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TITLE: Enhancing the Anti-Tumor Activity of ErbB Blockers with Histone
Deacetylase (HDAC) Inhibition in Prostate Cancer Cell Lines

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14. ABSTRACT PURPOSE: characterize the capacity of HDAC inhibitors to enhance the anti-tumor activity of anti-ErbB agents in prostate cancer cell lines. SCOPE: Interactions between these agents will be examined at both the cell signaling level, as well as through biologic end-points, including cellular proliferation, impact on cell cycle kinetics, invasion, and angiogenesis. MAJOR FINDINGS: HDAC inhibitors attenuate ErbB expression and the combination of HDAC inhibition and ErbB blockade resulted in near complete abrogation of EGFR and AKT signaling in the prostate cancer cell lines. HDAC inhibitors enhanced anti-proliferative effects and apoptosis induction of ErbB blockade in multiple cell lines. Preliminary gene expression profiles using cDNA arrays suggests multiple levels of potential synergy between ErbB and HDAC inhibitors. NEXT STEP: Continuing work with additional prostate cancer cell lines and examining other biologic end-points, including cell cycle kinetics, angiogenesis, and invasion. Promising results will then be evaluated in vivo.					
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Introduction

The treatment of patients with locally advanced or recurrent prostate cancer remains a major challenge in oncology. Several novel targeted therapies, including anti-ErbB agents and HDAC (histone deacetylase) inhibitors show promise for clinical benefit in these patients. Despite high enthusiasm for these approaches, early clinical data with ErbB inhibitors, for example, suggests that only 10-20% patients respond favorably to treatment. Therefore, considerable effort is currently being focused toward increasing the anti-tumor activity of ErbB inhibitors by using them in combination with other agents. One particular class of agents with strong potential for enhancing the effects of ErbB inhibitors are HDAC inhibitors. HDAC inhibitors are a promising new class of anticancer agents which independently demonstrate activity in a wide variety of solid and hematologic tumors. During preliminary screening studies using cDNA microarrays, we identified the potential of HDAC inhibition to attenuate the transcription of ErbB family oncoproteins in human prostate cancer cell lines. Furthermore, our preliminary data suggests that when used cooperatively, HDAC inhibitors can enhance growth inhibition and apoptosis induction of ErbB receptor blockers in prostate cancer.

A potential relationship between HDAC inhibition and ErbB expression has only recently been reported and the mechanism responsible for this phenomenon has not been defined. Combining anti-ErbB blockers with HDAC inhibitors may hold strong promise for enhancing anti-tumor activity and overall clinical response. The primary objective of this grant proposal is to characterize the capacity of HDAC inhibitors to enhance the anti-tumor activity of anti-ErbB agents in prostate cancer cell lines. Interactions between these agents will be examined at both the cell signaling level, as well as through biologic end-points, including cellular proliferation, impact on cell cycle kinetics, invasion, and angiogenesis. Promising data would provide rationale for future clinical trial investigations.

Body

The initial tasks, as described in the approved Statement of Work, involve determining the capacity of HDAC inhibitors to abrogate ErbB expression and AKT activation. Our results are shown in Figures 1 and 2. As preliminary studies suggest,

- SAHA demonstrates the capacity of down-modulate both EGFR and ErbB2 expression in a panel of prostate cancer cell lines (Fig 1A/B) although did not seem to impact ErbB3/4 expression (Fig 1 C/D).
- Similarly, SAHA did not impact AKT expression, although did have the capacity to inhibit its activation in multiple prostate cancer cell lines (Fig 2 A/B).

Our next task was to conduct in vitro experimentation to characterize the capacity of HDAC inhibitors to enhance the effects of anti-ErbB agents in a panel of prostate cancer cell lines. Initial studies involved determining the impact of the single agent SAHA (HDAC inhibitor), Tarceva (EGFR inhibitor), and CI-1033 (pan-ErbB tyrosine kinase inhibitor) on cellular proliferation.

- The HDAC inhibitor SAHA demonstrated the capacity to inhibit cellular proliferation in a panel of prostate cancer cell lines (Fig. 3).
- Tarceva, on the other hand, had very little impact on cellular proliferation (Fig. 4).

- The pan-ErbB inhibitor CI-1033 demonstrated a significant impact on cellular proliferation (Fig. 5) in 2 of the 4 prostate cancer cell lines tested (DU145 and PC3).

The impact of HDAC inhibition on cellular proliferation was further evaluated by cell cycle analysis.

