Metabolic characterization of an Imatinib-resistant chronic myelogenous leukemia cell model

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INTRODUCTION: Philadelphia-positive chronic myelogenous leukemia (CML) is identified by the fusion of BCR with the Abl tyrosine kinase resulting in constitutive activity and uncontrolled myeloid cell proliferation [1]. Imatinib (Gleevec or STI-571) is a small molecule inhibitor of BCR-Abl and a therapeutic agent for the treatment of CML. Resistance to Imatinib has been observed both in patients and experimental cell models [2] although the mechanisms underlyng resistance are not fully understood. Recently, an Imatinib-resistant CML cell model, MyLR was generated from normal CML cells, MyL. These Imatinib-resistant cells are independent of BCR-Abl overexpression or mutations [3] and display a multi-drug resistant phenotype [4,5]. NMR spectroscopy is a unique method that identifies and quantitates multiple metabolites in crude cell extracts and can be used for non-invasive metabolite assessment in whole, live cell preparations. Here we examined the global metabolic differences between MyL and MyLR cells. Several metabolites were decreased in the MyLR cells, however the most dramatic change was a ~7-fold increase in the total creatine (Cr). Real time in vivo 31P NMR experiments were conducted to determine the significance and role the elevated Cr plays in drug resistance.

METHODS: MyL and MyLR cells were cultured in T-175 flasks and grown to high density. 10^6 cells were collected, added to fresh culture media and incubated 2 hours at 37°C. Cells were then collected and metabolites extracted with ice-cold methanol. Lyophilized extracts were dissolved in D2O containing 1.5 mM TSP as a concentration and chemical shift reference. 1H NMR spectra were obtained on a narrow-bore 1H ST Varian INOVA (125 MHz, 13C, Varian Instruments) equipped with a 5 mm inverse detect probe. For the in vivo studies a custom designed 10 mm NMR compatible bioreactor system, which allows for continuous media flow and temperature regulation was used [6]. Approximately 2.5 x 10^6 MyLR cells were electrostatically encapsulated into ~500 μm alginate beads and loaded into the bioreactor system. The flow rate during the experiment was 4 mL/min and the O2 concentration was maintained in the media using a Gas Exchange Module (GEM), which was filled with 95% Air/ 5% CO2. Bioreactor 31P NMR spectra were acquired on a 14.1T Varian INOVA. Data from both 1H and 31P experiments were processed using ACD/Labs 1D and 2D NMR processing software, version 7.0 (Advanced Chemistry Development, Inc. Toronto). In some cases spectra were binned and analyzed by Principal Component Analysis and Mutual Information Analysis to determine what metabolites were significantly different between cell types. Additionally, individual metabolite concentrations were determined for 1H spectra using Chenomx software (Chenomx, Inc. Alberta).

RESULTS: Figure 1 shows representative 1H NMR spectra from MyL and MyLR cell extracts highlighting the significant increase in Cr. 2D 1H-13C HSQC of the MyLR extract demonstrated that the resonances in the 1D 1H spectrum are representative of Cr (Fig. 1 - Insert). Several other metabolites were found to be decreased in MyLR cells compared to MyL cells, including, choline, phosphocholine, myo-inositol, taurine, and acetate. These data suggest that maintenance of the Cr pool is highly coupled to mitochondrial ATP production in MyLR cells.

DISCUSSION AND CONCLUSIONS: We examined the metabolic profile of MyL and MyLR cells and found that drug resistant cells display an altered metabolic phenotype compared to their non-resistant counterparts. These data suggest that drug-resistant MyLR cells have decreased glycolytic flux and display a near 7-fold increase in total Cr. While others have observed an increase in PCr in adriamycin-resistant breast cancer, this is the first such example in drug-resistant leukemia cells. We propose that enhanced Cr synthesis provides an additional energy reserve in the form of PCr thereby giving a selective advantage to MyLR cells and possibly facilitating drug resistance.

REFERENCES: