A decade in prostate cancer: from NMR to metabolomics

Elita M. DeFeo, Chin-Lee Wu, W. Scott McDougal and Leo L. Cheng

Abstract | Over the past 30 years, continuous progress in the application of nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance spectroscopic imaging (MRSI) to the detection, diagnosis and characterization of human prostate cancer has turned what began as scientific curiosity into a useful clinical option. In vivo MRSI technology has been integrated into the daily care of prostate cancer patients, and innovations in ex vivo methods have helped to establish NMR-based prostate cancer metabolomics.

Evaluation of the technique for human imaging became clear. This advance, which led to a new wave of radiological studies, followed the 1970s transformation of planar radiography into three dimensional CT. The capacity of the NMR signal to generate, via magnetic field gradients, three dimensional (3D) images to visualize physiological and pathological structures inspired the rapid development of an independent radiological discipline—MRI (the term 'nuclear' was dropped, possibly to reduce public panic and patient resistance). MRI (emphasizing the type of image collected), rather than NMR (indicating the site of signal origin), is now the standard term recognized by patients as a diagnostic imaging technique. In this Review, unless specified otherwise, NMR and MRS or MRI will be used interchangeably to refer to the spectroscopy and imaging of nuclear protons.

The systematic investigation of tissues and biofluids, using regular NMR tubes and conventional solution NMR methods for disease detection and diagnosis, also began in the mid-1980s, parallel to the development of MRI for in vivo diagnosis. Ironically, the seminal study in the area, which proposed the detection of breast cancer through a blood-based NMR test, is now considered highly problematic.

Although the study itself was not reproducible, the ensuing debate and discussion meant that the concept it described—using NMR to measure cellular metabolites in tissue and biofluids to characterize disease—took hold within the NMR community. During the late 1990s, an important milestone was achieved when solid-state NMR methodology—magic angle spinning (MAS)—was applied to the analysis of intact biological tissue. MAS subjects samples to fast mechanical rotation at a specified angle from the magnetic field. This technique, now further refined as high resolution magic angle spinning (HRMAS) is different from its solid-state NMR origin, as it permits the quantification of cellular metabolites within intact tissue at a much higher spectral resolution than could be achieved with tissue placed in an NMR tube and measured with solution NMR methods. Furthermore, it preserves tissue architecture, histological and molecular pathology analysis. The application of HRMAS to the evaluation of disease directly contributed to the introduction and establishment of NMR-based cancer metabolomics as the collective evaluation of the global variations of metabolites and the measurement of their global profiles from various metabolic pathways, under the influence of oncological developments and progressions.

Introduction

Although it was predicted on the basis of advances in quantum mechanics in the 1920s, it was not until the 1940s that nuclear magnetic resonance (NMR) was finally observed, with the measurement of proton nuclear relaxation times for biological tissue following soon afterwards. Bloch and Purcell shared the Nobel Prize in 1952 for their respective contributions to these achievements, just as NMR was gaining widespread use as a tool for analytical chemistry. Another two decades passed, during which physicists and biologists occasionally visited the field, but this era came to an abrupt halt in 1973 when Mansfield’s mathematical analysis and Lauterbur’s gradients together permitted image production from NMR signals. In 1973, NMR images showing test tubes of heavy water surrounded by ordinary water were published, and the implications of the technique for human imaging became clear. This advance, which led to a new wave of radiological studies, followed the 1970s transformation of planar radiography into three dimensional CT. The capacity of the NMR signal to generate, via magnetic field gradients, three dimensional (3D) images to visualize physiological and pathological structures inspired the rapid development of an independent radiological discipline—MRI (the term ‘nuclear’ was dropped, possibly to reduce public panic and patient resistance). MRI (emphasizing the type of image collected), rather than NMR (indicating the site of signal origin), is now the standard term recognized by patients as a diagnostic imaging technique. In this Review, unless specified otherwise, NMR and MRS or MRI will be used interchangeably to refer to the spectroscopy and imaging of nuclear protons.

