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Magnetic resonance spectroscopy: A promising tool for the diagnostics of human prostate cancer?★

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Abstract

Background—Prostate Cancer (CaP) is one of the topmost diagnosed malignant diseases worldwide. In developed countries, early cancer detection methods have led to an increase of incidence rates over the last decades; however, with great variance of the prognosis. There is no diagnostic tool for an exact prediction of tumor aggressiveness, thus there is a lack of adequate and optimal treatment planning.

Methods—Electronic databases (Medline, PubMed) were scanned for scientific literature. Basic concepts of magnetic resonance spectroscopy (MRS), important results and its clinical applications were extracted and reviewed in this article.

Conclusions—MRS provides crucial information about the metabolic status of human prostate samples while preserving the specimens for further investigations. Single metabolites and metabolomic profiles can be quantified to distinguish benign from malignant tissue and to predict aggressiveness, such as the recurrence rates of CaP. Studies are also anticipating that MRS might be beneficially applicable for in vivo investigations in the future.

Keywords

Human prostate cancer; Metabolomics; Spectroscopy; MRS; MRSI; HRMAS

Introduction

Prostate Cancer (CaP) is the second most common malignant disease and the sixth leading cause of cancer death in men worldwide. The distribution of incidence is very heterogeneous and the highest rates were found in developed regions such as Australia, Western and Northern Europe, and Northern America [1].

Thus CaP is the most frequently diagnosed malignant disease of adult males in the U.S.A. and the latest cancer statistics estimate 217,730 new cases for 2010. With more than 32,050 prostate cancer associated deaths expected for 2010, it is furthermore the second leading

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cause for cancer associated mortality in men in the U.S.A. [2]. The most important reason for higher incidence rates seems to be the widely held implementation of particular early cancer detection strategies in developed countries. Screening has been found to be rising rapidly in the early 1990s, after the prostate specific antigen blood test (PSA) was established [1].

Tumor screening requires diagnostic tests to be performed in the absence of any symptoms or indications of disease. The aim of these tests is to identify cancers at an early and well treatable stage and therefore increasing the chances of successful treatment. However, it is still under discussion if the patient's benefit from PSA screening justifies possible harms from further examinations or treatment. Acknowledging that there is no true PSA cutoff point distinguishing cancer from non-cancer, the traditional PSA level of 4.0 ng/mL is currently recommended as a reasonable threshold for further evaluation. Nevertheless, benign prostate hyperplasia (BPH) and chronic prostatitis are also able to increase PSA serum levels, leading to diagnostic uncertainty. High false positive rates and inequalities are currently leading to overdiagnosis and overtreatment [3]. Recent systematic reviews of American studies failed to find any significant decreases in death rates for screened or not screened CaP Patients [4,5]. Even though a European trial indicated that PSA-based screening might reduce mortality rates, they also highlighted a high risk of overdiagnosis [6,7].

If the PSA screening reveals abnormal findings, it is usually followed by trans-rectal ultrasound (TRUS) guided biopsy and subsequent histopathologic evaluation of these prostate needle biopsies (PNBs). Ultrasound ensures only that the randomly taken biopsy samples are from different zones within the prostate. It is not yet utilizable to detect cancerous lesions with an adequate certainty. Given the heterogeneous distribution of cancer foci in the organ, those biopsy results are affected by high false negative rates [8].

Treatment for biopsy proven prostate cancer includes active surveillance/watchful waiting but also procedures with a high potential for side effects such as conventional surgery, cryosurgery, radiotherapy, brachytherapy, hormonal treatment, high-intensity focused ultrasound (HIFU), chemotherapy, or a combination of these treatments. Today, more than 150 prostatectomies are performed each day in the U.S.A. Moreover, it is estimated that about 67% of the screening detected cancers would not have led to the patient's death if it had been left undetected. Depending on the treatment, the adverse effects are serious and may lead to an impairment of life quality as well as to a reduced life expectancy [9–11].

However, the current recommendation of the American Cancer Society includes PSA testing for asymptomatic males older than 50 years after a process of informed decision-making with their health care provider. Men at higher risk, including African Americans and men with positive family history, should receive this information and the screening to follow beginning at age 45. Early cancer detection is not recommended for men whose life expectancy is less than 10 years [12].

Latest research indicates that magnetic resonance is a promising tool for both detection and characterizing of lesions within the prostate. MRS might lead to the ability to distinguish

accurately between indolent and aggressive cancer types. It might also be an efficient in vivo method for an exact localization of malignant areas in human prostates. Spectroscopy will therefore hopefully contribute to reduce the adverse effects of prostate cancer treatment sufficiently to tip the balance clearly in favor of early cancer detection.

Basics of NMR techniques

Cancer in general, and prostate cancer in particular, often shows a great variety in rate of growth, tendency for dissemination, capability for therapeutic approaches, and recurrence rates. A major trend in oncology is therefore personalized treatment protocols. An individual-based treatment planning requires an exact evaluation of the cancer type and localization, profound knowledge about cancer metabolism, and a sensitive treatment monitoring.

Magnetic resonance (MR) in medicine provides morphologic information as well as information about the specimen's metabolic status. It is a noninvasive tool used in vivo and in contrast to traditional X-ray or computer tomography (CT) scans it does not apply any ionizing radiation. Both MR imaging (MRI) or MR spectroscopy (MRS) are based on the principles of nuclear magnetic resonance (NMR), where protons in strong magnetic fields are stimulated by radio frequency pulses (rf). In the clinical MRI and in most studies, hydrogen (^1H) and sometimes carbon (^{13}C) are used. The rf stimulation causes a change in the vector of their precession movement which induces a change in their absorption frequency that can be detected by the scanner. In MRI, it is this signal and the protons relaxation time, which differ in different types of tissue, and which is being transformed into an image by encoding signal strength and time into different levels of gray. In MRS, the signal intensities associated with different frequencies are being displayed in a spectrum after converting signal decays in the time domain into individual spectra using Fourier's transformation in the frequency domain. The property that a single and "naked" proton would resonate at lower field strengths than the nuclei of covalently bonded hydrogen is hereby used to distinguish between different molecular structures. The MR spectrum of nuclei in an idealized strong and homogeneous magnetic field would be shaped like a single line. Under real conditions, the spectral resolution is affected by an increased width of those peaks with rising inhomogeneity and decreasing strength of the external magnetic field. Also, the location of different NMR resonance signals is dependent on both field strength and rf frequency. Since 2 magnets will never have the exact same field, resonance frequencies vary accordingly. The resulting chemical shift is represented on the x-axis in a diagram, while the signal intensity is usually displayed on the y-axis.

Initial demonstration of HRMAS ^1H MRS for human prostate cancer analysis

It has been demonstrated, 10 years ago, that histopathologic and biochemical data can be obtained from the exact same prostate tissue specimen. In this particular study, the critical observation was spermine. Spermine, a member of the polyamine family, has already been reported to play a role in carcinogenesis of CaP. With its symmetrical structure containing 5 types of methylene groups, spermine in tissue has as yet been hard to detect with

conventional MRS due to overlaps with signals from different amino acids, creatine, and choline compounds.

Under ex vivo conditions with small tissue samples, it is possible to minimize the line width and to maximize spectral resolution. Very high field strengths (7–14.1 Tesla) are applied, and the specimen is rotated around an axis, which is tilted in an angle of 54.74° in respect of the field's direction. This particular method is called high resolution magic angle spinning (HRMAS) and was invented for intact tissue analysis in the late 1990s [13]. It was the first time that a solid state NMR method was used in biological semisolid samples, which allowed an assessment of metabolite status while preserving tissue architecture for subsequent histopathology analysis.

Reducing the spectral line widths with HRMAS, this was the first time when spermine could be quantified with MRS for tissue samples. Its influence on CaP has been studied by investigating malignant ($n = 3$) and benign ($n = 13$) human prostate samples. The results presented a significant linear correlation between spermine and citrate ($P < 0.0001$), a secretory product of the prostate that is produced under androgenic control. Also as a premiere statistical significant correlation for both spermine ($P < 0.018$) and citrate ($P < 0.001$) with the volume percentage of benign prostatic epithelial glands of CaP patients were reported [14].

Assessing cancer-specific metabolites in human prostate needle biopsies using HRMAS ^1H MRS

Assessing the metabolite status of biopsy samples is an important matter of clinical concern. Two studies were using tissue obtained from PNB. Single metabolites and metabolite ratios were analyzed for their potential to distinguish malignant from benign tissue [15,16]. A Dutch collaboration examined samples from 48 patients performing HRMAS MRS on a 500 MHz spectrometer. Immediately after defrosting the tissue, deuterium oxide (D_2O) and phosphate buffered saline (PBS) were added, and the specimen was scanned at a 5 kHz spinning rate. The tissue was again snap-frozen, cut, fixed, and stained with hematoxylin and eosin (H and E) for subsequent histopathology. Possible damages of the samples by magic angle spinning did not interfere with the pathologic diagnosis, and the tissue was either classified as benign ($n = 30$) or malignant ($n = 18$). The group found a significant increase in the ratios of choline/citrate, choline/creatine, lactate/alanine, and glycerophosphocholine/phosphoryl choline in malignant compared to benign cases ($P < 0.05$), while a decrease was found for citrate/creatine ($P < 0.05$), and the ratio of choline + creatine/citrate was also reported to be different in cancerous versus benign samples ($P < 0.05$). Furthermore, a relation between spectroscopy results and the tumor grading could have been shown in this article. Using Spearman's rank analysis, positive significant correlation coefficients could have been found for the ratios of glycerophosphocholine + phosphocholine/creatine, choline/creatine, citrate/creatine, total choline/citrate, and choline + creatine/citrate ($P < 0.05$) and the Gleason score. By analyzing linear regression, significant correlations were described between the ratios of choline/creatine and citrate/creatine and the Gleason score. The linear regression correlation between lactate/alanine and citrate + choline/creatine was found to be minimal and not significant ($P = 0.37$ and $P =$

0.95). Nevertheless, they indicated that a contamination of the tissue sample with ethanol or ultrasound gel might disturb the spectra analysis (Fig. 1) [16].

A larger population was examined in another study, where 96 out of 130 PNB samples obtained from 82 patients were scanned at 11.7 T in a 500 MHz spectrometer to determine the concentrations of lactate and alanine. Here, 25% of the samples were unusable for investigation due to contamination with periprostatic fatty tissue or a local anesthetic (Hurricane, Beutlich, Waukegan, IL), which was applied prior to the PNB procedure. They added 3 μ l deuterium oxide and 0.75 wt% sodium-3-trimethylsilylpropionate (D_2O + TSP) before scanning the sample with a 2250 Hz spin rate. The tissue was then prepared as described above, and subsequent pathology revealed 82 benign and 16 malignant biopsies. While the concentrations of lactate and alanine were found to be very low in benign samples, the findings are remarkable for a highly significant increase for both lactate and alanine in malignant tissue ($P < 0.0001$) (Fig. 2). Due to the low total case number and the fact that only 6 out of 16 malignant samples revealed a Gleason score greater than 6, it was not possible for Tessem and colleagues to determine a correlation between lactate and/or alanine concentrations and cancer grade. Also there were no significant differences between benign samples taken from patients with ($n = 38$) and without ($n = 44$) at least 1 positive clinical BNP detected. These results indicate that biopsies without cancerous glands can be used as controls even if they were taken from prostates with cancer proven biopsies. This study also examined the metabolite levels in the different kinds of benign prostate tissue, and there were no significant differences between alanine levels ($P > 0.05$) and between lactate levels ($P > 0.05$) and predominantly glandular ($n = 54$) vs. stromal ($n = 28$) tissue. However, they discussed if benign pathologic processes such as BPH or prostatitis are confounding the results [15].

Further studies with higher numbers of cases and first of all more cancer cases in relation to non-cancer cases may be required to separate confounding factors by their metabolic patterns. Due to the small size and the heterogeneous distribution of malignant areas within the prostate, an important step might be the detection of suspect lesions prior to the biopsy. For a more effective treatment planning, a longterm investigation might be interesting to gain insight into the relationships between metabolism and tumor aggressiveness.

Developments of the technology

Since 2001, multiple publications evaluated individual metabolites and metabolic profiles in human prostate tissue samples obtained from prostatectomy. Studies were performed to get a better understanding of metabolism in benign and malignant prostates, to improve the technique, and to develop a tool that is useful in clinical routine.

To preserve a better quality of the tissue for further pathologic examination, it has been attempted to reduce the sample spinning rates. The aim was to test if metabolic information obtained with scanning at low spinning rates (600 Hz, 700 Hz) is as accurate as scans at higher spinning rates, such as 3 kHz and above, and if tissue histology quality would be better preserved with the low frequency method. Thirty-five samples were scanned with slow and high speed spinning, with subsequent histopathology performed on each sample.

Although it has been shown that the tissue was more affected by high speed than by slow speed spinning, a differentiation between benign and malignant tissue was in either way still possible (Fig. 3). At slow spinning rates, HRMAS single pulse spectra of tissue samples are dominated by large water peaks and its spinning side bands (SSB). The authors of the study were able to show that SSBs could be ignored by selecting different spectral regions measured from spectra of different spinning rates [17]. In a more recent study, it was shown that SSBs could be successfully suppressed by editing the 2 spectra obtained at different slow spinning rates with a simple minimum function. Thirty-one postsurgical tissue specimens and solutions of common metabolites as standards were again examined with HRMAS MRS at different spinning rates from 250 Hz to 3.2 kHz. Statistically significant linear correlations were observed from the edited spectra measured at low spinning rates with concentrations measured at 3 kHz. The slow spinning spectra were reported to be sensitive enough for the purpose of disease diagnosis [18].

The signal to noise relation was studied by comparing rotor-synchronized adiabatic pulses (WURST-8 mixing scheme) with the conventional hard pulse scheme (MLEV-17) in total correlation spectroscopy (TOCSY) for solutions and solid prostate tissue. According to the authors, WURST-8 pulses produced significantly greater absolute cross peak signal intensities. In addition, it has been reported that WURST-8 was able to display cross peaks that were not observed in MLEV-17 schemes, including cross peaks corresponding to choline- and ethanolamine containing metabolites [19]. To develop a straightforward approach for the MRS analysis of prostate tissue, researchers of the same group for the first time published the T1 and T2 relaxation times for the most common prostate metabolites after scanning 60 samples in a 11.7 spectrometer. Polyamines such as spermine and citrate were reported to have the shortest relaxation times, while taurine and choline presented the longest relaxation times. Also the relaxation times for all metabolites were measured at different temperatures (1, 20, and 37°C); they were found to be increased with the temperature. This indicates that HRMAS MRS might be performed with faster repetition and acquisition times at lower temperatures. Similar to this, it was found in serial measurements that metabolite degradation was minimized at the coldest possible temperature without freezing [20].

To evaluate possible freeze-thaw effects on NMR measurements, 2 studies investigated the impact of different storage habits on the quality of the samples. In the first study, 12 samples were analyzed fresh and after they were frozen overnight (12–16 hours). The samples were scanned on a 14.1 Tesla spectrometer at 600 and 700 Hz with and without using rotor-synchronized DANTE pulse-sequences. Also, subsequent histopathology was performed to detect possible freezing-thawing effects on histologic evaluation. It was reported that tissue storage impacts tissue quality for both pathology and spectroscopy. The spectral line widths were found to be identical for all metabolites except citrate and acetate. The differences of mean intensities of the signals for each metabolite were in this study found to be less than 30%. However, the relative concentrations were less affected by storage than the absolute concentrations. While the quality of frozen tissue was compromised, fresh tissue that has been spun first at 600 Hz and later at 700 Hz were reported to produce histologic images that were similar to those produced from original fresh tissue [21].

The impact of storage duration was tested in the study that followed. Taken from the exact same cases as in the study cited above, 15 samples were stored at -80°C for 32 months and analyzed in 2005 following the same protocol as in 2002 [21]. These findings indicate that long-term storage of prostate tissue does not lead to metabolite alterations, which are detectable with current MRS techniques. Even though there were no cancerous glands found in the examined tissue, the likelihood seems to be high that malignant samples can also be quantitatively analyzed by HRMAS-MRS after long term storage without concerns regarding changes in metabolite profiles [22].

Prostate tissue following HRMAS MR spectroscopy is still utilizable for quantitative histopathology. Although it has been reported that biopsy tissue is more fragile than post-surgical tissue, it was also shown that the mRNA integrity is not significantly affected. The samples can therefore still be used for genetic microarray analysis [23]. Whilst still putting a lot of effort in improving MRS techniques, several researchers also investigated single metabolites and metabolite profiles in tissue samples obtained from prostatectomy. Two groups used two-dimensional (2D) HRMAS-MRS designs to assess cancer specific characteristics in prostate metabolism. On an 11.7 Tesla magnet, 47 samples (benign $n = 32$, malignant $n = 15$) were examined at 2250 Hz addressing choline and ethanolamine containing metabolites. All the samples were histopathologically interpreted within about 1 hour following the spectroscopy procedure. To quantify metabolite concentrations in tissue, a phantom solution was also scanned. In this 2D setting TOCSY was reported to be more capable in resolving and quantifying overlapping peaks compared with conventional correlation spectroscopy (COSY). In 73% of all malignant cases, but only in 19% of all benign tissues, phosphocholine was found. Furthermore, phosphocholine ($z = 3.5$) was found to be significantly increased in cancerous v noncancerous samples. Contrastingly, ethanolamine ($z = 3.3$) was significantly lower in malignant tissue. Glycerophosphocholine, phosphoethanolamine, and glycerophosphoethanolamine were elevated in malignant samples, as well as the ratios of phosphocholine/glycerophosphocholine, phosphocholine/phosphoethanolamine, phosphoethanolamine/ethanolamine, and glycerophosphoethanolamine/ethanolamine [24].

