Rosiglitazone suppresses human lung carcinoma cell growth through PPARγ-dependent and PPARγ-independent signal pathways

ShouWei Han1 and Jesse Roman1,2
1Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine, Emory University School of Medicine and 2Atlanta Veterans Affairs Medical Center, Atlanta, Georgia

Abstract
Peroxisome proliferator-activated receptors γ (PPARγ) exert diverse effects on cancer cells. Recent studies showed that rosiglitazone, a synthetic ligand for PPARγ, inhibits cell growth. However, the exact mechanisms underlying this effect are still being explored, and the relevance of these findings to lung cancer remains unclear. Here, we report that rosiglitazone reduced the phosphorylation of Akt and increased phosphatase and tensin homologue (PTEN) protein expression in non–small cell lung carcinoma (NSCLC) cells (H1792 and H1838), and this was associated with inhibition of NSCLC cell proliferation. These effects were blocked or diminished by GW9662, a specific PPARγ antagonist. However, transfection with a CMX-PPARγ2 overexpression vector restored the effects of rosiglitazone on Akt, PTEN, and cell growth in the presence of GW9662. In addition, rosiglitazone increased the phosphorylation of AMP-activated protein kinase α (AMPKα), a downstream kinase target for LKB1, whereas it decreased phosphorylation of p70 ribosomal protein S6 kinase (p70S6K), a downstream target of mammalian target of rapamycin (mTOR). Of note, GW9662 did not affect phosphorylation of AMPKα and p70S6K protein. The inhibitory effect of rosiglitazone on NSCLC cell growth was enhanced by the mTOR inhibitor rapamycin; however, it was blocked, in part, by the AMPKα small interfering RNA. Taken together, these findings show that rosiglitazone, via up-regulation of the PTEN/AMPK down-regulation of the Akt/mTOR/p70S6K signal cascades, inhibits NSCLC cell proliferation through PPARγ-dependent and PPARγ-independent signals. [Mol Cancer Ther 2006;5(2):430–7]

Introduction
Peroxisome proliferator-activated receptors (PPAR; isoforms α, β/δ, and γ) are ligand-inducible nuclear transcription factors (1). PPARs heterodimerize with retinoid X receptors (2) and bind to PPAR response elements located in the promoter region of PPAR target genes. These lipid-sensitive receptors can be activated in a variable isotype-specific manner by natural fatty acids, leukotrienes, prostaglandins, and some synthetic agonists, including antidiabetic drugs, such as rosiglitazone, ciglitazone, and pioglitazone. These drugs are also effective in regulating cell activation, differentiation, proliferation, and/or apoptosis (3, 4).

The role of PPARγ, one PPAR isoform, has been extensively studied during the past several years. Synthetic PPARγ agonists specifically bind to PPARγ isoforms, and induce its activity, which in turn, results in altered expression of PPARγ target genes. The efficacy of these compounds as anticancer agents has been examined in a variety of cancers, including colon, breast, and prostate (5). PPARγ expression has also been shown in non–small cell lung carcinoma (NSCLC) cells and was correlated with tumor histologic type and grade (6). Activation of PPARγ by troglitazone, ciglitazone, and pioglitazone caused growth inhibition and apoptosis of NSCLC cells (7, 8). Recently, studies in animal models of tumorigenesis showed that treatment of A549 tumor-bearing severe combined immunodeficient mice with troglitazone or pioglitazone inhibited primary tumor growth by 66.7% and significantly inhibited the number of spontaneous lung metastatic lesions. These observations suggest that PPARγ ligands may serve as potential therapeutic agents in the management of NSCLC (9).

Thus far, the mechanisms by which PPARγ ligands suppress NSCLC cell growth have not been fully elucidated. Here, we explore the effects of the PPARγ ligand rosiglitazone on NSCLC proliferation, and how its effects might relate to the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt and the AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR)/p70 ribosomal S6 kinase (p70S6K) pathways. PI3K is a heterodimeric enzyme involved in the regulation of mitogenesis, apoptosis, cell adhesion, and motility (10). This enzyme has been suggested as a proto-oncogene in human cancer. mTOR has been shown to be a key kinase acting downstream of the activation of the PI3K, through which it regulates an array of cellular processes. Signaling via
mTOR controls cell size and growth as well as other functions, and this pathway represents a potential therapeutic target in graft rejection, certain cancers, and disorders characterized by inappropriate cell or tissue growth (11). mTOR signaling induced by hormones or growth factors and amino acids regulates the phosphorylation of several proteins, including p70S6K and eIF-4E binding protein (4E-BP1), which are key regulators of translation, and are among the most well-characterized targets of mTOR (12). This pathway is down-regulated by AMPK and the tumor suppressor LKB1 (13–18), which is an upstream signal of mTOR. AMPK is the central component of a protein kinase cascade that plays a key role in the regulation of energy control. Several findings point to a link between AMPK and the growth and/or survival of some cancer cells (14, 15). It has been shown that the tumor suppressor LKB1 is a kinase that has a major role in phosphorylating and activating AMPK (13). In addition, other studies indicate that mammalian homologue of mTOR, which has been implicated in the pathogenesis of insulin resistance and many types of cancer, is inhibited by AMPK (14).

We found that rosiglitazone inhibited the Akt/mTOR/ p70S6K pathway and stimulated phosphatase and tensin homologue (PTEN) and AMPK signals in NSCLC cells through PPARγ-dependent and PPARγ-independent pathways. These events were responsible for the growth-inhibitory effects of rosiglitazone.

Materials and Methods

Culture and Chemicals

The human NSCLC cell lines H1838 and H1792 were obtained from the American Type Culture Collection (Manassas, VA) and grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, HEPES buffer, 50 IU/mL penicillin/streptomycin, and 1 μg amphotericin (complete medium) as previously described (19). Rapamycin and polyclonal antibodies specific for PTEN, AMPKα, p70S6K, Akt, and their respective phosphorylated active forms were purchased from Cell Signaling (Beverly, MA). GW9662, rosiglitazone, and all other chemicals were purchased from Sigma Aldrich (St. Louis, MO), unless otherwise indicated.

Transient Transfection with a CMX-PPARγ2 Plasmid Construct

The CMX-PPARγ2 overexpression vector was obtained from Dr. Neil Sidell (Department of Gynecology and Obstetrics, Emory University, Atlanta, GA) and has been described previously (20). For functional studies, H1792 cells were seeded at a density of 1 x 10^5 per well in six-well dishes and grown to 60% confluence. For each well, the plasmid DNA containing CMX-PPARγ2 (1.3 μg/mL) was transfected into cells using FUGENE 6 lipofection reagent as described in our earlier work (21). Control cells were transfected with FUGENE 6 lipofection reagent only. After 24 hours of incubation, cells were treated with or without GW9662 (20 μM) for 1 hour before exposing the cells to rosiglitazone (10 μM) in the presence or absence of 1 μCi/mL [methyl-3H]thymidine (Amersham, Arlington Heights, IL; specific activity = 250 Ci/mmol) for an additional 24 hours. Afterwards, the cells were processed for Western blot analysis and the [methyl-3H]thymidine incorporation assay as described below.

Western Blot Analysis

The procedure was done as previously described (22). Briefly, protein concentrations were determined by the Bio-Rad protein assay. Equal amounts of protein from whole-cell lysates were solubilized in 2× SDS-sample buffer and separated on SDS/8% polyacrylamide gels. Blots were incubated with antibodies raised against AMPKα and phosphorylated AMPKα, p70S6K and phosphorylated p70S6K, Akt and its activated form, and PTEN (1:1,000). The blots were washed and followed by incubation with a secondary goat antibody raised against rabbit IgG conjugated to horseradish peroxidase (1:2,000; Cell Signaling). The blots were washed, transferred to freshly made enhanced chemiluminescence solution (Amersham) for 1 minute, and exposed to X-ray film. In controls, the antibodies were omitted or replaced with a control rabbit IgG.

Treatment with AMPK Small Interfering RNA

The AMPKα small interfering RNA (siRNA, ID no. 772) was purchased from Ambion (Austin, TX). Nonspecific control siRNA was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). For the transfection procedure, cells were grown to 50% confluence, and AMPKα or control siRNAs were transfected using the LipofectAMINE 2000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Briefly, oligofectamine reagent was incubated with serum-free medium for 10 minutes. Subsequently, a mixture of siRNA was added. After incubation for 15 minutes at room temperature, the mixture was diluted with medium and added to each well. The final concentration of siRNA in each well was 100 nmol/L. After culturing for 48 hours, cells were washed and resuspended in new culture media in the presence or absence of rosiglitazone for up to 48 hours for Western blot and cell growth assays.

[Methyl-3H]Thymidine Incorporation Assay

Human lung carcinoma cells (10^5 per well) were cultured with GW9662 (20 μM) for 1 hour or transfected with AMPKα siRNA (100 nmol/L) for 48 hours before exposing the cells to rosiglitazone (10 μM) or rapamycin (10 nmol/L) followed by incubation with 1 μCi/mL [methyl-3H]thymidine (Amersham; specific activity = 250 Ci/mmol) for up to 48 hours. The medium was removed, and the attached cells were washed with 1× PBS. Afterwards, the attached cells were treated with ice-cold 6% trichloroacetic acid at 4°C for 20 minutes and washed once with 6% trichloroacetic acid. The cells were then solubilized with 0.1 N NaOH and counted in a liquid scintillation counter in 4 mL of scintillation fluid.

Statistical Analysis

All experiments were repeated a minimum of three times. All data collected from Western blot and [methyl-3H] thymidine incorporation assays were expressed as means ± SD. The data presented in some figures are from a
representative experiment, which was qualitatively similar in the replicate experiments. Statistical significance was determined with Student’s t test (two tailed) comparison between two groups of data sets. Asterisks shown in the figures indicate significant differences of experimental groups compared with the corresponding control condition (P < 0.05; see figure legends).

Results

Rosiglitazone Inhibits the Phosphorylation of Akt Protein in a Dose-Dependent and Time-Dependent Manner

The PI3K/Akt signal pathway has been implicated in the regulation of cell cycle progression and cell proliferation. In certain cell types (e.g., human embryonic kidney 293 cells and intestinal epithelial cells), this pathway was blocked by rosiglitazone (23, 24). Therefore, we tested the effects of rosiglitazone in NSCLC cells. We found that rosiglitazone reduced the phosphorylation of the PI3K downstream signal Akt in a time-dependent and dose-dependent manner with maximal reduction at 10 µmol/L for 3 hours, whereas no effects were noted in total Akt protein (Fig. 1A-B). Conversely, rosiglitazone stimulated the expression of PTEN protein in a dose-dependent and time-dependent manner with a most effective dose of 10 µmol/L at 24 hours; higher concentrations had no further effect (Fig. 1C-D). Similar results were obtained in H1838 cells (data not shown).

Rosiglitazone Inhibits Akt Phosphorylation and Stimulates PTEN Expression through PPARγ

Rosiglitazone has been shown to activate PPARγ in several studies (7, 8, 25, 26). Therefore, we tested whether the effects of rosiglitazone on Akt and PTEN were mediated through the activation of PPARγ by treating the cells with GW9662, a specific PPARγ antagonist. As shown in Fig. 2A, the effects of rosiglitazone on Akt phosphorylation and PTEN expression were eliminated in the presence of GW9662. However, cells transfected with a PPARγ overexpression vector showed restoration of the effect of rosiglitazone on Akt and PTEN expression. These findings suggest that PPARγ-dependent signals mediate the effect of rosiglitazone on Akt and PTEN.

To evaluate the relevance of the above findings to NSCLC proliferation, we treated NSCLC cells with rosiglitazone and evaluated proliferation. We found that rosiglitazone inhibited the proliferation of NSCLC cells in a dose-dependent manner (Fig. 2B). The inhibitory effect of rosiglitazone was diminished by the PPARγ antagonist GW9662 (Fig. 2C). Cells transfected with the PPARγ overexpression vector antagonized the effect of GW9662 on cell growth (Fig. 2C). Of note, GW9662 only inhibited the effect of rosiglitazone partially suggesting that PPARγ-independent signals were also involved. Similar results were obtained in H1838 cells (data not shown).

