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In Vitro Proton Magnetic Resonance Spectroscopy of Four Human Prostate Cancer Cell Lines

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ABSTRACT: There is accumulating evidence that some biochemical pathways observable by magnetic resonance spectroscopy, e.g., citrate acid and phospholipid metabolism, are altered in human prostate cancer. Four well-established human prostate cancer cell lines were therefore studied with magnetic resonance spectroscopy to compare differences in metabolic content with tumor biological behavior. Herein we demonstrate that, although each cell line has its own metabolic profile, relative creatine and citrate levels can be used to discriminate the androgen-dependent LNCaP cell line from the androgen-independent DU-145, TSU, and PC-3 cell lines.

KEY WORDS: **^{1}H MRS, human prostate cancer lines, metabolism, prostate cancer**

INTRODUCTION

The recent development and clinical introduction of transrectal probes not only improved the quality of magnetic resonance imaging (MRI) of the human prostate but also made this organ accessible to magnetic resonance spectroscopy (MRS) [1–4]. Transrectal MRI and MRS, basically employing the same instrumental setup, are potential powerful noninvasive methods for monitoring the diseased prostate, first, to identify with MRI the suspected lesion, and secondly, to monitor with MRS the metabolism of this lesion. Changes in various metabolic pathways associated with several cancers have been reported [5–7]. For prostate cancer, metabolic derangements might provide markers that improve diagnosis and allow more accurate prediction of its clinical behavior. In vitro and in vivo studies employing ^{31}P, ^{1}H, and ^{13}C MRS have shown that differences in the metabolic content of human prostate tissue samples correlated with tumor grade [3,4,8–14]. Moreover, for the Dunning R-3327 rat tumor model, it has been demonstrated that the relative levels of phosphocreatine, glycerophosphorylglycerol, glycerophosphoethanolamine, and glycerophosphocholine, obtained by ^{1}H and ^{31}P MRS, can be used to differentiate sublines by differentiation grade, androgen sensitivity, and metastatic capacity [15,16].

However, interpretations of differences in MRS spectra between human prostate tissues with different biological behavior are hampered by tissue heterogeneity and the lack of a complete understanding of biochemical pathways. MRS examinations of well-defined human model systems are therefore needed to allow correlations between metabolic patterns and biological behavior. For this reason, studies of human prostate (cancer) cell strains are of value. Yacoe et al. [17] investigated cell strains derived from human prostates to investigate whether ^{1}H MRS could reliably distinguish normal prostate epithelium from prostate cancer.[17] In the present study we attempted to identify differences in metabolites which correlated with different biological behavior, e.g., androgen responsiveness and morphology, in *human* prostate cancer. Four well-established human prostate cancer

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TABLE I. Characteristics of the Four Human Prostate Cancer Cell Lines Used

Cell line	Origin	Histology of original tumor	Doubling time (hr)	Androgen responsiveness	PSA-producing
LNCaP[15]	Lymph node	Moderately different	50.1 ± 8	+	+
DU-145[16]	Brain	Poorly different	28.7 ± 2.9	−	−
PC-3[17]	Bone	Poorly different	35.2 ± 6.7	−	−
TSU-PR1[18]	Lymph node	Poorly different	26.4 ± 4.5	−	±

cell lines were characterized with in vitro proton MRS, and metabolic differences were correlated with their biological behavior.

MATERIALS AND METHODS

Tumor Cell Lines

Four established human prostatic carcinoma cell lines, PC-3, DU-145, TSU-Pr1, and LNCaP, were used in this study, [18–21]. The characteristics of the four cell lines are summarized in Table I. Cells were maintained in RPMI 1640 culture medium (Life Science, Breda, The Netherlands) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 250 nM dexamethasone at 37°C in 6% CO_2 at 99% relative humidity.

Preparation of Tissue Extracts

After reaching a confluency of 70–80%, cells were harvested for perchloric acid (PCA) extraction as described previously [14]. In short, single-cell suspensions were obtained by trypsinization (0.25% trypsin/0.1% EDTA) and immediately washed three times with ice cold 0.9% NaCl to remove all medium components. The total number of cells varied between $4–6 \times 10^6$ cells. Viability, as determined by the trypan blue dye exclusion test, was always ≥95%. After centrifugation of the single-cell suspension, the cell pellet was frozen in liquid nitrogen and stored at −80°C until time of extraction.