The application of HRMAS to the evaluation of disease directly contributed to the introduction and establishment of NMR-based cancer metabolomics as the collective evaluation of the global variations of metabolites and the measurement of their global profiles from various metabolic pathways, under the influence of oncological developments and progressions.
After the brain, the prostate is the human organ most frequently studied using NMR and MRI, which is largely due to the diagnostic uncertainties that have surrounded prostate cancer treatment since the introduction of serum PSA testing. NMR prostate studies aim to identify cancer-suspicious regions for targeted biopsy, determine pathological stage before prostatectomy, monitor treatment efficacy, and search for metabolic markers to more accurately reflect cancer aggressiveness than current histopathology techniques. These common research goals form the backdrop for this Review, which will discuss \textit{ex vivo} measurements of human materials and \textit{in vivo} evaluations of human prostate. We will review the developments in NMR prostate cancer investigations during 2000–2011, and consider current research and future directions in NMR technology.

**Human prostate NMR—moments of transition**

The early milestones in MR studies of prostate cancer, reached in the last decades of the 20th century, seem somewhat primitive by today’s standards (Table 1). However, they established the foundations of \textit{ex vivo} and \textit{in vivo} imaging, providing insight and direction for the further research and clinical implementation of MRI in the new century. These early advances in NMR and MRI occurred concurrently with the development of PSA testing for prostate cancer. As newly emerging MR technologies increasingly facilitated observation of MR signals within \textit{in vivo} settings, the effort to characterize human prostate cancer from human prostate specimens redoubled, to further both the development of \textit{in vivo} protocols and the interpretation of the resulting images.

In the early 2000s, NMR evaluation of human prostate tissue samples was performed by using conventional liquid NMR methods, whereby tissue samples were packed in standard NMR tubes and immersed in phosphate buffered solutions. The analysis of tissue samples obtained from 14 prostatectomies, treated using this method and measured on a 14.1 T (600 MHz) NMR spectrometer, revealed a relationship between reductions in NMR-observable polyamine and the presence of prostate cancer.\(^{13}\) By combining high-performance liquid chromatography (HPLC) with NMR on standard compounds, it was confirmed that the polyamines observed in prostate tissue were mostly spermine.\(^{15}\) Polyamines have an important role in supporting eukaryotic cell growth and, interestingly, spermine has been identified as an endogenous inhibitor of prostate cancer growth from cell-line studies.\(^{16}\)

Chemical extraction and whole tissue NMR generally require >0.1 g of tissue for analysis, which can present a serious obstacle when dealing with human samples, such as a prostate biopsy core, which weighs ~0.005 g. Unlike measurements using tissue chemical extraction solutions, analysis of the whole tissue resembles \textit{in vivo} observations and preserves the tissue architecture for histopathological

<table>
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<th>Year</th>
<th>Topic</th>
<th>Results</th>
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<tr>
<td>1975</td>
<td>NMR technique to distinguish cancer from benign tissue</td>
<td>Malignant tissue in humans has a longer spin-lattice relaxation time than normal tissue</td>
<td>Eggleston et al.(^{155})</td>
</tr>
<tr>
<td>1982</td>
<td>NMR imaging of human prostates</td>
<td>Images produced are comparable to first generation CT</td>
<td>Steyn et al.(^{156})</td>
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<td>1988</td>
<td>\textit{In vivo} (^{13})C-NMR of malignant and normal prostates</td>
<td>Citrate detected in prostate</td>
<td>Sillerud et al.(^{157})</td>
</tr>
<tr>
<td>1991</td>
<td>\textit{In vivo} (^{31})P-NMR of normal benign and malignant neoplasm of prostate</td>
<td>Malignant cells contain less citrate than benign cells, but results were not statistically significant</td>
<td>Narayan et al.(^{158})</td>
</tr>
<tr>
<td>1993</td>
<td>\textit{In vivo} localized proton MRS with Helmholz coil at 1.5 T</td>
<td>Detection of citrate signal in prostate</td>
<td>Schick et al.(^{159})</td>
</tr>
<tr>
<td>1993</td>
<td>Ex \textit{vivo} proton NMR of tissue extracts</td>
<td>Adenocarcinoma is characterized by low citrate levels compared with normal peripheral zone and BPH tissues</td>
<td>Schiebler et al.(^{160})</td>
</tr>
<tr>
<td>1996</td>
<td>\textit{In vivo} 3D MRSI at 1.5 T with endorectal coil</td>
<td>MRSI can accurately define presence and spatial extent of prostate cancer</td>
<td>Kurhanewicz et al.(^{46})</td>
</tr>
<tr>
<td>1997</td>
<td>Proton MRS analysis of prostatic fluid</td>
<td>Strong correlation between secretion of citrate and spermine in cancer patients and controls</td>
<td>Lynch et al.(^{161})</td>
</tr>
<tr>
<td>1997</td>
<td>Ex \textit{vivo} proton MRS of prostate tissue and multivariate analysis of spectral data</td>
<td>Differentiation of malignant from benign tissues with overall accuracy of 96.6%, sensitivity 100% and specificity 95.5%</td>
<td>Hahn et al.(^{162})</td>
</tr>
</tbody>
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Abbreviations: 3D, three dimensional; BPH, benign prostatic hyperplasia; CT, computed tomography; MRS, magnetic resonance spectroscopy; MRSI, magnetic resonance spectroscopic imaging; NMR, nuclear magnetic resonance.
evaluation after NMR measurement. This advantage has proven to be extremely important for early-stage prostate cancer studies, especially given the known heterogeneity of this disease, as samples obtained from patients with prostate cancer might not contain malignant tissue. Verifying tissue pathology after NMR analysis is currently the only way to ensure the accurate interpretation of the MR spectra and the images produced.

