Investigation of Small Molecule Inhibitors of PHGDH and Endocrine Therapies in Endocrine Resistant ER+ Breast Cancer

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Abstract

Breast Cancer is currently the most commonly diagnosed form of cancer among women in the United States (NCI). In these cases, about two-thirds express the Estrogen Receptor (ER) and are classified as Estrogen Receptor positive (ER+) breast cancers. Binding of Estrogen to these receptors causes the activation of mitotic and growth genes, and ultimately leads to cell proliferation. Hormone therapy is the first line treatment for this form of breast cancer. Using selective estrogen receptor modulators (SERMS) such as Tamoxifen or pure antiestrogens such as ICI, breast cancer growth can be inhibited. However, many ER+ breast cancers possess either *de novo* or acquired resistance to these drugs, leading to lower efficacy of Tamoxifen and ICI. One mechanism that has been implicated in resistance to endocrine therapy is changes in cellular metabolism, including the serine synthetic pathway (SSP) (Martens et al 2005)**,** De Marchi et al 2017)**.** Serine contributes to massive changes in the genome of the cancer cell via its role in one-carbon metabolism and producing the DNA methylation donor S-adenosylmethionine. Based on these previous findings, and data generated in the laboratory, we wanted to determine if inhibition of the serine synthetic pathway would alter the sensitivity of endocrine resistant cells (LCC9) to various endocrine therapies. To inhibit this pathway, small molecule inhibitors, NCT-503 and CBR 5884, of phosphoglycerate dehydrogenase (PHGDH), were used in combination with common ER+ breast cancer therapies, Tamoxifen and ICI. After determining the dose-response of both CBR and NCT, the effective range of both small molecule inhibitors were tested in combination with the other endocrine therapies. The data demonstrates that these concentrations of the PHGDH inhibitors, NCT-503 and CBR 5884, were insufficient to produce an anti-proliferative effect. In addition, results are inconclusive regarding the effect of combinatorial effect of endocrine therapies and inhibitors of PHGDH. This work intended to highlight the role of the serine synthetic pathway in resistance to endocrine therapy and requires further investigation into combination therapies of SSP inhibitors and endocrine therapies.

Methods

Cell Lines

- LCC9 cells were cultured in IMEM medium supplemented with 5%FBS and 0.1% gentamicin. Cells were incubated at 37°C and 5% CO_2.

Plating

- LCC9 cells were seeded at a concentration of 2,500 cell per well for each experiment.
- Cell counts were performed via trypan-blue exclusion to ensure accurate cell seeding

Chemicals

- (Z)-4-Hydroxytamoxifen was obtained from Sigma (H7904) and was dissolved in 100% pure Ethyl Alcohol (Sigma – E7023).
- ICI (1047) and CBR 5884 (5836) were obtained from TocrisBioscience and were dissolved in dimethyl-sulfoxine (Fisher BioReagents – BP231).
- NCT-503 was obtained from Selleckchem.com (S8619) and was dissolved in dimethyl-sulfoxine (Fisher BioReagents – BP231).
- Concentrated stock solutions were prepared, aliquoted and stored at -20°C (CBR, ICI, 4-OHT) or -80°C. Diluted working stocks were prepared fresh for each treatment.

Treatment

- Cells were treated with either the PHGDH inhibitors alone, 4OHT alone, ICI alone, or in combination of the PHGDH inhibitors with the endocrine therapies at the indicated concentrations. All treatments were applied at various doses for a period of four days.

Analysis

- Cell proliferation was analyzed via the FluoReporter Blue Fluorometiric dsDNA Quantification Kit (F-2962) In brief, all the liquid from the plates were removed and the cells were lysed by a series of freeze-thaw cycles. Hoechest stain was added and fluorometric analysis was performed. Fluorescence was measured and responses are reported as percent decrease from vehicle control.

Acknowledgments

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Results

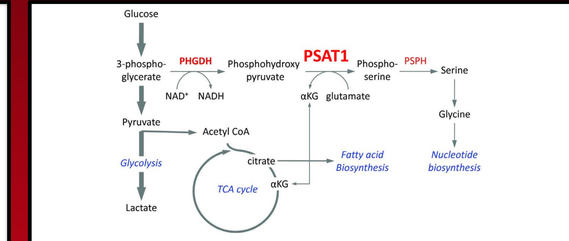

Figure 1: Schematic of Serine Synthesis Pathway. Serine synthesis is shunted off from the glycolytic pathway at 3-phosphoglycerate and involves three enzymatic reactions catalyzed by phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), and phosphoserine phosphatase. The rate-limiting step is catalyzed by PHGDH. Adapted from Luo, 2011, Breast Cancer Research.

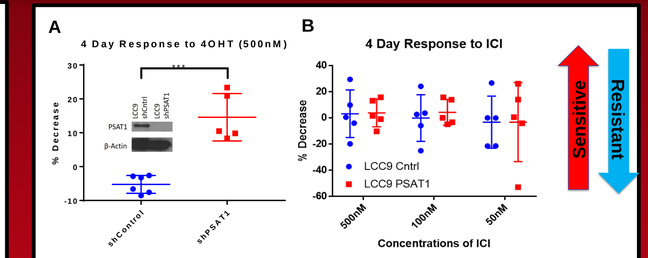

Figure 2: Genetic suppression of PSAT1 altered sensitivity to 4OHT treatment but not ICI. A) Response to 500nM Tamoxifen in resistant LCC9 cells following PSAT1 knockdown, measured by percent decrease in cell viability. P<0.05. B) Response to ICI treatment following PSAT1 knockdown, measure by percent decrease in cell viability.

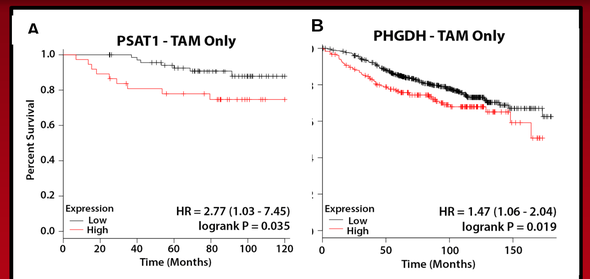

Figure 3: Clinical relevance of SSP enzymes in ER+ patients treated with tamoxifen. A.) Progression-free survival of ER+ breast cancer patients treated with Tamoxifen stratified by PSAT1 expression. B.) Progression-free survival of ER+ breast cancer patients treated with Tamoxifen stratified by PHGDH expression.

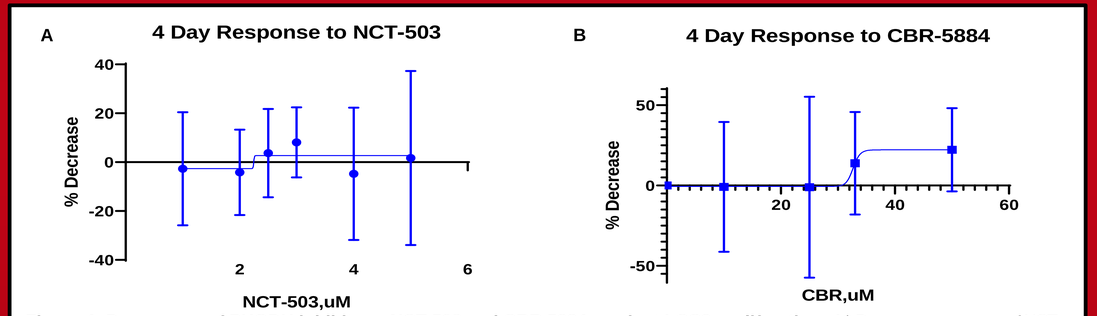

Figure 4: Dose curve of PHGDH inhibitors, NCT 503 and CBR 5884, against LCC9 proliferation. A.) Dose response curve of NCT 503 as demonstrated by percent decrease in cell viability; concentrations were 1uM, 2uM, 2.5uM, 3uM, 4uM, and 5uM. B.) Dose response curve of CBR 5884 as demonstrated by percent decrease in cell viability; concentrations are 10uM, 25uM, 33uM, and 50uM.

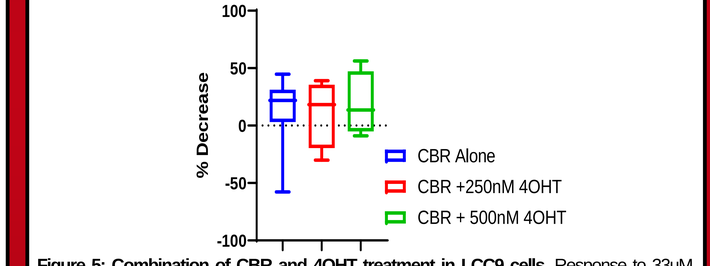

Figure 5: Combination of CBR and 4OHT treatment in LCC9 cells. Response to 33uM CBR 5884 alone or in combination with 250nM or 500nM 4OHT. 500nM, measured by percent decrease in cell viability.

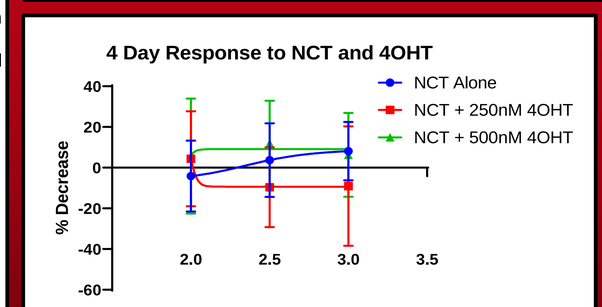

Figure 6: Combination of NCT and 4OHT treatment in LCC9 cells. Response to NCT-503 alone or in combination with 250nM or 500nM 4OHT, measured by percent decrease in cell viability. Cells were treated with NCT-503 at concentrations of 2uM, 2.5uM, or 3uM.

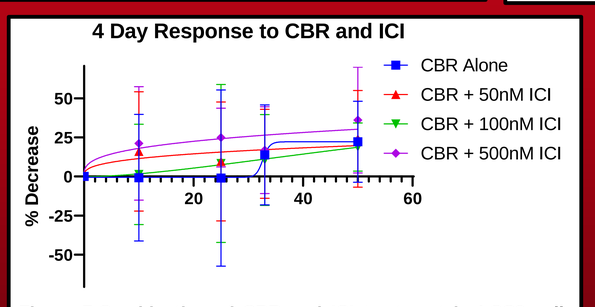

Figure 7:Combination of CBR and ICI treatment in LCC9 cells. Response to CBR alone or in combination with 50nM, 100nM, or 500nM ICI, measured by percent decrease in cell viability. Cells were treated with CBR 5884 at concentrations of 10uM, 25uM, 33uM, or 50uM.

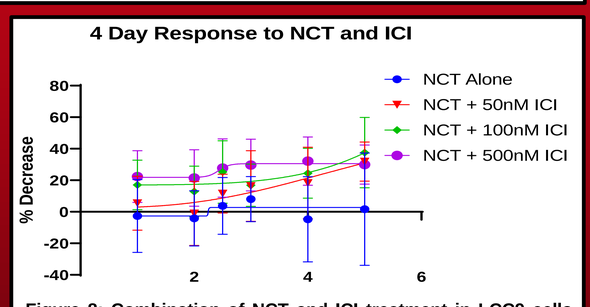

Figure 8: Combination of NCT and ICI treatment in LCC9 cells. Response to NCT alone or combination with 50nM, 100nM, or 500nM ICI, measured by percent decrease in cell viability. Cells were treated with NCT-503 at concentrations of 1uM, 2uM, 2.5uM, 3uM, 4uM, or 5uM.

Conclusions

- Prior to this project, it was determined that there was a coorelation between SSP enzymes and resistance to Tamoxifen.
- Concentrations of PHGDH inhibitors, NCT-503 and CBR 5884, were insufficient to produce an anti-proliferative effect.
- Effect of combinations of PHGDH inhibitors and endocrine therapies are inconclusive due to technical variation.

Future Directions

- Future Experiments
  - Further investigation into combination treatments of endocrine therapy and PHGDH inhibitors, using higher concentrations of the inhibitors.
  - Extend analysis to other endocrine resistance cell types.
  - Investigation into the metabolic consequences of combination treatment, especially on the Serine Synthesis Pathway.