The cell pellet was transferred into an all-glass homogenizer, and 4 ml ice-cold 0.25 M PCA were added dropwise. Cell homogenization was achieved at −80°C in approximately 30 min. After centrifugation (12,000 *g*, 15 min, 4°C) of the cell extract, the pellet was discarded and the pH of the supernatant was immediately adjusted to 7.5 with 2.5 M KOH. The PCA precipitate was centrifuged (12,000 *g*, 15 min, 4°C), and the supernatant was passed through a Chelex sample preparation disc (Bio-Rad Laboraties, Richmond, VA) and lyophilized. Lyophilizates were dissolved in 500 μl 40 mM potassiumphosphate, pH 7.0. The pH was corrected to pH 6.90–7.10 if necessary by addition of HCl or KOH. The samples were lyophilizated again and stored at −20°C.

Three-ml samples of culture media of all prostate cancer cell lines investigated were lyophilizated and stored at −20°C.

Just before the ^{1}H MRS measurements, the lyophilizate was carefully thawed and dissolved in 500 μl D_2O containing 1.6 mM 3-(trimethylsilyl) propionic acid-d4 sodium salt (TSP).

^{1}H NMR Measurements

^{1}H NMR spectra were acquired on a 500 MHz spectrometer (Bruker AM500) and were recorded with a standard 5 mm ^{1}H NMR probe. The spectra were recorded employing a 45° flip angle (6 μs) and a 7.28 sec pulse repetition time. The resonance of H_2O was suppressed by low-power continuous wave presaturation. For the spectrum of each PCA-extracted cell sample, 900 scans were accumulated. The chemical shifts were referenced with respect to the chemical shift position of the TSP resonance.

^{1}H NMR spectra were further evaluated employing the NMR1 package (New Methods Research, Inc., Last Syracuse, NY) on a SUN Sparc station 330 (Sun Microsystems, Inc., Mountain View, CA). Free induction decays (FIDs) were Fourier transformed after zero-filling from 16 K to 32 K and after the application of a Lorentzian to Gaussian transformation filter. The spectra were semiautomatically fitted to Gaussian lineshape model functions. Relative resonance integrals of proton metabolites of interest are expressed as ratios to the integral resonance of TSP.

Statistical Analyses

Differences in ratios of metabolites between the different cell lines were investigated by the Wilcoxons rank test. Due to multiple testing, $P<0.01$ was considered as statistically significant.

