Efficacy of Three Novel Compounds on Cell Viability

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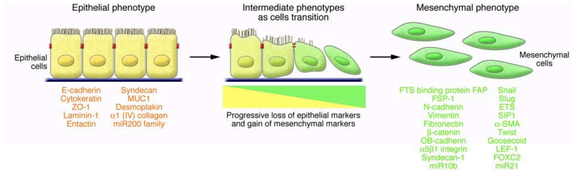
Abstract

Triple negative breast cancer is a form of breast cancer which accounts for 10-20% of breast cancer diagnoses in which the receptors for progesterone, estrogen, and the hormone epidermal growth factor receptor 2 (HER-2/neu) gene, are not present. Since they are not present, drugs that target these receptors, like Tamoxifen, Megace, and Herceptin, are ineffective. Chemotherapy may be an effective treatment, especially in early stages, but its side effects are often painful and demoralizing. These studies focus on analyzing the efficacy of three novel compounds, CES-X-29D, CES-I-61, and ACB-111-163, on the proliferation and viability on various triple-negative metastatic breast cancer cell lines. Two lines, MDA-MB-231 and MDA-MB-468, are human in origin, while the other line, MMTV-PyVMT, are murine. Through experimentation, we have found that the compounds ACB-111-163 and CES-I-61 have a significant negative effect on the viability of cells, while the compound CES-X-29D seems to have little or no impact on cell viability. In the future, we aim to determine the effect of these compounds on the epithelial-mesenchymal transition (EMT), which is an early indicator of invasion and metastasis.

Introduction

Breast cancer is the most common cancer among women worldwide, accounts for 10-20% of all cancer diagnoses, and is a frequent cause of cancer deaths in women. Breast cancer is notoriously difficult to treat once traditional cancer therapy treatments have been attempted, especially if patients develop metastasis, or secondary tumors at a site separate from the original malignancy. In metastasis, cancer cells often undergo a process called epithelial-mesenchymal transition (EMT), in which epithelial or surface level cells stop expressing cell-cell adhesion molecules, such as E-cadherin, and gains mesenchymal cell traits. Mesenchymal cells have increased mobility, invasiveness, and resistance to apoptosis, and thus, cancerous mesenchymal cells become increasingly more difficult to treat. EMT can be determined by monitoring several biomarkers, such as the aforementioned E-cadherin. E-cadherin is an intercellular adhesion molecule that combines with other molecules, called catenins, to form an E-cadherin/beta-catenin/alpha-catenin complex that links further to the cytoskeleton. E-cadherin also helps moderate cell polarity and support epithelial tissue function and structure. N-cadherin is a cell adhesion molecule that discourages cell-cell adhesion and encourages invasion. Because of E-cadherin's important role in EMT, its is a useful biomarker for determining whether the process has taken place. This study aims to assess the effect of the novel compounds CES-X-29D, ACB-111-163, and CES-I-61, synthesized by Chad Stephens from Augusta University, on the epithelial-mesenchymal transition in human (MDA-MB-231 and MDA-MB-468) and mouse (MMTV-PyVMT) epithelial mammary cancer cells.

Figure 1



Kalluri, Raghu, and Robert A Weinberg. "The basics of epithelial-mesenchymal transition." *The Journal of clinical investigation* vol. 119,6 (2009): 1420-8. doi:10.1172/JCI39104

Methods and Materials

Thus far, I have conducted cell viability assays with all three compounds with each cell line. With the MDAM-B231 and MMTV-PyVmT lines, there were cytotoxicity assays at three timepoints: 24 hours, 48 hours, and 72 hours. For the MDAM-468 line, there was a cytotoxicity assay at 72 hours. For each assay, cells were plated at a 2.2 x 10^5 cells per mL concentration. The next day, the cell line was treated with 0 µM, 0.78125 µM, 1.5625 µM, 3.125 µM, 6.25 µM, 12.5 µM, 25 µM, 50 µM, and 100 µM concentrations of each compound. The plates were incubated at 37˚C for the given timepoint, then the CellTiter 96 Non-Radioactive Cell Proliferation Assay Protocol was carried out, using the MTT dye. Additionally, an immunofluorescence assay was carried out with the MDAM-B231 cell line at 48 hours, in which protocol was essentially the same as for the cytotoxicity assays, except immunofluorescence reagents were added instead of the CellTiter96 Dye Reagent. Most recently, protein was extracted from MDA-MB-231 cells treated with 0 µM, 3.125 µM, 6.25 µM, and 12.5 µM concentrations of ACB-111-163, and a Western Blot analysis will be conducted for N-cadherin, E-cadherin, and actin.

Figure 2

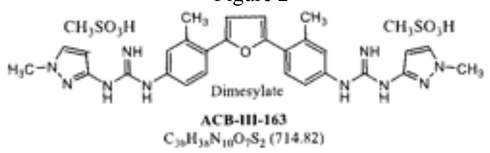


Figure 3

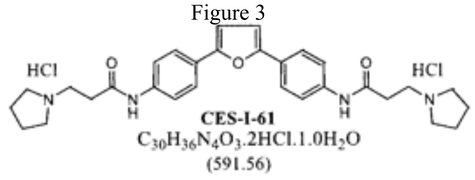
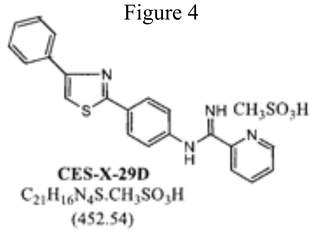


Figure 4



Figures 2-4: Compounds used in this project.

