## Multi-drug resistant breast cancer cells exhibit high choline kinase and stem-like markers

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### Introduction:
The availability of molecular markers such as CD44+/CD24- of breast cancer stem-like cells (1) have provided the opportunity to investigate the metabolism of cells with these markers with magnetic resonance spectroscopy (MRS). These stem-like cancer cells are thought to lead to recurrence and metastasis, and therefore it is critically important to characterize the function of these cells to uncover novel therapeutic targets. Stem-like breast cancer cells are also characterized by drug resistance (2) and in this study we have investigated breast cancer stem-like cell markers and choline metabolism in MCF-7 cells resistant to Adriamycin (MCF-7/Adr) that display a MDR phenotype. The MDR phenotype was validated by determining cell viability/proliferation following treatment with 5-Fluorouracil (5-FU) using an MTT assay, and by determining rhodamine efflux from the cells.

### Methods:
MCF-7 wild-type and MCF-7/Adr cells (originally obtained by Dr. M. Gamcsik from Dr. B. Teicher) were cultured in EMEM with 10% FBS. For the MCF-7/Adr cells, the medium was supplemented with Adriamycin. Rhodamine was used at a final concentration of 0.5 μg/ml for 1×10⁶ cells. Cells were incubated for 45 minutes at 37°C then washed in cold buffer before performing the analysis. The MTT assay was performed by using an ATCC kit. 5x10⁵ cells were plated in 96 well plates. The cells were treated with 2.5 μg/ml 5-FU for 24h. Cells were grown for 48h before processing the assay. For the analysis of the expression of CD24 and CD44, we used anti-CD44 and anti-CD24 antibodies conjugated with fluorescein isothiocyanate (FITC) and phycoerythrin (PE) respectively (Pharmingen). Cells were incubated for 40 minutes at 4°C then washed in cold buffer before analysis. Flow cytometry was performed on a FacsCalibur (Becton Dickinson) and data were analyzed using Cellquest software (Becton Dickinson). High-resolution 1H MRS was performed on a vertical 11.7 T Bruker spectrometer. Spectra were obtained from comparable cell numbers (~ 2 x 10⁶ cells). For the immunoblot analysis, we used a custom-made Chk antibody (3).

### Results:
Rhodamine 123 (Rho123) is a fluorescent substrate for P-gp that can be used as a molecular probe to study the MDR phenotype. We confirmed that the MCF-7/Adr cells exclude more Rho123 that the wild-type as shown in Figure 1. Rho123 fluorescence intensities were quantified for both cell lines and the values were 273.99 ± 92.85 for MCF-7 wild-type and 109.98 ±22.08 for MCF-7/Adr. MTT assays performed on these cells showed that MCF-7/Adr cells displayed a higher resistance to treatment with 5-Fluorouracil (5-FU) (Figure 2). The expression of CD44 and CD24 was significantly different between the cell lines. As demonstrated in Figure 2, MCF-7 wild-type cells do not express CD44 whereas MCF-7/Adr showed significantly higher expression of CD44. The pattern of expression of CD24 was also different in both cell lines. The fraction of breast cancer stem-like cells characterized by CD44+/CD24- was only 8.22 % for MCF-7/Adr cells (Figure 3).

Expression of choline kinase was significantly lower in MCF-7 wild-type cells compared to MCF-7/Adr cells (Figure 4). In Figure 5, proton spectra with signal from choline region are displayed for both cell lines. We quantified glycerophosphocholine (GPC) and phosphocholine (PC) concentrations. The values were 1.77 ± 0.5 mM (GPC) and 2 ± 0.25 mM (PC) for the MCF-7 wild-type cells and 0.85 ± 0.5 mM (GPC) and 4.19 ± 0.1 mM (PC) for MCF-7/Adr cells. The Adr resistant cells showed a higher concentration of PC.

### Conclusions:
Consistent with the MDR phenotype MCF-7/Adr cells showed an increased exclusion of Rho123 as well as a higher resistance to 5-FU. Here we have shown that this drug resistant phenotype was associated with a high level of Chk expression and increased PC as well as a higher fraction of cells with breast cancer stem-cell like markers. These data are also consistent with our previous studies showing that small interfering RNA (siRNA) mediated downregulation of Chk increased the effect of 5-FU in breast cancer cells (4) suggesting Chk as an important target in these cells. While there is some controversy surrounding the parental origins of the MCF-7/Adr cell line (5), we are currently performing studies with additional drug resistant and stem-like cell lines to support our observations.

### References: