Reactivation of Suppressed RhoB is a Critical Step for the Inhibition of Anaplastic Thyroid Cancer Growth


1Department of Cancer Biology, Mayo Clinic Comprehensive Cancer Center and 2Division of Endocrinology, Internal Medicine Department, Mayo Clinic, Jacksonville, Florida; 3Daiichi Sankyo Co., Ltd., Edogawa-ku, Tokyo, Japan; Departments of 4Laboratory Medicine/Pathology, 5Surgery, and 6Medicine, 7Endocrine Malignancy Working Group, Mayo Clinic, Rochester, Minnesota; and 8Department of Otolaryngology-Head and Neck Surgery, Eastern Virginia Medical School, Norfolk, Virginia

Abstract

Anaplastic thyroid carcinoma (ATC) is a highly aggressive form of the disease for which new therapeutic options are desperately needed. Previously, we showed that the high-affinity peroxisome proliferator–activated receptor γ (PPARγ) agonist, RS5444, inhibits cell proliferation of ATC cells via induction of the cyclin-dependent kinase inhibitor p21WAF1/CIP1 (p21). We show here that up-regulation of RhoB is a critical step in PPARγ-mediated activation of p21-induced cell stasis. Using multiple independently derived ATC cell lines, we found that treatment with RS5444 leads to the up-regulation of RhoB and subsequent activation of p21, and that silencing of RhoB by RNAi blocks the ability of RS5444 to induce p21 and to inhibit cell proliferation. Our results show that transcriptional regulation of RhoB by the nuclear transcription factor PPARγ is responsible for the induction of p21 mRNA and protein. We further implicate RhoB as a key signaling effector for the growth inhibition of ATC, as treatment with a histone deacetylase inhibitor shown to increase RhoB expression in lung cancer cells caused the up-regulation of RhoB in ATC cells accompanied by increased expression of p21 and inhibition of cell proliferation; this effect occurred even in ATC cells that were unresponsive to RS5444 due to a lack of expression of PPARγ. Our results implicate RhoB as a novel intermediate in critical signaling pathways and as an additional target for therapeutic intervention in ATC. [Cancer Res 2009;69(4):1536–44]

Introduction

Thyroid cancer is the most common endocrine cancer with an approximate incidence rate of 33,550 newly diagnosed cases per year in the United States (1). Despite a generally good prognosis, more than 1,500 patients with thyroid cancer die of their disease annually. Anaplastic thyroid carcinoma (ATC) is the most poorly differentiated, highly aggressive form of thyroid cancer with a median survival of ~4 months, a 1-year survival rate of <10%, and 99% lethality overall. If detected early, extensive surgery combined with radiotherapy offers the best chance of local disease control. To date, chemotherapy (usually doxorubicin plus cisplatin or paclitaxel) has shown only palliative benefit in advanced ATC (reviewed in ref. 2), but neither agent has shown curative potential. More effective targeted therapies based on a better understanding of the molecular and cellular signaling pathways disrupted in ATC are therefore needed.

Peroxisome proliferator–activated receptor γ (PPARγ) agonists have chemopreventive and antivirus activity against a variety of human cancers associated with transcriptional activation of PPARγ (3–6). These findings are in keeping with the hypothesis that PPARγ can act as a tumor suppressor in several cancers including thyroid cancer (7, 8). Mechanisms of PPARγ-mediated antitumor activity include the induction of differentiation, promotion of cell cycle arrest, antiangiogenic effects, and induction of apoptosis (9). In more differentiated thyroid cancers, PPARγ agonists have been shown to induce apoptosis through cytochrome c/caspase 3 and PTEN-Akt pathways (10).

We and others have recently shown that the PPARγ agonist, RS5444, inhibits cell proliferation and induces tumor differentiation (9, 11, 12). In our previous work, we found that RS5444 inhibited ATC tumor growth in vitro and in vivo but did not induce apoptosis as a single agent (9). We showed that RS5444 is dependent on PPARγ for its antitumor activity because GW9662, a pharmacologic antagonist of PPARγ, blocked the inhibition of cell growth by RS5444 (9). We also found that the cyclin kinase inhibitor p21WAF1/CIP1 (p21) was up-regulated by RS5444. To date, p21 has been implicated as a modulator of PPARγ-mediated inhibition of cell proliferation, but this evidence has been limited to correlative observations (13–16). In our recent study, we found that p21 was required for PPARγ-mediated growth inhibition by RS5444 in ATC cells, and that combinatorial treatment of ATC cells with RS5444 and paclitaxel resulted in apoptotic synergy. Silencing experiments showed the requirement of p21 for this observed synergy (9), but the mechanism by which PPARγ agonists might up-regulate p21 remains unknown.

RhoB is a member of the Ras superfamily of isoprenylated small GTPases, which regulate actin stress fibers and vesicle transport (17, 18). Membrane association of RhoB occurs through either geranylgeranylated (RhoB-GG) or farnesylated (RhoB-F) modifications. RhoB is required for apoptosis in transformed cells that are exposed to farnesyltransferase inhibitors, DNA-damaging agents, or paclitaxel (19). In cancer cells, RhoB modulates proliferation, survival, invasion, and angiogenic capacity (17). RhoB is not mutated in cancer, but its altered expression and activity seem...
crucial to cancer progression and therapeutic responses. Farnesyltransferase inhibitors up-regulate RhoB levels and this up-regulation of RhoB can mediate phenotypic reversion, growth inhibition, cytoskeletal actin reorganization, and apoptosis (20).

We now define a sequential pathway whereby the thiazolidinedione RS5444 acts via a PPARγ-dependent mechanism to up-regulate RhoB leading to increased expression of p21 followed by attenuation of cell proliferation. The elaboration of this novel signaling pathway triggered by PPARγ agonists provides insight into how to target such agents for treatment of ATC. We now show that the high-affinity histone deacetylase (HDAC) inhibitor, FK228 (a.k.a. romidepsin), previously shown to stimulate RhoB expression in lung cancer cell lines (21), also inhibits ATC cell proliferation via p21 in a RhoB-dependent fashion. These results identify RhoB up-regulation as a key step for targeting ATC cell proliferation and tumor progression.

Materials and Methods

**Chemicals.** PPARγ agonists RS5444 and troglitazone were kindly provided by Daiichi Sankyo, Inc. GW9662 was purchased from Sigma-Aldrich, FK228 (NSC 630176, depsipeptide or romidepsin) was a gift from Glueckshore Pharmaceuticals, Inc. (Cambridge, MA) and the Division of Cancer Treatment and Diagnosis, National Cancer Institute. Rosiglitazone was obtained from ChemPacific.

**Cell culture.** DRO90-1 (DRO) and ARO81 (ARO) ATC cell lines were kindly provided by Dr. G.J. Juillard (University of California, Los Angeles, CA) as were KTC2 and KTC3 ATC cell lines by Dr. Junichi Kurebayashi of the Institut Claudius Regaud, Toulouse, France) was also examined for luciferase assay. An 1876 bp RhoB promoter-luciferase (Daniel Tovar, R. Evans, Salk Institute, La Jolla, CA) by RS5444 was shown by dual luciferase expression in lung cancer cell lines (21), also inhibits ATC cell proliferation via p21 in a RhoB-dependent fashion. These results identify RhoB up-regulation as a key step for targeting ATC cell proliferation and tumor progression.

**Plasmids.** pcDNA3.1 was purchased from Invitrogen and pcDNA3.1-dominant negative RhoB was kindly provided by Séverine Steune (Free University of Brussels, Brussels, Belgium). The RhoB promoter linked to a luciferase reporter gene plasmid (hBB rhB promoter/luciferase, 1876 bp) was kindly provided by Dr. Daniel Tovar (Institut Claudius Regaud, Toulouse, France). For transient transfection, cells were plated in 60 mm plates and transfected using LipofectAMINE 2000 (Invitrogen) for 24 h followed by an additional 24 h, after which cells were treated with 10 nmol/L of RS5444, 1 ng/mL of FK228, or DMSO. Cells were then collected and mRNA was isolated using RNAqueous (Ambion).
mean ± SD and were analyzed by ANOVA. \( P < 0.05 \) was considered statistically significant.