Rosiglitazone Induces AMPK Phosphorylation and Inhibits p70S6K Phosphorylation through PPARγ-Independent Signals

We then investigated the potential PPARγ-independent signals that mediate the effects of rosiglitazone on NSCLC cell proliferation. We focused our attention on the mTOR pathway. We started by examining the effect of rosiglitazone on AMPK, an upstream modulator of mTOR. As shown in Fig. 3A and B, rosiglitazone induced the phosphorylation of AMPKα protein in a dose-dependent and time-dependent manner, whereas it had no effect on total content of AMPKα protein as determined by Western blot analysis in

Figure 1. The effect of rosiglitazone on Akt phosphorylation and PTEN protein expression. **A**, rosiglitazone inhibition of Akt phosphorylation is dose dependent. Cellular protein was isolated from H1792 cells that were cultured with increasing concentrations of rosiglitazone for up to 24 h. Then, Western blot analysis was conducted using antibodies against phosphorylated Akt (p-Akt) and total Akt. **B**, rosiglitazone inhibition of Akt phosphorylation is time dependent. Cellular protein was isolated from H1792 cells that were cultured with rosiglitazone (10 µmol/L) for indicated periods of time followed by Western blot analysis with antibodies against phosphorylated Akt and total Akt protein. **C**, rosiglitazone stimulation of PTEN is dose dependent. Cellular protein was isolated from H1792 cells that were cultured with increasing concentrations of rosiglitazone for up to 24 h followed by Western blot analysis with antibodies against PTEN. **D**, rosiglitazone stimulation of PTEN is time dependent. Cellular protein was isolated from H1792 cells that were cultured with rosiglitazone (10 µmol/L) for indicated periods of time followed by Western blot analysis with antibodies against PTEN protein. Actin served as internal control for normalization purposes. Columns, mean of phosphorylated Akt or PTEN/actin of at least three independent experiments; bars, SD. *, P < 0.05, significant differences compared with untreated cells.
and incubation with 1 (20 24 h. Afterwards, the cells were treated with or without GW9662 or without CMX-PPAR mediated through PPAR incubation with 1 treated with increasing concentrations of rosiglitazone followed by A, on lung carcinoma cell growth.

Figure 2. PPARα-dependent signal pathways mediate the effects of rosiglitazone on phosphorylation of Akt and PTEN protein expression and on lung carcinoma cell growth. A, PPARα-dependent signal pathways mediate the effects of rosiglitazone on phosphorylation of Akt and PTEN protein expression. Cellular protein was isolated from H1792 cells transfected with CMX-PPARα2 vector using FUGENE 6 lipofection reagent for 24 h. After transfection, cells were cultured for 1 h in the presence or absence of GW9662 (20 μmol/L) before exposing the cells to rosiglitazone (10 μmol/L) for an additional 24 h, then subjected to Western blot analysis with antibodies against phosphorylated Akt (p-Akt), total Akt, PTEN, and actin. Columns, mean of at least three independent experiments; bars, SD. Actin served as internal control for normalization purposes. *, P < 0.05, significant differences compared with untreated cells. B, rosiglitazone inhibits cell growth in a dose-dependent manner. H1792 cells were treated with increasing concentrations of rosiglitazone followed by incubation with 1 μCi/mL [methyl-3H]thymidine for up to 48 h and cell number determination. C, effects of rosiglitazone on cell growth are mediated through PPARα signals. H1792 cells were transfected with or without CMX-PPARα2 vector using FUGENE 6 lipofection reagent for 24 h. Afterwards, the cells were treated with or without GW9662 (20 μmol/L) for 1 h before exposing the cells to rosiglitazone (10 μmol/L) and incubation with 1 μCi/mL [methyl-3H]thymidine for up to 48 h and cell number determination. *, P < 0.05, significant differences compared with the untreated cells. **, P < 0.05, significance of combination treatment compared with rosiglitazone alone. ***, significance of combination treatment compared with rosiglitazone plus GW9662. Con, untreated cells.

