARγ expression (by transient transfection) on pioglitazone- or rosiglitazone-mediated up-regulation of PPRE activity in A549 cell line. The basal and TZD-mediated PPRE activity was significantly suppressed by dn.PPARγ expression ($*$, $P < 0.05$). Arachidonic acid (15 μM) was added before the collection of the medium for PGE_2 assay. The overexpression of dn.PPARγ did not abrogate the suppression effect of pioglitazone (B) and rosiglitazone (C) on PGE_2 levels in A549 cell line. GW9662, a PPARγ antagonist, was applied to A427 cells. One hour before adding pioglitazone (D) or rosiglitazone (E), GW9662 (10 μM) was applied to the medium. One representative experiment of three independent experiments is shown here. $*$, $P \leq 0.05$. ARA, arachidonic acid; Pio, pioglitazone; Rosi, rosiglitazone.

ing other prostaglandins such as PGI_2. This is potentially important because the latter has been noted to have antitumor properties (Keith et al., 2004). It has been suggested that a ratio of PGs may be important in regulating the malignant phenotype. Thus, inhibiting COX-2 activity would diminish both PGE_2 and PGI_2, whereas selective induction of 15-PGDH could lead to a more favorable PGI_2/PGE_2 ratio. Second, the suppression of PGE_2 levels without alteration in COX-2 may limit some of the cardiovascular toxicities associated with COX-2 inhibition. Finally, unlike COX-2 inhibition, which may lead to up-regulation of certain leukotrienes that favor malignant progression (Mao et al., 2004), 15-PGDH induction may lead only to a decrement of PGE_2. This speculation will require further investigation. Ultimately, these findings will allow strategies for developing PGE_2 inhibitors in the treatment and prevention of lung cancer.

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Address correspondence to: Saswati Hazra, UCLA Lung Cancer Research Program of the Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, 37-131 CHS, 10833 Le Conte Avenue, Los Angeles, CA 90095-1690. E-mail: shazra@mednet.ucla.edu