- The HDAC inhibitor SAHA demonstrated a dose dependant G2/M phase cell cycle arrest in the prostate cancer cell line DU145 (Fig. 6).

Initial studies examining the interaction between HDAC and ErbB inhibitors were performed at the level of cellular proliferation. Of the ErbB inhibitors, CI-1033 was chosen, as Tarceva demonstrated little activity in cell lines tested.

- The combination of CI-1033 and SAHA demonstrated an additive/synergistic impact on cellular proliferation in the DU145 and PC3 cell lines (Fig. 7).
- To determine if this interaction was additive or synergistic, serial concentrations of each agent was used to create an isobologram (Fig. 8) which demonstrated synergy with the combination of these agents in the DU145 cell line.

In addition to cellular proliferation, the interaction between HDAC and ErbB inhibitors was further evaluated at the level of apoptosis.

- The combination of the HDAC inhibitor SAHA and CI-1033 demonstrated a supra-additive induction of apoptosis, evaluated by caspase activity (Fig. 9A) and PARP cleavage (Fig. 9B).

The mechanism underlying these favorable interactions between HDAC and ErbB inhibitors was evaluated. Initial experiments involved the impact of HDAC inhibition on ErbB signaling.

- Both CI-1033 and SAHA demonstrated modest inhibition of EGFR and AKT activation. Combination of the two agents demonstrated a near complete abrogation of signaling (Fig. 10).

We are continuing work with additional prostate cancer cell lines and examining other biologic end-points, including cell cycle kinetics, angiogenesis, and invasion. Promising results will then be evaluated in vivo, as described in our final task.

Figure 1

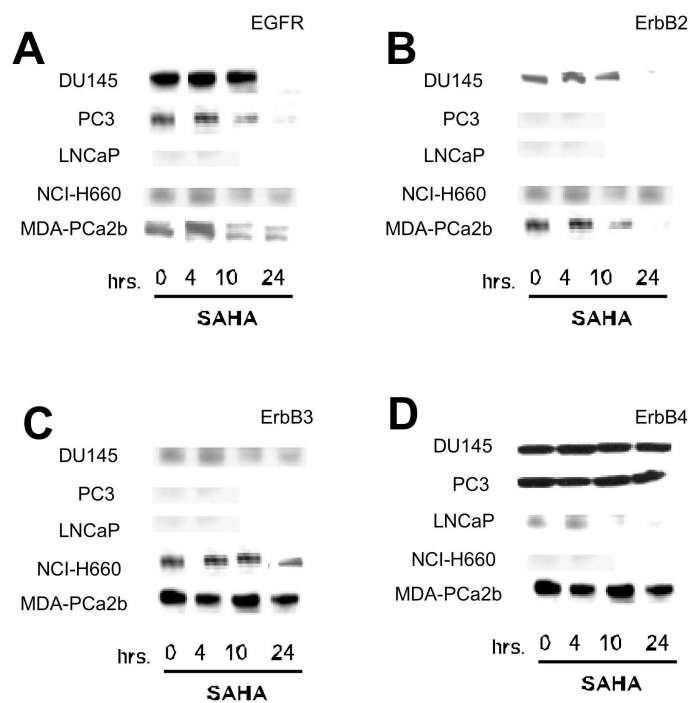


Figure 2

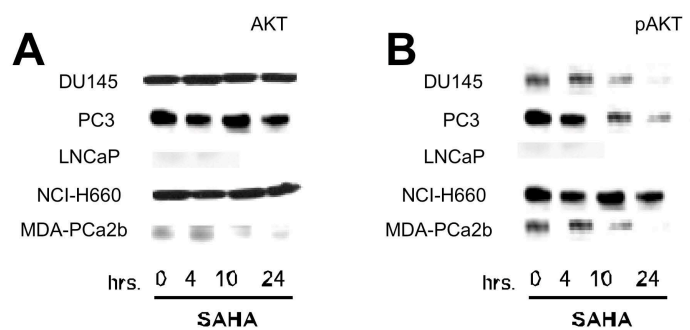


Figure 3

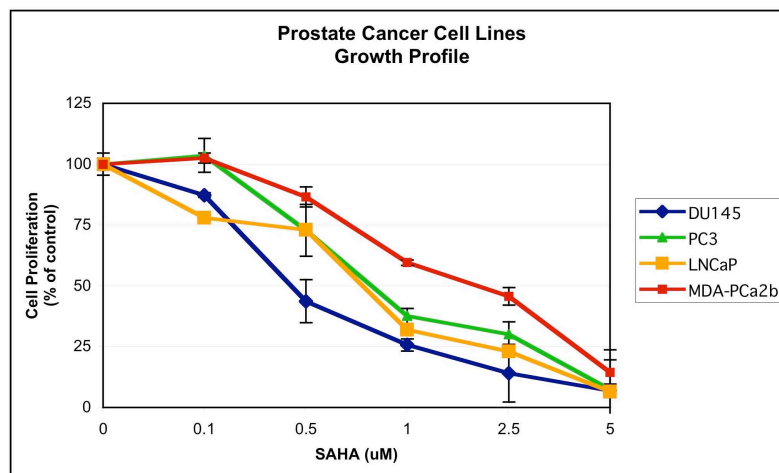


Figure 4

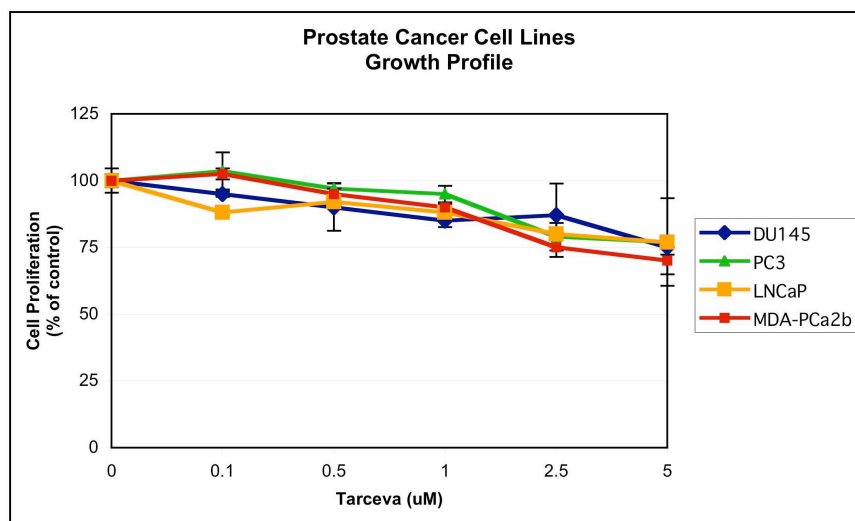


Figure 5

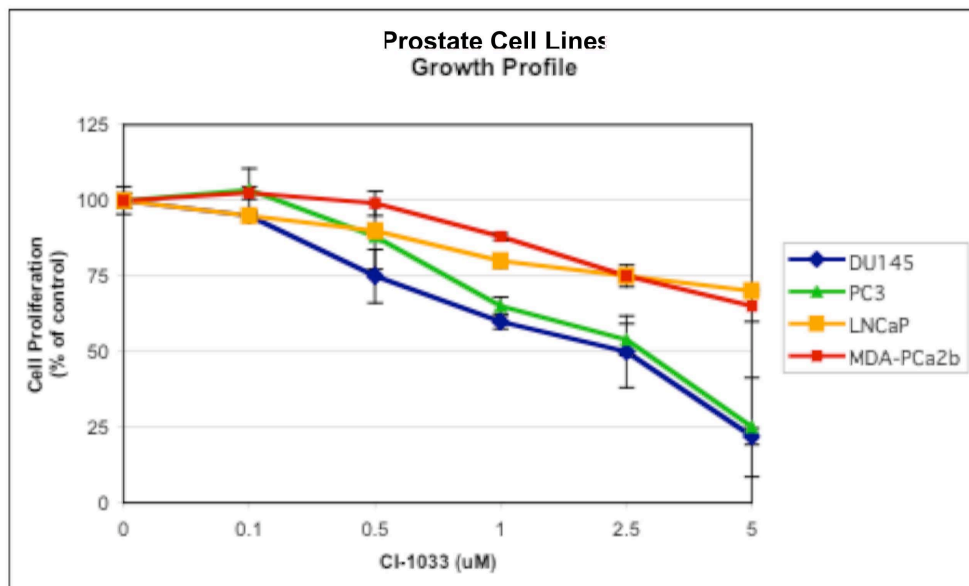