The advantage of NMR in preserving tissue architecture was illustrated by the study of a larger patient population (55 peripheral zone and 16 transition zone specimens from 41 patients, most with prostate tumors, but a few without cancer) with an 8.5 T (360 MHz) spectrometer (Figure 1d). In this study, intact tissue samples were measured in the NMR tube with 0.3 ml of a solution of phosphate buffered saline (PBS) and deuterium oxide (heavy water). Histology after NMR analysis revealed that 27 of the 71 specimens contained prostate cancer cells, while 44 specimens represented benign prostatic hyperplasia (BPH) of glandular and stromal origins.17

Quantitative analysis of spectra from whole tissue poses a challenge, because conventional NMR (developed for measuring aqueous solutions) produces limited spectral resolution when used on intact whole tissue, and individual metabolites are impossible to identify from these broad-line spectra. Thus, this study, like its predecessors, relied on visual inspection of the MR signals to identify spectral regions that coincided with abnormal histology, such as those areas with altered choline/creatinine and lipid/lysine ratios (choline and its compounds have been closely associated with numerous cancers). The use of such spectral region ratios, in connection with complete sections and evaluation of tissue pathology, resulted in an impressive NMR sensitivity and specificity of 97% and 87%, respectively, for distinguishing BPH from prostate cancer.17 These ex vivo results, which are measured under similar conditions to in vivo analysis, are of great value when applied to the evaluation and interpretation of in vivo MRS. However, they present difficulties for quantitative cross-validation, owing to the uncertainty associated with broad-line features of the spectra from which the spectral baseline is determined, so the results can be ambiguous.

The development of microchambers for NMR analysis of small sample volumes enabled NMR to be used on biopsy cores. Using this technology, viable cancer cells were identified in 140 biopsy cores from 35 patients who had received radical radiotherapy for prostate malignancies, with a sensitivity and specificity of 88.9% and 92.0%, respectively.18 However, although this approach demonstrated the feasibility of NMR for microgram tissue analysis, it did not improve spectral resolution and metabolite identification, largely owing to intrinsic magnetic susceptibility interference (Figure 1e). The authors divided spectra of 0.5–3.5 ppm into 450 subregions and performed multivariate analysis on 65 of the resulting spectral regions. The collective inclusion of multiple spectral subregions for disease characterization was an early step towards the development of metabolomics.

The transition moment for the ex vivo NMR analysis of prostate tissue arrived in early 2000 when HRMAS was first applied to prostate cancer. HRMAS evaluation of intact prostate tissue samples of ~10 mg permitted the acquisition of high-resolution spectra and the subsequent assessment of the samples using serial-section histology. HRMAS provided complete metabolite and pathology quantifications, in order to differentiate...
identified in vivo by MRS (Figure 1a). This study, which used ex vivo MRS as a bridge between in vivo MRS and histology, reported 81% diagnostic accuracy for in vivo MRI and MRSA. It also demonstrated the compatibility of immunohistochemical staining of the cell proliferation marker MIB-1 for tissue samples after HRMAS analysis, reporting a significant correlation between MIB-1 and choline levels ($P = 0.01$) measured using NMR. Such proof-of-concept studies soon led to the use of HRMAS for the analysis of actual patient prostate biopsy cores.

HRMAS spectra of biopsy cores from patients with and without prostate cancer have demonstrated a significant correlation between tumor Gleason scores and metabolic ratios for choline/creatinine, total choline/creatinine, and citrate/creatinine ratios ($P = 0.014, 0.011$, and 0.049, respectively). A further study combined the high resolution of HRMAS spectra—which permits measurement of metabolites at low concentrations—with an external electronic reference, and found that differential lactate and alanine concentrations could help distinguish prostate cancer from benign tissue.

The high-resolution spectra achieved with HRMAS enables the use of reduced sample sizes and improves spectral signal-to-noise ratios. As a result, two-dimensional homonuclear or heteronuclear spectra can be measured in shortened experimental intervals. This increased speed has resulted in the detection of polyunsaturated omega-6 fatty acid, a suspected prostate cancer promoter, in prostate tissue and compounds containing choline and ethanolamine, as well as other human prostate tissue metabolites, have been quantified. The observation that levels of ethanolamine-containing compounds could differentiate between prostate cancer and benign tissue (with ethanolamine concentration found to be substantially lower in cancerous tissue than in benign tissue) has been supported by a recent report of $^{13}$P-NMR analysis of prostate cancer tissue extracts. The incorporation of electronic references is only one of the ways in which HRMAS has been modified for prostate cancer applications. To better preserve tissue pathology structures, slow spinning schemes have been introduced, and the effects of tissue frozen on the measured spectra have been evaluated. Rotor-synchronized adiabatic pulses have been introduced to improve metabolite quantification, and attempts have been made to optimize HRMAS for short T2 metabolites and quantitative pathology evaluation procedures have been proposed. The technical maturity represented by HRMAS has led to a paradigm shift in prostate cancer research and investigation. Metabolite alterations initiated by cancer cannot be isolated within any single pathway, so, inspired by genomics and proteomics, the concept of prostate cancer metabolic profiles was first suggested in 2005. Prostate tissue samples ($n = 199$) obtained from 82 biopsy-proven prostate cancer patients after prostatectomy were measured with HRMAS on a 14.1 T (600 MHz) spectrometer (Figure 1f). Histology evaluations after MRS revealed that, among these 199 samples, only 20 contained prostate cancer. Of the 20 prostate cancer samples, both prostate cancer and histologically

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**Figure 2** | 400 MHz HRMAS proton MR spectra (water-suppressed, T2-filtered) of human prostate tissues. **a** | Normal human prostate, containing 45% epithelial cells and 55% stroma. **b** | Tumor-bearing prostate, containing 23% malignancy, 17% epithelial cells and 60% stroma, as determined by histopathological quantification of the specimen after NMR treatment. Permission obtained from Elsevier Ltd © Cheng, L. FEBS Lett. 494, 112–116 (2001). Abbreviations: CHO, choline; CHO-P, phosphocholine; Cit, citrate; Cr, creatine; Glu, glutamate; HRMAS, high-resolution magic angle spinning; Lac, lactate; MR, magnetic resonance; NMR, nuclear magnetic resonance; Spm, spermine.

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benign samples had been found in 13 of these patients. The metabolomic profiles were calculated from the 36 most common and intensive metabolite resonances and spectral regions of curve-fitted MRS, using the statistical tool principal component analysis (PCA), and principal components obtained were then correlated with quantitative pathology of the same specimens. Tissue metabolomic profiles, correlated with patient serum PSA levels ($P < 0.006$), could differentiate malignant from histopathologically benign samples from the same patient with an overall accuracy of 98%. Furthermore, metabolomic profiles obtained from histologically benign prostate tissue samples from patients with Gleason scores of 6 and 7 delineated a subset of less aggressive tumors ($P < 0.008$), and indicated the potential existence of metabolic field effects, meaning that cancer-associated metabolic alterations extended beyond cancerous areas into the histologically benign tissue. This study, which used tissue from a reasonably large patient population, illustrated the clinical potential of HRMAS during the early development of the technique. However, it should be noted that, although the study proposed a hypothesis and reported on a training cohort, like many other early reports, it lacks the verification of the hypothesis through a testing cohort.

