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Inhibiting HDAC6 and HDAC1 upregulates RhoB with divergent downstream targets, BIM_{EL} or $p21^{WAF1/CIP1}$, leading to either apoptosis or cytostasis

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ABSTRACT

Background: Anaplastic thyroid carcinoma (ATC) is a highly aggressive undifferentiated carcinoma with a mortality rate near 100%. This high mortality rate is due to a multiplicity of genomic abnormalities resulting in the lack of effective therapeutic options. In order to find and apply effective targeted therapies, new molecular targets need to be discovered. Our lab has previously identified that the upregulation of RhoB is therapeutically beneficial in ATC and can serve as a molecular target. **Methods**: For studying RhoB and its epigenetic regulation, HDAC inhibitors and HDAC shRNAs are used to examine the downstream effects of upregulated RhoB. **Results**:RhoB is repressed by HDAC1 and, for the first time, we identify HDAC6 as another repressor of RhoB. Both HDAC1 and HDAC6 are overexpressed in ATC and we find that RhoB has divergent downstream targets depending upon which HDAC is inhibited. When HDAC1 is inhibited by romidepsin or by shRNA, RhoB upregulates p21 leading to cytostasis. However, HDAC6 inhibition by belinostat, vorinostat, or shRNA leads to RhoB mediated upregulation of BIM_{EL} resulting in apoptosis. Interestingly, these divergent pathways can be flipped between the two, through the bilateral regulation of RhoB. When p21 is silenced, romidepsin can now induce apoptosis through RhoB→BIM_{EL}. When BIM_{EL} is silenced, the effects of belinostat and vorinostat now shift to RhoB→p21 leading to cytostasis. The combination of these HDAC inhibitors with paclitaxel also yields divergent results. Belinostat and vorinostat in combination with paclitaxel results in synergy and this can be reversed when BIM_{EL} is silenced. Romidepsin in combination with paclitaxel has no synergy, but when p21 is silenced, synergy is invoked. **Conclusions:** Thus, to attain optimal therapeutic benefit, drugs that alter RhoB to favor BIM_{EL} induced apoptosis should be employed. This study shows that the combination of either belinostat or vorinostat with paclitaxel may prove to be an effective therapeutic option in ATC patients via de-repression of HDAC6 suppressed RhoB.

INTRODUCTION

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. No effective therapy or standard of care exists for ATC patients. It is a rapidly progressing cancer with median survival of 3 – 5 months upon diagnosis (Smallridge et al 2009). Using genomic profiling, our work previously detailed a novel signaling pathway, RhoB, leading to antitumor synergy with the microtubule stabilizer, paclitaxel, in ATC. RhoB is a member of the Ras superfamily of isoprenylated small GTPases and is suppressed, but not mutated in numerous cancers. RhoB can induce apoptosis in transformed cells, but we previously found that RhoB upregulates p21 and induces cell cycle arrest (Marlow et al 2009). Knowing that HDAC1 suppresses RhoB mRNA via an inverted CCAAT box in the RhoB promoter and the use of a class I HDAC inhibitor led to apoptosis, we reasoned that HDAC inhibitors combined with paclitaxel may be an effective combinatorial therapy. HDAC inhibitors have shown considerable potential as new antitumor agents which modulate acetylation by targeting histone deacetylases for inducing differentiation and apoptosis via transcriptional modulation. HDACs are divided into 4 classes encompassing HDACs 1-11. In our current investigation comparing clinically relevant HDAC inhibitors, we delineated novel RhoB-mediated signaling pathways dependent upon the class of HDAC inhibitor used that resulted in either cell cycle arrest or apoptosis. We can now predict apoptosis versus cell cycle arrest when using HDAC inhibitors as well as antitumor synergy of an HDAC inhibitor with paclitaxel in ATC.

RESULTS

Comparison of class I and I/II HDAC inhibitors

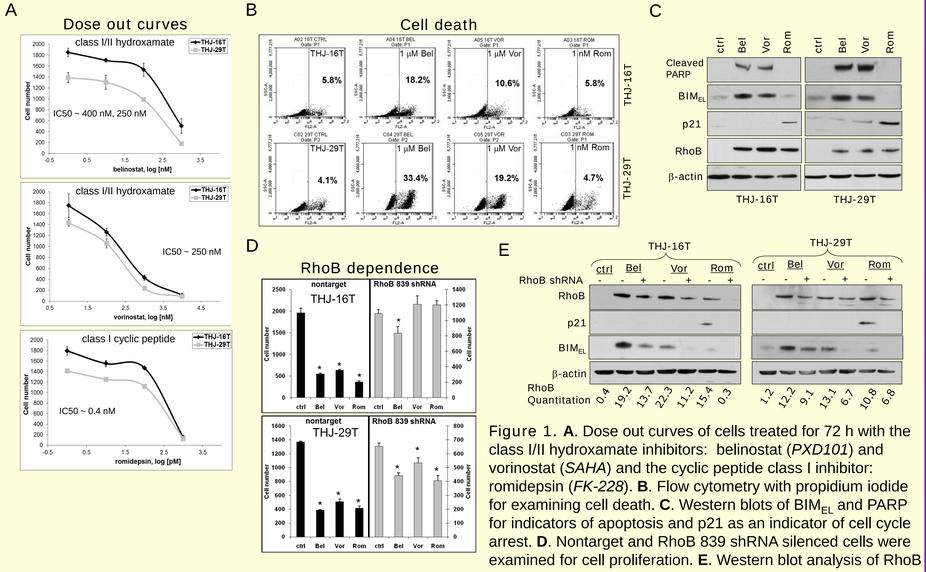

Figure 1. **A**. Dose out curves of cells treated for 72 h with the class I/II hydroxamate inhibitors: belinostat (*PXD101*) and vorinostat (*SAHA*) and the cyclic peptide class I inhibitor: romidepsin (*FK-228*). **B**. Flow cytometry with propidium iodide for examining cell death. **C**. Western blots of BIM_{EL} and PARP for indicators of apoptosis and p21 as an indicator of cell cycle arrest. **D**. Nontarget and RhoB 839 shRNA silenced cells were examined for cell proliferation. **E**. Western blot analysis of RhoB 839 shRNA cells for downstream effects.

