MS-275 and Letrozole Treatments Inhibit Tumor Growth and Reduce Phosphomonoesters in Triple Negative MDA-MB-231 Tumors

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Introduction: Histone deacetylase (HDAC) inhibitors have been found to reverse the epigenetic profile of some genes, including estrogen receptor (ER), epidermal growth factor receptor (EGFR), and retinoic acid receptor (RAR) -β2 (1). Here we have studied the metabolic effects of the HDAC inhibitor MS-275, the aromatase inhibitor letrozole, and their combination *in vivo* in the ER/PR/Her-2 negative (triple negative) MDA-MB-231 human breast cancer xenograft in SCID mice. The highest tumor growth inhibition was achieved with the combination treatment supporting the possibility that reactivation of the transcription of the ER by MS-275 rendered MDA-MB-231 human cell xenografts sensitive to aromatase inhibition *in vivo*. We observed a significant decrease of phosphocholine (PC) and total choline (tCho) with MS-275 as well with combination treatment. However, a significant decrease of phosphoethanolamine (PE) was observed for the combination treatment

Materials and Methods: Twenty mice were injected with $5x10^6$ MDA-MB-231 cells subcutaneously in the mammary fat pad of female SCID mice. Tumors were allowed to grow to approximately 5 mm before treatment was initiated. Mice were randomized and divided in 4 groups consisting of a control group which received saline alone, a second group which received MS-275 alone, a third group which received letrozole alone, and a fourth group which received both MS-275 and letrozole. MS-275 was dissolved in 30% hydroxypropyl-β-cyclodextrin to achieve the target concentration of 0.75 mg/ml. Each mouse received 100 µl (2.5 mg/kg/day) of 0.75 mg/ml of MS-275 daily for 2 week via oral administration using a feeding tube. Letrozole was dissolved in 0.3% hydroxypropyl cellulose in saline. For each treatment mice were injected daily *via* intraperitoneal (i.p.) injection with 100 µl (400 µg/kg) of 100 µg/ml letrozole. Tumor volume was measured twice a week for the duration of the study. Relative tumor growth was determined and compared between the groups. Once tumors reached 10 mm in size in approximately two weeks, mice were sacrificed and the tumors excised and snap-frozen in liquid nitrogen. Tumor extracts were made using dual phase chloroform-methanol method as described (2). Metabolites were estimated and compared between the groups. The Mann-Whitney U test was performed to test for statistically significant differences ($p < 0.05$) between the groups

Results and Discussion: Combination treatment with MS-275 and letrozole resulted in a significant reduction ($p<0.05$) of tumor growth (Figure 1). While letrozole or MS-275 alone also resulted in a reduction of tumor growth, this was to a lesser extent than the combination treatment.

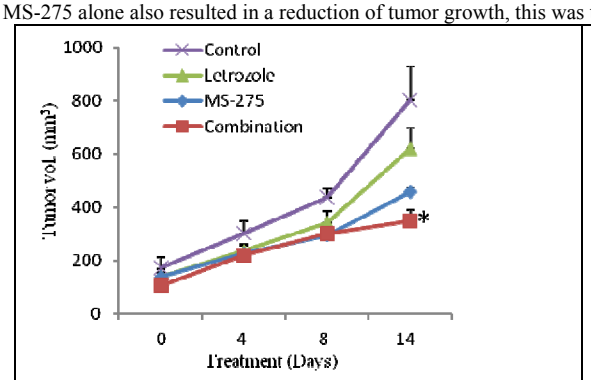

Figure 1. Tumor growth (in mm^3) of MDA-MB-231 tumors in the mammary fat of female SCID mice, following the different treatment protocols ($p < 0.05$).