With a similar study design 54 nonmalignant and 27 malignant samples were used to investigate the presence of polyunsaturated fatty acids (PUFAs). PUFAs have previously been shown to promote prostate cancer which indicates an effect of dietary habits on cancer formation. In the cited study HRMAS NMR was proven to be a powerful method to identify and characterize PUFAs in tissue samples. Omega-6 PUFAs could be thereby found in 15% of the examined tumors [25].

Furthermore, spermine, the mRNA expression levels of enzymes in the spermine metabolic pathway, and the PSA-velocity were analyzed using MR spectroscopy, histopathology, laser-capture microdissection, and real-time polymerase chain reaction (rt-PCR) [26].

Towards clinical application

Most of the prostate tumors are not palpable or macroscopically visible within the organ. In addition, cancerous regions are, as already described above, often small sized, wide spread,

and heterogeneously distributed. With the aim to get a better ratio of benign vs. cancerous tissue samples, in another publication *in vivo* 3-D-MRSI was used prior to prostatectomy to identify cancerous spots. Immediately after surgery, the prostates were inked and sectioned before the samples ($n = 54$) were taken from the targeted foci. The overall accuracy for the MRI/3D-MRSI targeting of the biopsy was reported to be 81%. Subsequent histopathology revealed 33 samples without cancer and 20 malignant tissue specimens. Using HRMAS spectroscopy at 9.4 Tesla prior to the pathologic examination, histobenzene tissue could have been discriminated from cancer cases. The levels of citrate ($P = 0.04$) and polyamines ($P = 0.01$) were found to be decreased in cancerous tissue, while the levels of choline containing compounds ($P = 0.02$) such as choline, phosphocholine, and glycerophosphocholine were elevated. The ratios of total choline, free choline, and glycerophosphocholine plus phosphocholine over creatine were reported to be significantly increased in tissue containing more than 20% malignant areas vs. healthy predominantly glandular ($P = 0.0163$, $P = 0.0406$, $P = 0.0298$), and healthy predominantly stromal tissue ($P = 0.0069$, 0.0024 , 0.0442). Choline and its components were also found to be elevated in Gleason 7 vs. Gleason 6 tumors although the results were not significant, which, according to the authors, is due to the small number of cases. Lack of citrate and polyamines could also be found in predominantly stromal tissue, which was nevertheless distinguishable from cancer by significantly lower levels of choline compounds ($P = 0.01$) (Fig. 4). The citrate/creatine ratio was reported to be significantly higher for healthy predominantly glandular versus healthy predominantly stromal tissue ($P = 0.0011$), samples containing less than 20% cancerous glands ($P = 0.017$) and samples containing more than 20% prostate cancer ($P = 0.05$). There were no significant differences between citrate/creatine in tumors with Gleason 6 vs. Gleason 7. Also the ratios of polyamine/creatine were found to be elevated in healthy glandular tissue compared with healthy stromal tissue ($P = 0.0013$), tissue containing <20% cancer ($P = 0.017$), and tissue with more than 20% cancer ($P = 0.013$). Furthermore, a correlation of larger increases in choline and decreases in citrate and polyamines ($P = 0.05$) with increased cancer aggressiveness was present. However, they were also reporting problems with the interpretation of cancer spectra, due to cancer heterogeneity, different amounts of glands and stroma, as well as nonmalignant pathologic conditions like chronic and acute prostatitis [27]. The findings for changes in metabolite concentrations and metabolite ratios as measured with HRMAS-MRS are summarized in Table 1.

The knowledge about individual metabolites led to the analysis of large ensembles of metabolites. These metabolomic profiles of prostate cancer have the potential to be effective tools for the characterization of prostate cancer, and might be useful even at low magnetic field strengths.

