Anti-tumorigenic properties of HDAC inhibition on mammary tumorigenesis
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INTRODUCTION
Histone deacetylases (HDACs) are enzymes that epigenetically modify nucleosomes repressing the transcription of certain genes. HDACs can also modify non-histone target proteins altering their function. Understanding the diverse functions of HDACs has lead to an in depth study of the relationship between HDACs and cancer. Since the increased deacetylation of histones leads to increased cell proliferation, angiogenesis and invasion, the use of HDAC inhibitors (HDACi) may be a new therapeutic strategy. HDACi promotes cell cycle arrest, apoptosis and cell differentiation, while preventing angiogenesis. Previous studies have shown a decrease in cell viability, proliferation, and motility with the use of HDACi. Recent investigations are directed towards elucidating the mechanism through which misregulation of HDACs promotes invasion. Currently, we are investigating the role of HDACi on non-histone target Hsp90 and its subsequent effect on the Her2 signaling pathway in mammary tumorigenesis.

Figure 1. The effect of HDAC3 on tumor incidence in mice. Mice carrying a PMT transgene and wild type, heterozygous, or null HDAC3 genotype were monitored for development of mammary gland tumors measured in weeks from birth. On average, wild type mice typically develop tumors at 13 weeks of age and heterogeneous mice developed heterogeneous tumors at 24 weeks. Data show a statistically significant difference between wild type and heterogeneous mice. (A) Images of the effects of Depsi on MCF10A motility. (B) The movement of MDA-MB 231 cells was significantly decreased by HDAC3 specific inhibitor 966-7 at 0.5µM, 1µM, and 2µM and pan-inhibitor Depsi at 0.1 nM, 1.0 nM, and 10.0 nM. (C) Images of the effects of 966-7 on MDA-MB 361 motility.

Figure 2: The effect of HDACi on proliferation of ZR-75-1 cells. ZR-75-1 cells were plated in 96 well plates (n=3 per inhibitor and timepoint) and treated with 10µM Belinostat, 2.5µM SAHA, and 10µM 966-7. WST-1 reagent was added and proliferation recorded at 0, 12, and 24 hours. Data show a statistically significant difference between control cells and cells treated with Belinostat in change in proliferation between 12 and 24 hours (p<0.03).

Figure 3. HDAC inhibitors decrease the motility of mammmary cell lines. Confluent monolayer of cells were infected with a wound, treated with HDACi, and photographed. (A) The movement of MCF 10A cells was significantly decreased by pan HDAC inhibitor Depsi at 0.1nM, 1.0nM, and 10.0nM. (B) The movement of MDA-MB 231 cells was significantly decreased by HDAC3 specific inhibitor 966-7 at 0.5µM, 1.0µM, and 2.0µM and pan-inhibitor Depsi at 0.1 nM, 1.0 nM, and 10.0 nM. (D) Images of the effects of 966-7 on MDA-MB 361 motility.

SUMMARY
We hypothesized that increased HER2+ signaling induces the release of TGF-β1 from CAFs. HER2+ cancer cells are able to promote paracrine TGF-β1 signaling to which it responds. While some studies have alluded to HER2 mediated increase of autocrine TGF-β signaling, lack of significant change in p-ΣMDA levels in monolucutie along with no alteration of the production of TGF-β1 in response to HDACi negates this idea. Therefore, we suggest that TGF-β signaling is mediated through paracrine signaling between CAFs and cancer cells. HER2+ promoted increase in TGF-β signaling in late stage tumors prevents apoptosis. Due to its implications with metastasis in HER2+ gastric cancers, we believe that MMP-9 is involved. Increased TGF-β signaling has been found to induce the transcription of MMP-9, which cleaves and activates latent TGF-β1. HER2 is a client protein of heat shock protein 90 (Hsp90) meaning that Hsp90 facilitates proper folding. HDAC6 has been shown to regulate the acetylation and subsequent activation of Hsp90. In the context of HER2+ breast cancers, HDAC6 prevents proper folding and aggregation of HER2 and Her2+ signaling in late stage tumors prevents apoptosis. As a result, misfolded HER2 will be ubiquitinated and degraded by the cell, decreasing the amount of HER2 present and its overall signaling. By decreasing the amount of HER2, HDAC1 could potentially decrease tumorigenic properties of cancer cells through preventing TGF-β signaling.

Future studies will include analyzing the response of TGF-β signaling in cells treated with exogenous TGF-β to more accurately represent the microenvironment breast cancer cells are in . We also plan to investigate how HDAC1 may decrease in vitro invasion even in the presence of exogenous TGF-β signaling.

Literature Cited

Figure 5: Pan-HDAC inhibition decreases HER2 levels in HER+ cancer cells. HER2+ MDA MB 361 cells were cultured in 10% FBS and treated with increasing concentrations of Belinostat for 24 hours. Extractions from each concentration were prepared and subjected to immunoblot analyses using antibodies for (A) HER2. GAPDH was used for a positive loading control. (B) Fold decrease in HER2 levels was calculated using pixel density.HER2+ MDA MB 361 cells were cultured in 10% FBS and treated with increasing concentrations of Belinostat for 24 hours. Extractions from each concentration were prepared and subjected to immunoblot analyses using antibodies for (A) HER2. GAPDH was used for a positive loading control. (B) Fold decrease in HER2 levels was calculated using pixel density.

Figure 6: Pan-HDAC inhibition does not affect autocrine TGF-β signaling in HER2+ MDA MB 361 cells. HER2+ MDA MB 361 cells were cultured in 10% FBS and treated with increasing concentrations of Belinostat for 24 hours. Extractions from each concentration were prepared and subjected to immunoblot analyses using antibodies for (A) p-SMAD2 and (B) p-SMAD2. GAPDH was used for a positive loading control. Fold changes in p-SMAD2 levels relative to SMAD2 was calculated using pixel density.

Figure 7: Various HDACi treatments do not significantly affect the production of TGF-β1 in HER2+ MDA MB 361 cells. HER2+ MDA MB 361 cells were seeded in 96 well plates were serum starved during a 24 hours incubation with 10µM SAHA, 0.25nM, and 966-7 µM. Sera were isolated and presence of TGF-β1 was recorded.

Figure 8: HDACi treatments do not significantly affect the production of TGF-β1 in HER2+ MDA MB 361 cells. HER2+ MDA MB 361 cells were seeded in 96 well plates were serum starved during a 24 hours incubation with 10µM SAHA, 0.25nM, and 966-7 µM. Sera were isolated and presence of TGF-β1 was recorded.

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SAHA, 0.25nM, and 966-7 µM.

Figure 9: HDACi treatments do not significantly affect the production of TGF-β1 in HER2+ MDA MB 361 cells. HER2+ MDA MB 361 cells were seeded in 96 well plates were serum starved during a 24 hours incubation with 10µM SAHA, 0.25nM, and 966-7 µM. Sera were isolated and presence of TGF-β1 was recorded.