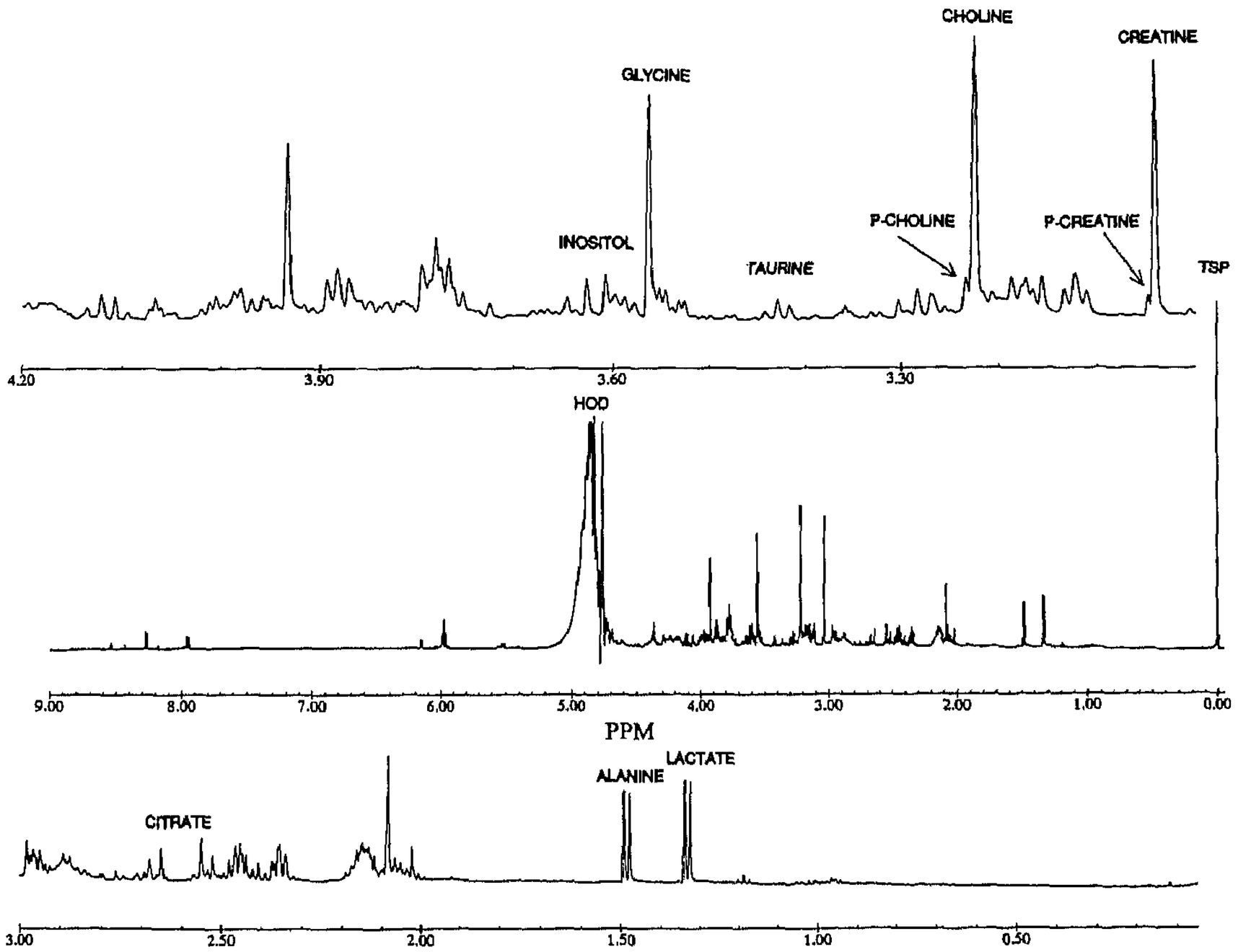

Fig. 1. In vitro ^{1}H MR spectrum with peak assignments of PCA extract of LNCaP human prostate cancer cell line.

RESULTS

PCA-Extracted Cell Lines

MR spectra of PCA extracts of four human prostate cancer cell lines were recorded, and for each cell line at least five different passages were investigated. A 500 MHz proton MR spectrum of a PCA extract of the LNCaP cell line, with two expanded parts, is shown in Figure 1. Resonances of some main metabolites could be identified with data from the literature [14, 22].

For each compound, the best resolved resonance(s) were selected for quantitation purposes. Table II lists relative peak area integral ratios involving the doublet of the CH3 group of lactate (Lac) at 1.33 ppm, the doublet of the CH3 group of alanine (Ala) at 1.49 ppm, the quartet of the CH2 group of citrate (Cit) at 2.54 ppm, the singlet of the CH3 group of (phospho)creatine at 3.04/3.05 ppm, singlets of the CH3 groups of phosphocholine (PC) and choline (Chol) at 3.23 and 3.22 ppm respectively, the triplet of the CH2 group of taurine (Tau) at 3.42 ppm, the singlet of the CH2 group of glycine (Gly) at 3.59 ppm, and the triplet of the CH group of inositol (Ino) at 3.63 ppm.

Several differences were found among MRS spectra of the PCA-extracted human cell lines.

The first prominent observation was of the relatively high amount of total creatine (TCr = PCr + Cr) in the PCA extracts of LNCaP cells (Table II). The TCr/TPS ratio was significantly higher for the LNCaP cells samples when compared with the other three cell lines ($P < 0.01$).

Secondly, citrate resonances were absent in the MR spectra of PCA extracts of the PC-3, DU-145, and TSU cell lines, whereas in only one of the five samples of the LNCaP cell line could citrate be detected in the MR spectra. Since citrate can rapidly diffuse from the prostate cell into the culture media, all media were also spectroscopically examined. ^{1}H MR spectra of the culture media of the LNCaP cells showed citrate resonances, whereas in all the media of the other three cell lines no citrate resonances were detected (Fig. 2).

Finally, several differences in metabolic content of PCA extracts of DU-145, TSU, and PC-3 cells were found. The relative amount of taurine was significantly higher for the DU-145 PCA cell extracts when compared with the PC-3 and TSU cell extracts (all $P<0.01$). PC-3 and TSU cell extracts differed significantly from each other for inositol and creatine content, whereas the creatine content of DU-145 PCA extracts was also different from that of TSU PCA extracts ($P<0.01$, Table II).

TABLE II. Mean of Metabolite Ratios (Range Between Parentheses) of Four Human Prostate Cancer Cell Lines

	LNCaP	DU-145	TSU-PR1	PC-3
Iac/tsp	94 (18–169)	34 (11–67)	25 (14–46)	12 (6–56)
Ala/tsp	84 (18–164)	11 (7–23)	6 (4–19)	5 (1–9)
Citr/tsp	0 (0–72)	0	0	0
TCr/tsp	60 (36–132)	9 (2–11)	0	10 (3–37)
Chol/tsp	40 (18–69)	12 (9–23)	22 (15–26)	16 (7–56)
PChol/tsp	6 (3–9)	5 (4–9)	4 (0–7)	3 (1–10)
Ino/tsp	0 (0–140)	156 (6–406)	0 (0–20)	44 (21–53)
Tau/tsp	11 (0–43)	84 (20–224)	0 (0–5)	0 (0–13)
Gly/tsp	48 (0–107)	7 (0–13)	5 (0–9)	6 (3–10)

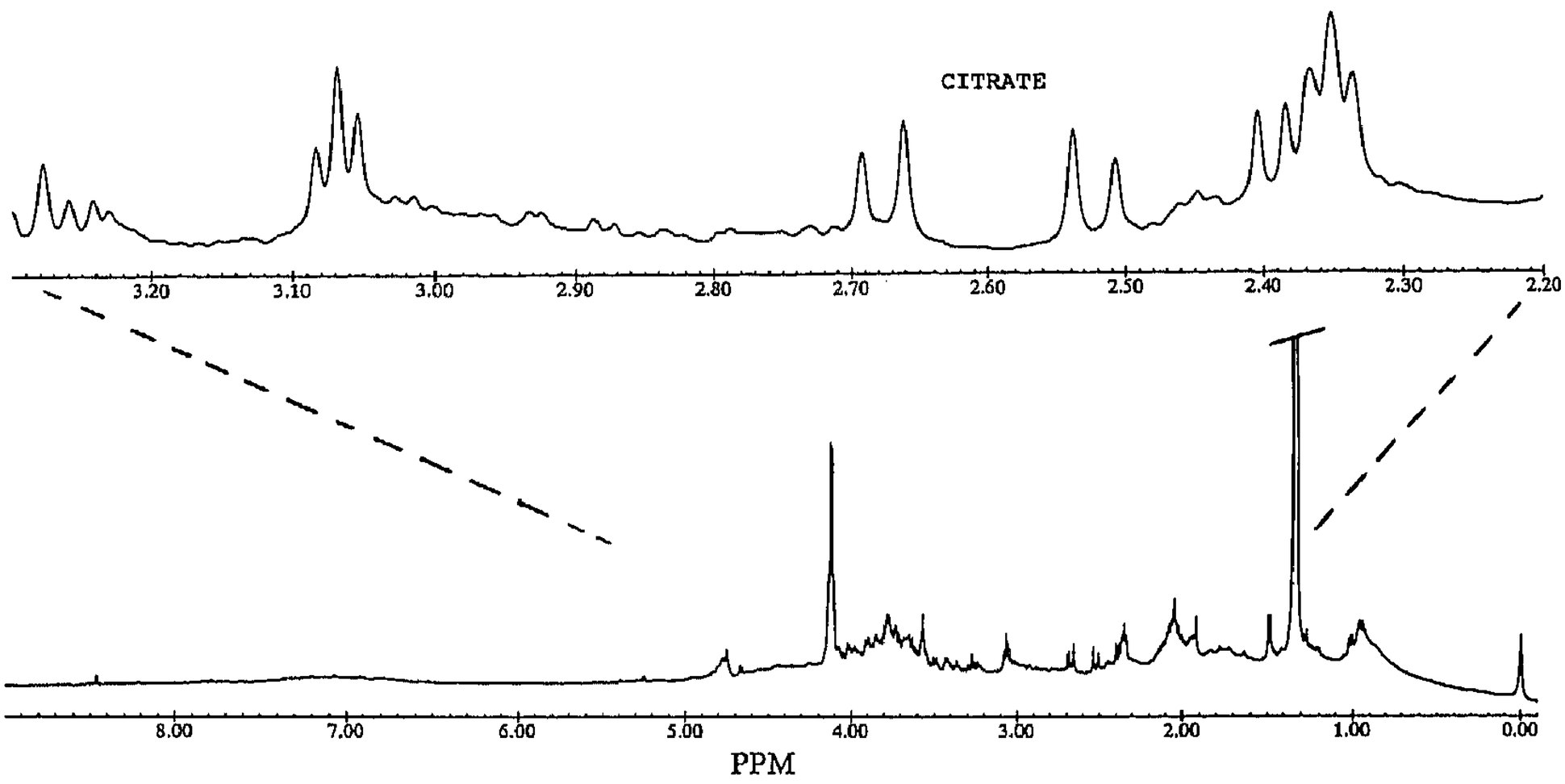

Fig. 2. In vitro ^{1}H MR spectrum of 3 ml culture medium of LNCaP human prostate cancer cell line. Citrate resonances are indicated.

DISCUSSION

Prostate tumors present sharp interindividual variations in biological behavior and response to therapy. At least 20–25% of the patients where hormonal treatment is the first choice therapy will not react, and an equal percentage become resistant to the treatment within 2 years [23,24]. Therefore, new diagnostic methods able to predict more accurately the biological behavior of the tumor are needed, since these may lead to a better basis for individual treatment. Several in vitro MRS studies have suggested a possible role for this noninvasive technique for improving diagnosis of prostate cancer [10–15]. Moreover, correlations were found for several metabolites and for the biological behavior of Dunning R-3327 rat prostate tumor sublines. In this study, four well-characterized human prostate cancer cell lines were studied with ^{1}H MRS to substantiate these findings. An in vitro approach was chosen because ^{1}H MRS of biological material may produce complex spectra, which are difficult to resolve in vivo. Assignments for the same main metabolites were made as found earlier for human prostate cancer [14].

In this panel of PCA-extracted human prostate cancer cell lines, the relative level of total creatine was found to be significantly increased for the hormone-sensitive LNCaP cell line compared to the three hormone-nonresponsive ones. This suggests a correlation for hormone sensitivity and the creatine content of the tumor. Vigneron et al. [16] found by in vivo ^{31}P MRS, an increased PCr/ATP ratio in one hormone-sensitive Dunning tumor subline when compared to one hormone-insensitive subline. Androgen deprivation (orchiectomy) resulted in a significant decrease of this ratio [16]. However, in a recent in vitro MRS study, where three different hormone-sensitive and four different hormone-insensitive Dunning sublines were investigated, no specific correlation for hor-

