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The Classification of Benign and Malignant Human Prostate Tissue by Multivariate Analysis of ^{1}H Magnetic Resonance Spectra

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ABSTRACT

^{1}H magnetic resonance spectroscopy studies (360 MHz) were performed on specimens of benign (n = 66) and malignant (n = 21) human prostate tissue from 50 patients, and the spectral data were subjected to multivariate analysis, specifically linear-discriminant analysis. On the basis of histopathological assessments, an overall classification accuracy of 96.6% was achieved, with a sensitivity of 100% and a specificity of 95.5% in classifying benign prostatic hyperplasia from prostatic cancer. Resonances due to citrate, glutamate, and taurine were among the six spectral subregions identified by our algorithm as having diagnostic potential. Significantly higher levels of citrate were observed in glandular than in stromal benign prostatic hyperplasia (P < 0.05). This method shows excellent promise for the possibility of *in vivo* assessment of prostate tissue by magnetic resonance.

INTRODUCTION

Prostate cancer is the most common malignancy in males in the United States and Canada, accounting for about 335,000 new cases in 1996 (1, 2).[2] The best results for treatment of prostate cancer are for patients whose tumors are clinically confined to the prostate and who are able to have definitive treatment with radical prostatectomy or radical irradiation therapy. It seems that treating prostate cancer in its early stage is an effective means of achieving long-term survival, and a combination of diagnostic methods detects more early-stage malignancies. However, despite their ability to detect prostate cancer in more patients at an early stage, the noninvasive diagnostic tests currently available, *i.e.*, digital rectal examination, transrectal ultrasound, and measurements of PSA[3] are all relatively insensitive and are incapable of detecting small, well-differentiated cancers (3). These tests can detect only those tumors large enough to be palpable, visible on ultrasound, or capable of elevating the serum PSA level. PSA, the most commonly used diagnostic test in prostate cancer, has several limitations. Although PSA is specific to the prostate, it is not specific to prostate cancer. Other prostatic abnormalities/diseases, such as BPH and prostatitis (prostatic inflammation), may also cause an elevation in the PSA value (3). Over the years, there have been attempts to improve the use of PSA as a prostate cancer marker (4, 5). However, all these techniques have their limitations (4), and the search for a better diagnostic tool continues.

MRS provides biochemical and metabolic information associated with tumor growth and development, and is thus in a position to detect early, premorphological changes in tissue. Furthermore, it is conceivable that patient prognosis may be directly reflected in the MR-detectable biochemistry. MRS of cultured cells and of biopsy specimens from humans and animals have demonstrated sensitivity to the onset of malignancy (6–11). We report here our recent progress using ^{1}H MRS to characterize and classify human prostatic tissue. There have been several nuclear magnetic resonance studies reported on prostate tissue extracts with very small sample sizes (12–17). We believe that an *ex vivo* MRS study of whole-tissue biopsy specimens provides information that is more representative of the *in vivo* situation because some of the tissue biochemistry may be morphology- and architecture-dependent.

The prostate MRS results reported thus far suggest that resonances from metabolites such as citrate, choline, creatine, and inositol may have some diagnostic value (12–17). However, because most of these studies were performed on only a few cases, definitive conclusions cannot be drawn. Of these resonances, that of citrate has been pursued with the greatest interest because citrate levels were found to be present at significantly lower concentrations in prostate adenocarcinoma than in both normal and BPH specimens. However, this is true only for BPH of glandular tissue origin. Stromal BPH has been found to have citrate levels as low as levels found in cancer and hence the distinction between the two, based solely on the citrate intensity, becomes less marked. Thus, instead of relying on a particular resonance or a set of arbitrarily selected resonances, as is done in the conventional methods of analysis, every piece of information in the spectra should be considered. Multivariate analysis methods, by virtue of their ability to use all of the information in the spectra, have been very successful for analyzing MRS data in a robust, nonsubjective, and reliable manner (11, 18–20). Here, we present a preliminary analysis of benign and malignant prostatic tissue using these methods.

