Absolute quantitation of metabolites in human prostate cancer biopsies by HR-MAS ^{1}H NMR spectroscopy

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Target Audience: This study will be useful to basic science and clinical researchers working in the field of prostate cancer metabolism and MR spectroscopy.

Purpose: Human prostate cancer is the main cancer that affects the male population in the western world. As PSA, the principal diagnostic method, is not very accurate or specific, there is a need for new diagnostic methods such as HRMAS ^{1}H NMR analysis of biopsy samples[1-4]. Relative metabolite concentrations or metabolite ratios give ambiguous results, so several methods have recently been proposed for obtaining absolute concentrations[3, 4]. LCModel has been widely used for estimating metabolites from *in vivo* ^{1}H MRS data of brain, and we have used it on data from mouse colon tissues[5]. In order to get absolute concentrations of metabolites from human prostate biopsies analysed by HRMAS ^{1}H NMR we have developed a modified LC-model basis set and used it to analyse samples of benign, low and high grade human prostate biopsies.

Methods: Sample collection: Informed consent has been obtained from the patients. Surgical samples were snap frozen in liquid nitrogen and preserved at -80°C until the NMR analysis.

Metabolite data acquisition and analysis: HRMAS ^{1}H NMR data acquisition was performed on a Bruker 600MHz, with a 4mm HRMAS probe. All the spectra were obtained at a spin rate of 3000Hz and a sample temperature at 4°C. LCModel software was used on water-suppressed spectra to estimate the metabolite concentrations. A modified LCModel basis set was used. Since these were not brain tumours, NAA & NAAG were omitted from the analysis; instead, citrate and phosphocreatine (PCr) signals were simulated. The absolute metabolite concentrations were quantified relative to the water signal observed in each individual experiment and then student's t-test (two tailed) was carried out. The methodology for estimation of metabolite concentrations was validated with phantoms containing known concentrations of metabolites. In this preliminary study, HRMAS ^{1}H NMR data from benign (n=10) and low grade (n=7) and high grade (n= 7) were obtained.

RESULTS:

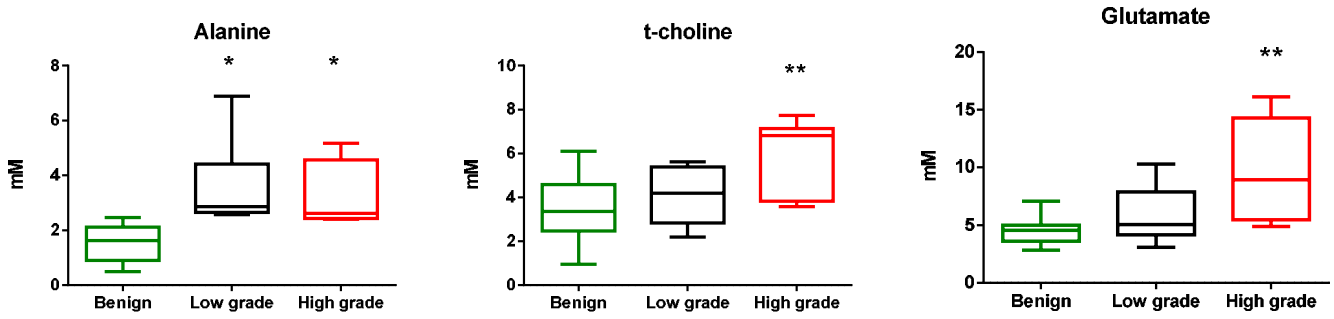

Figure 1 shows significant changes in metabolites of benign and malignant prostate biopsies (low and high grade)

We could reliably estimate the absolute concentrations of alanine, lactate, glutamine, glutamate, citrate, choline, phosphocholine(PC) + glycerophosphocholine(GPC) , creatine + phosphocreatine (t-creatine), taurine, myo-inositol and spermine (polyamines) in benign prostate and in low and high grade prostate cancer samples. Alanine levels were elevated (almost two-fold) in low and high grade prostate cancers comapared to the benign samples (Figure 1). High grade prostate biopsy samples showed significantly increased glutamate, t-choline (choline +PC+GPC) concentrations compared to benign samples. The concentrations changes of citrate and spermine between the benign and cancer samples were not statistically significant. None of the macromolecule (0.9ppm, 2.0ppm) or lipid signals (0.9ppm, 1.3ppm and 2.0ppm) showed any statistical differences between the benign and cancer samples.

DISCUSSION: Elevated alanine might indicate enhanced glycolysis in high grade cancer samples. Increased levels of choline and choline containing compounds have frequently been observed, both by MRS *in vivo* and by *ex vivo* studies on cancer biopsies and cell extracts. Similarly, the t-choline content of the high grade prostate cancer samples was significantly higher than in benign prostate. The significantly higher glutamate in high grade cancer biopsies might be due to increased glutaminolysis; however, although glutamine levels were higher in these samples (not shown), they were not statistically significant.

CONCLUSIONS: Absolute concentrations of alanine, lactate, glutamine, glutamate, citrate, t-choline, t-creatine, taurine, myo-inositol and spermine (polyamines) metabolites were measured in samples of benign prostate, low grade and high grade prostate cancer. Alanine was significantly elevated in both low and high-grade prostate cancer biopsies; t-choline and glutamate were significantly higher in high-grade malignant prostate.

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