H1792 cells. However, rosiglitazone had no effects on LKB1, an upstream signal of AMPK (data not shown). Similar results were obtained in H1838 cells (data not shown).

To examine whether the effects of rosiglitazone on AMPK and p70S6K phosphorylation were mediated through PPARα, we inactivated PPARα with GW9662. As shown in Fig. 5A, GW9662 did not block the effects of rosiglitazone on either AMPK or p70S6K phosphorylation, indicating that these effects were PPARα independent. In view that rosiglitazone regulated mTOR-related proteins AMPK and p70S6K, we tested whether the mTOR inhibitor rapamycin influenced the NSCLC growth inhibition induced by rosiglitazone. As shown in Fig. 5B, rapamycin reduced NSCLC cell growth and further enhanced cell growth inhibition in the presence of rosiglitazone. In contrast to its effects on rosiglitazone-mediated cell growth inhibition, GW9662 did not block the effect of rapamycin on NSCLC cell growth reduction, suggesting that the inhibition of cell proliferation by rosiglitazone and rapamycin was mediated through different pathways. Similar results were obtained in H1838 cells (data not shown). Blocking the AMPK Signal Abrogated, in Part, the Effect of Rosiglitazone on Cell Growth Inhibition

Because rosiglitazone activated the phosphorylation of AMPK, we therefore determined whether blocking the expression of AMPK could affect the inhibitory effect of rosiglitazone on cell growth. We first depleted AMPKα expression in cells using the small RNA interference method. As shown in Fig. 6A, AMPKα siRNA greatly diminished endogenous AMPKα protein production; no changes were noted in cells transfected with control siRNA. Next, H1792 cells were transfected with AMPK siRNA.

Figure 3. Effects of rosiglitazone on AMPK protein. A, rosiglitazone stimulation of AMPKα phosphorylation is dose dependent. Cellular protein was isolated from H1792 cells that were cultured with increasing concentrations of rosiglitazone for up to 24 h. Afterwards, Western blot analysis was done using antibodies against phosphorylated AMPKα (pAMPKα) and total AMPKα. B, rosiglitazone stimulation of AMPKα phosphorylation is time dependent. Cellular protein was isolated from H1792 cells that were cultured with rosiglitazone (10 μmol/L) for indicated periods of time followed by Western blot analysis with antibodies against phosphorylated AMPKα and total AMPKα. Actin served as internal control for normalization purposes. Columns, mean of AMPKα/actin of at least three independent experiments; bars, SD. * P < 0.05, significant differences compared with the untreated cells.
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Afterwards, the cells were exposed to rosiglitazone for an additional 48 hours followed by evaluation of cell proliferation by thymidine incorporation assay. As shown in Fig. 6B, AMPK siRNA significantly abrogated rosiglitazone-reduced cell proliferation; the control siRNA had no effect. This suggests that activation of AMPK also plays a role in mediating the effect of rosiglitazone on NSCLC cell growth inhibition. Similar results were obtained in H1838 cells (data not shown).

Discussion

Rosiglitazone, one of the thiazolidinedione derivatives, is the most potent and selective synthetic ligand of PPARγ. It binds to PPARγ with a $K_d$ of ~ 40 nmol/L and is known to have marked adipogenic effects on preadipocyte and mesenchymal stem cells in vitro and to have dramatic antidiabetic effects (27). In this study, we showed that rosiglitazone inhibits human lung carcinoma cell growth in vitro. These effects were dose dependent, and the concentrations used here were consistent with those reported by others. For example, Valentiner et al. found that rosiglitazone inhibited in vitro growth and viability of human neuroblastoma cell lines in a dose-dependent manner, showing considerable effects only at high concentrations (10 and 100 nmol/L; ref. 28). In another study, rosiglitazone inhibited both the proliferation and invasiveness of the human adrenocortical cancer cell line H295R in a dose-dependent manner with the maximal effect (about 50% inhibition) obtained at 20 nmol/L (26).