A more rigorous retrospective study of metabolomics has since been reported. 16 patients with recurrent prostate cancer with an initial PSA increase of $0.69 \pm 0.26$ ng/ml and monitored at around 48 months after prostatectomy were paired by age and Gleason score with 32 patients of the same clinical stage (the training cohort) or pathological stage (the testing cohort), but who had no recurrence. HRMAS measured at the time of prostatectomy identified 27 of the most common intense spectral metabolic regions for statistical analysis. These spectral regions from cases of clinical-stage-matched groups were analyzed with PCA, through which four principal components related to pathology were defined. Canonical correlation analyses were then performed to establish metabolomic profiles able to indicate the likelihood of prostate cancer recurrence among clinical-stage-matched groups. Applying the coefficients derived from PCA and canonical analysis results to the pathological-stage-matched groups resulted in an overall 71–78% accuracy in identifying recurrence in the testing cohort of patients. This level of accuracy exceeds the ability of existing clinical nomograms, and could enable patients with suspicious metabolic profiles to explore additional treatment options before prostatectomy, and perhaps even as early as the time of prostate biopsy.

In the past decade, NMR studies of prostate tissue aimed at interpretation of in vivo observations have led to the proposal and development of prostate cancer metabolomics. The evolution of this concept is extremely important: the measurement of each metabolite may be sensitive to certain disease conditions, but out of context of the entire metabolome, these individual measurements are less meaningful. As such, the view of the entire profile is required to provide the insight needed to accurately characterize disease states.

**MRSI of prostate: diagnostic imaging**

Unlike MRI, which measures the water content of tissues under different physiological and pathological conditions, MRSI measures concentrations of cellular metabolites. In soft tissue, water concentrations range from 40 to 50 mmol/l, whereas metabolite concentrations are <10 mmol/l, and are shadowed by water signals. The MRSI equipment for observing low concentration metabolites must, therefore, be more sophisticated than that used for water. Furthermore, higher field strengths are needed for MRSI because separations between different metabolite signals and between metabolites and water are proportional to the strength of the magnetic field. Major technological developments for in vivo MRSI including water and lipid suppression and endorectal coils were developed specifically for prostate imaging, and, along with the establishment of 1.5 T field strength as standard, have made it possible for prostate MRSI to flourish. Out of 90 reports related to MRSI studies using 1.5 T scanners, about 68 reports applied MRSI for prostate cancer diagnosis and monitoring of patient populations from more than 35 institutes around the world. As a measure of the status of the field, we estimated the number of patients reported annually by these articles, and saw an increase from around 60 per year in the early 2000s to >1,000 per year in 2010 (Figure 3). This survey indicated that two institutions, the University of California, San Francisco and Memorial Sloan–Kettering Cancer Center, are particularly noteworthy for their early engagement and continued pursuit of clinical MRSI on prostate cancer. Both institutions have produced more than 15 reports across the decade and have established use of the ratio between spectral regions from choline to creatine over citrate and the “live point scale” (in which 1 corresponds to “benign”, 2, “probably benign”, 3, “equivocal”, 4, “probably malignant”, and 5, “likely malignant”) as prostate cancer evaluation criteria. Of note, the spectral region from choline to creatine, which is increased in cancerous tissue, also includes the resonance of the polyamines, which, conflictingly, has been shown to correlate with benign tissue in ex vivo studies. Unfortunately, although the clinical importance of polyamines to prostate cancer is recognized, limited spectral resolution and short T2 relaxation times prohibit the measurement of polyamines between choline and creatine resonances. Most reports have found that MRSI was of clinical benefit. Exceptions included reports on combined MRSI with MRI for differentiating stage II from stage III prostate cancer, where no advantage was shown over MRI alone, and on diffusion-weighted imaging (DWI), which outperformed MRSI for guiding biopsy. Over the past decade, MRSI evaluation of prostate cancer clinical characteristics has grown from simple tumor detection and volume estimation to the determination of MRS features for high-grade prostatic intraepithelial neoplasia (PIN), corroboration with other MR tests, and potential contributions to active surveillance programs.

A direct test of the ability of MRSI to reveal prostate cancer has been conducted in association with prostate biopsy. In these studies, MRSI-identified metabolically suspicious
regions were fused onto transrectal ultrasonography (TRUS) images to guide biopsies or compared with extended biopsy patterns. A report of 648 biopsy samples obtained from 54 patients found that combined MRI–MRSI could detect biopsy-proven prostate cancer with 72.3% accuracy if multiple biopsy cores from the same patient were treated as a single case, or 76.8% if evaluating each biopsy core independently, and concluded that combining MRI with MRSI could increase the accuracy of TRUS-guided biopsy for the transition zone of the prostate. This potential has inspired the development of MR-compatible biopsy systems during the past decade.

