MAYO CLINIC Cancer Center RhoB as a novel molecular target for therapy in human anaplastic thyroid carcinoma



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ABSTRACT

Anaplastic thyroid carcinoma (ATC) is one of the most aggressive and poorly differentiated carcinomas with a mortality rate near 100%. With the need of better treatment options, we have now identified a novel PPARy agonist, RS5444, which exhibits antiproliferative activity in human ATC both in vitro and in vivo. RS5444 is dependent upon functional PPARy as evidenced by silencing of PPARy in ATC cells. We have also discovered that activation of PPARy upregulates RhoB, a known inhibitor of tumor growth typically via apoptosis. Induction of RhoB is not unique to RS5444 since other PPARy agonists also induce its expression. Silencing of PPARy demonstrates that upregulation of RhoB is dependent upon transcriptionally active PPARy. Furthermore, primary cells from ATC tissue identify that PPARy expressing cells treated with RS5444 induce RhoB suggesting that patients treated with a PPARy agonist may respond to this therapeutic regimen. When silencing RhoB in ATC cells, RS5444 loses its growth inhibitory activity illustrating that growth inhibition of ATC is dependent upon functional RhoB. We have also identified 2 other classes of compounds, an HDAC inhibitor (FK-228) and FTI inhibitor (FTI-277) that induced RhoB dependent growth inhibition in ATC cells. Thus, we are currently investigating the molecular mechanism of RS5444 induction of RhoB and identifying potential combinatorial therapies focused on RhoB as a molecular target.



Figure 1. RS5444 induction of RhoB is PPARy dependent.

A. Athymic nude mice were treated for 4 weeks with RS5444 after ectopic implantation of ATC cells demonstrate inhibition of tumor growth.

B. Western analysis demonstrates upregulation of RhoB expression in ATC tumors from mice following 4 wks of 0.025% RS5444 treatment (+) as compared to the vehicle control (-). C. ATC cells were treated with 10 nM RS5444, 100 nM rosiglitazone, 1 µM troglitazone, and 10 μ M GW9662 for 24 hrs. RhoB protein expression was induced most strongly by RS5444 and was abolished by GW9662, a PPARy antagonist.

D. Immunoblotting of ATC cells transfected for 72 hrs with scrambled and PPARy siRNA illustrate lack of RhoB upregulation in the absence of PPARy despite the presence of 10 nM RS5444 (+) with no effects on RhoA

E. Western analysis of THJ-11T cells that express PPARy have upregulation of RhoB by RS5444. THJ-16T cells that lack PPARy do not have the ability to upregulate RhoB protein expression upon exposure to RS5444.



Figure 2. RhoB upregulation by RS5444 is necessary for RS5444-induced inhibition of ATC proliferation.

A. Three ATC cell lines were examined by Western analysis to verify that RhoB expression remained silenced even in the presence of RS5444. Cells were transfected for 24 hrs with scrambled (scr) and RhoB siRNA and treated with 10 nM RS5444.

B. Cellular proliferation was examined by transfecting ATC cell lines with RhoB or scrambled siRNA for 24 hrs and at 48 hr intervals cells were treated with 10 nM RS5444 for 6 days beginning 24 hr after the first RNAi treatment. Cell numbers were counted and data are plotted as percent of scrambled control ± S.D. * indicates p<0.01 when comparing scrambled control to RS5444 treatment.

RESULTS



A. Western analysis of ATC cells transfected with

Rho

either scrambled or RhoB siRNA treated with 1 ng/ml FK-228 and 10 mM FTI-277 for 24 hours demonstrate induction of RhoB can be blocked by siRNA.

B. Cell proliferation of ATC cells transfected with either scrambled or RhoB siRNA treated with the indicated compounds for 6 days with a media/drug change every 48 hours. Growth inhibition by FK-228 and FTI-277 is dependent upon RhoB upregulation.

C. Graphic representation of DAPI stained ATC cells that stably express Tet-ON constitutively active RhoB show overexpression of active RhoB leads to apoptosis, FK-228 and FTI-277 to a lesser extent also induces apoptosis via RhoB.



Scr. siRN

RhoB siRN

В.

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CONCLUSIONS AND FUTURE DIRECTIONS

- RS5444 induces RhoB expression both in vitro and in vivo
- Induction of RhoB by RS5444 is PPARγ dependent
- RhoB expression is also induced by HDAC inhibitors and farsenvl transferase inhibitors
- Growth inhibition in ATC by PPARγ agonists, HDAC inhibitor and FTIs are dependent upon RhoB upregulation
- Investigate the mechanism of RS5444-induced RhoB growth arrest and lack of apoptosis in ATC cells
- Investigate the use of HDAC inhibitors and farnesyl transferase inhibitors in combination with paclitaxel to determine optimal anti-tumor activity



Anaplastic thyroid carcinoma (ATC) represents only 1-2 % of all malignant thyroid diseases while accounting for over half of thyroid carcinoma related deaths in the United States. ATC is characterized as poorly differentiated and highly aggressive with mortality rates near 100% and median survival of 3 to 7 months after diagnosis (Sherman 2003). The attempt to manage ATC has been very unsuccessful and to date, only paclitaxel and doxorubicin have shown any benefit despite no curative potential (Ain 2000). As a result, molecularly targeted therapies are being investigated to improve management of persistent and recurrent anaplastic thyroid carcinomas by interfering with signal transduction pathways to inhibit tumor growth and angiogenesis (Baudin and Schlumberger 2007).

RS5444, a peroxisome proliferator activated receptor gamma (PPARy) agonist, has been found to arrest ATC tumor growth both in vitro and in vivo. Also, RS5444 (PPARy agonist) in combination with paclitaxel showed apoptotic synergy that was dependent upon the upregulation of p21, a cyclin kinase inhibitor (Copland et al 2006). However, the mechanism by which a PPARy agonist upregulates p21 has yet to be demonstrated and it could provide information about additional therapeutic targets in ATC.

RhoB is a member of the Ras superfamily of isoprenylated small GTPases that normally regulates actin stress fibers and vesicle transport. Although RhoB is not mutated in cancer, it has altered expression and activity which modulates proliferation, survival, invasion and angiogenesis. RhoB's activity appears crucial for potentially regulating cancer progression and therapeutic responses (Prendergast 2001).