**Results**

**Regulation of cell proliferation by RS5444 is PPAR\( \gamma \) dependent.** We previously showed that DRO cells were growth-inhibited by 10 nmol/L of RS5444 through a PPAR\( \gamma \)-dependent mechanism (9). We now confirm and extend this finding to four human cell lines (DRO, ARO, KTC2, and KTC3) that have been engineered to stably express lentiviral shRNA against PPAR\( \gamma \) (Fig. 1A). Cells silenced for PPAR\( \gamma \) expression (PPAR\( \gamma \)-shRNA) expressed little or no PPAR\( \gamma \) as compared with nontarget lentiviral shRNA control cells (Fig. 1B). Cells were then tested for transcriptional response to PPAR\( \gamma \) by transient transfection of PPRE-tk-luciferase as a transcriptional reporter for PPAR\( \gamma \) and showed loss of transcriptional response to 10 nmol/L of RS5444 (Fig. 1C). These results show that PPAR\( \gamma \)-induced growth inhibition is a general property of ATC cells.

PPAR\( \gamma \) agonists up-regulate RhoB expression, which is dependent on PPAR\( \gamma \). We observed altered cellular morphology following treatment with 10 nmol/L of RS5444 (Fig. 2A), which led us to examine the potential involvement of members of the RhoGTPase family of small GTPases, as these are known to play roles in regulating the actin cytoskeleton (17). Because the primary effects of PPAR\( \gamma \) are at the level of transcriptional regulation of target genes, we used real-time PCR to rapidly screen for changes in mRNA levels of the members of the RhoGTPase family, and found that RS5444 consistently up-regulated RhoB expression in all four cell lines examined (Fig. 2A). Up-regulation of RhoB protein was shown in DRO and KTC2 cells treated for 24 h with 10 nmol/L of RS5444 or two related thiazolidinedione PPAR\( \gamma \) agonists, 100 nmol/L of rosiglitazone, and 1 \( \mu \)mol/L of troglitazone (Fig. 2B). These effects were confirmed to be dependent on PPAR\( \gamma \), as pretreatment of cells with 10 \( \mu \)mol/L of GW9662, an irreversible pharmacologic inhibitor of PPAR\( \gamma \), blocked the induction of RhoB (Fig. 2B), as did shRNA silencing of PPAR\( \gamma \) (Fig. 2C). We also investigated these effects in mouse xenograft models in which we previously identified tumor growth inhibition of established tumors and up-regulation of p21 as a result of 0.025% RS5444 treatment (9), and found that RhoB expression was strongly up-regulated in DRO and ARO tumors excised from mice chronically treated with 0.025% RS5444 in the diet for 4 weeks (Fig. 2D). We confirmed this finding in an ATC cell line (THJ-11T) created in our laboratory (Fig. 2D). These results show that increased expression of RhoB is a general response to the activation of PPAR\( \gamma \) in ATC cells.

**RS5444 growth arrest is mediated via RhoB.** As RhoB has been shown to possess antiproliferative and proapoptotic activity in other types of cancer cells (26), we assessed the role of RhoB in the inhibition of ATC cell growth by PPAR\( \gamma \) (Fig. 3). Cells transfected with scrambled siRNA respond to 10 nmol/L of RS5444 with the up-regulation of RhoB protein (Fig. 3A). Cells transfected

