Lactate and alanine as metabolic biomarkers for prostate cancer in TRUS- guided biopsies measured by ^{1}H HR-MAS spectroscopy

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Introduction

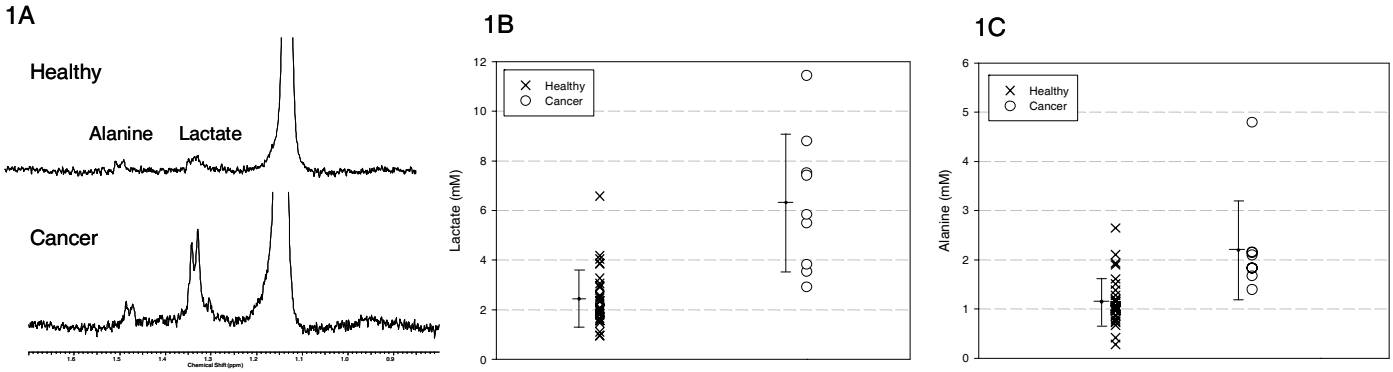
Tumor malignancy is associated with an increase in glycolytic flux and quantitative studies of human cancers have indicated positive correlation between lactate concentration and incidence of metastasis already in an early stage of disease.[1] Primary carcinomas in cervix[2], head and neck[3] and rectal regions[4] have shown that high concentrations of lactate is associated with metastasis and can actively enhance the degree of tumor malignancy. Lower lactate concentrated tumors have shown an overall longer and disease free survival.[1] The amino acid alanine is another important end product of glucose utilization and is an important precursor of gluconeogenesis and protein synthesis.[1] The purpose of this study was to establish the potential of lactate and alanine as metabolic biomarkers for prostate adenocarcinoma using snap frozen transrectal ultrasound (TRUS) guided biopsies.

Methods

Forty-one TRUS guided prostate biopsies (18 gauge needle, ~15mm x 1mm) were obtained from 32 different patients. Nine of the collected biopsies were categorized as 10-60% cancer (Gleason scores 3+3, 3+4 and 4+4), and 33 of them as healthy glandular and stromal tissue. After the TRUS procedure, biopsies were rapidly (≈15 sec) frozen on dry ice and stored on -80°C. High resolution proton magic angle spinning (^{1}H HR-MAS) spectroscopy was performed on an 11.7 T (500MHz) Varian Inova NMR spectrometer equipped with a gHX nanoprobe. Spectra were acquired at 1°C and with a spin rate of 2250 Hz. In addition to a standard presat sequence, proton spectra were obtained using a Carr-Purcell-Meiboom-Gill (CPMG) spin echo sequence (TE=144 and 288 msec, 256 and 512 transients, respectively, TR=6 sec), to suppress signals from overlapping lipids and macromolecules. After HR-MAS analysis, the biopsy tissue was embedded in Tissue-Tek® optimal cutting temperature (OCT), cryo-sectioned and stained (hemoatoxylin & eosin) for pathology reading. Lactate and alanine were quantified by integrating the peak areas and corrected for their T_2 relaxation times using ACD Labs 1D NMR processor (Toronto, Canada). The concentrations were calculated relative to the peak area of the ERETIC (Electronic Reference To access In Vivo Concentration) signal,[5] with ERETIC concentration calibrated using standard solutions of lactate. The concentrations were compared using a Student t-test assuming a significance level of $P<0.05$.

Results

Figure 1A shows representative 1D CPMG HR-MAS spectra from the lactate and alanine area (0.8-1.7 ppm) of a healthy sample (top) and a cancer sample (bottom). A significant increase in lactate (2.43±1.14 mM vs 6.31±2.77 mM, $P<0.0000001$) and alanine (1.14±0.48 vs. 2.20±1.00, $P< 0.00006$) concentrations was found for prostate cancer compared to healthy tissues. The concentrations of lactate and alanine in each biopsy are shown in Figure 1B and 1C. The standard deviation is shown as error bars. While there was significant overlap of individual alanine concentrations (1C), there was minimal overlap of individual lactate concentrations (1B) between healthy and cancer samples.



Discussion

The concentration of lactate in healthy biopsy tissue were found to be much lower (≈2.4 mM) than surgical samples (≈45 mM)[6] indicating very little anaerobic glycolysis occurred during biopsy collection. The very significant increase in lactate concentration (≈6.3 mM) found in prostate cancer biopsies is in agreement with previous studies of lactate in human cancers.[1] From a clinical standpoint it is also important that there was minimal overlap of individual concentrations of lactate between healthy and malignant tissues. The present observation of significantly increased alanine concentration also supports that tumor malignancy is associated with an increase in glycolytic flux, and consistent with the need for increased protein synthesis in tumors.[7] The observed changes in lactate and alanine could be used to improve the clinical diagnosis and characterization of prostate cancer using lactate and alanine edited ^{1}H or hyperpolarized ^{13}C spectroscopic imaging sequences.

References

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