

Correlation of Phospholipid Metabolites with the Proliferation Marker Ki-67 in Prostate Cancer Tissues

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Introduction: The amount of nuclear antigen Ki-67 staining has been associated with cancer grade [1] and metastases [2]. Elevated levels of phospholipid metabolites have also been correlated with the presence of prostate cancer [3]. However, a direct correlation between the levels of individual phospholipid metabolites with cancer proliferation and Gleason grade has not been determined. This is of great clinical significance since most prostate cancers are indolent and will never metastasize, but there is currently no accurate way to identify these patients at diagnosis. HR-MAS two-dimensional total correlation spectroscopy (TOCSY) can be used to quantify choline and ethanolamine phospholipid metabolites in intact human prostate tissues prior to pathology and immunohistochemistry of the same tissues [3]. The purpose of this study is to establish the relationship between phospholipid metabolite levels, Ki-67, and Gleason Grade.

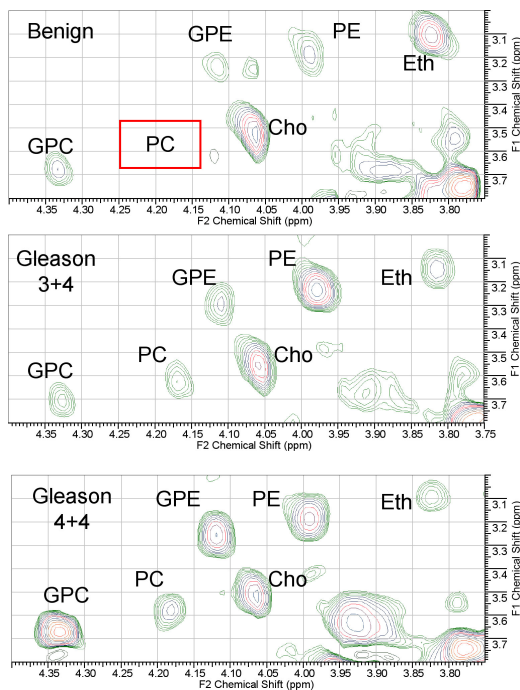


Fig 2. A. Benign, Ki-67: neg, Cancer: Neg, PC: 0.
B. Gleason 3+4, Cancer: 50% Ki-67 <1% PC: 0.17
PC+GPC: 0.35 **C.** Gleason 4+4 Cancer: 40% Ki-67: >10% PC: .426 PC+GPC: 5.3

and concentrations of PC and GPC. Clinically, cancers with a dominant Gleason pattern 4 are considered to be aggressive with a high likely of metastases, and are therefore treated aggressively. This study suggests that increased concentrations of PC and GPC would predict for aggressive disease. This will need to be tested in a larger cohort of surgical patients with clinical outcome data.

References: (1) M R Feneley, et al. *J Clin Pathol.* 49:741-748, 1996
(2) A. Pollack, et al. *J Clin Oncology* 22:2133-2140, 2004
(3) M Swanson, et al. *Magnetic Resonance in Medicine* 2008
(4) R Li, K Heydon, et al. *Oncology.* 10:4118-4124, 2004

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Methods: TOCSY's were acquired from snap frozen tissue samples obtained at radical prostatectomy in order to resolve the choline (choline - Cho, phosphocholine - PC, glycerophosphocholine - GPC) and ethanolamine-containing compounds (ethanolamine - Eth, phosphoethanolamine - PE, glycerolphosphoethanolamine - GPE), that overlap in 1-D HR-MAS tissue spectra. NMR spectra were acquired using a 11.7T (500MHz for ¹H), 1°C, and a 2250 Hx spin rate using a Varian INOVA spectrometer equipped with a 4-mm gHX nanoprobe (Varian, Palo Alto, CA) and processed as previously described to generate absolute concentrations [3]. After HR-MAS, tissues were imbedded in OCT and 5 mm sections were obtained using a Leica CM1850 cryostat. For each sample, adjacent sections were stained with hematoxylin and eosin (H&E) and Ki-67 [1] and the % of the sample that was prostate cancer as well as the % of the cancer that stained positive for Ki-67 was determined by two experienced pathologists who were blinded to the clinical and metabolic findings. For the purpose of the abstract we divided cancers into low grade (≤ 3+4) and high grade (≥ 4+3).

Results: A total of 43 surgical samples were studied; 11 high grade (6 4+3, 5 4+4), 22 low grade (18 3+3, 4 3+4), and 10 benign. The mean percent of Ki-67 staining significantly increased between benign tissue and low grade cancer (0.4 ± 0.8 versus 1.9 ± 1.4 , $p < 0.01$) and between low and high grade cancers (1.9 ± 1.4 versus 3.8 ± 1.6 , $p < 0.03$). All of the high grade samples stained positive for Ki-67, with the 14 samples that did not stain positive being Gleason 3+3 (N=6) and benign (N=8). As seen in figure 1, the mean concentration of GPC and PC significantly increased going from benign to low grade cancer ($.2 \pm 0.2$ versus 2.2 ± 2.5 , $p < 0.03$) and between low and high grade cancer (2.2 ± 2.5 versus 5.3 ± 6.2 , $p < 0.05$). There was also a trend of increasing PC and GPC with increasing Ki-67 staining but no clear correlation. Interestingly, GPC increased the most in high grade tumors (Figure 2). PE and GPE also significantly increased between benign prostate tissue and low grade cancer but was not different between low and high grade cancer (Fig. 2).

Conclusions: The results of this study demonstrate that high grade prostate cancers, i.e. cancers having a dominant Gleason pattern 4, have significantly higher ki-67 staining

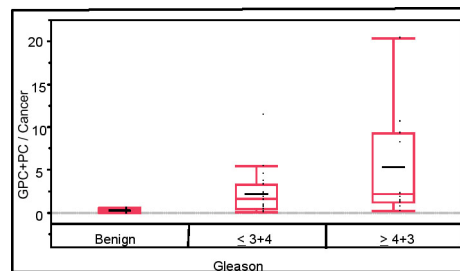


Fig 1. PC+GPC corrected for cancer % versus Gleason