The diagnostic utility of these profiles has been studied with prostatectomy tissue samples ($n = 199$), obtained from 82 CaP patients. These tissue samples were measured with HRMAS-MRS on a 14.1 Tesla NMR scanner and subsequent histopathology. Cancerous glands were found in 20 of the 199 cases. The remaining samples ($n = 179$) were histologically benign specimens taken from cancerous prostates. The metabolite profiles were evaluated using principal component analysis of the spectroscopy results. They were later correlated with the results of the pathology reading by using linear regression analysis. The metabolomic

profiles calculated from resonances of 36 different single metabolites were found to be sensitive in differentiating benign from malignant tissue ($P < 0.0001$) with an overall accuracy of more than 98%. It has also been reported that histobenzign samples, which were obtained from cancerous prostates with a Gleason score of 6 and 7, might be able to encircle a subset of less aggressive neoplasia ($P < 0.008$) and to predict a perineural invasion of the tumor ($P < 0.03$). Selected principal components calculated with spectral data from benign samples were thereby able to distinguish between prostate confined cancer cases and cancer cases that invaded extraprostatic tissue with statistical significance. Furthermore, a correlation of metabolite profiles and PSA-blood levels could be detected ($P < 0.006$) [28].

Prostate cancer biochemical recurrence (BCR) after prostatectomy is defined as an increase of serum PSA to the detectable level of > 0.2 ng/ml after initial surgical treatment. A recent retrospective study has demonstrated that the assessment of metabolomic profiles may be used for the prediction of prostate cancer BCR. Spectroscopy and pathology data of CaP samples ($n = 48$) were obtained from prostatectomy cases ($n = 183$ cases). The cases of BCR ($n = 16$) were matched with cases ($n = 32$) without biochemical recurrence. The 27 most intense resonance peaks were reported to be used for principal component analysis calculations. The components were correlated to the amount of epithelium, cancer, and stroma, and matched in order of their clinical stage to correlate these groups with the BCR group. With an accuracy of 71%–78% depending on the components, the results indicated that HRMAS-MRS might be useful to estimate the CaP recurrence potential [29].

The new frontier

To implement the CaP metabolomics for in vivo examinations in clinic a downgrade of the magnetic field strength would be necessary. A preliminary test has been presented with prostatectomy-removed whole prostates ($n = 5$) in a 7 Tesla whole body magnet. Localized, cross sectional multivoxel spectra were measured and analyzed according to CaP metabolomic profiles acquired in previous studies of tissue specimens with HRMAS-MRS in a 14 Tesla scanner. A malignancy index calculated from these data was compared with pathology results of the whole mounted organs at the approximately same location. This study demonstrated a linear correlation of a calculated malignancy index with the size of the cancerous lesion ($P < 0.013$) and a 93%–97% overall accuracy for the detection of prostate cancer foci (Fig. 5). The validity of the method was tested with the phantom of known metabolite concentrations [30].

Along the same line of reasoning as metabolomic imaging concept presented above, where the emphasis is on the map of diseases rather than the images of individual physical or chemical parameters, a recent trend of imaging researches has evolved towards the concept of multiparametric or multimodal MRI. By including T2-weighted and dynamic contrast enhanced (DCE) MRI with MRSI with other MRI methodologies, results showed great clinical potential for prostate cancer detection and characterization [31–37]. Thus, the final images from such multimodal approach will produce the maps of medical conditions rather than the images reported by individual modal.

Conclusions and future perspectives

Magnetic resonance spectroscopy is a unique tool that provides biochemical information of specimens while preserving the tissue for further investigations, and it is applicable for in vivo examinations. MRS is noninvasive and does not apply ionizing radiation.

The results of all cited studies suggest that MRS might deliver useful additional information for the diagnosis of prostate cancer. It can be applied ex vivo for the evaluation of prostate needle biopsies and of samples obtained from post-prostatectomy organs. It has been shown that MRS is able to distinguish between benign and malignant human prostate tissue. In addition, there is a high potential for the measurement of single metabolite concentrations, metabolite ratios, and metabolomic profiles to provide useful information concerning the estimation of cancer growth, cancer aggressiveness, and recurrence potentials. Further investigations should focus on new techniques for the detection of malignant foci prior to the biopsy procedure to increase the number of samples containing cancerous glands at different stages.

