

Assessing Prostate Cancer Growth with Intact Tissue MRS and mRNA of Spermine Anabolic Enzymes

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Introduction: The implementation of serum prostate specific antigen (PSA) as a screening test for prostate cancer (PCa) has increased the disease detection at an early stage. While PCa early detection may present patients with the opportunity for treatment, the inability of current pathology to distinguish between a latent form of the disease and a fast growing tumor often leads to unnecessarily aggressive treatments for some patients. As a result, assays that can accurately reflect tumor biological activity are urgently needed in this era of personalized medicine [1, 2]. In a search to better understand the latent characteristics of PCa, spermine was identified as an endogenous inhibitor of PCa cell growth [3]. Inspired by this report, and encouraged by our ability to measure spermine in intact prostate tissue with high-resolution magic angle spinning (HRMAS) proton magnetic resonance spectroscopy (¹HMRM) [4-6], we investigated the proposed inhibitory effect of spermine with selected prostate cancer cases. HRMAS technology is able to generate detailed tissue metabolic compositions while preserving the pathological structures needed to accurately correlate metabolic changes with pathological alterations [7]. Without direct means to quantify PCa growth in vivo, we resorted to evaluate patients with a surrogate marker. For all patients in the study we measured spermine concentration of tissues from prostatectomy with HRMAS ¹HMRM, and quantified the expression levels of mRNA for enzymes in the spermine synthesis and degradation pathways for different pathological features.

Methods: *Tissue samples.* The population recruited represents a group of PCa patients (n=9) with multiple elevated PSA values, but negative biopsy results until the final positive biopsy that led to prostatectomy and presented tissue for the study. With PSA elevation over time we were able to determine PSA velocities (V_{psa} , ng/ml/day) for these patients. Although we recognize V_{psa} values have been a topic of debate for multiple reasons, in our case they serve as a potential marker that can be used to measure the progression of PCa. Two samples were collected from each patient: one containing both cancer and benign glands, and the other containing only benign glandular epithelium. *MR Spectroscopy.* All tissue was analyzed with HRMAS ¹HMRM on an AVANCE system (Bruker Biospin, Billerica, MA) operating at 600 MHz (14.1T). Spectroscopic data were processed with commercial software NUTS according to the following procedure: 1Hz apodization before Fourier transformation, baseline correction, and phase adjustment. Resonance intensities used in the study were integrals of curve fittings with Lorentzian-Gaussian line shapes measured from the composed spectra from 600 and 700 Hz HRMAS spectra based on a previously reported scheme [8]. *Quantitative Pathology.* A pathologist with no knowledge of the spectroscopic results visually estimated % area representing cancer cells, normal epithelial cells, and stroma in each cross section to the nearest 5%. *Real-time Quantitative PCR.* Expression of polyamine pathway related mRNA for enzymes Ornithine decarboxylase (ODC1), S-adenosylmethionine decarboxylase (AMD1), Spermidine/spermine N1-acetyltransferase (SAT1), antizyme (OAZ1) and oncogene c-MYC were analyzed using rt-q-PCR using gene specific human primers and the SYBR Green dye as the fluorescent reporter.

Results: From our previous study of human prostate tissue with HRMAS technique, we found a statistically significant correlation between the concentration of spermine and the volume percentages of benign prostate epithelial glands quantified from the same tissue samples after spectroscopy analysis. The tissue spermine concentration was normalized by the volume percentages of benign epithelial glands measured from the same tissue samples in order to consider spermine correlation with tumor growth, but the evaluation of spermine concentrations against V_{psa} did not reveal any apparent correlation. This result inspired us to further hypothesize that the inhibition of cancer growth in the prostate may employ a signaling pathway initiated by the presence of PCa, to synthesize spermine on demand. The critical factor for PCa inhibition may not depend on the static levels of spermine concentrations, but rather on the rate of biosynthesis, the spermine anabolic processes. In order to consider potential differences in spermine synthesis among different pathological features, we conducted analysis of spermine pathway enzymes [9] for different prostate pathological features. Rt-q-PCR analysis revealed that the measurable expression levels of spermine anabolic enzyme ODC1 in cancer and benign epithelia surrounding cancer glands were 3.0 fold upregulated when compared with strictly benign epithelia in tissue samples PCa patients, and 1.4 fold upregulated when compared with stroma near cancer glands (Fig 1.). Furthermore, we found the expression levels of ODC1 and AMD1 in benign epithelia surrounding cancer glands were linearly correlated with each other, possibly indicating that signaling for spermine production to inhibit PCa growth simultaneously activates both signaling pathways (Fig. 2). Expression levels of both spermine anabolic enzymes ODC1 and AMD1 in benign epithelia surrounding cancer glands logarithmically reduced with the increase of V_{psa} , indicating that mRNA upregulating activity dynamically varied during the course of disease progression. The observed upregulation of ODC1 mRNA in benign epithelia proximal to cancer glands, and the reduction of this upregulation with progression of tumor growth suggest a mechanism that the presence of PCa may activate signaling processes of spermine production to delay PCa progression. To further investigate these dynamic mRNA changes, we analyzed additional mRNAs: SAT1, OAZ1 and c-MYC for different pathological components. Analysis of c-MYC mRNA revealed a statistically significant downregulation in expression levels (1.7±0.9 fold) for the surrounding benign glands, and no significant downregulation of c-MYC for stromal and benign epithelia more distant from cancer. This observation seemed to indicate the activities of these benign epithelial glands in downregulating c-MYC levels in order to preserve spermine biosynthesis.

Conclusions: Although these results are clinically important, the inability to quantify the enzymes and their role in spermine biosynthesis for individual prostate pathological components, suggests this study remains explorative and can only present circumstantial evidence that there seems to be a correlation between mRNAs in spermine anabolic pathways and PCa progression status. Despite limitations, the observed correlations are still of critical clinical significance. These mRNA results may be able to indicate the progression rate of PCa, an extremely important parameter used in determining therapy strategies that is currently unavailable in the PCa clinic. In future studies, the PCa inhibitory mechanism reported here may be incorporated into clinical practice for newly diagnosed PCa patients at the time of prostate biopsy prior to prostatectomy. By evaluating the expression levels of these enzyme related mRNAs, patients with slowly growing and less aggressive tumors may elect active surveillance, while those with confirmed lethal PCa could opt for immediate radical prostatectomy and adjuvant therapies.

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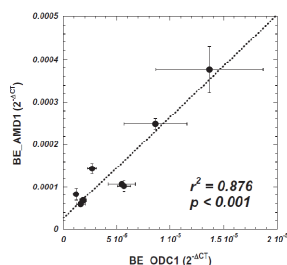


Fig. 2. Expression levels of ODC1 and AMD1 are linearly correlated.

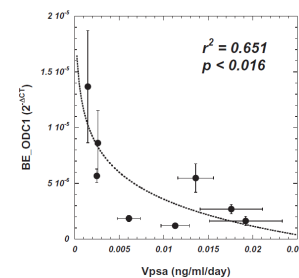


Fig. 1. Spermine anabolic enzyme ODC1 is upregulated in benign tissue.