The mechanisms responsible for the effects of rosiglitazone seem to involve both PPARγ-dependent and PPARγ-independent signals. Specifically, we found that rosiglitazone inhibited the phosphorylation of the downstream signal of PI3K, Akt, and increased PTEN expression in NSCLC cells. These changes were inhibited by GW9662, suggesting that they depend upon PPARγ activation. The PI3K/Akt signaling axis plays an important role in cellular proliferation and growth signaling (23). Akt, also known as protein kinase B, is a critical enzyme in several signal transduction pathways that regulate cell survival, growth, differentiation, angiogenesis, migration, and metabolism. This pathway is frequently

![Image](https://example.com/image1.png)

**Figure 4.** Effect of rosiglitazone on p70S6K protein. A, rosiglitazone inhibition of p70S6K phosphorylation is dose dependent. Cellular protein was isolated from H1792 cells that were cultured with increasing concentrations of rosiglitazone for up to 24 h. Afterwards, Western blot analysis was done using antibodies against phosphorylated p70S6K (p-p70S6K) and total p70S6K. B, rosiglitazone inhibition of p70S6K phosphorylation is time dependent. Cellular protein was isolated from H1792 cells that were cultured with rosiglitazone (10 nmol/L) for indicated period of time followed by Western blot analysis with antibodies against phosphorylated p70S6K and total p70S6K protein. Actin served as internal control for normalization purposes. Columns, mean of phosphorylated p70S6K/actin of at least three independent experiments; bars, SD. ***, $P < 0.05$, significant differences compared with the untreated cells.

![Image](https://example.com/image2.png)

**Figure 5.** PPARγ-independent signal pathways mediate the effects of rosiglitazone on AMPK and p70S6K expression and on lung carcinoma cell growth. A, PPARγ-independent signal pathways mediate the effects of rosiglitazone on protein expression. Cellular protein was isolated from H1792 cells cultured for 1 h in the presence or absence of GW9662 (20 nmol/L) before exposing the cells to rosiglitazone (10 nmol/L) for an additional 24 h, then subjected to Western blot analysis with antibodies against phosphorylated AMPKα (p-AMPKα), total AMPK, phosphorylated p70S6K (p-p70S6K), total p70S6K, and actin. Columns, mean of at least three independent experiments; bars, SD. Actin served as internal control for normalization purposes. B, effects of rapamycin on cell growth are not mediated by PPARγ signals. H1792 cells were cultured with GW9662 (20 nmol/L) for 1 h before exposing the cells to rapamycin (10 nmol/L) and incubated with 1 μCi/mL [methyl-3H]thymidine for up to 48 h. Afterwards, the cells numbers were determined. **, $P < 0.05$, significance of combination treatment compared with rosiglitazone alone. ***, $P < 0.05$, significance of combination treatment compared with rapamycin alone. Con, untreated cells.
The PPARγ antagonist GW9662 abolished the effects of rosiglitazone on phosphorylation of Akt and PTEN expression, suggesting that PPARγ mediated, at least in part, the effects of rosiglitazone. Rosiglitazone has been shown to inhibit the growth of several human cancer cells through PPARγ-dependent signals (26, 35, 36). Ferruzzi et al. (26) found that rosiglitazone inhibited the growth and invasiveness of human adrenocortical cancer cells through activation of PPARγ. Results obtained with the CMX-PPARγ overexpression vector further suggest the involvement of PPARγ-dependent signals in mediating the effects of rosiglitazone on AKT and PTEN expression and cell growth inhibition.

As stated above, we found that GW9662 abrogated the effects of rosiglitazone on cell growth. However, GW9662 did not completely block the effects of rosiglitazone suggesting the involvement of PPARγ-independent signals. Rosiglitazone has been shown to activate AMPK, a multisubstrate enzyme activated by increase in AMP during metabolite stress caused by exercise, hypoxia, and lack of cell nutrients as well as hormones (15, 16, 18). We found that rosiglitazone activated the phosphorylation of AMPK in NSCLC cells. This fuel-sensing enzyme, which is an upstream signal of mTOR, plays a major role in the regulation of cellular lipid and protein metabolism in response to stimuli, such as exercise, changes in fuel availability, and the adipocyte-derived hormone leptin (13). Many cancers are characterized by increased expression and/or activity of enzymes that are inhibited by AMPK. Recent studies showed that activation of AMPK inhibits protein synthesis by phosphorylation of several important regulators. AMPK phosphorylation negatively regulated protein synthesis by directly phosphorylating and inhibiting mTOR (17). Rosiglitazone antagonized the stimulatory effects of androgen on fatty acid and protein synthesis in prostate cancer cells by down-regulating fatty acid synthesis, acetyl-COA carboxylase, and mTOR (15). The tumor suppressor LKB1 has been identified as an upstream activator of AMPK. Of note, rosiglitazone did not affect LKB1 expression in the current study, and this corresponds with other reports showing that an AMPK activator does not affect LKB1 expression (37, 38).

