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Pioglitazone and Rosiglitazone decrease PGE2 in non-small cell lung cancer cells by upregulating 15-hydroxyprostaglandin dehydrogenase

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Running title: TZD mediated suppression of PGE2 in NSCLC

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A list of nonstandard abbreviations:
NSCLC-Non small cell lung Cancer,
TZDs- Thiazolidinediones,
PPARγ-Peroxisome Proliferator-Activated Receptor gamma,
15-PGDH-15-hydroxyprostaglandin dehydrogenase

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Abstract:

Lung cancer cells elaborate the immunosuppressive and anti-apoptotic mediator PGE2, a product of COX-2 enzyme activity. Because PPARγ ligands, such as thiazolidinediones (TZDs) inhibit lung cancer cell growth, we examined the effect of TZDs (pioglitazone and rosiglitazone) on PGE2 levels in NSCLC (A427 and A549 cells). Both TZDs inhibited PGE2 production in NSCLC cells via a COX-2 independent pathway. In order to define the mechanism underlying COX-2 independent suppression of PGE2 production, we focused on other enzymes responsible for the synthesis and the degradation of PGE2. The expression of all three Prostaglandin synthases (mPGES1, cPGES and mPGES2) were not downregulated by the TZDs. Importantly, 15-hydroxyprostaglandin dehydrogenase (15-PGDH), an enzyme that produces biologically inactive 15-keto-prostaglandins from active PGE2, was induced by TZDs. The TZD-mediated suppression of PGE2 concentration was significantly inhibited by si-RNA to 15-PGDH. Studies utilizing dominant-negative PPARγ overexpression or GW9662 (a PPARγ antagonist) revealed that the suppressive effect of the TZDs on PGE2 is PPARγ independent. Collectively, these findings indicate that it is possible to utilize a clinically available pharmacological intervention to suppress tumor-derived PGE2 by enhancing catabolism rather than blocking synthesis.

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Introduction:

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 PGE2 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 iso-enzymes of cyclooxygenase (COX) have been described: a constitutive enzyme COX-1 and an inducible enzyme COX-2. Elevated expression of COX-2 is found in a variety of malignancies including colon, gastric, esophageal, prostate, breast, and lung carcinomas (Dannenberg and Zakim, 1999; Huang et al., 1998; 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, which converts arachidonic acids to PGG2 and a peroxidase activity, which converts PGG2 to PGH2. PGH2 is then converted to Prostaglandin E 2 (PGE_2), PGD_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2 (TXA_2) by their respective synthases (Smith et al., 1991).

PGE_2 may promote malignant growth by stimulating angiogenesis, tumor invasiveness, apoptosis resistance and inhibiting immune surveillance in human non-small cell lung cancer (NSCLC) (Heuze-Vourc'h et al., 2003; Krysan et al., 2004; Pold et al., 2004;

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Stolina et al., 2000). One strategy for inhibiting carcinogenesis or treating established disease is to prevent the overproduction of PGE2 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 the tumor growth, leading to prolonged survival (Stolina et al., 2000).

There is considerable evidence suggesting 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 (Schror 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 PGE2 may lead to increased tumor growth (Ding et al., 2005). Thus augmentation of 15-PGDH expression and activity could limit PGE2 without impacting COX-2.

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Thiazolidinedione (TZDs), also known as peroxisome proliferator-activated receptor-gamma (PPAR-gamma) 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). Here we investigated the effect of TZDs (pioglitatzone and rosiglitazone) on COX-2 and PGE2 levels in non-small cell lung cancer cell lines. We report that both TZDs suppress PGE2 levels in a COX-2 expression independent and 15-PGDH dependent manner**.**

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Methods and materials:

Cell Culture and Reagents: Human A427 (obtained from Dr. J.A. Radosevich, Northwestern University) and A549 (American Type Culture Collection, Bethesda, MD) are maintained in Dulbecco's modified Eagle's medium (Mediatech, Herndon, VA) supplemented with 4.5 g/liter glucose and 4 mM L-glutamine, 100units/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 utilized in these studies were pioglitazone (Actos, Eli Lilly, Ontario, Canada) and rosiglitazone (Cayman Chemical, Ann Arbor, MI). DMSO was utilized as diluents. The cells were incubated with 1-10μM pioglitazone or rosiglitazone for 24 hours for PGE2 ELISA Assay. The concentration of 10μM of pioglitazone and rosiglitazone were used for the rest of the experiments. To assess the role of PPARγ, experiments were performed in the presence of the PPARγ antagonist GW9662 (10μM) (Sigma-Aldrich Co, St. Louis, MO) or dn.PPARγ.

PGE2 EIA Assay: Cells were plated in 6-well plates in RPMI containing 10% FBS medium and cultured overnight. Next day, cells were treated with pioglitazone and rosiglitazone (10μM) for 24h. Arachidonic acid (final 15μM) was added to the culture one hour before collecting the culture medium. Therefore, TZDs and arachidonic acid were both in the medium for only one hour before collecting the supernatant for PGE2 assays. PGE_2 levels were determined by PGE_2 EIA kits (Cayman Chemicals, Ann Arbor, MI). Cells were lysed with RIPA buffer and the COX-2 levels were determined by human COX-2 ELISA kits (Assay Designs, Ann Arbor, MI).

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COX-2 ELISA Assay: Cells were plated in 6-well plates in RPMI 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, Ann Arbor, Michigan) using 30μg of each protein sample.

Western Blot Analysis of Cellular Proteins: NSCLC cells were cultured in a 6-well plate for 24 hours. Cells were washed with PBS once and lysed with a lysis buffer containing 50 mM Tris-HCl, pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1mM EDTA, 1mM PMSF, 1x complete protease inhibitor mixture (Roche Diagnostics Corp., Indianapolis, IN)], 1mM Na3Vo4, 1mM NaF. The protein content was measured using Bradford reagent (Biorad, Hercules, CA). An equal amount (20 μg) of the whole cell protein was run and separated by SDS-PAGE and transferred on polyvinylidene difluoride membranes (Millipore Corp., Bedford, 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 0.2–2 μg/10 ml in TBS (100 mM Tris-HCl, 1.5 M NaCl, pH 7.4) with 5% nonfat milk. For determining the expression of all three prostaglandin E synthase expression and 15-PGDH expression in pioglitazone and rosiglitazone treatment, polyclonal antibodies (Cayman Chemical, Ann Arbor, MI) against Prostaglandin E Synthase-1 (microsomal), 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)

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at 1 µg/10 ml. Detection of proteins on Western blots was achieved using the Amersham ECL Detection System (Amersham Biosciences, Piscataway, NJ). Equal protein loading was confirmed by immuno-detecting the membranes with anti-GAPDH antibody. **Inhibition of 15-PGDH Expression by Small Interfering RNA (siRNA):** Cells were plated in 24-well plates at 6 x 10^4 cells per well and grown overnight in RPMI + 10% fetal bovine serum. Cells were transfected with 15-PGDH or negative control (comprised of a 19 bp scrambled sequence) Silencer 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% to 90% inhibition) by utilizing 15-PGDH polyclonal antibody (Cayman Chemical, Ann Arbor, MI) in western blotting. For additional experiments, transfection was performed in serum-free RPMI using 1.6 µg of 15-PGDH or control siRNA, 3.2 µL of Enhancer R, and 8 µL of TransMessenger reagent for 3 hours followed by the replacement of transfection medium with fresh RPMI supplemented with 10% fetal bovine serum and incubation for an additional 24 hours. After 24 hours of incubation, the cells were treated with TZD for another 24 hours. Arachidonic acid (15µM) was added 1 hour before collecting the medium for PGE2 ELISA.

Transient transfection/PGE2 assay: The dn.PPARγ construct was a generous gift of Dr. VK Chatterjee (Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge) (Gurnell et al., 2000). Cells were seeded at a density of 3.10^6 cells/well (A549) in a 6 well plate in RPMI medium containing 10% FBS and cultured overnight. For each well, 1 µg of the PCDNA3 control vector or 1µg of dn.PPARγ vector was transfected with the Effectene transfection reagent (Qiagen, Valencia, CA) according

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to the manufacturer's protocol. The TZDs (10μM) were added 24 hours after the transfection in a serum free medium for another 24 hours. Arachidonic acid (15μM) was added 1 hour before harvesting the medium. Culture medium was collected and PGE2 levels were determined using PGE2 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 $P < 0.05$.

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Results:

Pioglitazone and rosiglitazone inhibit PGE2 production in NSCLC cell lines. Two NSCLC cell lines, A427 and A549 were utilized to access the impact of TZDs on PGE2 production. These cells were incubated with 1-10μM pioglitazone or rosiglitazone for 24 hours. The greatest inhibition of PGE2 by these TZDs was observed at the 10μM concentration level in both A427 (1A) and in A549 (1B) cells when 1, 5 and 10μM of TZDs were used for the treatment.

Suppression of PGE2 by pioglitazone and rosiglitazone is mediated via COX-2 expression independent pathway. To determine whether this inhibition of PGE2 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 figure 2B and 2C.

TZDs do not suppress the expression of PGE synthases but upregulate 15-PGDH expression in NSCLC cells. In order to understand the mechanism underlying COX-2-independent suppression of PGE2 by the TZDs in NSCLC cells, the downstream events that regulate PGE2 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 while cytosolic PGES (cPGES) is

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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 if these three enzymes (mPGES1, CPGES, mPGES2) were altered by TZDs in A427 and A549 cell lines (Fig 3A). 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 PGE2. As described previously, the first step of metabolism of PGE2 is 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-hour treatment with pioglitazone (10μM) (Fig 3E) or rosiglitazone (Fig 3F) upregulated 15-PGDH protein expression in both NSCLC cell lines.

15-PGDH mediates the inhibition of PGE2 by TZDs in NSCLC cells. To determine the role of 15-PGDH in the reduction of PGE2 concentrations by pioglitazone and rosiglitazone, A549 cells were transiently transfected with si-RNA targeting the 15-PGDH gene. Following transfection with 15-PGDH si-RNA, western blot analysis revealed significant suppression of 15-PGDH protein while it did not affect GAPDH protein expression in A549 cells (Fig 4A). Following 24-hour treatment with pioglitazone or rosiglitazone, cell free culture supernatants were harvested for PGE2 assays. In the absence of TZDs, 15-PGDH siRNA treated cells showed a marked increase in PGE2 concentrations compared to that of control si-RNA. (Fig 4B, 4C). While significant

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reductions in PGE2 concentrations (approximately 50% for both pioglitazone and rosiglitazone) were demonstrated in the control siRNA-transfected cells following 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 PGE2 levels.