Since 2000, MRSI has been used to direct and monitor prostate cancer treatments, including brachytherapy, hormone deprivation therapy, and external beam radiotherapy, in addition to its use in detecting and characterizing prostate cancer. The capacity of MRSI to predict and detect prostate cancer recurrence after radical prostatectomy and external beam radiation therapy is being evaluated. MRI combined with dynamic contrast-enhanced MRI has been used to improve accuracy in detecting local prostate cancer recurrence in patients who had previously undergone radical prostatectomy. Furthermore, MRSI data collected from 202 patients before radical prostatectomy have shown that this technology is able to predict the time of prostate cancer recurrence after prostatectomy. There have been a large number of developments and new clinical implementations of 1.5 T MRSI in prostate cancer clinics over the past decade (Table 2). The MRSI clinical trial ACRIN 6659, which showed a similar accuracy of MRI and MRI–MRSI for localizing peripheral zone cancers, is particularly notable.

Around the middle of the decade, MRSI advanced from the 1.5 T platform—which was, by now, widely available—to 3 T scanning (Figure 1b), with concomitant increases in spatial, temporal and spectral resolution. Later reports confirmed the clinical advantage of this higher field strength, via studies showing that 3 T provided a high degree of accuracy in prostate cancer staging, relative choline content and tumor volume were significant parameters for localizing prostate cancer, and demonstrating the use of eight-channel external receiver coils for metabolic quantification. Moreover, the use of an external surface coil, rather than endorectal coils, has been developed for use with 4 T and 7 T scanners, and the inflation of endorectal coils with magnetic susceptibility-reducing materials to improve coil performance has also been tested. Thus, although MR scanners with field strengths of 3 T and above are not as widely available as 1.5 T platforms for clinical use, and this might remain the case in the foreseeable future, research using high field strengths continues to flourish.

In vivo MRSI moved from concept development to clinical implementation in only 10–15 years, with numerous technological advances in the available hardware, such as increases in field strength, and in the software, including those developed for data preprocessing and automated computerized decision making. These developments have encouraged further clinical implementation, both in the available technology and in concept, further supporting the use of MRI before prostate biopsy. However, these advances notwithstanding, the daunting reality remains that for a patient diagnosed with early-stage prostate cancer by PSA testing, diagnostic radiology techniques, including MRI and MRSI, still have limitations in reliably and reproducibly detecting small prostatic lesions. Although the use of higher magnetic field strengths could certainly result in more accurate prostate cancer detection and diagnosis, the reality is that MR studies at >1.5 T are not likely to become cost-effective for widespread clinical use in the foreseeable future.

The future of prostate cancer imaging
Over the past decade, two primary clinical challenges have arisen from the dramatic increase in early-stage prostate cancer diagnoses that followed the introduction of routine annual serum PSA tests. These challenges concerned the imaging of prostate cancer for targeted biopsies, and the prediction of prostate cancer biological activity for better patient prognostication. To address these challenges, NMR studies of human prostate tissues went in two directions: MRSI was proposed to address the challenge of prostate cancer imaging, and tissue-based prostate cancer metabolomics was developed to address that of biomolecular characterization.

By the end of the decade, these two efforts had combined, with the advent of prostate cancer metabolomic imaging. In 2010, it was reported that, by applying prostate cancer metabolomic profiles obtained by 14 T...
spectrum studies on intact tissue to 7 T MR-scanner MRSI findings (Figure 1c), specific metabolomic profiles were associated with prostate cancer lesions with an overall 93–97% accuracy.26

Prostate cancer molecular biology and metabolism
Preclinical cell-line and animal model NMR studies have greatly increased our knowledge of prostate cancer biology,97–109 and MRSI acquisition pulse-sequence techniques for the optimal quantification of prostate metabolites are developing.80,100–109