monal sensitivity with relative metabolite levels could be made [15]. A correlation of increased PCr concentration and hormone sensitivity has also been suggested for rat breast cancer [25]. Creatine levels can presently be detected in ^{1}H MR spectra recorded with transrectal probes; hence, in vivo human MRS studies can now test the relevance of these in vitro observations [26].

Previous biochemical and MRS studies have suggested that the metabolism of citrate may be altered in prostate cancer [12–14,27–30]. Citrate is a secretory product of the prostate, and its production and secretion are under androgenic control [28]. Testosterone stimulates the net citrate production and might also play an role in citrate secretion [28]. Normally citrate is present in human prostate tissue in high concentrations [27,28,30]. In culture, however, citrate can rapidly diffuse from the prostate cell in the culture media [27]. It is therefore obligatory to measure both citrate levels in prostate (cancer) cells, and in their culture media in studies with cultured prostate (cancer) cells. In the present study, citrate resonances were absent in the MR spectra of PCA extracts of DU 145, PC-3, and TSU cells, and in their culture media. Kurhanewicz et al. [12] have already shown that citrate signals could not be observed in ^{1}H MR spectra of DU-145 xenografts, either in vivo or in PCA extracts. In contrast, in one of the five LNCaP extracts, an MRS-detectable level of citrate was found, and all the media of LNCaP cells showed citrate resonances in the MR spectra, suggesting that the hormone-dependent LNCaP cells have a higher relative level of citrate as compared to the other three hormone-insensitive cell lines. Earlier studies already suggested that citrate is reduced in early prostate cancer and nearly absent in advanced disease, both of which are known to have only a small portion of hormone-sensitive cells [28–30]. Possibly there is a difference in citrate content between hormone-responsive and -nonresponsive prostate cancers. Our present findings seem to fit well with this hypothesis and the aforementioned studies. However, further in vivo MRS studies should be performed to compare the citrate concentrations in tumors of patients which are escaped from hormonal treatment with patients who do respond well, to validate this finding.

Finally, each prostate cancer cell line has its own metabolic fingerprint, i.e., several differences in metabolic content were found irrespective of their biological behavior. The most striking difference was the high taurine content of the DU-145 cell extracts as compared to both the PC-3 and the TSU cell extracts. Kurhanewicz et al. [12] also showed large taurine resonances in the in vivo and in vitro ^{1}H MRS spectra of the DU-145 tumor. The difference in taurine content might be explained by differences in origin of the metastasis [18–21].

In conclusion, this MRS study of PCA extracts of four human prostate cancer cell lines demonstrates that, although each cell line has its own metabolic fingerprint, the total creatine/tsp and citrate/tsp ratios can be used to discriminate the androgen-dependent LNCaP cell line from the other androgen-independent cell lines. The extrapolation of these findings to the clinic will not be so easy. Clinical MRS studies with transrectal probes are ongoing, but are complicated by several difficulties. The minimum volumes (voxels) within the prostate which can be measured with transrectal MRS are relatively large. Heterogeneity of the measured lesion, i.e., contamination of the cancer with normal prostate tissue and/or BPH, cannot be excluded. Smaller volumes and spectroscopic imaging methods will probably solve these problems. Secondly, a proper absolute quantification of the metabolites measured with in vivo MRS is still not possible.

The question remains as to whether MRS will be clinically applicable in the detection and staging of prostate cancer. Our results suggest that MRS might possibly play a role in the identification of hormone-responsive prostate cancers.

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REFERENCES

1. Schnall MD, Lenkinski RE, Pollack HM, Imai Y, Kressel HY: Prostate: MR imaging with a transrectal surface coil. Radiology 172:570–574, 1989.
2. Schnall MD, Lenkinski RE, Milestone B, Ressel HY: In "Society of Magnetic Resonance in Medicine, Ninth Annual Meeting, 1990," p 288.
3. Narayan P, Vigneron DB, Jajodia P, Anderson CM, Hedgcock MW, Tanagho EA, James TL: Transrectal probe for ^{1}H MRI and ^{31}P MR spectroscopy of the prostate gland. Magn Reson Med 11:209–220, 1989.
4. Narayan P, Jajodia P, Kurhanewicz J, Thomas A, MacDonald J, Hubesch B, Hedgcock M, Anderson CM, James TL, Tanagho EA, Weiner M: Characterization of prostate cancer, benign prostatic hyperplasia and normal prostates using transrectal ^{31}P magnetic resonance spectroscopy: a preliminary report. Urol 146:66–74, 1991.