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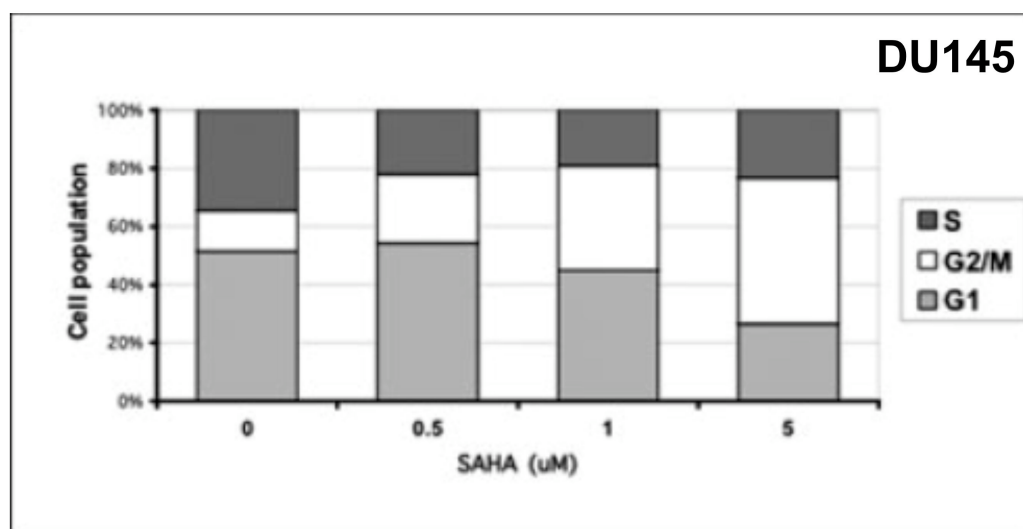


Figure 7

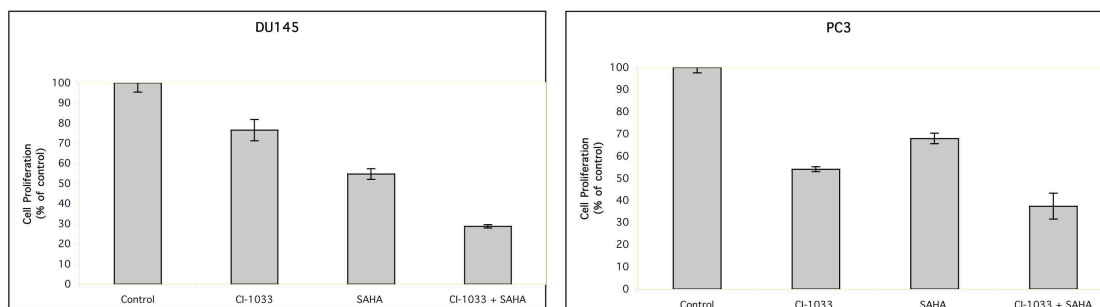


Figure 8

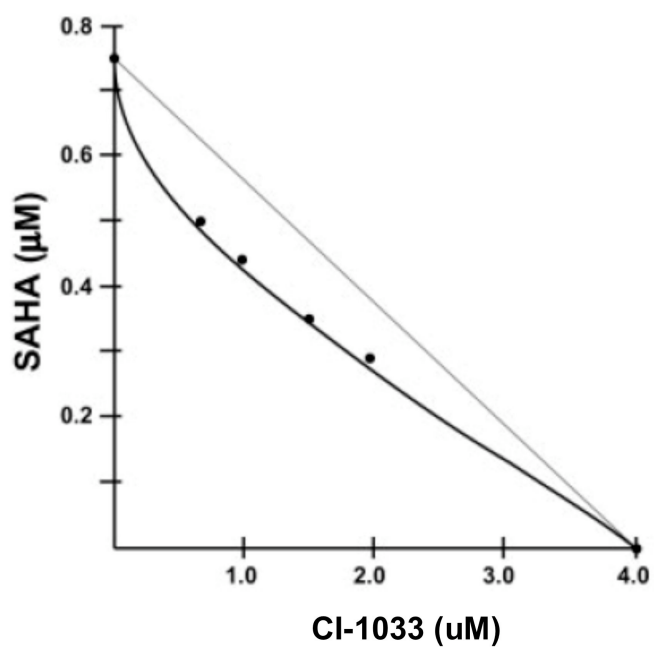
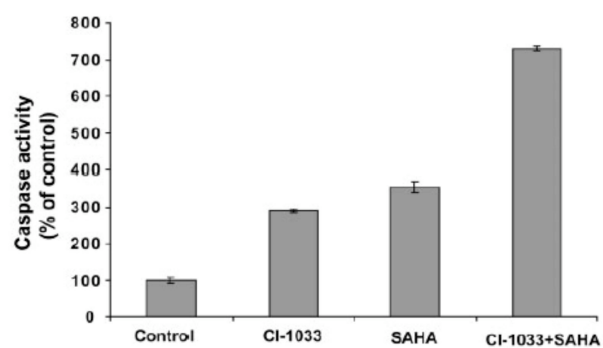


