Assessing Prostate Cancer Growth with Citrate Measured by Intact Tissue Proton Magnetic Resonance Spectroscopy

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Introduction: Since the implementation of Prostate Specific Antigen (PSA) annual screening, the number of prostate cancer (PCa) cases diagnosed has increased significantly. Early detection saves lives of patients harboring lethal PCa, however, PSA testing has also led to the overtreatment of many patients with slow-progressing or clinically insignificant tumors. Although the critical role of Gleason score and the incidence of PCa recurrent potential has been recognized, current pathology is often unable to distinguish latent from aggressive PCa. To improve clinical ability in assessing PCa grade, stage, and malignant potential, tumor biology studies have correlated these clinical parameters with various molecular biomarkers. They have shown that the depletion of zinc in prostate cells correlated with cell-line growth rates, and may therefore relate to the progression of PCa. Furthermore, since zinc is normally an inhibitor of citrate oxidation, the reduction of zinc in PCa may cause a decrease in citrate secretion levels in the glandular epithelia of PCa patients. In this study, we test the relationship between the levels of secreted citrate in the glandular epithelia of PCa patients and the progression of PCa. We measured citrate concentrations in intact tissue samples with high-resolution magic angle spinning proton magnetic resonance spectroscopy (HRMAS 1HMRS), followed by quantitative histopathology of the same specimens. Using these data, we evaluated correlations between citrate concentrations and PCa growth rate, as represented by PSA velocity (V_{PSA}), PSA density (D_{PSA}), and percent free PSA (FreePSA%).

Methods: We conducted 27 individual measurements of prostate peripheral zone tissue spectroscopy and quantitative pathology for 18 PCa patients. *MR Spectroscopy*. Samples and 1.0µl of D2O were loaded into 4mm Zirconia rotors with spherical inserts. Spectroscopy measurements were carried out on a Bruker AVANCE spectrometer, pre-cooled to 4ºC and operating at 600 MHz (14.1T). Spectra were acquired using slow spinning rates of 600 and 700Hz and post-spectral edited with Min(A, B) scheme. Spectroscopic data were processed using Nuts software (Acorn NMR Inc., Livermore, CA). *Histopathology*. After spectroscopy, tissue samples were fixed in formalin, embedded in paraffin, cut into sets of 5µm sections at 100µm intervals, and stained with hematoxylin and eosin. Volume percentages of histological features (cancer, benign epithelium, and stroma) were analyzed and quantified by a pathologist.

Results: We observe different linear patterns between citrate concentrations and benign glandular epithelia from patients of different PSA velocities. In Figure 1, we arranged data of citrate vs. benign epithelia in three groups, with samples from the low V_{PSA} group presented in small dots (n=12), median V_{PSA} group in median dots (n=9), and fast V_{PSA} group in large open circles (n=6). This data arrangement reveals that, while a linear correlation between citrate and benign epithelia is measurable for the entire data set with statistical significance (the dashed line, p=0.023, r2=0.19), the scattering is mostly caused by the data dispersion of the fast V_{PSA} group. Thus evaluating the slow and median groups revealed much stronger linearity and statistical significance (the solid line, p<0.0001, r2=0.56) of a relationship between citrate concentrations and volume percentages of benign epithelia. More importantly, we obtain a significant correlation between PSA velocity, density, and percent free PSA, and citrate concentrations in unit volume of benign epithelial glands of the peripheral zone, as shown in Figure 2.

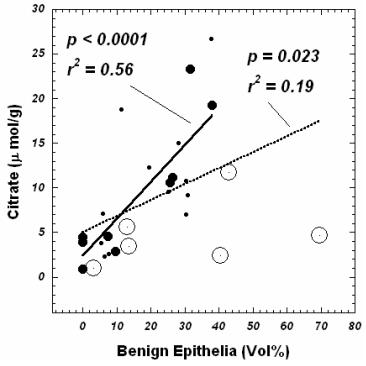

Figure 1. Correlations between citrate concentrations and volume percentages of benign epithelia.

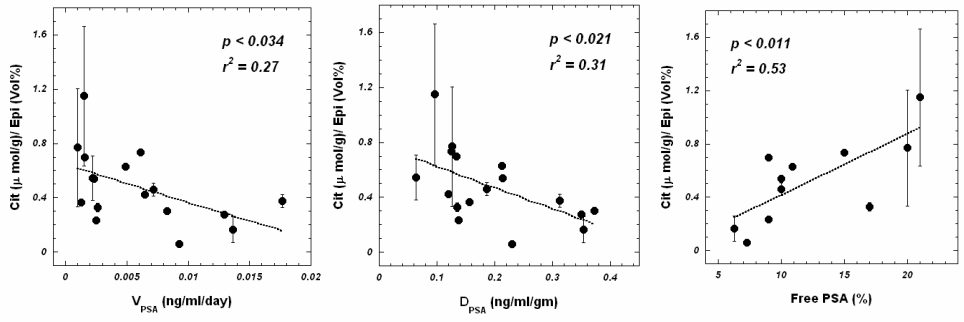

Figure 2. Relationships between levels of citrate in benign epithelia PSA velocity, density and percent free PSA.

Conclusions: We demonstrated that the citrate concentrations of benign epithelial glands in the peripheral zone of PCa patients correlate to PSA velocity, density, and percent free PSA, such that low levels of citrate represent rapidly increasing PSA values, and therefore, likely fast growing cancer. Thus tissue samples obtained at the time of biopsy may be evaluated for their citrate concentrations for the prediction of PCa growth rates, allowing for the implementation of alternative treatment options and reducing overtreatment.

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