MATERIALS AND METHODS

MRS Specimens. Prostate tissue specimens were collected from transurethral resection of the prostate and radical prostatectomy from 50 patients. Two chips were selected randomly from each transurethral resection specimen (40–60 chips on average). A needle biopsy was obtained from radical prostatectomy specimens at the time of surgery from the removed prostate. These biopsies were randomly obtained from the posterior aspect of the prostate. All biopsies were obtained at one time point only, *i.e.*, there were no patients who were rebiopsied. Three patients were receiving hormonal therapy. Prostate tissue specimens (n = 87) were placed in D_2O-PBS solution, snap frozen in liquid nitrogen within 30 min of excision, and stored at −70°C. The frozen specimens were thawed, mounted in small capillary tubes, and subjected to ^{1}H MRS (Bruker Instruments, 360 MHz) at 37°C with presaturation of the water signal (21). Acquisition parameters included: 90° pulse of 6.5 μs, number of scans = 256 or 640, spectral width = 5000 Hz, recycle delay = 2.41 s, and time domain data points = 4K. Immediately following the MRS experiments, all samples were fixed in 10% formalin and subjected to routine histopathological assessment. Sections of 5 μm were cut and stained with H&E and were then examined by a pathologist. In cases of cancer, the Gleason grades (scores) were given to indicate the degree of differentiation. Gleason's tumor grading is the classification of adenocarcinoma of the prostate by the evaluation of the pattern of glandular differentiation. The Gleason score is the sum of the dominant (primary) and secondary patterns, each numbered on a scale of 1–5 with 1 representing a well-differentiated tumor and 5 representing a poorly differentiated tumor (22). Eighty five % of the malignant biopsies had a Gleason score of 3 + 3. Three specimens had each Gleason scores of 2 + 2, 3 + 4, and 4 + 3; it was impossible to give an accurate Gleason score on one of the biopsies due to poor preservation. In cases of BPH, the percentages of

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[2] American Cancer Society data also available at http://www.cancer.org/cff7.html.

[3] The abbreviations used are: PSA, prostate-specific antigen; MRS, magnetic resonance spectroscopy; BPH, benign prostatic hyperplasia.

glandular and stromal tissue components were provided. The ratio of the glandular to stromal component was less than 1:1 in 69% of the BPH specimens, equal to 1:1 in 17%, and greater than 1:1 in 14% of the specimens.

Data Analysis. Peak intensities (areas) per unit mass of tissue were determined for the following five spectral regions using standard Bruker integration routines. These regions are: 3.3–2.9, 2.8–2.45, 2.2–1.87, 1.85–1.09, and 1.09–0.5 ppm. Resonances were assigned via COSY (two-dimensional correlation spectroscopy) spectra and by comparison with chemical shift values of standard substances and literature values (7, 8). Peak intensity values reported are mean ± SD. Mean values of the intensities were compared by using the *t* test ($P \leq 0.05$; EXCEL 5.0) and were considered significant at the 95% confidence level.

Before commencing the multivariate analysis, the spectra were partitioned into a training set (33 BPH, 13 tumors) and a test set (33 BPH, 8 tumors). Each magnitude spectrum was normalized by dividing every data point by the total spectral area and then was aligned on the reference peak (p-amino benzoic acid) at 6.81 ppm. Normalization to the sample mass was not required for this analysis. The 0.5–3.55 ppm region of each spectrum was selected to minimize the influence of spectral artifacts created by the suppression of the water peak at 4.6 ppm and of the resonance at 3.6 ppm due to the glycine-containing solution normally used during the surgical procedure. This region of 450 data points was divided into 50 equal subregions by averaging nine consecutive data points. The optimal set of six subregions was determined by our region selection algorithm (18) using linear-discriminant analysis with the leave-one-out method on the training set (19, 20).

RESULTS

Typical ^{1}H MR spectra obtained from benign and malignant prostatic tissue are depicted in Fig. 1*A* and Fig. 1*B*. Table 1 shows the mean values of the intensities of the spectral regions and the statistical significance of the differences between the two groups. All values presented in this table should be regarded as semi-quantitative (as discussed in Ref. 21). Table 2 shows the sensitivities and specificities obtained from the conventional analysis, *i.e.*, peak height/area analysis, of the MR spectral data. Because the most statistically significant differences were obtained in the comparison of ratios of metabolites (see Table 1), the sensitivities and specificities shown in Table 2 are only for those spectral ratios.

Table 1 *Mean (±SD) peak areas (arbitrary units per unit mass of tissue)*

Spectral region (ppm)	BPH (n = 66)	Cancer (n = 21)	P
3.3–2.9 (I)	20.6 ± 16.5	10.1 ± 10.5	0.001
2.8–2.45 (II)	64.1 ± 7.2	27.1 ± 5.3	0.015
2.2–1.87 (III)	23.3 ± 14.7	20.1 ± 23.7	0.56
1.85–1.09 (IV)	47.8 ± 59.2	51.8 ± 53.6	0.77
1.09–0.5 (V)	33.3 ± 19.5	30.6 ± 30	0.71
II/I	0.28 ± 0.21	0.22 ± 0.22	0.27
II/III	0.26 ± 0.17	0.12 ± 0.13	0.0003
II/IV	0.16 ± 0.11	0.05 ± 0.08	0.00002
II/V	0.19 ± 0.16	0.076 ± 0.085	0.00006

Table 2 *Sensitivities and specificities of spectral ratios*[a]

	II/I	II/III	II/IV	II/V
Sensitivity	62	81	76	81
Specificity	67	68	80	62

[a] Numbers represent percentages.