We also found that rosiglitazone inhibited the phosphorylation of p70S6K. The link between the AMPK and mTOR signaling pathway has been reported in other studies (15, 17, 39). AMPK activators, AICAR and rosiglitazone, inhibited p70S6K activity and phosphorylation in human corneal epithelial and other cancer cells (14, 15, 17, 18). The inhibition of protein synthesis by pharmacologic activation of AMPK may be a key regulatory mechanism by which hypertrophic growth can be controlled (40). Our results suggest that regulation of AMPK and p70S6K by rosiglitazone might contribute to growth inhibition of NSCLC cells.

As mentioned above, we found that the effects of rosiglitazone on phosphorylation of AMPK and p70S6K were not blocked by the PPARγ antagonist, suggesting...
that PPARγ-independent signals were involved. PPARγ-independent signals mediating the effect of rosiglitazone have been shown in several other studies (41–43). Galli et al. found that rosiglitazone and pioglitazone inhibit invasiveness of pancreatic cancer cells via PPARγ-independent mechanisms that involve metalloproteinases-2 and plasminogen activator inhibitor-1 expression (41). Shiau et al. (42) showed that the two prostate cancer cell lines PC-3 (PPARγ expressing) and LNCaP (PPARγ deficient) cells are sensitive to apoptosis induction by troglitazone and its PPARγ-inactive analogue irrespective of their PPARγ expression status. This is, in part, related to their ability to inhibit the antiapoptotic functions of Bcl-xL and Bcl-2 rather than through activation of the PPARγ signal.

We found that rosiglitazone enhanced the effect of the inhibitor of mTOR, rapamycin, in reducing NSCLC cell proliferation. The effect of rosiglitazone on cell growth was not completely blocked by the PPARγ antagonist GW9662 suggested that other signals were involved in mediating the effect of rosiglitazone on cell growth inhibition. The effect of rapamycin on reduction of cell growth was not affected by inactivation of PPARγ signal, suggesting that different signal pathways were involved. Interestingly, interfering with rosiglitazone-induced activation of AMPK by decreasing the expression of the enzyme using siRNA, abrogated, in part, the inhibitory effect of rosiglitazone on NSCLC cell growth, suggesting AMPK signal is also involved in the NSCLC cell growth inhibition by rosiglitazone. This finding is in agreement with other studies showing that the effects of rosiglitazone in increased glucose flux in skeletal muscle cells via activation of AMPK signal were blocked by the AMPK siRNA (44); in addition, the reduction of AMPK expression by AMPK siRNA reversed the inhibitory effects of AMPK activators, such as rosiglitazone and 15d-PGJ₂, which are also PPARγ ligands, on inducible nitric oxide synthase expression and nitric oxide production in myocytes (45). In line with other studies showing that activation of AMPK inhibited growth of several types of cancer cells in vitro (14, 15, 46), these observations establish a causative role for AMPK in rosiglitazone-mediated inhibition of NSCLC cell growth.

Taken together, our observations show that rosiglitazone can inhibit NSCLC growth through PPARγ-dependent signals that inhibit Akt and stimulate PTEN. Through PPARγ-independent signals, rosiglitazone up-regulates AMPK, thereby down-regulating the mTOR/p70S6K pathway, which further contributes to growth inhibition (Fig. 7). These data provide insight into potentially additive interventions for anticancer therapies against NSCLC.

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References

38. Lizzano JM, Goransson O, Toth R, et al. LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. EMBO J 2004;23:833 – 43.
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ShouWei Han and Jesse Roman

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