![Figure 1. RS5444-mediated growth inhibition is PPAR\( \gamma \) dependent. A, cell proliferation assay as described in Materials and Methods of PPAR\( \gamma \)-shRNA lentivirally infected cell lines which were dependent on PPAR\( \gamma \) for RS5444-mediated growth inhibition. Columns, cell numbers were counted on day 7 and plotted as a percentage of nontarget control; bars, SD. *, \( P < 0.05 \) when compared with untreated controls. B, Western analysis confirms that these cell lines were silenced for PPAR\( \gamma \) protein expression. Nontarget shRNA controls showed endogenous PPAR\( \gamma \) expression that is equivalent to that of noninfected cells. C, PPAR\( \gamma \)-shRNA functionally blocked PPAR\( \gamma \)-mediated transcription as shown in these same cell lines that were transiently transfected with a PPRE3-tk-luc reporter and treated with 10 nmol/L of RS5444 for 24 h. Firefly luciferase activity was normalized to Renilla luciferase activity and graphed as relative light units (RLU) as compared with nontarget control ± SD.](https://www.aacrjournals.org/cancerres/article-pdf/69/4/1538/26329769/cancerres-2009-3718.pdf)
with RhoB siRNA and treated with RS5444 showed RhoB levels similar to that of scrambled untreated controls (Fig. 3A). Silencing RhoB expression revealed that all four lines were RhoB dependent for RS5444-induced growth arrest (Fig. 3B). Scrambled siRNA-treated cells were growth inhibited by 70% to 80% after exposure to 10 nmol/L of RS5444 over 6 days with media changes and the addition of 10 nmol/L of RS5444 every 48 hours (Fig. 3B). Conversely, cells silenced for RhoB expression showed significantly less growth inhibition when exposed to RS5444 (Fig. 3B). These results show that RhoB is an essential intermediate in PPARγ-mediated growth inhibition of ATC cells.

**p21 up-regulation is RhoB dependent.** We previously discovered that RS5444 up-regulated p21 protein levels in a PPARγ-dependent fashion and that growth arrest was p21 dependent (9). Here, we tested whether the induction of p21 by RS5444 was dependent on RhoB. We confirmed in all four cell lines that a 24-hour treatment with 10 nmol/L of RS5444 up-regulates p21 mRNA and protein (Fig. 4A). These events were rapid because RhoB mRNA levels in DRO cells were elevated 2-fold after 2 hours and were higher than 8-fold by 24 hours post-RS5444 treatment compared to time-matched vehicle controls (Fig. 4B); p21 mRNA levels were elevated 2-fold at 4 hours post-RS5444 treatment and 7-fold at 24 hours compared to controls (Fig. 4C) indicating that RS5444 transcriptionally up-regulates RhoB and p21. RhoB protein levels are elevated after 4 hours whereas p21 is elevated by 6 hours, and remain elevated 48 hours after a single treatment.

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*RhoB in ATC*

Figure 2. Changes in morphology and induction of RhoB is PPARγ dependent. A, live microscopic images of KTC2 and KTC3 cell lines treated with and without 10 nmol/L of RS5444 for 24 h (magnification, ×40) revealed altered morphologies. Real-time PCR for multiple RhoGTPases in cell lines treated with RS5444 for 24 h showed an increase of RhoB mRNA with no appreciable alteration in mRNA levels of the other RhoGTPases. Experiments were performed in triplicate. * P < 0.05 when compared with untreated control. B, three thiazolidinedione PPARγ agonists (10 nmol/L, RS5444, 100 nmol/L rosiglitazone, 1 mmol/L troglitazone) induced RhoB protein expression in DRO and KTC2 cells examined 24 h after treatment. When the irreversible PPARγ antagonist GW9662 was added 5 min prior to thiazolidinedione treatment, induction of RhoB was blocked, demonstrating specificity for PPARγ. C, Western analysis of lentivirally infected DRO and KTC2 cells silenced for PPARγ illustrates a lack of RhoB up-regulation in the absence of PPARγ when treated with RS5444. D, Western analysis showed induction of RhoB expression in tumors (DRO, ARO, and THJ-11T) grown in athymic nude mice following chronic oral treatment (4 weeks) with 0.025% of RS5444 versus that of vehicle control. Results from three random animals from each group.