5. Ross BD: The biochemistry of living tissues: examination by MRS. NMR Biomed 5:215, 1992.
6. Ruiz-Cabello J, Cohen JS: Phospholipid metabolites as indicators of cancer cell function. NMR Biomed 5:226–233, 1992.
7. Negendank W: Studies of human tumors by MRS: a review. NMR Biomed 5:303–324, 1992.
8. Sillerud LO, Halliday KR, Griffey RH, Fenoglio-Preiser C, Sheppard S: In vivo ^{13}C NMR spectroscopy of the human prostate. Magn Reson Med 8:224–230, 1988.
9. Sillerud LO, Halliday KR, Freyer JP, Griffey RH, Fenoglio-Preiser C: ^{13}C and ^{31}P NMR studies of prostate tumor metabolism. In Evelhoch JL, Negendank W, Valeriote FA, Baker LH (eds): "Proceedings of the 21st Annual Detroit Cancer Symposium." 1989, pp 149–179.
10. Fowler AH, Pappas AA, Holder JC, Finkbeiner AE, Dalrymple GV, Mullins MS, Sprigg JR, Komoroski RA: Differentiation of human prostate cancer from benign hypertrophy by in vitro ^{1}H NMR. Magn Reson Med 25:140–147, 1992.
11. Schick F, Bongers H, Kurz S, Jung W-I, Pfeffer M, Lutz O: Localized proton MR spectroscopy of citrate in vitro and of the human prostate in vivo at 1.5 T. Magn Reson Med 29:38–43, 1993.
12. Kurhanewicz J, Dahiya R, MacDonald JM, Chang L-H, James TL, Narayan P: Citrate alterations in primary and metastatic human prostatic adenocarcinomas: ^{1}H magnetic resonance spectroscopy and biochemical study. Magn Reson Med 29:149–157, 1993.
13. Schiebler ML, Miyamoto KK, White M, Maygarden SL, Mohler JL: In vitro high resolution ^{1}H-spectroscopy of the human prostate: benign prostatic hyperplasia, normal peripheral zone and adenocarcinoma. Magn Reson Med 29:285–291, 1993.
14. Cornel EB, Smits GAHJ, Oosterhof GON, Karthaus HFM, Debruyne FMJ, Schalken JA, Heerschap A: Characterization of human prostate cancer, benign prostatic hyperplasia and normal prostate by in vitro ^{1}H and ^{31}P magnetic resonance spectroscopy. J Urol 150:2019–2024, 1993.
15. Cornel EB, Heerschap A, Smits GAHJ, Oosterhof GON, Debruyne FMJ, Schalken JA: Magnetic resonance spectroscopy detects metabolic differences between seven Dunning rat prostate tumor sublines with different biological behavior. Prostate 25:19–28, 1995.
16. Vigneron DB, Hricah H, James TL, Jajodia PB, Nunes L, Narayan P: Androgen sensitivity of rat prostate carcinoma studied by ^{31}P NMR spectroscopy, ^{1}H MR imaging, and ^{23}Na MR imaging. Magn Reson Med 11:152–160, 1989.
17. Yacoe ME, Sommer G, Pheel D: In vitro proton spectroscopy of normal and abnormal prostate. Magn Reson Med 19:429–438, 1991.
18. Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA, Murphy GP: LNCaP model of human prostatic carcinoma. Cancer Res 43:1809–1818, 1983.
19. Stone KR, Mickey DD, Wunderli H, Mickey GH, Paulson DF: Isolation of a human prostate carcinoma cell line (DU 145). Int J Cancer 21:274–281, 1978.
20. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW: Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest Urol 17:16–23, 1979.
21. Iizumi T, Yazaki T, Kanoh S, Kondo I, Koiso K: Establishment of a new prostatic carcinoma cell line (TSU-PR1). J Urol 137:1304–1306, 1987.
22. Evanochko WT, Sakai TT, Ng TC, Rama Krishna N, Kim HD, Zeidler RB, Ghanta VK, Brockman RW, Schiffer LM, Braunschweiger PG, Glickson JD: NMR study of in vivo RIF-1 tumors: analysis of perchloric acid extracts and identification of ^{1}H, ^{31}P and ^{13}C resonances. Biochim Biophys Acta 805:104–116, 1984.
23. Catalona WJ, Scott WW: Carcinoma of the prostate. In Walsh PC, Gittes RF, Perlmutter AD, Stamey TA (eds): "Campbells Urology," Vol 2. Philadelphia: W.B. Saunders, 1986, pp 1463–1534.
24. Resnick MI, Grayhack JT: Treatment of stage IV carcinoma of the prostate. Urol Clin North Am 141–161, 1978.
25. Rodrigues LM, Midwood CJ, Coombes C, Stevens AN, Stubbs M, Griffiths JR: ^{31}P-Nuclear magnetic resonance spectroscopy studies of the response of rat mammary tumors to endocrine therapy. Cancer Res 48:89–93, 1988.
26. Heerschap A, De Jager G, De Koster A, Barentz J, De la Rosette J, Debruyne F, Ruijs J: ^{1}H MRS of prostate pathology. In: "Book of Abstracts, Annual Meeting, Society of Magnetic Resonance in Medicine, New York 14–20 August 1993." p 213.
27. Franklin RB, Costello LC, Littleton GK: Citrate uptake and oxidation by fragments of rat ventral prostate. Enzyme 22:45–51, 1977.
28. Costello LC, Littleton GK, Franklin RB: Regulation of citrate-related metabolism in normal and neoplastic prostate. In Sharma RK, Criss WE (eds): "Endocrine Control in Neoplasia." New York: Raven Press, 1978, pp 303–314.
29. Marberger H, Marberger E, Mann T, Lutwak-Mann C: Citric acid in human prostatic secretion and metastasizing cancer of the prostate. Br Med J [Clin Res] 1:835, 1962.
30. Cooper JF, Farid I: The role of citric acid in the physiology of the prostate: III. Lactate/citrate ratios in benign and malignant prostatic homogenates as an index of prostatic malignancy. J Urol 92:553, 1964.