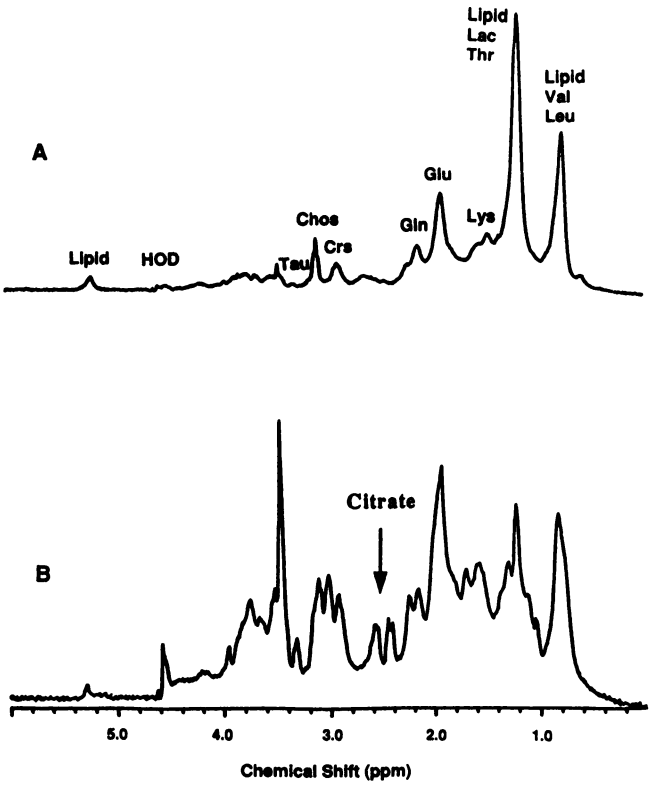

Fig. 1. ^{1}H MR spectra (360 MHz, 37°C) of prostate tissue specimens. *A*, cancer (Gleason grade: 3 + 3). *Chos*, cholines; *Crs*, creatines; *Lac*, lactic acid; *Tau*, taurine; HOD, partially deuterated water. Although certain substances are assigned on figure, this does not imply that these are the only substances contributing to a particular resonance. *B*, BPH

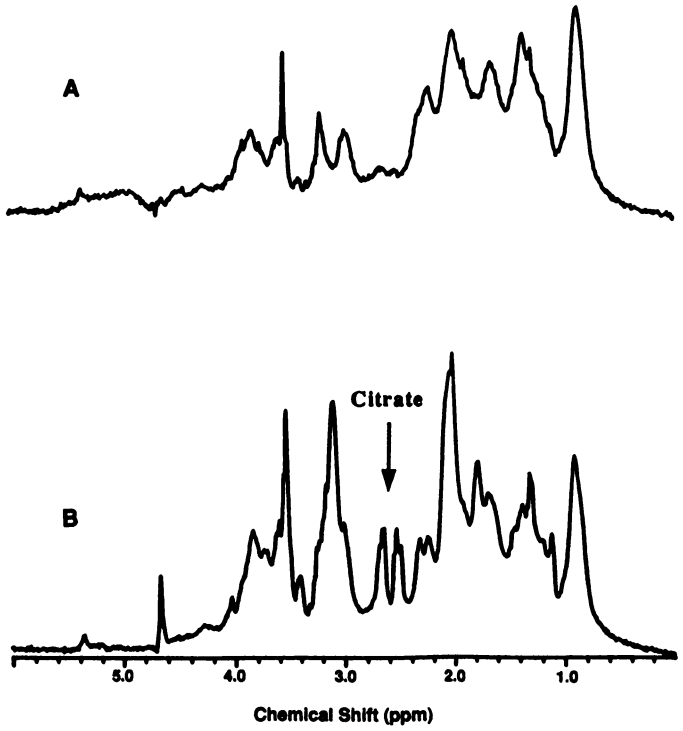

Fig. 2. ^{1}H MR spectra (360 MHz, 37°C) of BPH tissue specimens. *A*, 95% stromal-5% glandular. *B*, 90% glandular-10% stromal.