Suppression of PGE2 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 is increased in the presence of both pioglitazone and rosiglitazone in NSCLC (5A). Expression of dn.PPARγ significantly suppressed the induction of basal as well as TZDs mediated PPRE activity in A549 cell lines (Fig 5A), indicating that this dn.PPARγ significantly suppress PPARγ expression. In presence of overexpression of dn.PPARγ (Fig 5B & 5C), pioglitazone and rosiglitazone maintained the capacity to decrease PGE2 suggesting that this effect of the TZDs is PPARγ-independent. This observation was further verified by using GW9662, a PPARγ antagonist (Fig 5D & 5E). GW9662 (10μM) was added 1 hour before addition of pioglitazone or rosiglitazone in A427 cell ine. The experimental results utilizing dn.PPARγ and GW9662 suggest that the suppression of PGE2 by these TZDs is PPARγ independent.

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Discussion

Here we report that pioglitazone and rosiglitazone have the capacity to reduce PGE2 production in NSCLC. In order to avoid the potential cardiovascular toxicities of COX-2 inhibition, we evaluated pharmacologic agents for their capacity to regulate tumor derived PGE2 by modulating arachidonic acid pathway elements downstream of COX-2. Because PGE2 was decreased without changes in COX-2 levels, we concluded that these TZDs reduce PGE2 in a COX-2 expression independent manner. We found that PGES protein levels were not suppressed by the TZDs in NSCLC cells. In contrast, 15-PGDH expression was upregulated by TZDs.

It has been recently observed that colon cancers manifest very limited expression of 15-PGDH; Backlund *et. al* also reported that 15-PGDH is down-regulated in colorectal cancer (Backlund et al., 2005). Furthermore, levels of 15-PGDH are reduced in several other malignancies including NSCLC (Ding et al., 2005); Ding et al found that in comparison to 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 PGE2, 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 upregulated 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 si-RNA increases PGE2 production in A549 cells. Here we report that the capacity for

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pioglitazone and rosiglitazone to decrease PGE2 was significantly decreased following 15-PGDH si-RNA transfection.

TZDs are currently utilized for the treatment of type 2 diabetes mellitus (Durbin, 2004) and have been shown to have a broad array of biological activities. More recently, some of these TZDs were shown to exert anti-inflammatory (Consoli and Devangelio, 2005), anti-proliferative (Schmidt et al., 2004) and anti-angiogenic 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.

While 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 utilizing the embryonic stem cells from PPARγ null mice, Chawla et al found that neither macrophage differentiation nor anti-inflammatory effects of synthetic PPARγ ligands are PPARγ receptor dependent (Chawla et al., 2001). In order to understand whether the suppression of PGE2 by pioglitazone and rosiglitazone is PPARγ dependent in NSCLC, we performed experiments utilizing either

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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 PGE2 in a PPARγ independent manner.

It is well known that PGE2 plays an important role in tumorigenesis. However, the precise role of the 15-PGDH enzyme, which regulates the biological activity via degradation of PGE2, has not yet been well defined in the pathogenesis of lung cancer. The potential benefits of inhibiting PGE2 levels in a COX-2 independent manner include the following. First, promoting 15-PGDH activity could decrease PGE2 without modifying other prostaglandins such as PGI2. This is potentially important because the latter has been noted to have anti tumor 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 PGE2 and PGI2 whereas selective induction of 15-PGDH could lead to a more favorable PGI2/PGE2 ratio. Second, the suppression of PGE2 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 upregulation of certain leukotrienes that favor malignant progression (Mao et al., 2004), 15-PGDH induction may lead only to a decrement of PGE2. This speculation will require further investigation. Ultimately, these findings will allow strategies for developing PGE2 inhibitors in the treatment and prevention of lung cancer.

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Footnotes:

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Figure legends.

Fig 1 (A-B): Pioglitazone and rosiglitazone decrease PGE2: A427 and A549 cells were treated with 1-10μM of either pioglitazone or rosiglitazone for 24 hours in 6 well plates in serum free medium. Arachidonic acid (15μM) was applied one hour prior to collecting the conditioned medium. PGE2 concentrations in the medium were then assessed by ELISA. PGE2 was significantly decreased by pioglitazone and rosiglitazone (10μM) by approximately 2 fold and 3 fold respectively in the A427 cell line (Fig 1A)(* $P < 0.05$, when compared to the control "DMSO+ARA"). Pioglitazone and rosiglitazone (10 μM) decreased PGE2 by approximately 2 fold in A549 cells (Fig 1B)(* $P < 0.05$, when compared to the control "DMSO+ARA"). All data are representative of four independent experiments.

Pio = Pioglitazone, Rosi=Rosiglitazone, ARA= Arachidonic Acid

Fig 2 NSCLC cells were pretreated with IL-β (280 U/ml) for 30 min followed by treatment with 10μM of either pioglitazone or rosiglitazone for 24 hours in 6 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 (2A). This was further confirmed by COX-2 ELISA assay. Neither pioglitazone (Fig 2B) nor rosiglitazone (Fig 2C) affected basal or IL1-induced COX-2 levels in NSCLC cell lines. Data are representative of four independent experiments.

Pio=Pioglitazone, Rosi=Rosiglitazone

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Fig 3: Neither pioglitazone nor rosiglitazone suppress PGE synthase (PGES) expression in NSCLC while they upregulate 15-PGDH protein expression: The cells were treated with TZDs or DMSO for 24 hours in serum free medium. Both Pioglitazone (10μM) and rosiglitazone (10μM) failed to inhibit mPGES1 (Fig 3A), cPGES (Fig 3B) and mPGES2 (Fig 3C) expression as compared to the DMSO control in both A427 and A549 cells suggesting that the TZDs suppressed PGE2 via PGES independent way. The bottom panel of GAPDH expression (Fig 3D) 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-hour TZD treated cells. Treatment with pioglitazone (10μM) shows a significant increase in the 15-PGDH protein expression in both A427 and A549 cell lines (Fig 3E). Rosiglitazone treatment (10μM, 24 hour) up-regulated 15-PGDH protein expression in both A427 and A549 cell lines (Fig 3F). Blots were stripped and re-probed with anti-GAPDH antibody to verify equal loading (Fig 3E and 3F, bottom panels). (Data are representative of three independent experiments).

Fig 4 (A-C): 15-PGDH inhibition by si-RNA prevents the TZD mediated suppression of PGE2 levels in A549 cells. Silencing of 15-PGDH by si-RNA transfection shows a significant suppression of 15-PGDH protein while no change in control GAPDH protein expression was demonstrated by western blot analysis (Fig 4A). In the control si-RNA transfected cells, PGE2 levels were increased in the presence of arachidonic acid (15μM) while pioglitazone (Fig 4B) or rosiglitazone (Fig 4C) treatment significantly reduced

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PGE2 concentrations (* $P < 0.05$). Inhibition of 15-PGDH by si-RNA led to a marked increase in PGE2 levels compared to the control si-RNA transfection in the presence of arachidonic acid (15μM) (Fig 4B and 4C). In the presence of 15-PGDH siRNA, pioglitazone and rosiglitaozne did not significantly (NS, non significant) decrease PGE2 levels (Fig 4B and 4C). Data are representative of three independent experiments
ARA=Arachidonic acid, Pio=Pioglitazone, Rosi=Rosiglitazone

Fig 5 (A-D): PPARγ antagonists do not alter PGE2 levels in NSCLC cell lines: The cells were first transiently transfected with dn.PPARγ for 24 hours followed by incubation with 10μM pioglitazone and rosiglitazone for another 24 hours. Fig 5A shows the effect of a dn.PPARγ expression (by transient transfection) on pioglitazone or rosiglitazone mediated upregulation of PPRE activity in A549 cell line. The basal and TZDs mediated PPRE activity was significantly suppressed by dn.PPARγ expression (* $P < 0.05$).
Arachidonic acid (15μM) was added prior to the collection of the medium for PGE2 assay. The overexpression of dn.PPARγ did not abrogate the suppression effect of pioglitazone (5B) and rosiglitazone (5C) on PGE2 levels in A549 cell line .
GW9662, a PPARγ antagonist, was applied to A427 cells. One hour prior to adding pioglitazone (Fig 5D) or rosiglitazone (Fig 5E), GW9662 (10μM) was applied to the medium. One representative experiment out of three independent experiments is shown here. *, $P \leq 0.05$
ARA=Arachidonic acid, Pio=Pioglitazone, Rosi=Rosiglitazone

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Figure 1

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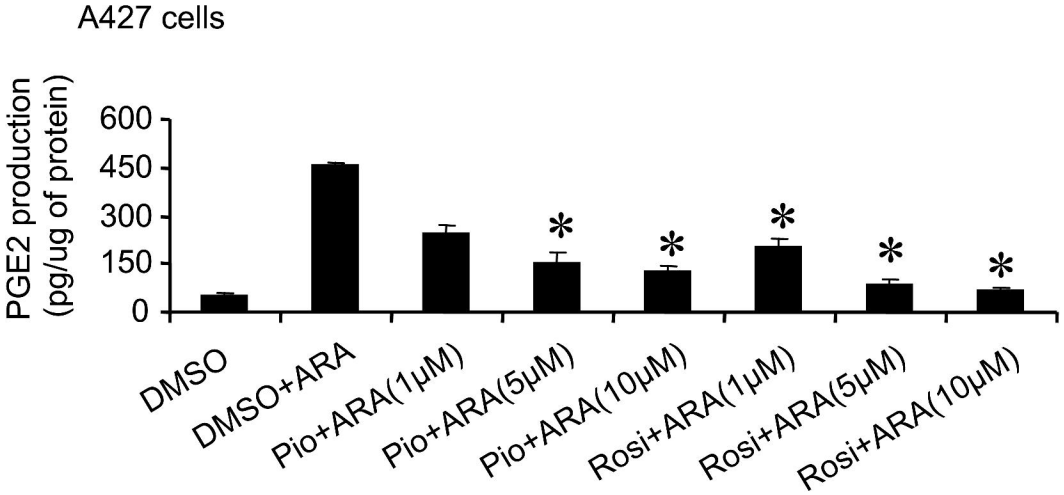
1A



1B

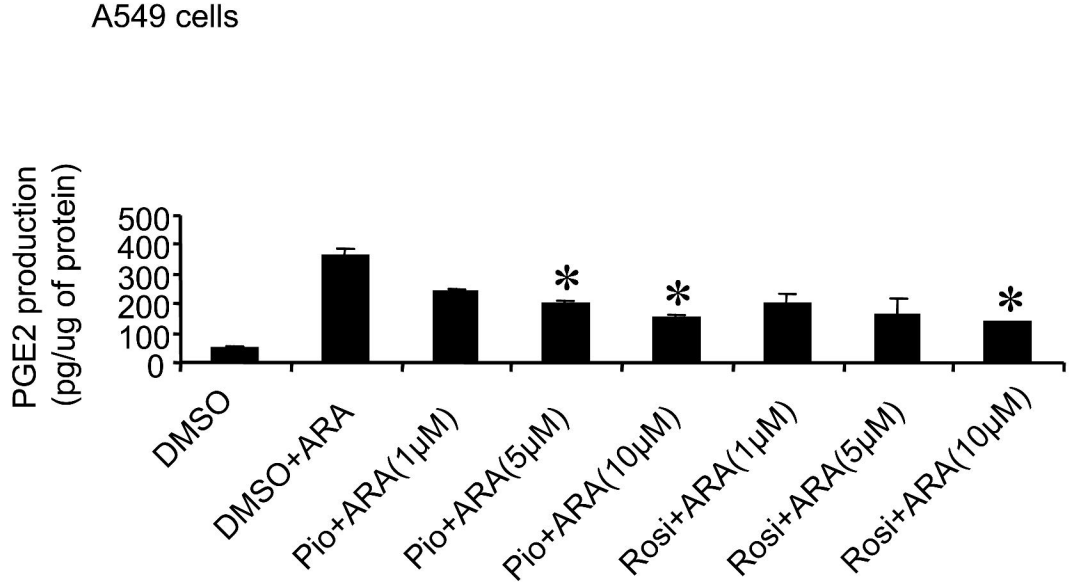


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Figure 2

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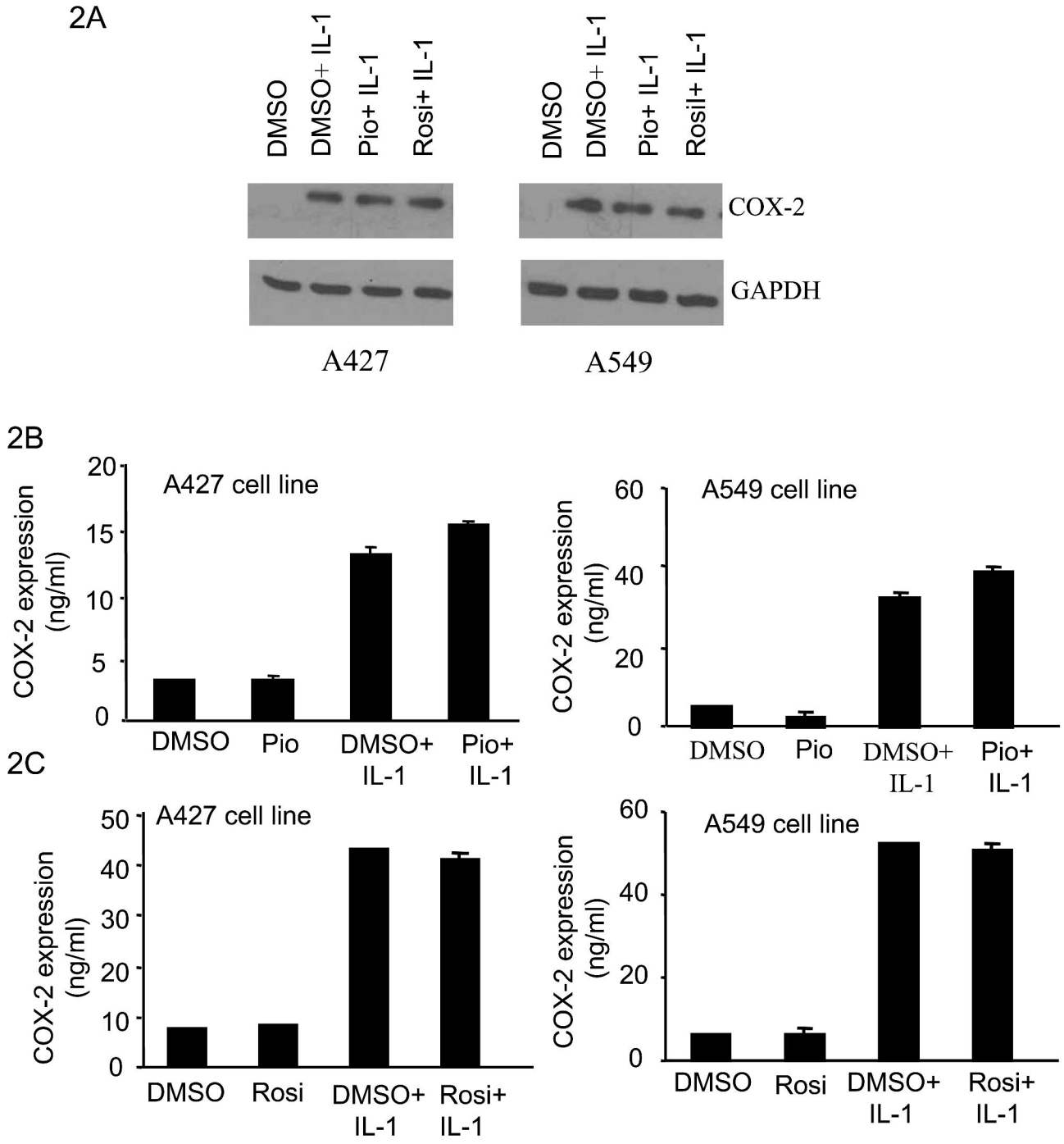