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**Table 1.**

<table>
<thead>
<tr>
<th>mRNA fold change after RS5444 exposure</th>
<th>DRO</th>
<th>ARO</th>
<th>KTC2</th>
<th>KTC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RhoB</td>
<td>4.31 ± 0.02*</td>
<td>4.02 ± 0.04*</td>
<td>2.18 ± 0.07*</td>
<td>2.21 ± 0.03*</td>
</tr>
<tr>
<td>RhoA</td>
<td>0.89 ± 0.02</td>
<td>1.24 ± 0.03</td>
<td>1.04 ± 0.03</td>
<td>1.05 ± 0.02</td>
</tr>
<tr>
<td>RhoC</td>
<td>1.37 ± 0.05</td>
<td>0.97 ± 0.04</td>
<td>0.97 ± 0.09</td>
<td>0.94 ± 0.02</td>
</tr>
<tr>
<td>Rac1</td>
<td>1.07 ± 0.01</td>
<td>1.97 ± 0.02*</td>
<td>1.23 ± 0.06</td>
<td>1.23 ± 0.03</td>
</tr>
<tr>
<td>cdc42</td>
<td>1.09 ± 0.04</td>
<td>0.79 ± 0.06</td>
<td>1.02 ± 0.03</td>
<td>1.08 ± 0.02</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SEM. * P < 0.05 compared with untreated control.
of RS5444 in DRO cells (Fig. 4D). Collectively, these data show that RhoB mRNA and protein are up-regulated by RS5444 before that of p21, suggesting that increased expression of RhoB is upstream of p21 up-regulation. Consistent with this interpretation, we found that silencing RhoB in DRO and KTC2 cells significantly blocked the up-regulation of p21 mRNA by RS5444 (Fig. 5A). Similarly, expression of dominant-negative RhoB also blocked RS5444-induced up-regulation of p21 mRNA (Fig. 5D).

We next evaluated the effect of RhoB silencing on protein expression levels of RhoB, p21, and RhoA (Fig. 5C). We found that silencing RhoB had no effect on protein expression for RhoA, demonstrating siRNA specificity, and that stimulation with RS5444 in scrambled siRNA transfected cells showed up-regulation of RhoB and p21 protein (Fig. 5C). Moreover, we found that RhoB siRNA inhibited the induction of p21 protein levels by RS5444. These results show that RhoB is necessary for p21 mRNA and protein up-regulation by RS5444 (Fig. 5C).

HDAC inhibitor FK228 also up-regulates RhoB. Our results showed that increased expression of RhoB is an essential upstream step for PPARγ/p21-dependent inhibition of ATC proliferation, and in doing so, we identified RhoB as a novel point of therapeutic intervention in ATC. We next wished to test whether this information could be used to identify PPARγ-independent therapeutic approaches for targeting ATC. Previous investigators found that HDACs suppress RhoB expression in human non–small cell lung carcinoma cell lines and that HDAC inhibitors up-regulate RhoB (21, 27). We found that KTC3 cells transfected with scrambled or three different RhoB siRNAs showed RhoB siRNA 4 almost completely inhibited the pan-HDAC inhibitor, FK228, from inducing RhoB mRNA whereas RhoB siRNA 3 showed modest inhibition and RhoB siRNA 1 showed no inhibition (Supplementary Fig. S1). Cell proliferation of KTC3 cells using these same siRNAs illustrated differential growth effects when treated with 1 ng/mL of FK228 based on the ability of the siRNA to silence RhoB (Supplementary Fig. S1). Using scrambled siRNA, cells respond to FK228 with up-regulation of RhoB and p21 protein whereas cells transfected with RhoB siRNA 4 show silenced RhoB and lack of p21 up-regulation in response to FK228 (Fig. 6A), and that knockdown of RhoB significantly inhibited FK228-induced growth arrest (Fig. 6B).

These results suggest that HDAC inhibitor–based therapies could be effective against ATC that are unresponsive to PPARγ agonists. To test this possibility, we evaluated the response to FK228 in THJ-16T cells, which is a cell line derived from a primary ATC tumor in our laboratory. THJ-16T cells express no PPARγ and do not show up-regulation of RhoB or inhibition of cell proliferation in response to RS5444 (Fig. 6C). However, THJ-16T cells do show up-regulation of RhoB and p21 in response to FK228 and silencing of RhoB expression attenuates FK228-dependent inhibition of cell proliferation (Fig. 6C).