Identification of HDAC1 and HDAC6 as repressors of RhoB

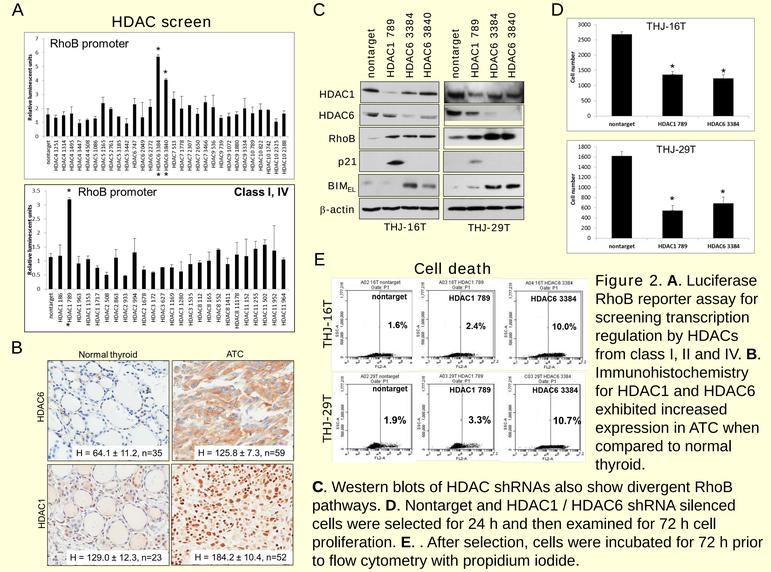

Figure 2. **A**. Luciferase RhoB reporter assay for screening transcription regulation by HDACs from class I, II and IV. **B**. Immunohistochemistry for HDAC1 and HDAC6 exhibited increased expression in ATC when compared to normal thyroid. **C**. Western blots of HDAC shRNAs also show divergent RhoB pathways. **D**. Nontarget and HDAC1 / HDAC6 shRNA silenced cells were selected for 24 h and then examined for 72 h cell proliferation. **E**. . After selection, cells were incubated for 72 h prior to flow cytometry with propidium iodide.

Flipping the RhoB→p21 and RhoB→BIM_{EL} pathway switch

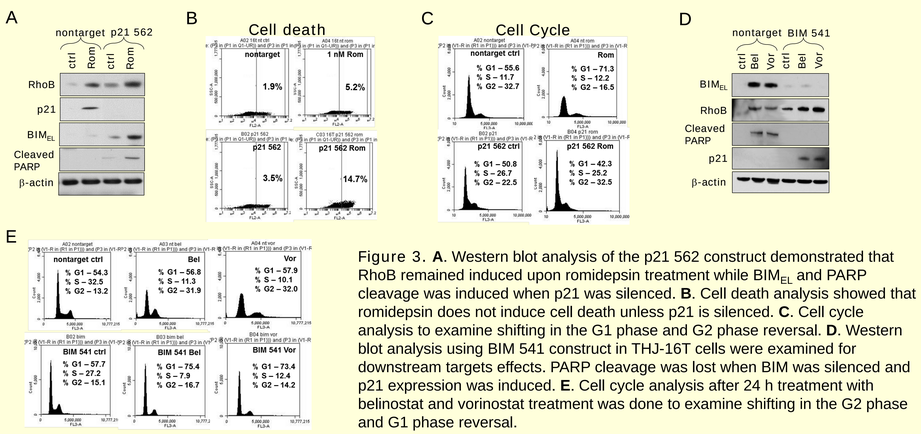

Figure 3. **A**. Western blot analysis of the p21 562 construct demonstrated that RhoB remained induced upon romidepsin treatment while BIM_{EL} and PARP cleavage was induced when p21 was silenced. **B**. Cell death analysis showed that romidepsin does not induce cell death unless p21 is silenced. **C**. Cell cycle analysis to examine shifting in the G1 phase and G2 phase reversal. **D**. Western blot analysis using BIM 541 construct in THJ-16T cells were examined for downstream targets effects. PARP cleavage was lost when BIM was silenced and p21 expression was induced. **E**. Cell cycle analysis after 24 h treatment with belinostat and vorinostat treatment was done to examine shifting in the G2 phase and G1 phase reversal.

SUMMARY

* Belinostat and vorinostat are class I/II hydroxamate HDAC inhibitors that are RhoB-dependent leading to induction of BIM_{EL} for promoting G2/M cell cycle arrest and apoptosis.

* Romidepsin is a class I cyclic peptide HDAC inhibitor that is RhoB-dependent leading to G1 cell cycle arrest.

* HDAC1 and HDAC6 suppress RhoB and they are overexpressed in ATC patient tissues.

* HDAC1 and HDAC6 divergently regulate BIM and p21 through RhoB.

* When p21 is silenced, the pathway is shifted to the BIM_{EL} apoptotic pathway.

* When BIM_{EL} is silenced, the pathway is shifted to the p21 cytostatic pathway.