Abstract:
Multi-drug resistance (MDR) occurs when cancer cells become resistant to a diverse array of chemotherapeutics and xenobiotics. Among the many mechanisms of MDR, one of the most prominent is the overexpression of ATP-binding cassette (ABC) transporters. ABC transporters harness the energy from ATP hydrolysis to transport xenobiotics, drugs, and other toxic compounds out of the cell. When ABC transporters are overexpressed in cancer cells, their efflux activity can lower the intracellular concentrations of chemotherapeutics. However, recent studies have explored the potential for overcoming breast cancer cell line. Here we compare the effectiveness of the inhibitors at re-sensitizing the cells to mitoxantrone relative to the parental cell line which does not overexpress ABC. The experimental compounds were also assessed with regard to toxicity to non-cancerous HFL1 cells.

Materials and Methods

Cell Culture: The MCF7 and MCF7-M100 breast cancer cell lines were grown in complete RPMI-1640 with L-glutamine media supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. To maintain a multidrug (MDR) profile, the MCF7-M100 cell lines were grown in the presence of 100 nM mitoxantrone (MNT). The non-cancerous HFL1 cells were grown in F12K media supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. HFL1 cells were grown in collagen-coated flasks (0.01 mg/ml, collagen I in PBS for 10 minutes, rinsed with PBS). All cells were stored in a humidified incubator at 37°C and 5% CO2.

MTT Cell Viability: The MTT assay measures the reduction of yellow solution 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to blue, insoluble formazan crystals by cellular redox processes in living cells. Cells were seeded at 5000 cells per well in a 96 well plate and incubated for 24 hours. After 24 hours, MCF7 and MCF7-M100 cells were then treated with experimental compound (10μM), mitoxantrone (MNT, 200nM), and the known BCRP inhibitor Ko143 (10μM). After 72 hours, media was replaced and MTT solution was added (20 μl/well of 5 mg/ml MTT in PBS). After incubation for 4 hours, the supernatant was removed, and the formazan crystals were dissolved in 100 μl DMSO. Plates were shaken for 10 minutes at 500 rpm. The absorbance was measured at 570 nm using a Bio-Tek ELISA plate reader (Bio-Tek, Winooski, VT). The measured absorbance value per well correlates with the number of metabolically active cells in that well. The percent of cell viability was calculated using the following equation:

\[ \text{Viability} = \frac{\text{Absorbance of test well} - \text{Absorbance of blank well}}{\text{Absorbance of control well} - \text{Absorbance of blank well}} \times 100 \]

Table 1: Summary of Experimental Compounds against MCF7. Increased MNT toxicity is reported as the difference between the mean viability of MCF7 in the presence of 10 μM compound alone and the mean viability of the presence of 10 μM compound and 200 nM MNT. Top compounds: 96, 70, 117, 83, 50, 124 (ranked by sensitivity, best to worst).

Table 4: Table of In silico Predicted BCRP Inhibitors against MCF7 Parental Cells, MCF7-M100 MDR Cells, and HFL1 Cells. Compounds 96 and 124 (green) are known MNT inhibitors and are non-toxic to HFL1 cells.

Figure 2: Characterization of Mitoxantrone Resistance in the Parental MCF7 and MDR MCF7-M100 Cell Lines. In both (A) and (B), cells were treated with varying concentrations of the chemotherapeutic mitoxantrone (MNT) only (black), or in combination with the BCRP inhibitor Ko143 at 1 μM (orange). Data represent mean ± SD, n = 8, two trials. In (A), the parental MCF7 cells show little change in viability in the presence of Ko143. In (B), the MCF7-M100 cell lines are re-sensitized to MNT in the presence of the potent BCRP inhibitor Ko143.

Figure 3: Screening of Potential BCRP Inhibitors against the MCF7 Cell Line. The parental mitoxantrone (MNT) sensitive MCF7 cells were incubated for 72 hours with 10 μM compounds alone (black) or with 10 μM compounds and 200 nM MNT (grey bars). Data represent the mean ± SD, n=8.

Figure 4: Screening of Potential BCRP Inhibitors against the MCF7-M100 Cell Line. The multidrug resistant MCF7-M100 cell lines were incubated for 72 hours with 10 μM compounds alone (black) or with 10 μM compounds and 200 nM MNT (grey bars). Data represent the mean ± SD, n=8.

Figure 5 and Table 3: Testing Experimental Compounds for Intrinsc Toxicity using the HFL1 Cell Line. HFL1 cells were incubated for 72 hours in the presence of 10 μM compounds alone. Data represent mean ± SD, n=8, two trials.

Conclusions and Future Directions

All of the tested compounds from in silico screens against BCRP resulted in increased MNT toxicity in the MDR breast cancer cell line MCF7-M100. Four of the compounds (Compounds 53, 70, 96, 124) are also non-toxic to the non-cancerous HFL1 cell line. Compounds will continue to be tested in MDR cell lines that overexpress BCRP, alongside the parental cell line in the MDR lines. Variants of the top performing and non-toxic compounds will be designed in silico using Chembl to optimize efficacy as potential BCRP inhibitors.

References