SPERMINE AND CITRATE AS METABOLIC BIOMARKERS FOR ASSESSING PROSTATE CANCER AGGRESSIVENESS

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Purpose: Currently there are no accurate diagnostic tools for discriminating aggressive from harmless types of prostate cancer. In this study, high resolution magic angle spinning (HR-MAS) MRS was used to provide the metabolite profiles of human prostate cancer and normal adjacent tissues. The purpose was to identify metabolic biomarkers for prostate cancer aggressiveness.

Methods:
Using a new harvesting method (Bertilsson 2011), high quality prostate tissue samples (n=162 samples, 48 patients) were obtained from normal tissue and cancer tissue with different Gleason scores (score 6-9, where 9 is the most aggressive) and analyzed by HR-MAS MRS (Bruker avance DRX600). Multivariate analysis (PLS, PLS-DA) and absolute quantification (LCModel) were used to examine the metabolic changes and predict cancer aggressiveness by comparing normal, low grade (Gleason score=6) and high grade (Gleason score≥7) cancers. The multivariate models were validated by double cross-validation, and differences in metabolite concentrations were examined using linear mixed models.

Results and discussion:
Based on the metabolite profiles, normal tissue, low grade and high grade tissue were discriminated with a classification accuracy of 85%, 66% and 77%, respectively, using PLS-DA. Out of 23 quantified metabolites, 17 metabolites were significantly changed in cancer samples compared to normal adjacent tissue ($p<0.05$). The metabolite profiles could be related to Gleason score ($r=0.71$) by PLS analysis (Fig. 1a-b). High grade cancer tissues were distinguished from low grade cancer tissues by decreased concentrations of spermine ($p=0.0044$) and citrate ($p=7.73\cdot10^{-4}$), suggesting spermine and citrate as metabolic biomarkers for prostate cancer aggressiveness (Table 1).

Metabolite	Low grade (n=29) median (IQR)	High grade (n = 77) median (IQR)	p-value
Spermine	1.96 (1.23-3.72)	1.05 (0.54-1.57)	0.0044
Citrate	8.45 (7.20-14.82)	4.76 (2.95-7.78)	0.0008

Table 1: Metabolite concentrations in low and high grade prostate cancer. Concentrations are reported as mmol/kg wet weight. The reported p-values are results from linear mixed models and corrected for multiple testing by Benjamini-Hochberg correction.

Conclusions:
HR-MAS MRS provides detailed metabolite profiles distinguishing cancer and normal adjacent tissues. The metabolite profiles are related to prostate cancer aggressiveness, and spermine and citrate are promising biomarkers for separating indolent from aggressive prostate cancers. HR-MAS MRS can be used as an additional diagnostic tool, and the results support the benefit of MRS in future *in vivo* investigations of prostate cancer patients.

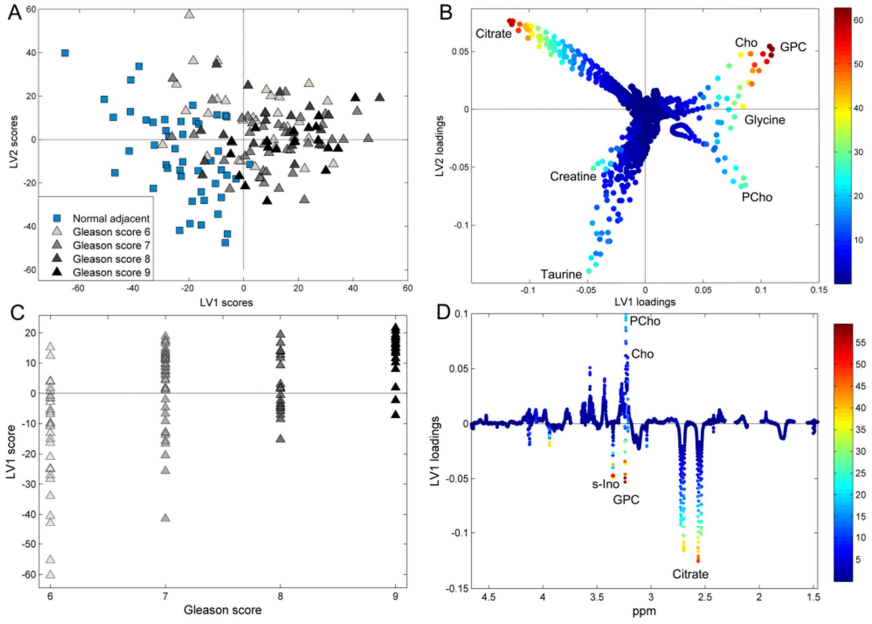

Figure 1: A) PLS scores and (B) loadings of LV1 and 2 from PLS regression correlating the metabolite profiles to Gleason score with a correlation coefficient r = 0.71. The cancer samples are separated from the normal adjacent samples in the score plot, with the loadings showing metabolic alterations related to malignancy. (C) PLS scores and (D) the corresponding loading profile of LV1 from PLS regression of the cancer samples only, correlating the metabolite profiles to Gleason score with a correlation coefficient r = 0.45. The loadings in (B) and (D) are colored according to their variable importance in projection (VIP) score.

References: Bertilsson et al (2011) The Prostate 71: 461-469