Inhibiting HDAC6 and HDAC1 upregulates RhoB with divergent downstream targets, BIM\textsubscript{EL} or p21\textsuperscript{WAF1/CIP1}, leading to either apoptosis or cytostasis

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\section*{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, which 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\textsubscript{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\textsubscript{EL}. When BIM\textsubscript{EL} is silenced, the effects of belinostat and vorinostat or shRNA 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\textsubscript{EL} is silenced. Romidepsin in combination with paclitaxel has no synergy and this can be reversed when p21 is silenced, synergy is invoked. Conclusions: To attain optimal therapeutic benefit, drugs that alter RhoB to favor BIM\textsubscript{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.

\section*{RESULTS}

Comparison of class I and I/II HDAC inhibitors

\begin{figure}[h]
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\includegraphics[width=0.8\textwidth]{fig1.png}
\caption{A. Dose out curves of cells treated for 72 h with the class II/II hydroxamate inhibitors: belinostat (Bel), vorinostat (Vor) and the cyclic peptide class I inhibitor: romidepsin (Rom). B. Flow cytometry with propidium iodide for examining cell death. C. Western blot analysis using BIM 541 construct in THJ-16T cells were examined for downstream effects. E. Western blot analysis of RhoB 839 shRNA silenced cells for downstream effects.}
\end{figure}

\section*{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, we 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 predict apoptosis versus cell cycle arrest when using HDAC inhibitors as well as antitumor synergies of an HDAC inhibitor with paclitaxel in ATC.

\section*{SUMMARY}

* Belinostat and vorinostat are class I/II hydroxamate HDAC inhibitors that are RhoB-dependent leading to induction of BIM\textsubscript{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\textsubscript{EL} apoptotic pathway.

* When BIM\textsubscript{EL} is silenced, the pathway is shifted to the p21 cytostatic pathway.