There have been attempts to develop methods for correlating molecular pathology parameters of prostate cancer with NMR measurements from the same cases and tissues, and protocols have been designed to provide fresh frozen prostate specimens for molecular biology and HRMAS NMR measurement of adjacent sections of similar pathology.120 These studies have made use of technologies including genechip microarray analysis and quantitative PCR on tissue fragments obtained from tissue-print micropeel techniques after presurgery MRSI,121 and on tissue specimens after HRMAS NMR analysis.122 A study of MRI, MRSI and immunohistochemical (IHC) analysis of Ki67, phospho-Akt and androgen receptor expression from 82 prostate cancer patients before surgery demonstrated a potential improvement in diagnostic accuracy if IHC is carried out at the same time as biopsy.123 The function of spermine as an endogenous inhibitor for prostate growth16 inspired a study in which spermine from intact tissue was quantified with HRMAS NMR and the levels of mRNA expression for enzymes in the spermine metabolic pathways were evaluated with real-time quantitative PCR.124 Although the relationship between spermine and tumor growth rate (represented by PSA velocity) was inconclusive, correlations between reduced levels of ornithine decarboxylase (ODC) or S-adenosylmethionine decarboxylase (AMD) expression in benign epithelia surrounding the cancer glands, and increased PSA velocity (ODC, P < 0.016; AMD, P < 0.048), and between increases in antizyme expression in cancer cells and PSA velocity (P < 0.001), were found to be significant.124

Imaging technology developments
The accuracy of in vivo MRSI can be evaluated using imaging-guided biopsies followed by histological verification. Unavoidable and unpredictable heterogeneous deformation of tissue during histology preparations, as well as nonlinear deformation of the prostate under the pressure of an endorectal coil, have made relating imaging presentations with pathology a challenge. A seemingly obvious answer to the problems associated with fixation is to avoid the procedure entirely by using automatic, freeform deformation algorithms,125 or on other computerized, semi-automatic, freeform deformation algorithms,131 along with automated prostate segmentation methods.132

### Table 2 | Annual prostate cancer MRSI milestones on 1.5 T scanners, 2000–2010

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<th>Year</th>
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<tr>
<td>2000</td>
<td>Integrating MRSI data into brachytherapy treatment planning</td>
<td>MRSI may help to safely escalate doses and improve patient outcome</td>
<td>Zelefsky et al.48</td>
</tr>
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<td>2001</td>
<td>Using MRI and MRSI to study metabolism of hormone deprivation therapy</td>
<td>MRSI provided measure for residual cancer and time-course response</td>
<td>Mueller-Lisse et al.73</td>
</tr>
<tr>
<td>2002</td>
<td>MRSI in tumor volume measurement</td>
<td>Addition of spectroscopy increases accuracy of cancer detection</td>
<td>Coakley et al.24</td>
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<tr>
<td>2003</td>
<td>Defining MRSI metabolic signature for TZ tumors</td>
<td>TZ tumor profile is different to that of benign tissue, but a single metabolite ratio cannot differentiate cancer from benign tissue</td>
<td>Zakian et al.48</td>
</tr>
<tr>
<td>2004</td>
<td>Using DCE and MRSI to differentiate healthy PZ from carcinoma</td>
<td>DCE and MRSI combination has potential for localization and characterization of PZ tumors</td>
<td>van Dorsten et al.130</td>
</tr>
<tr>
<td>2005</td>
<td>Evaluate accuracy of TRUS-guided biopsy with MRSI</td>
<td>Metabolic information can be used to direct biopsy sampling</td>
<td>Prando et al.80</td>
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<td>2006</td>
<td>Assess MRI and MRSI for staging nomograms in organ-confined prostate cancer</td>
<td>MRI and MRSI contribute substantially to staging nomograms for prediction of organ-confined prostate cancer</td>
<td>Wang et al.154</td>
</tr>
<tr>
<td>2007</td>
<td>Test of PSA as tumor marker with MRI and MRSI for active surveillance patients</td>
<td>Using serial MRI/MRSI to conclude serial PSA tests can be used as a longitudinal cancer marker</td>
<td>Coakley et al.125</td>
</tr>
<tr>
<td>2008</td>
<td>MRSI and DCE-MRI for detecting prostate cancer recurrence</td>
<td>Combined MRSI/DCE-MRI is an accurate method for detecting prostate cancer recurrence</td>
<td>Sciarra et al.17</td>
</tr>
<tr>
<td>2009</td>
<td>Prediction of prostate cancer recurrence by MRSI and molecular markers</td>
<td>MRSI results contribute to clinical variables for predicting recurrence</td>
<td>Shukla-Dave et al.186</td>
</tr>
<tr>
<td>2010</td>
<td>Role of MRI and DCE-MRI in detection of prostate cancer foci</td>
<td>MRSI provides high sensitivity, specificity and accuracy for detection</td>
<td>Sciarra et al.133</td>
</tr>
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Abbreviations: DCE, dynamic contrast enhanced; MRI, magnetic resonance imaging; MRSI, magnetic resonance spectroscopic imaging; PZ, peripheral zone; TRUS, transrectal ultrasonography; TZ, transition zone.
The inclusion of MRSI with other MRI methodologies, including T2-weighted and dynamic contrast-enhanced MRI to form multimodal MRI, is a conceptual development that has shown great clinical potential for prostate cancer detection and characterization.40,9,131–137 The final parameters reported by such multimodal imaging will form ‘maps’ of medical conditions, echoing the concept of metabolomic imaging, in which the overview of a disease is produced, rather than merely images of individual physical or chemical parameters, as would be reported by each imaging modality alone.