Figure 9

A



B

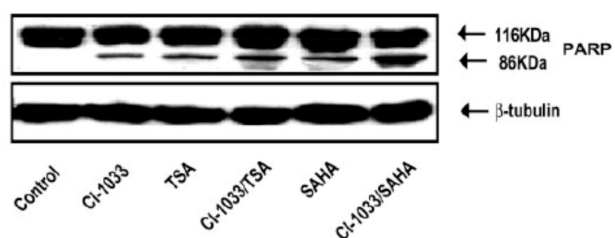
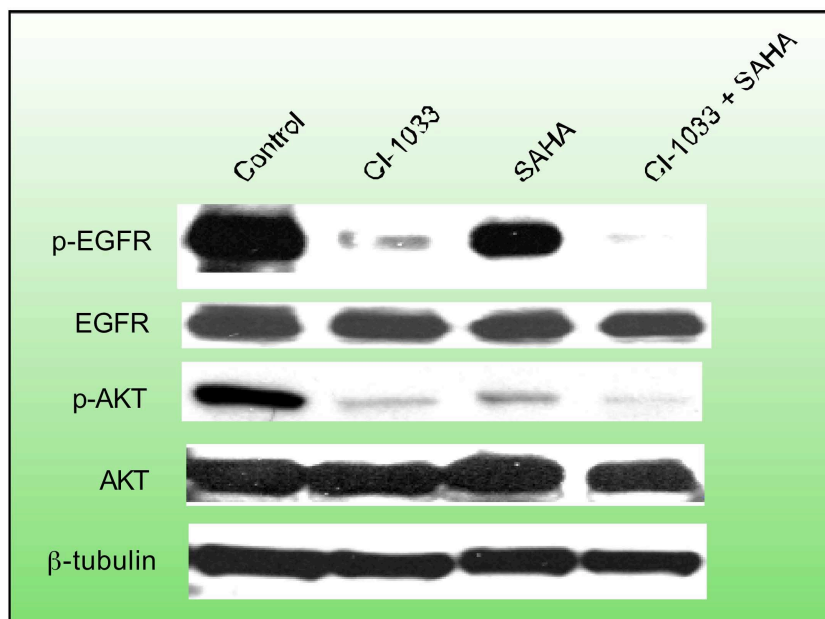


Figure 10



Key Research Accomplishments

Chinnaiyan P, Varambally S, Tomlins SA, Ray S, Huang S, Chinnaiyan AM, Harari PM. Enhancing the antitumor activity of ErbB blockade with histone deacetylase (HDAC) inhibition. *Int J Cancer*. 2006 Feb 15;118(4):1041-50.

List of Reportable Outcomes

The applicant applied laboratory knowledge and skills acquired during this grant period to initiate further inquiry involving interactions between HDAC inhibition, ErbB signaling, and radiation response. These concepts are the primary focus of a recently submitted NIH KO8 Physician Scientist Training Grant.

Conclusions

The results of the current study suggest the potential of HDAC inhibitors to enhance the antiproliferative and apoptotic effects induced by ErbB blockers in prostate cancer cell lines in vitro. Coadministration of these agents may represent a worthy strategy for more effective molecular targeting of the ErbB oncogenic pathway. Our findings demonstrate the capacity of HDAC inhibitors to down-modulate EGFR and ErbB2 expression in a variety of prostate cancer cell lines that moderately to significantly overexpress the ErbB oncoprotein. In addition, there appears to be a cooperative interaction involved in ErbB signaling, independent of protein expression. Further studies are designed to characterize these interactions in vivo and explore mechanisms of synergy.

References

Chinnaiyan P, Varambally S, Tomlins SA, Ray S, Huang S, Chinnaiyan AM, Harari PM. Enhancing the antitumor activity of ErbB blockade with histone deacetylase (HDAC) inhibition. *Int J Cancer*. 2006 Feb 15;118(4):1041-50.