Discussion

Our novel discovery that PPARγ up-regulates RhoB in ATC cells was prompted by RS5444-mediated alterations in cell morphology coupled with supporting literature demonstrating that enhanced RhoB activity was associated with growth inhibition and elevated p21 (17, 28, 29). RhoA and RhoC share ~86% amino acid homology with RhoB and also mediate actin stress fiber formation. Other RhoGTPases, which include Rac1 and Cdc42, have been found to promote oncogenesis, invasion, and metastasis (30, 31). However, RhoB is antiproliferative and proapoptotic in cancer cells (32), and overexpression of RhoB can inhibit cell migration, invasion, and metastasis (33). In head and neck, lung, and brain cancers, RhoB levels are decreased with tumor progression suggesting that
silencing this pathway is critical to tumor survival and progression (34–36). Our data show that RhoB mRNA and protein levels are low in ATC cell lines with up-regulation following exposure to RS5444 or FK228. We found that RS5444 regulates RhoB at the transcriptional level with rapid up-regulation of RhoB mRNA by 2 hours of treatment and in protein levels by 4 hours. We identified two putative PPARγ response elements (C01302 to C01282 and C01422 to C01402) in the RhoB promoter with 70% to 80% homology to a consensus PPARγ response element (PPRE). However, transient transfection of the RhoB promoter linked to a luciferase reporter (1876 bp promoter fragment) in DRO, KTC2, and KTC3 did not lead to the induction of luciferase activity after 24 hours of treatment with 10 nmol/L of RS5444, even though treatment with 1 ng/mL of FK228 induced luciferase activity 3-fold to 9-fold (Supplementary Fig. S2). Thus, the transcriptional regulation of RhoB by PPARγ may be regulated by another region of the RhoB gene or by a more complicated mechanism involving structured chromatin complexes not assayed using transfected RhoB promoter linked to luciferase. RhoB transcript levels have been observed to be transcriptionally suppressed by HDAC1 and found to be positively regulated when treated with an HDAC inhibitor (trapoxin A) via an inverted CCAAT element in the RhoB promoter 451 bp upstream of the transcriptional start site (27). RhoB is an early response gene responding to environmental stresses, such as UV irradiation, which under these circumstances, is mediated by the RNA-binding protein HuR stabilizing RhoB mRNA (37). Oppositely, oncogenic signals mediated by the Ras/PI3K/Akt pathway suppress RhoB expression (38). Thus, although multiple mechanisms seem to regulate RhoB expression in cancer, the important consequence of our work is that RhoB can be re-expressed in ATC through the use of drugs, resulting in biologically active protein that opposes tumor growth.

We describe a novel mechanistic signaling pathway leading to tumor growth inhibition by two classes of drugs via a RhoB-dependent mechanism that is upstream of p21. We show that

Figure 4. RhoB up-regulation by RS5444 occurs prior to p21 up-regulation. A, real-time PCR and Western analysis for p21 expression in all four cell lines showed p21 induction after 10 nmol/L of RS5444 treatment for 24 h. Fold change values were calculated between treated and control samples; columns, mean; bars, SD (n = 3). *, P < 0.01 when compared with controls. B, real-time PCR of RhoB mRNA in DRO cells upon exposure to RS5444 showed induction as early as 2 h. C, real-time PCR of p21 mRNA in DRO cells showed induction by 4 h. Data was normalized to 18S; points, mean; bars, SD (n = 4). *, P < 0.05 when compared with controls. D, immunoblotting of DRO cells treated with RS5444 showed up-regulation of RhoB protein by 4 h and p21 protein by 6 h. No change in RhoA protein occurred between control and RS5444-treated cells.
PPARγ-mediated up-regulation of p21 mRNA occurs through RhoB by using dominant negative RhoB and siRNA to silence RhoB. Two reports have identified a potential link between up-regulation of p21 and RhoB (28, 29). In one report, RhoB-GG induced p21 in a p53-dependent manner, although this was dispensable because RhoB-GG still inhibited the growth of p53-null cells that lacked p21 (28). The authors concluded that RhoB-GG suppressed human tumor cell proliferation by more than one mechanism and that it promoted apoptosis as well as inhibited cell cycle transit. In the other report, ectopic expression of RhoB was shown to up-regulate p21 (17, 28, 29). Our present study is the first to show that endogenous RhoB is directly responsible for the regulation of p21 mRNA and protein levels by RS5444 and FK228 in p53-wild-type (DRO and KTC2) as well as p53-mutant (ARO and KTC3) cells (39).

In a recent report, ciglitazone and rosiglitazone increased apoptosis in several ATC cell lines (40). The lack of induction of apoptosis by RS5444 (9) is striking because expression of RhoB induces apoptosis in cancer cells in cell culture (reviewed in ref. 32) and ectopic tumors grown in mice (41, 42). Even more surprising, DAPI staining revealed that treatment with FK228 also does not induce apoptosis in THJ-11T and THJ-16T, with only slight apoptosis (~ 12%) in DRO (Supplementary Fig. S3A). Using a LDH assay to examine overall cell death, FK228 treatment again only slightly induces cell death (~ 13%) in DRO with no effect on KTC2 and KTC3 cells (Supplementary Fig. S3B). One study showed that cyclin B1 is the target for suppression by RhoB causing apoptosis (43). It is possible that both RS5444 and FK228 could stimulate an antiapoptotic pathway antagonizing the proapoptotic properties of RhoB that is independent of the regulatory role of RhoB in cell proliferation. Alternatively, it may be that induction of apoptosis by rosiglitazone might be PPARγ/RhoB-independent. One additional consideration is that cellular localization and posttranslational modification (prenylation and sumoylation) of RhoB are critical regulators of its effects (32). Site-directed mutagenesis studies showed that RhoB-GG induced p21 and prenylated cysteine 192 for the tumor-suppressive and proapoptotic activities of RhoB (27, 28). Understanding these mechanisms could be important in the selection of the most potent PPARγ agonist or RhoB modulator for combinatorial therapy such as a thiazolidinedione and taxane against ATC (9). Importantly, we found that chronic daily treatment of mice harboring ATC tumors with 0.025% of RS5444 in the diet retains the ability to sustain elevated RhoB over 4 weeks of treatment (Fig. 2D). This also suggests that RhoB and p21 may be ideal molecular markers of response to therapy and easily measured in biopsy tissue following treatment.

In summary, we have identified a novel growth-inhibitory pathway regulated by PPARγ and identified a HDAC inhibitor that up-regulates RhoB mRNA and protein in human ATC cells (Fig. 6D). Elevated RhoB protein is necessary for these two drugs to up-regulate p21 mRNA and protein as well as inhibit cell proliferation (Fig. 6D). Thus, RhoB is implicated as a critical signaling node that could be therapeutically targeted in ATC. Importantly, it is a target that can be up-regulated by multiple classes of drugs. HMG CoA reductase inhibitors (statins; ref. 44), prenylation inhibitors (FTI, GGTI; ref. 32), HDAC inhibitors (FK228), and now, PPARγ agonists, up-regulate RhoB in various cancers. Although the connection to RhoB has not been made in ATC, the aforementioned classes of drugs inhibit growth in ATC cells (45–47), most likely acting in part through up-regulation of functional RhoB. We are currently investigating the role of these drugs in human ATC cell lines.
drugs that mediate RhoB up-regulation and growth inhibition in ATC. Posttranslational modification and cellular localization of RhoB as a result of treatment with each of these drugs will also most likely dictate optimal antitumor activity (32) and allow for rational selection of combinatorial therapy with maximum benefit to the patient.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References


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Laura A. Marlow, Lisa A. Reynolds, Alan S. Cleland, et al.

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