Metabolic Biomarkers in Blood Plasma of Tumor Bearing Mice Detected by ^{1}H NMR Spectra

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Introduction: High resolution ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy of blood plasma has a potential to characterize variety of metabolic processes. These spectra contain resonances from diverse groups of molecules, resulting in broad shapes (protein and fatty-acid long chains) and sharp peaks (small molecules). The 600 MHz ^{1}H NMR spectra of plasma from a xenograft mouse model suggest correlation of the succinate amplitude and the presence of the orthotopically grown prostate tumors. There are variations in the lipid content that also seem to correlate with tumor burden and suggest systematic alteration of lipid metabolism.

Methods: We acquired 18 spectra from plasma obtained at the point of sacrifice of nude mice upon completion of experiments with orthotopic tumors in the prostate. Tumors were developed using several LNCaP cell lines (wild-type, Luciferase, BST (bcl2-overexpressing)), and most of the mice were treated with series of antisense and/or androgen depravation therapies [1]. Ten of the mice had no tumors at the time of sacrifice: Eight failed to develop tumors and two were completely 'cured' following therapy. The tumors in the remaining mice (n=8) ranged in size from 2 to 514 mm^3 measured by Magnetic Resonance Imaging (MRI) [1]. NMR data was acquired on Varian INOVA 600 MHz spectrometer using water suppression and additional relaxation filtering to highlight small molecules. The methyl resonance of the alanine (1.46 ppm) was utilized for global alignment of the data. Further, the position of individual peaks were locally adjusted [2]. The spectral data was decomposed, using cNMF [3]. This algorithm is based on nonnegative matrix factorization, where the recovered spectral patterns and their corresponding amplitudes must be positive, thus these recovered spectral patterns are physically realizable. The entire analysis was performed by a software tool, *HiRes* [4], which is freely available for research purposes at http://hatch.cpmc.columbia.edu /highresmrs.html.

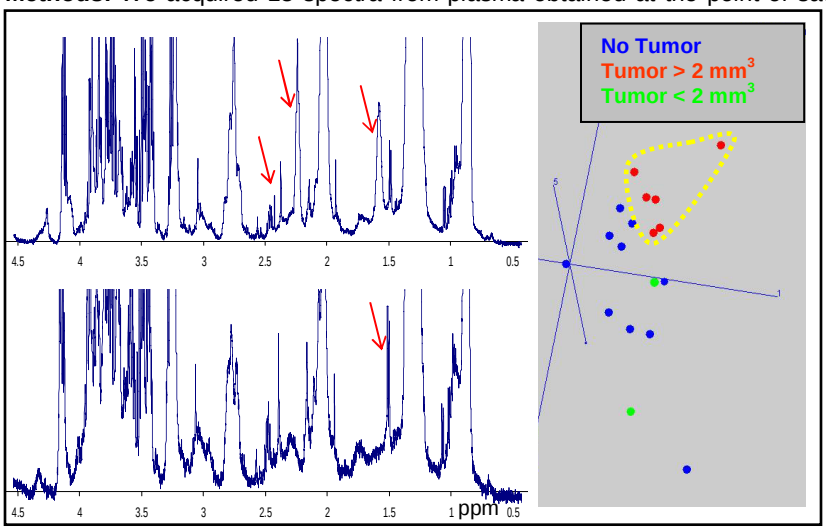

Figure 1. (left) Representative spectra from mouse without (upper) and with (lower) tumor. Arrows indicate specific differences between the two spectra in both broad (lipoproteins) and sharp (small molecules) peaks. (right) Principal Component Analysis (PCA) representation of the data, indicating clear clustering of the samples from mice with larger tumors.

Results: Specific differences between spectra from mice with and without a tumor are quite distinct, as can be seen from Figure 1 (left panel). However, the differences between the two groups of samples are much more complex and extensive. Principal Component Analysis (PCA) is often used for reducing the dimensionality of metabolomic datasets and when our data is represented in a space defined by the Principal Components, a clear clustering of the samples from animals with substantial tumors (>2 mm^3) can be observed (Figure 1, right panel). Using cNMF two patterns are uncovered in the spectral region between 2.2-2.65 ppm, as shown in Figure 2. The main observed difference is a decreased level of and/or complete absence of succinate in the blood of tumor bearing mice. It should be noted that both PCA and NMF analysis is applied to the entire spectral width, without the need for binning, which is traditionally used in complex spectral datasets.

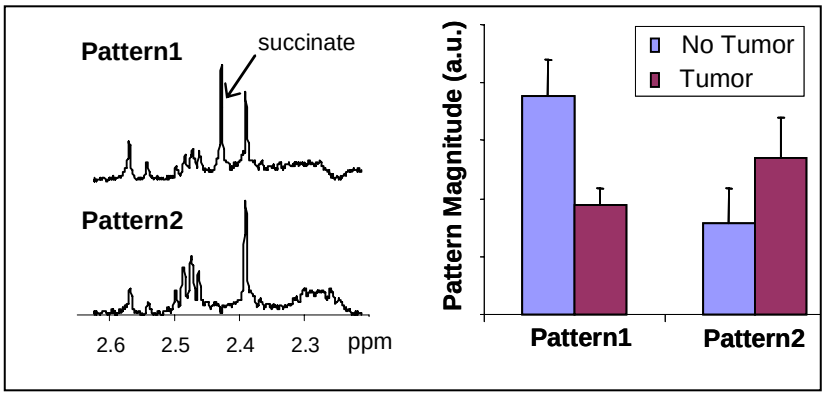

Figure 2. Spectral patterns (left) and their magnitudes (right) in the dataset from mouse plasma. The amplitude of the first pattern in mice without tumors is significantly higher ($p < 0.05$) than in the mice with tumors.

Discussion: The results from the analysis of this dataset are quite intriguing regardless of the fact that blood samples were collected under a variety of experimental conditions. Since most of the animals that did not harbor tumors had not been treated, it is unclear if the observed differences in spectral patterns are related to tumor burden or to treatment response. Longitudinal experiments during tumor development are under way and the analysis of this data will allow the comprehensive assessment of the metabolic processes related to tumor growth. The ultimate goal of the study is to apply the approach to human blood. In any case however, the ability to monitor tumor growth and/or treatment response on the basis of blood metabolic changes is significant from both scientific and clinical point of view.

Acknowledgement: National Cancer Institute CA101984; National Institutes of Health DK070301

Reference: [1] Stoyanova, R., Hachem, P., *et al, Int J Radiat Oncol Biol Phys.* 68,1151-60, 2007; [2] Stoyanova R., Nicholls A.W., *et al, J. of Mag. Res.*, 170,329-35, 2004; [3] Sajda, P., *et al*, *IEEE Trans. Med. Imaging,* 23,1453-65, 2004; [4] Zhao Q., Stoyanova R., *et al, Bioinformatics,* 22, 2562-64, 2006.