Results

These are the results from the cell viability assays we have conducted thus far. From the lack of impact CES-X-29D has on cell viability we can conclude that CES-X-29D does not have a significant impact on cell growth. In the future, we will concentrate on the other two compounds, ACB-111-163 and CES-I-61, and will be conducting more protein assays based on them in the future.

Figure 5

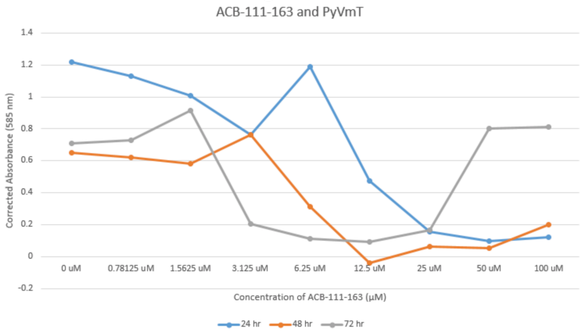


Figure 6

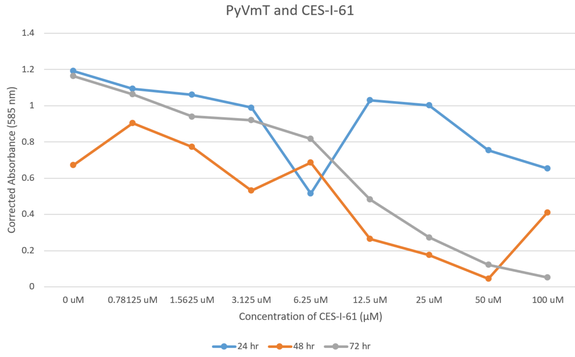
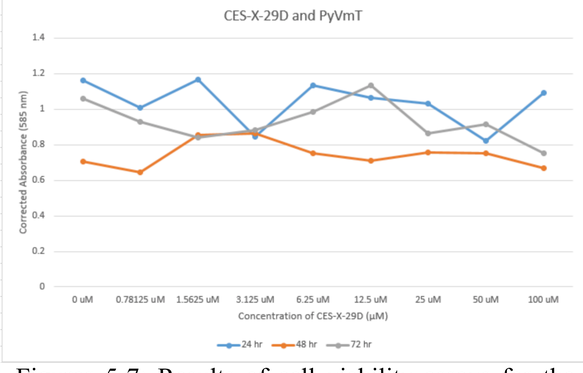


Figure 7



Figures 5-7: Results of cell viability assays for the PyVMT line.

Figure 8

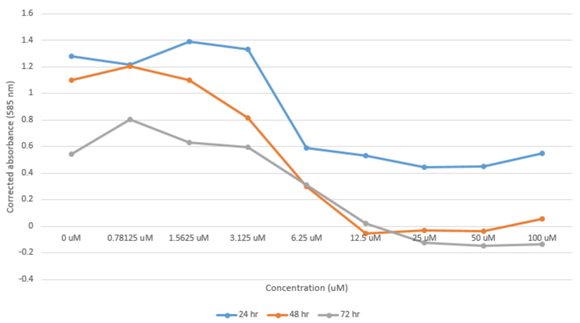


Figure 9

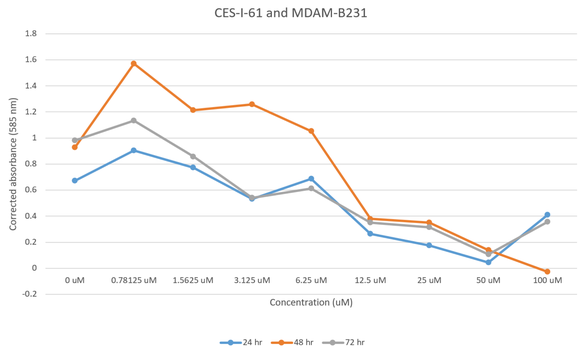
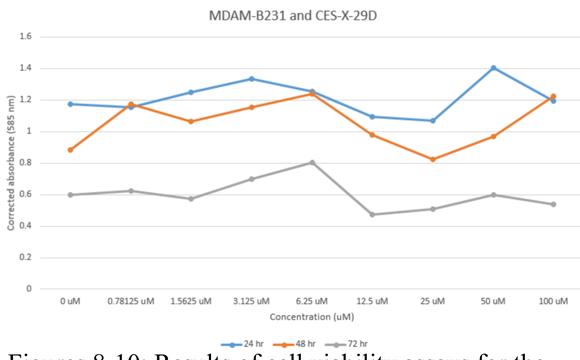


Figure 10



Figures 8-10: Results of cell viability assays for the MDA-MB-231 line.

Acknowledgements

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