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

B. J. Dewar[1], K. Keshari[1], R. E. Jeffries[1], L. M. Graves[2], and J. M. Macdonald[1]

[1]Biomedical Engineering, Univ. of North Carolina, Chapel Hill, North Carolina, United States, [2]Pharmacology, Univ. of North Carolina, Chapel Hill, North Carolina, United States

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 underlying 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* ^{31}P NMR experiments were conducted to determine the significance and role the elevated Cr plays in drug resistance.

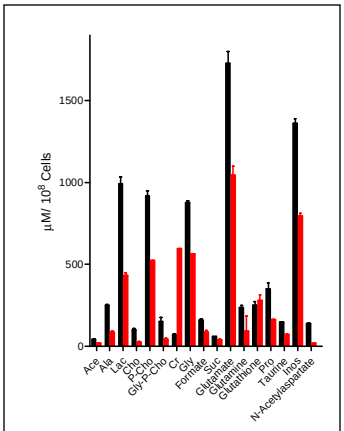

Fig 2. Quantification of select metabolites from MyL and MyLR cells. Conc. were determined from an internal Std. (TSP) and normalize to total cell number. MyL (■) and MyLR (■).

METHODS: MyL and MyLR cells were cultured in T-175 flasks and grown to high density. 10^8 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 D_2O containing 1.5 mM TSP as a concentration and chemical shift reference. ^{1}H NMR spectra were obtained on a narrow-bore 16.5T Varian INOVA (125 MHz ^{13}C, 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 10^7 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 O_2 concentration was maintained in the media using a Gas Exchange Module (GEM), which was filled with 95% Air/ 5% CO_2. Bioreactor ^{31}P NMR spectra were acquired on a 14.1T Varian INOVA. Data from both ^{1}H and ^{31}P 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 ^{1}H spectra using Chenomx software (Chenomx, Inc. Alberta).

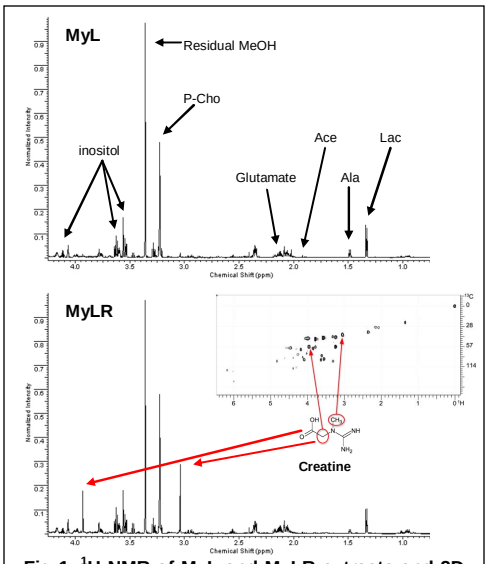

Fig 1. ^{1}H NMR of MyL and MyLR extracts and 2D ^{1}H-^{13}C HSQC of MylR cells. ^{1}H NMR spectrum of each cell type showing abundant metabolites and identifying the CH_3 and CH_2 peaks at 3.0 and 3.9 ppm, respectively of creatine and the 2D ^{1}H-^{13}C HSQC (inset). Lactate (Lac), alanine (Ala), acetate (Ace) phos-choline (P-Cho).

RESULTS: Figure 1 shows representative ^{1}H NMR spectra from MyL and MyLR cell extracts highlighting the significant increase in Cr. 2D ^{1}H-^{13}C HSQC of the MyLR extract demonstrated that the resonances in the 1D ^{1}H 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 the glycolytic related, alanine and lactate (Fig. 2). We also observed that glucose concentrations in MyLR media were higher, suggesting an alteration in glucose uptake and/ or utilization (glycolysis). As it is difficult to distinguish Cr from phosphocreatine (PCr) using ^{1}H NMR and due to the rapid degradation of PCr to Cr by creatine kinase, MyLR cells were encapsulated in alginate beads and a NMR-compatible bioreactor was used to examine PCr levels *in vivo*. Figure 3A shows ^{31}P NMR spectra collected over an 8 hr period clearly showing PCr in MyLR cells. Moreover, following the addition of 2,4-dinitrophenol (DNP), an uncoupler of mitochondrial oxidative phosphorylation, PCr levels were completely diminished, while βNTP levels remained relatively unaffected (presumably through glycolysis)(Fig. 3B). These data suggest that maintenance of the PCr pool is highly coupled to mitochondrial ATP production in MyLR cells.

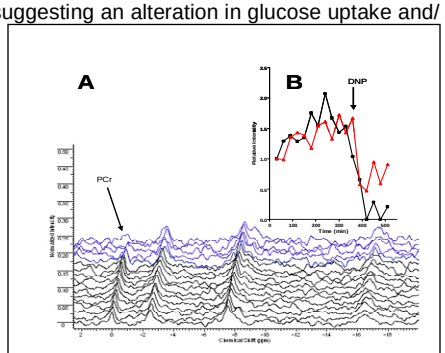

Fig 3. Detection of Phosphocreatine by ^{31}P Analysis. (**A**) Stacked spectra (summed data over 30 min) from a real-time perfusion experiment of MylR cells. Blue spectra represent data collected after addition of 100 uM DNP. (**B**) Relative concentrations of PCr (■) and βNTP (▲) over time.

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:

[1] Sherbenou, D.W. and B.J. Druker, J Clin Invest, 2007. 117(8): p. 2067-74.
[2] Shah, N.P. and C.L. Sawyers. Oncogene, 2003. 22(47): p. 7389-95.
[3] Ito, T., H. Tanaka, and A. Kimura. Eur J Haematol, 2007. 78(5): p. 417-31.
[4] Donato, N.J., et al. Cancer Res, 2004. 64(2): p. 672-7.
[5] Raguz, S., et al. Int J Cancer, 2008. 122(5): p. 1058-67.
[6] Jeffries, RE, et. al. Intern. Soc. Magn. Reson. Med. 2008.

ACKNOWLEDGEMENTS: National Institute of Health, GM075941-01.