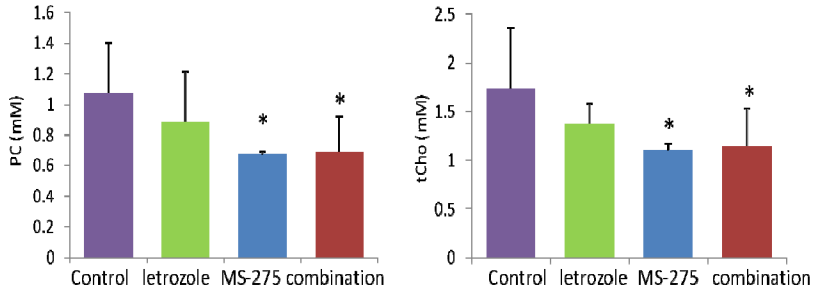

Figure 2. PC and tCho levels in MDA-MB-231 tumor extracts measured with ^{1}H spectroscopy for different treatment groups of MDA-MB-231 tumor bearing mice ($p < 0.05$).

Proton MR spectra showed significant differences in choline containing metabolites. Both phosphocholine (PC) and total choline (tCho) were significantly lower following MS-275 or combination treatment compared to control tumors ($p < 0.05$). Tumor metabolite levels in the different groups are summarized in Figure 2. Significant difference in PC and phosphoethanolamine (PE) were also detected with ^{31}P MR spectroscopy of tumor extracts. Representative ^{31}P spectra from water soluble extracts of MDA-MB-231 tumors in treated and untreated group are shown in Figure 3. Changes in PC showed similar trends to those observed with ^{1}H MR spectroscopy. Additionally, a significant decrease of PE ($p<0.03$) was observed, but only for the combination treatment. These data are summarized in Figure 4. Previous studies with a different HDAC inhibitor have reported an increase of PC following treatment (3). It will be important to determine the role of agent-specific effects *versus* mechanism-specific effects in the changes in metabolism observed following treatment with HDAC inhibitors.

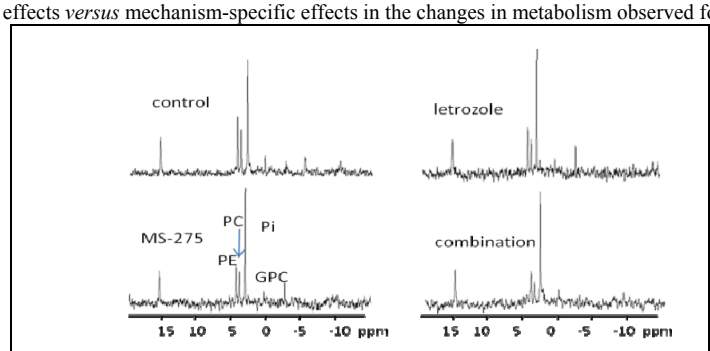

Figure 3. Representative ^{31}P spectra showing differences in metabolite levels in tumors treated with MS-275, letrozole, combined MS-275 and letrozole, and saline.

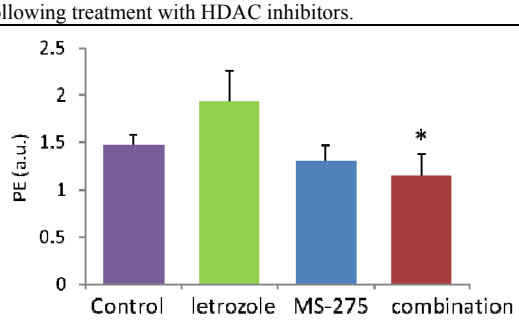

Figure 4. PE levels in arbitrary units for the treatment groups (* $p < 0.03$ compared to control).

Triple negative breast cancers are the most lethal cancers since the absence of receptors makes them resistant to hormonal treatment or to treatment with a Her-2/neu antibody. The ability to re-express these receptors following treatment with HDAC inhibitors opens new possibilities for treatment and MR spectroscopy can play an important role in monitoring response to these treatments.

References: (1) Hess-Stumpp H *et al* IJBCB 2007; 39: 1388-1405. (2) Glunde K *et al.* Cancer Res 2005;65:11034-11043. (3) Sankaranarayanpillai M *et al* Mol Cancer Ther 2006; 5:1325-34. **Acknowledgements:** This work was supported by P50 CA103175 and a USAMRMC W81XWH-04-1-0595 Center of Excellence (COE) grant.