Quantitative ^{1}H HR-MAS using LC Model shows glutamate, choline, glycerophosphocholine, and glucose as biomarkers of Prostate Cancer

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Introduction: ^{1}H HR-MAS MR spectroscopy gives detailed metabolic information of intact tissue samples and is capable of quantitative measures of metabolites. The metabolic profiles of prostate cancer have proven to give information on several important pathways such as the membrane turn-over [1] and glycolysis [2]. Using a previously reported technique [3] it is possible to take cores from surgically resected whole prostates and acquire a detailed analysis of their histology. The aim of this study was to collect a large data set of tissue samples using this technique, with histopathologically confirmed tumor and non-tumorous tissue and accurately quantify their ^{1}H HR-MAS spectra using LC Model [4]. This revealed positive and negative biomarkers of cancer.

Methods: Radically resected prostate tissue from 48 patients was subsampled to give 131 prostatectomy cores. These were histologically classified for their percentage content of benign tissue, stroma and tumour tissue. Two histopathological groups were defined: those containing no tumour tissue (n = 45) and those containing ≥50% tumour tissue (n = 86). ^{1}H HR-MAS of these cylindrical cores were performed at 600MHz, 4°C, and 5000Hz spinning rate with a presaturation-pulse acquire sequence (TR=6.3s) . Spectra were quantified using LCModel - with a baseline restricted to simulated macromolecules fit with prior knowledge of line width, chemical shift and relative concentration - and a novel basis set of 22 metabolites: alanine (Ala), choline (Cho), citrate (Cit), creatine (Cr), ethanolamine (Ethmn), glucose (Glc), glutamate (Glu), glutamine (Gln), glycerophosphocholine (GPC), glycerophosphoethanolamine (GPE), glycine (Gly), isoleucine (Ile), lactate (Lac), leucine (Leu), myo-inositol (mI), phosphocholine (PCh), phosphoethanolamine (PE), putriscine (Put), scyllo-inositol (sIns), spermine (Spm), succinate (Succ), and valine (Val), generated from careful assignment of 10 spectra selected from benign stroma and cancerous samples. Quantification was in reference to a formate standard solution added to each sample and reported as mmols/kg wet weight. Two sided t tests were performed, allowing unequal variances, with a statistical significance of $p<1x10^{-5}$. Pearson correlation coefficients were accepted at the $p<0.01$ confidence level. No corrections were made for multiple comparisons.

Results: LCModel gave robust metabolite fits (Figure A) with Cramér-Rao-Lower Bounds of less than 20% for 16 metabolites in ≥90% of the samples. Setting a strict significance limit revealed three positive markers for the tumour group Cho, Glu and GPC and one negative marker, Glc (Figure B). A significant-negative correlation was found in the no-tumour group between the percentage of stroma tissue and Cit (-0.47) and between stroma and Spm (-0.41).

Discussion: A robust quantification method has allowed accurate characterization of the metabolic contents of ^{1}H HR-MAS MRS samples from surgically resected prostates with prostate cancer. LCModel is a useful quantification tool for high throughput of data: once the basis set and other prior knowledge have been optimized (as here) analyses of further spectra can be fully automated for routine applications. Cho has been confirmed as being significantly higher in cancer tissue as reported previously [1,5]. We also found that GPC and Glu were higher in prostate cancer tissue while Glc was the only significant decrease between these groups at this strict confidence level; mean Cit amounts were also lower in tumour samples (p=0.017). The benign tissue samples do show that Cit is reduced when there is more stroma present in the sample and this is also the case for Spm.

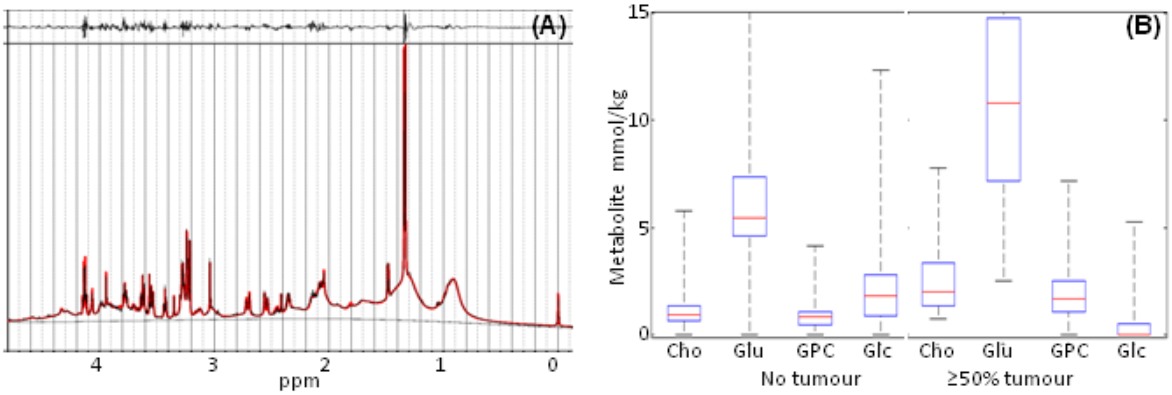

Figure. A) An example of a LCModel fit in red, with the baseline shown below (thin black line) and the residual in a separate box above. B) Box plots showing the difference in metabolite amounts between samples containing no tumour and samples containing ≥50% tumour.

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