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Brain-Penetrating Histone Deacetylase Inhibitor RG2833 Reduces the Viability of Human Malignant Melanoma Cell Lines SK-MEL-5 and SK-MEL-28 in vitro

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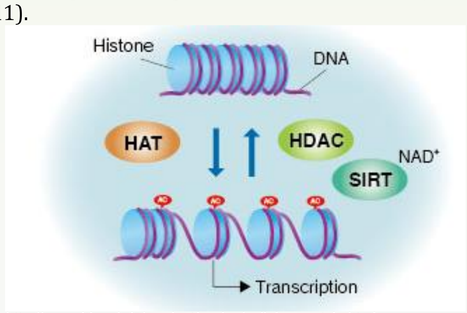
Abstract

Histone deacetylases (HDACs) play an important role in the epigenetic control of gene expression in both normal and cancer cells. Previous studies have demonstrated that pharmaceutical inhibition of HDACs can kill and/or suppress the growth of cancer cells. RG2833 is a HDAC inhibitor that targets specific HDACs known to be active in cancer cells. Melanoma cells have previously been shown to respond to HDAC inhibitors that are structurally similar to RG2833. **We hypothesized that the inhibition of HDAC activity by RG2833 would result in the reduced growth and/or death of cells from the malignant melanoma cell lines SK-MEL-5 and SK-MEL-28.** To test our hypothesis, we exposed SK-MEL-5 and SKMEL-28 cells to increasing concentrations of RG2833. We found that concentrations of RG2833 that effectively inhibited HDAC activity also resulted in reduced melanoma cell growth and viability. These results demonstrate the effectiveness of RG2833 in reducing the growth and viability of malignant melanoma cells in vitro and warrant further investigation of the potential therapeutic use of RG2833 and related compounds in the battle against cancer.

Introduction

Anticancer therapeutic studies targeting histone deacetylase inhibitors have shown promise in the suppression of cancer cell growth (Pan 2007; Boyle 2005; Munshi 2005). When histone tails are acetylated, the association between histones and DNA is loosened, and the DNA is more accessible to the transcriptional machinery. Histone deacetylases (HDAC) remove acetyl groups from histone tails, thereby tightening the association between DNA and histones (Dokmanovic *et al.* 2007). HDAC inhibitors promote acetylation by blocking HDAC activity. Cancer cell growth can be suppressed by HDAC inhibitors because of their effects on gene expression (Munshi *et al.* 2014). RG2833 is a recently developed HDAC inhibitor capable of penetrating the blood-brain barrier (Sandi *et al.* 2011).



http://ruo.mbl.co.jp/g/product/cyclex/cellular-acetyle.html

Methods

Cell Lines and Culture: The two human malignant melanoma cell lines SK-MEL-5 and SK-MEL-28, were obtained from the American Type Culture Collection (Manassas, VA). The cells were maintained under standard cell culture conditions.

HDAC Inhibitor. HDAC1 and HDAC3 inhibitor RG2833 (Figure 1) was obtained from Selleck Chemicals (Houston, TX).

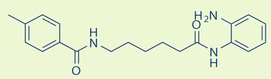


***Figure 1*:** Molecular Structure of RG2833

HDAC Activity Assay:
- Cayman Chemical: HDAC Cell-Based Activity Assay Kit
- Performed in live cells
- Evaluated HDAC inhibition at varying RG2833 concentrations

NCI-60 Assay / Sulforhodamine B (SRB) Assay:
- Measure relative number of cells by staining total protein
- Assay used by National Cancer Institute to screen potential anti-cancer compounds

CellTiter-Glo™ Viability Assay:
- Determined cell viability based on amount of ATP and metabolic activity
- Multiple time points

AlamarBlue® Viability Assay:
- Determined cell viability
- AlamarBlue® reagent is metabolized to colored product in living cells
- Multiple time points

Results

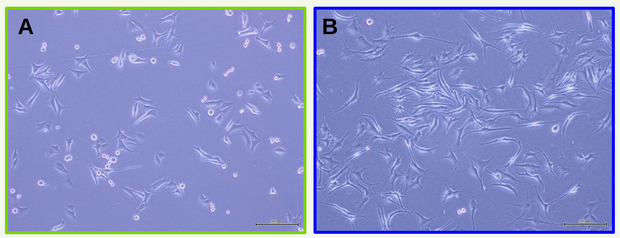


***Figure 2*:** Phase-contrast images of SK-MEL-5 (A) and SK-MEL-28 (B) cell lines.

HDAC Activity Assay:

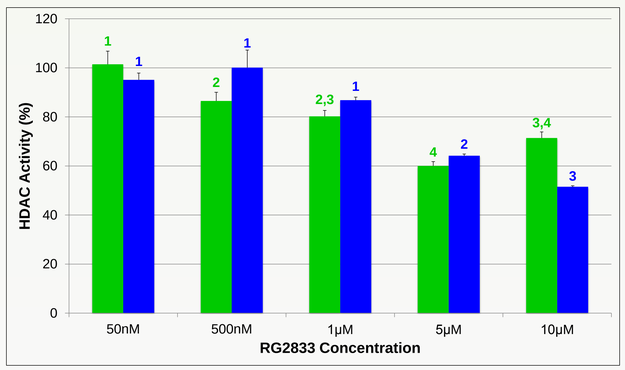


***Figure 3*:** RG2833 Inhibits HDAC Activity in SK-MEL-5 and SK-MEL-28 cell lines. Percentages are relative to untreated control. Numbers indicate statistically independent groups. $p < 0.05$

NCI-60 / SRB Assay (48 Hours):

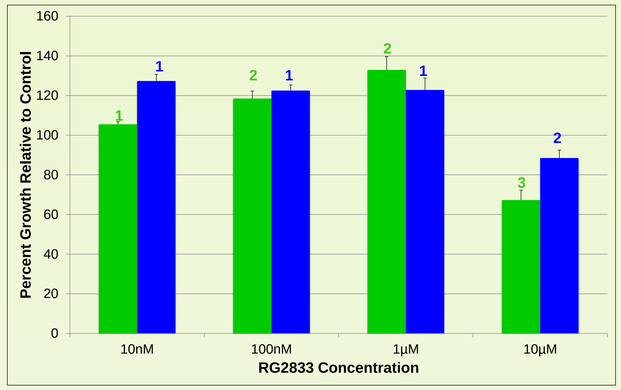


***Figure 4*:** Suppression of SK-MEL-5 and SK-MEL-28 growth is observed following treatment with 10uM RG2833 for 48 h. Numbers indicate statistically independent groups ($p < 0.05$).

CellTiter-Glo™ Viability Assay (48 Hours):

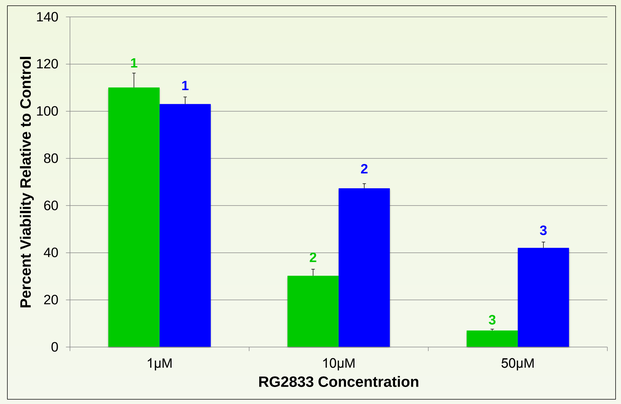


***Figure 5*:** Decreased viability of SK-MEL-5 and SK-MEL-28 cells is observed following treatment with 10uM RG2833 for 48 h. Numbers indicate statistically independent groups ($p < 0.05$).

Alamar Blue® Viability Assay (48 Hours):

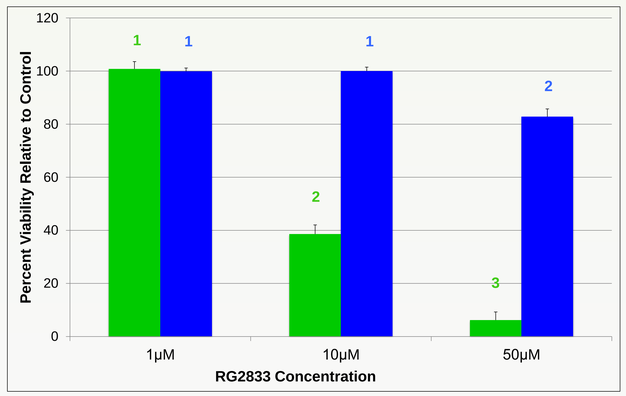


***Figure 6*:** Viability of SK-MEL-5 cells is reduced following treatment with 10uM RG2833 for 48 h. Note: SK-MEL-28 viability was significantly reduced with 10uM RG2833 at 72 h (not shown). Numbers indicate statistically independent groups ($p < 0.05$).

Conclusions

- RG2833 inhibited HDAC activity in SK-MEL-5 and SK-MEL-28 cells *in vitro*
- Concentrations of RG2833 that most effectively inhibited HDAC activity also reduced cell growth and viability of both cell lines
- We observed significant variability in results based on the the assay used.

Future Research

- Analyze gene expression using Real-Time RT-PCR
  - Qiagen RT² Profiler Array: Human Cancer Drug Targets
- Follow-up analysis of protein expression for select signaling pathways
- Introduce RG2833 to a control melanocyte cell line

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