Fig. 2*A* and Fig. 2*B* show representative spectra from BPH specimens of dominantly glandular and stromal tissue origin, respectively. As shown in the spectra, the citrate resonance is much more intense in glandular than in stromal BPH. Statistical comparisons of the mean intensities of the different spectral regions were made, and the only statistically significant difference ($P < 0.05$) found between the two groups was for citrate.

The variability of the intensities of the various resonances in the spectra of human tissue led us to seek a robust method of classification based more on overall pattern recognition than on intensity ratios. Multivariate analysis of the type used previously to classify brain (20)

Table 3 *Multivariate analysis results*[a]

Overall classification accuracy	96.6%
Training set	100%
Test set	92.7%
Sensitivity	100%
Specificity	95.5%

[a] $n_{total} = 87$; $n_{BPH} = 66$; $n_{cancer} = 21$.

and colon biopsies (11) gave excellent classification accuracies for both the training and the test sets, as indicated in Table 3. The sensitivity and specificity of the technique for cancer are also indicated in Table 3. Fig. 3 illustrates the two class average, reduced (0.5–3.5 ppm) spectra. Six optimally discriminatory regions with resonances at 3.49, 3.43, 2.53, 2.17, 1.87, and 1.15 ppm were selected by our algorithm. These include the resonances from taurine, citrate, and glutamate.

DISCUSSION

The ^{1}H MR spectra of human prostate tissue are dominated by signals from low molecular weight metabolites, such as choline-containing compounds (3.2 ppm), creatine (3.0 ppm), citric acid (2.5 ppm) glutamic acid (2.0 ppm), lysine (1.6 ppm), lactic acid (1.3 ppm), and amino acids (0.9 ppm). The resonances at 1.3 and 0.9 ppm have also intensity contributions from fatty acyl chains. In previous ^{1}H MRS studies of prostate tissue (12–17), there has been some discussion about several resonances that could be used as possible markers for prostate cancer. However, the resonances that received the greatest attention are those of choline and citrate. An increase in the choline peak intensity at 3.2 ppm was observed previously in cancerous human colon and prostate tissue (8, 14, 17). Because of peak overlaps in this region of the spectrum (particularly in the spectra from BPH specimens), it was difficult to integrate only the choline resonance. Thus, the entire spectral region between 3.3 and 2.9 ppm (which among other things includes the creatine resonance) was integrated. Although a statistically significant difference was observed between cancer and BPH in this region, the mean value for BPH was greater than that for cancer. This could be due to our inability to selectively integrate the region of interest, *i.e.*, choline.

Citrate has been found to be present in lower concentrations in prostate cancer than in both BPH and normal prostate (14, 15, 23). However, although the differences in citrate levels between cancer and BPH (mainly glandular) have been found to be statistically significant, those between cancer and normal were not (12, 13). For example, studies performed by Yacoe *et al.* (12) on cell strains derived from prostate indicate that cancer cells tend to have smaller amounts of citrate compared to normal prostate epithelium, but the difference is not statistically significant. Similarly, studies by Schiebler *et al.* (13) on prostate tissue extracts also show statistically significant differences between cancer and BPH but not between cancer and normal peripheral tissue. Citrate levels have also been found to be related to hormone dependency/sensitivity of prostate cancer cells. In studies reported by Cornel *et al.* (16), the relative creatine and citrate levels were found to be higher for hormone-dependent cell lines than for hormone-independent cell lines. Moreover, citrate levels were also found to be higher in primary cancer cells than in metastatic cells (17). Our findings are consistent with all of the above reports. Although the reason for the presence of citrate at very low levels in prostate cancer cells is not clearly known, several hypotheses have been put forward (24, 25). These include an increased amount of the enzyme aconitase in cancer cells, which helps break down citrate (24), and increased levels of the enzyme ATP-citrate lyase in malignant cells that use citrate in lipid synthesis (25).

Comparisons of benign and malignant prostate tissue based on MR spectral peak intensities (and/or ratios) are *ad hoc* and such individual parameters cannot fully summarize the information content of a spectrum. Accurate absolute quantification of metabolites in such experiments is difficult, and taking only the ratios of metabolites for comparison purposes could be misleading. The variability of spectra from specimens of the same histological type also mitigates against the use of simple spectral measurements.