Furthermore, it was shown that spectroscopy data can be obtained from scanning the whole organ and can be combined with morphology data at lower field strengths. These results indicate that in the future, MRS might also be applicable for in vivo diagnostics. However, further studies are still needed to move this bench-top technique to bed-side routine.

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Fig. 1. (A) ¹H HRMAS NMR spectrum of prostate needle biopsy contaminated with echo gel signals. (B) ¹H HRMAS NMR spectrum of echo gel. Reprinted with permission from van Asten JJA et al.¹⁶

Fig. 2.

Representative ^1H -MAS spectra and corresponding histopathologic sections of (A) benign predominantly glandular (40% glandular, 60% stroma) and (B) prostate cancer (70% Gleason 3+3) biopsy tissues. The major metabolites, TSP, and ERETIC resonances are shown. Lactate to alanine regions from 144-ms CPMG spectra are shown inset. Reprinted with permission from Tessem M-B et al.¹⁵ (Color version of figure is available online.)

Fig. 3.

The effects of HR-MAS stress on tissue morphology. (A) Human prostate tissue sample taken directly from a tissue bank without HR-MAS testing. The tissue showed highly organized ductal cellular structures with well-defined epithelial layers. (B) Another sample from the same patient but after an HR-MAS experiment, which involved spinning at 600 Hz for 45 min and then 700 Hz for 15 minutes. The tissue still exhibits a normal ductal structure that cannot be differentiated from the original sample (A). (C) Sample, again from the same clinical case, after MAS analysis at higher spinning frequency, 3.0 kHz, for 1 hour. The tissue ductal structures are visibly distorted compared with the natural specimens. (Images presented at the same magnification.) Reprinted with permission from Taylor JM et al.¹⁷ (Color version of figure is available online.)

Fig. 4.

Ex vivo ^1H HR-MAS spectra show the choline-to-citrate region and corresponding H&E staining patterns of excised tissue samples containing predominantly prostate cancer (A), benign predominantly stromal tissue (B), and benign predominantly glandular tissue (C). Reprinted with permission from Swanson MG et al.²⁷ (Color version of figure is available online.)

Fig. 5.

Correlations of metabolomic profiles with histology. (A) Sizes of T2-stage tumors correlate linearly and positively with values of the malignancy index ($r^2 = 0.975$, $P < 0.013$). Cancer lesions are shown as [filled square], other T2 tumors as [filled circle], and the T3 tumor as [filled diamond]. (B) Significant inverse linear relation exists between the average intensities of metabolomic profiles for voxels in the involved profile-elevated regions and the weighted distances (WD_{c-c}) ($r^2 = 0.998$, $P < 0.001$) for T2 tumors. This indicates that the closer the histologically and metabolically represented voxel centers, the stronger the cancer profile signal in the metabolomic map. (C) The malignancy index provides a threshold indication of malignant potential for profile-elevated regions with and without histologically identifiable cancer ($P < 0.008$, for all tumors; $P < 0.004$, for T2 tumors); overall accuracies are presented by the ROC curves, (D) for all tumors and for T2 tumors only. AUC = area under curve. Reprinted with permission from Wu C-L et al.³⁰

Table 1

Observed changes in metabolite concentrations and ratios in malignant vs. benign human prostate tissue samples

	Observed changes in CaP	Reference
Single metabolites		
Lactate	Increase	[16]
Alanine	Increase	[16]
Citrate	Decrease	[27]
Phosphocholine	Increase	[23]
Ethanolamine	Decrease	[23]
Choline	Increase	[27]
Phosphocholine	Increase	[27]
Glycerophosphocholine	Increase	[23,27]
Phosphoethanolamine	Increase	[23]
Glycerophosphoethanolamine	Increase	[23]
Polyamines	Decrease	[27]
Metabolite ratios		
Choline/citrate	Increase	[16]
Choline/creatine	Increase	[16]
Lactate/alanine	Increase	[16]
(Glycerophosphocholine + phosphocholine)/creatine	Increase	[16]
Citrate/creatine	Decrease	[16]
(Choline + Creatine)/Citrate	Decrease	[16]
Phosphocholine/glycerophosphocholine	Increase	[23]
Phosphocholine/phosphoethanolamine	Increase	[23]
Phosphoethanolamine/ethanolamine	Increase	[23]
Glycerophosphoethanolamine/ethanolamine	Increase	[23]