Another important conceptual development in prostate cancer metabolomics, although beyond the scope of this Review, is worthy of mention. Mass spectrometry is able to observe metabolites with much higher molecular weights than those measurable with NMR, and, given the feasibility of mass spectrometry imaging,138–140 a combination of both methodologies could advance prostate cancer metabolomic imaging to a new level of clinical importance. Sarcosine is an example of a metabolite that has been investigated in numerous mass spectroscopy studies of human tissue and biofluids, and mass-spectrometry-based metabolomics have suggested a potential role of sarcosine in prostate cancer progression,141 although this has been contradicted and is now hotly debated.142–144

Conclusions
The inclusion of nonradiological imaging modalities, such as MRI, MRSI and ultrasonography has resulted in the renaming of the traditional ‘Diagnostic Radiology’ specialty as ‘Medical Imaging’ in an increasing number of institutions. However, the original philosophy of using changes in specific surrogate markers of a disease, such as density for CT or the (choline + creatine)/citrate ratio for MRSI, rather than imaging disease directly, is still largely practiced. When evaluating complex disease, such as prostate cancer, it can be extremely difficult to achieve consistency within a technology, on the basis of a single parameter. It seems inconceivable, for instance, that the value of (choline + creatine)/citrate can accurately reflect the multiple clinical parameters associated with this disease, including Gleason score, PSA, and tumor stage, unless the parameters themselves are known to correlate with each other.

Longstanding radiological practices have been challenged by the new concept of metabolomic imaging, as well as by the fusion of images from various MR protocols. These approaches make use of all the available single parameters to construct profiles that reflect different aspects of the disease under study. During the construction of the chemically and physically abstract profiles, the real chemical and physical properties of individual parameters are converted to reflect different disease characteristics. A revolution in the philosophy of medical imaging is, therefore, occurring, emerging from these different MR technologies,145–150 and from other imaging modalities, including CT and PET.151–154 The processes reflected by these images are foremost in the minds of radiologists and clinicians during differential diagnosis, whether they are weighing spectral intensities of (choline + creatine)/citrate, using the five point scale, or viewing films of images on light boxes. We envision that, in the near future, these laborious and somewhat subjective practices could be replaced by automated computer algorithms that collectively consider and organize all available imaging data into 3D prostate cancer ‘characteristic maps’ that can be used for patient consultation and treatment planning, as well as during surgery.

Until then, however, missing pieces of the puzzles are numerous and remain to be assembled, and the knowledge that will emerge in solving them remains to be seen.

Review criteria
The PubMed database was searched using the following terms: "prostate AND ("NMR" OR "MR") OR "MRS" OR "MRI" OR "magnetic resonance" OR "mass spectrometry" OR "magic angle spinning" OR "HRMAS" OR "intact tissue" OR "metabolomics" OR "metabolomic"). The abstracts that were returned were manually read and appropriate articles were selected for their relevance to this Review.

68. Zielensky, M. J. et al. Intraoperative conformal optimization for transperineal prostate implantation using magnetic resonance
100. Al-Saffar, N. M. et al. The phosphoinositide 3-kinase inhibitor Ly103039 downregulates choline kinase alpha leading to phosphocholine and total choline decrease detected by magnetic resonance spectroscopy. Cancer Res. 70, 5507–5517 (2010).