The use of the ratios of intensities of the different spectral regions to discriminate between cancer and BPH resulted in significant overlaps between the range of values found for the two groups (Table 1). Therefore, we applied multivariate analysis (11, 20), specifically, linear-discriminant analysis, to classify the ^{1}H MR spectra of human prostate tissue. Six discriminatory regions were selected by our algorithm, one of them being the region due to citrate. Although a difference in the 1.3 ppm peak intensity is noted between the two spectra in Fig. 1, this region is not identified as discriminatory, probably due to intra-class variability in the peak intensity. The selection of the region near 3.4 ppm as optimally discriminatory is consistent with previously published reports of elevated levels of taurine (9, 10) in neoplastic tissue compared to normal tissue. The region near 2 ppm primarily due to glutamate, has also been selected as having discriminatory potential. Glutamate levels have been found to be significantly higher in both colon and gastric cancer tissues when compared to normal full-thickness or mucosal layers of the colon and the stomach (26). The classification of BPH *versus* cancer gave excellent agreement with histopathology, despite the problem of different tissue compositions in the BPH samples (*i.e.*, glandular and stromal); this is indicative of the high sensitivity and robustness of the technique. The robustness of this technique was also demonstrated previously by analyzing colon spectral data acquired by our group in Winnipeg, Canada and by another research group in Sydney, Australia (19). By interchangeably using the data from one site as the training set and the data from the other site as the test set, classification accuracies of 73–96% were achieved despite different freezing and spectral acquisition protocols. In the present study of prostatic tissue, the sensitivity (100%) and specificity (95.5%) obtained by multivariate analysis are remarkably higher than those obtained by the conventional methods (81 and 80%, respectively).

Because the Gleason scores of the majority of the malignant biopsies (85%) were the same (*i.e.*, 3 + 3), no attempt was made to correlate the Gleason score with any spectral features/characteristics.

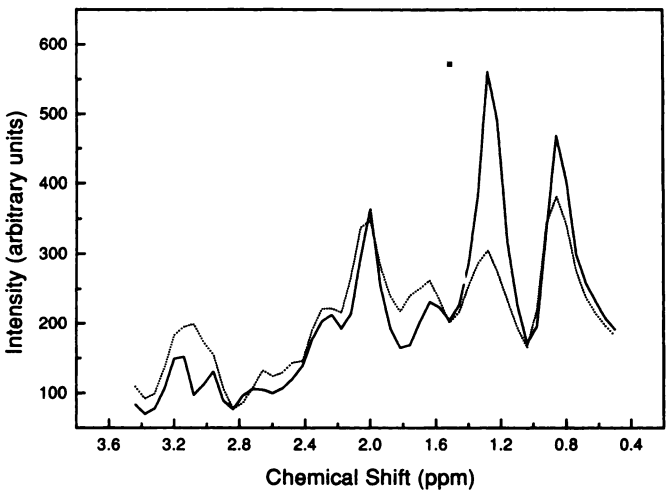

Fig. 3. Class averages of data point-reduced (450 to 50) ^{1}H MR spectra (360 MHz) of human prostate tissue. ·····, BPH; ——, cancer.

Prostatic intraepithelial neoplasia is believed by many to be a precursor of prostatic adenocarcinoma. There was only one case in our study in which low-grade intraepithelial neoplasia was observed. This case was purposely put in the test set and was classified as benign. The pathological report given on the case also indicated a benign diagnosis.

The above data were obtained on an analytical, high-resolution, 360 MHz MR instrument. Spectra of considerably lower resolution may be obtained *in vivo* on lower-frequency clinical MR instruments. A series of ^{1}H MR spectra of normal and cancerous prostate have been measured *in vivo* by Kurhanewicz *et al.* (23). They analyzed the data in terms of the relationships of the resonance due to citrate and the other resonances in the spectra. By using their methodology, shorter echo times to acquire more complete spectra, and the present analytical approach, it should be possible to characterize very accurately the state of abnormality of prostate tissue *in vivo*.

Conclusions. Citrate is a possible MRS marker for prostate cancer. Other regions of interest include the resonances due to taurine and glutamate. The application of sophisticated methods of data analysis to the MR spectra of prostate tissue yields a classification accuracy of 96.6% with a sensitivity of 100% and a specificity of 95.5% for cancer. The comparison of these results with those obtained from conventional analysis indicate the former to be superior. Moreover, ^{1}H MRS was also able to discriminate between BPH specimens of glandular and stromal tissue origins, a finding that may play a role in the proper management of BPH. Our results show that ^{1}H MRS, combined with multivariate analysis, gives very high sensitivity and specificity and thus can be reliably used to distinguish between benign and malignant prostatic tissue. Future plans include using this *ex vivo* information base to interpret/analyze *in vivo* prostate data.

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