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Figure 3

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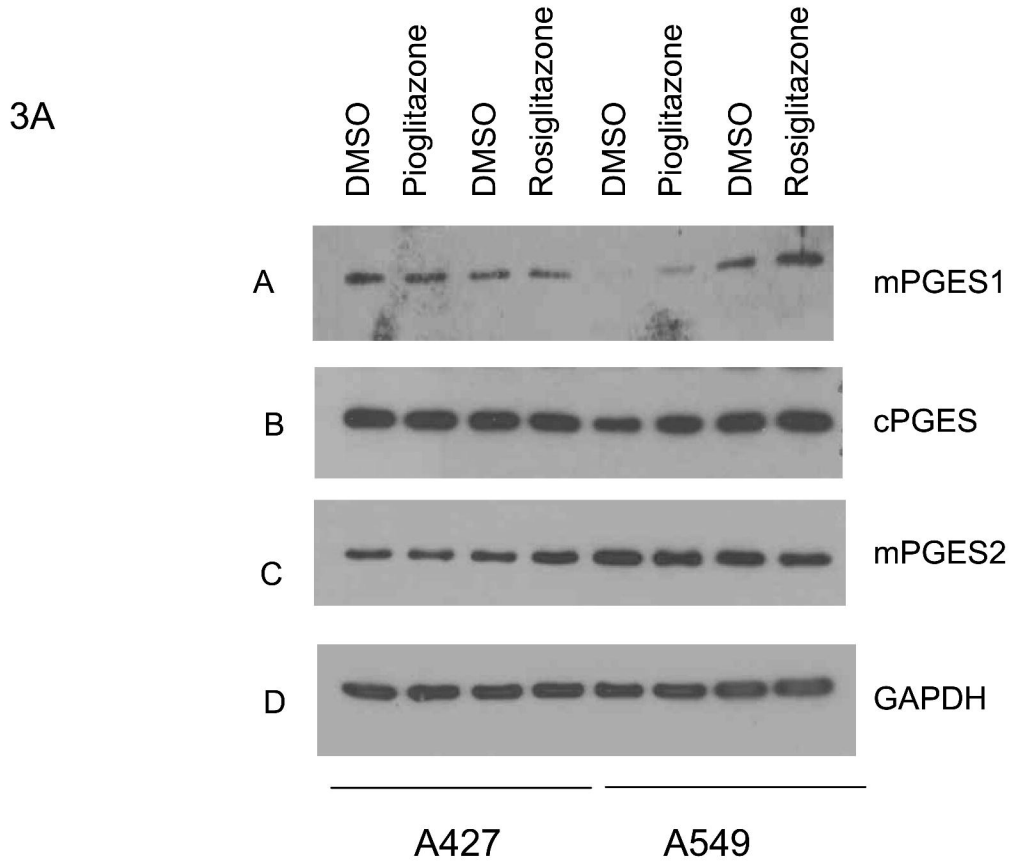

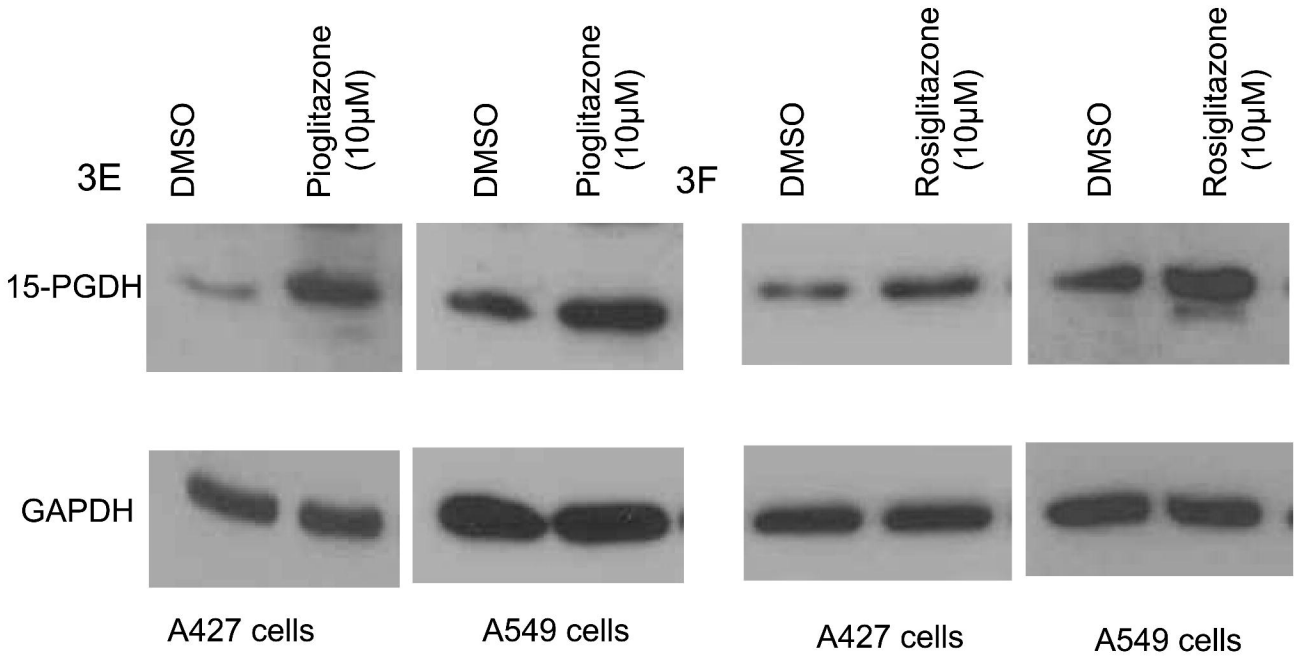

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Figure 4

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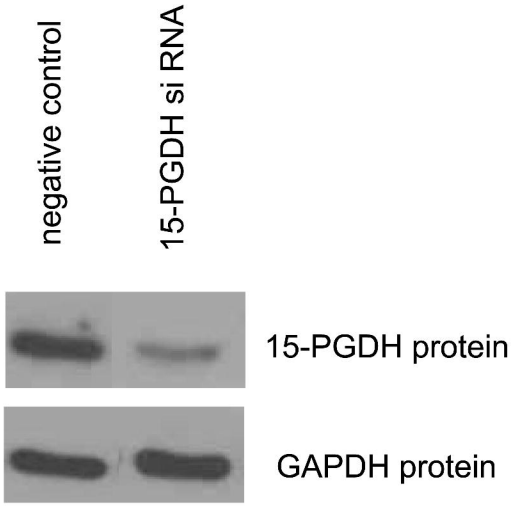

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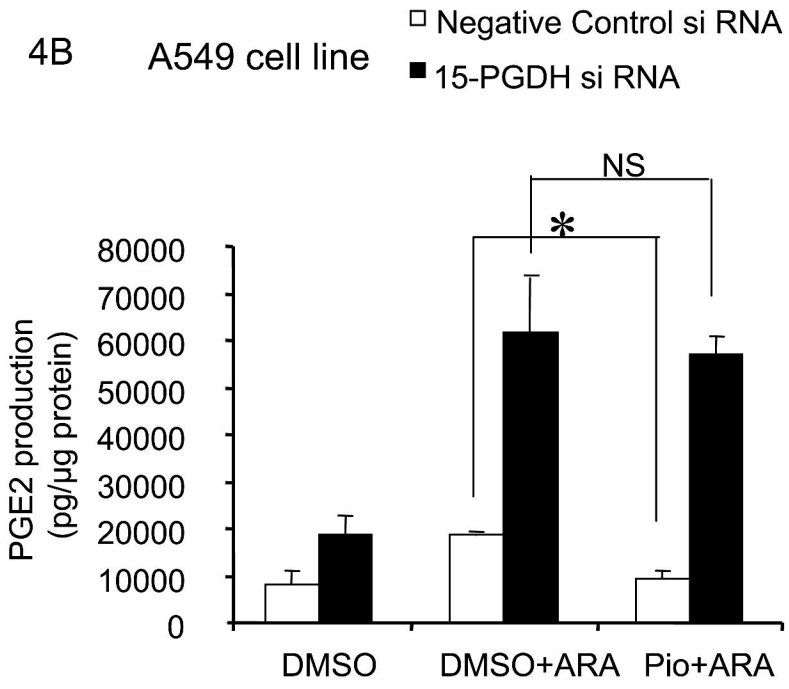

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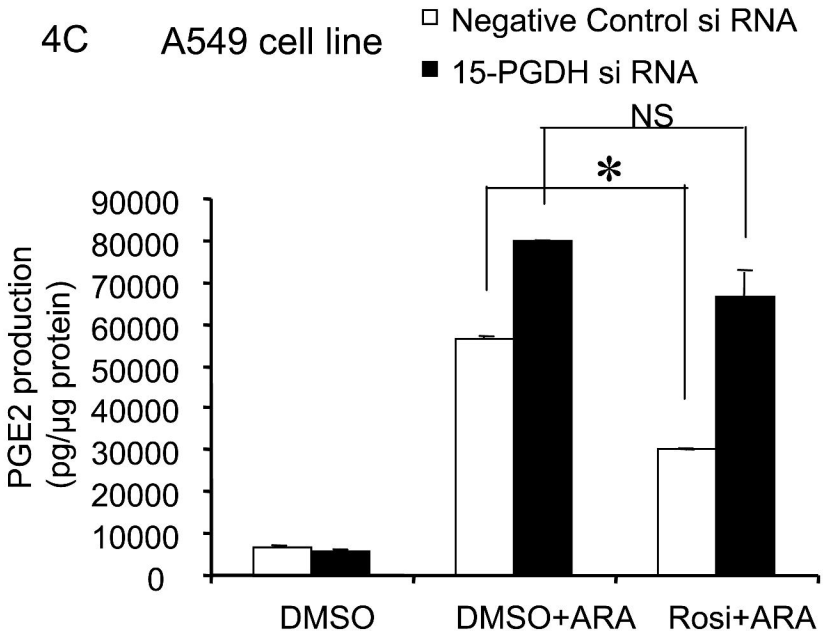

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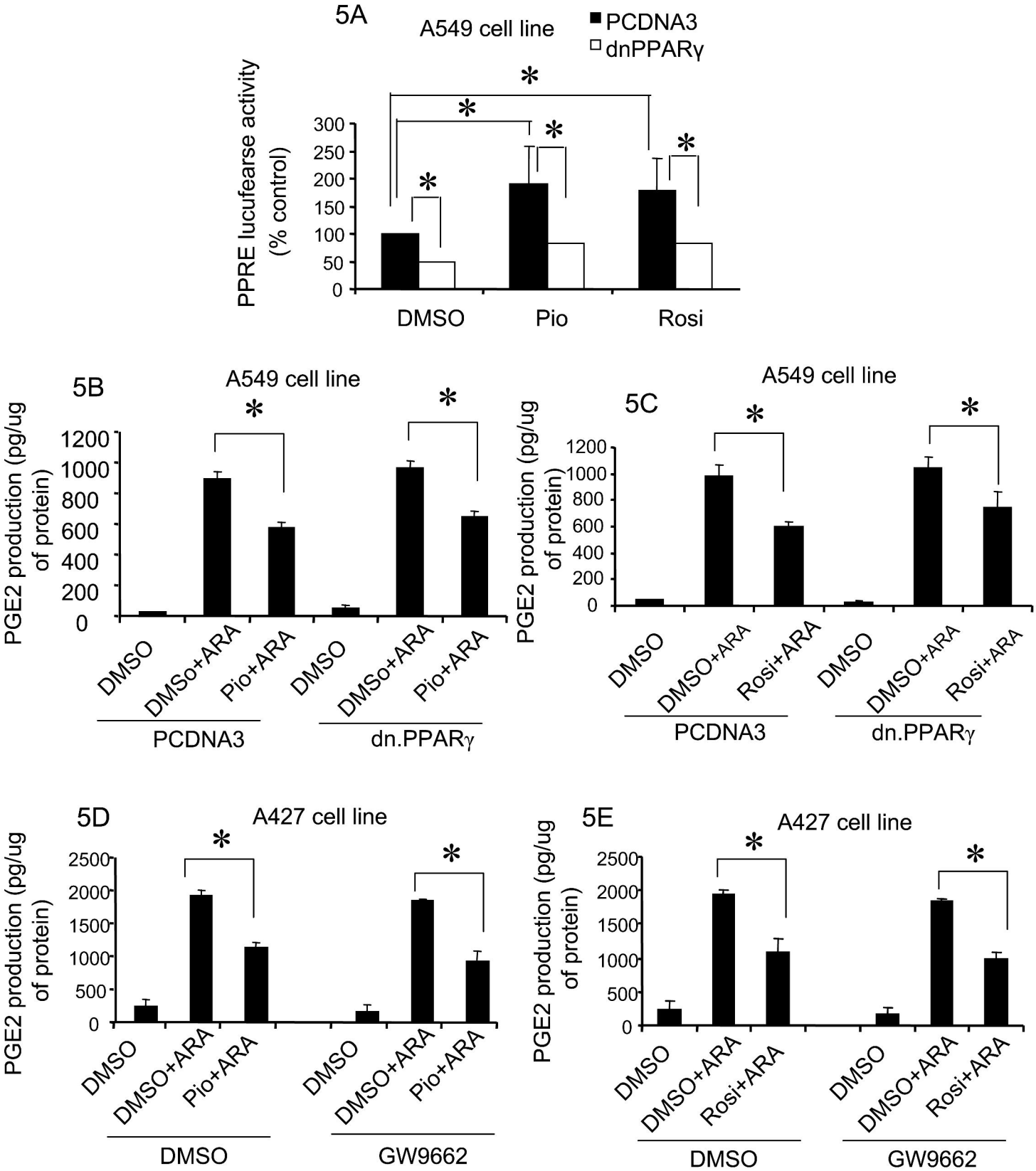
Figure 5



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