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Metabolomic Imaging for Human Prostate Cancer Detection

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Abstract

As current radiological approaches cannot accurately localize prostate cancer in vivo, biopsies are conducted at random within prostates for at-risk patients, leading to high false-negative rates. Metabolomic imaging can map cancer-specific biomolecular profile values onto anatomical structures to direct biopsy. In this preliminary study, we evaluated five prostatectomy-removed whole prostates from biopsy-proven cancer patients on a 7 Tesla human, whole-body magnetic resonance scanner. Localized, multi-cross-sectional, multi-voxel magnetic resonance spectra were used to construct a malignancy index based on prostate cancer metabolomic profiles obtained from previous, intact tissue analyses by a 14 Tesla spectrometer. This calculated Malignancy Index shows linear correlation with lesion size ($p < 0.013$) and demonstrates a 93–97% overall accuracy for detecting the presence of prostate cancer lesions.

Introduction

The Prostate Specific Antigen (PSA) blood test has greatly improved the detection of human prostate cancer at early and asymptomatic stages (1–3). However, PSA values, while prostate specific, are not cancer specific, and benign prostate conditions may elevate PSA readings. Thus, at present, prostate cancer can only be definitively diagnosed with a positive biopsy. Moreover, ultrasound-guided prostate biopsies currently ensure only that randomly conducted biopsies are from the prostate; they unfortunately cannot reveal cancer-suspicious locations. Given the extremely heterogeneous distribution of cancer in prostate, such biopsies carry high false-negative rates (4). New radiological paradigms, able to indicate

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suspicious regions for biopsy prior to prostatectomy, would alleviate many difficulties and controversies now involved in the clinical management of this disease (5).

Until recently, magnetic resonance imaging (MRI) obtained to detect, localize, and stage prostate cancer were largely based on T2-weighted studies. However, advanced imaging technologies, such as functional MRI and MR spectroscopy, have been increasingly employed in diagnostic radiology (6) to address limits in the clinical utility of T2-weighted images. Such functional MRI technologies as apparent diffusion coefficient (ADC) maps from diffusion-weighted images, K^{trans} maps from modeling of dynamic contrast enhancement (DCE) data, and metabolic ratios from MR spectroscopy are now being investigated to improve diagnostic capability (7). Still, these individual MR approaches reveal only selected parameters associated with cancer; none yet fully reveals the extent of prostate cancer as a complex disease. A clear need thus exists for multiparametrical imaging that can assess complicated disease processes.

At present, the production and interpretation of quantitative, multiple-parametric MRI maps aimed at improving differential diagnosis in the context of malignancy must involve the collaborative contributions of expert radiologists and MR scientists. Efforts are underway, however, to render useful findings from the multiparametric data sets obtained at MR imaging more accessible within clinical contexts and increasingly specific for parameters of particular interest. For example, combining T2-weighted images with ADC maps can help predict prostate cancer volume (8), while combining them with DCE-MRI can improve the sensitivity and specificity of cancer detection for patients who have previously had negative biopsies, but are of high suspicion for cancer (9). The relationship of MR spectroscopy metabolic ratios to tumor stages has also been explored (10,11). However, MR spectroscopy's particular contribution to cancer diagnosis, as distinct from other MR approaches, lies in the shift it entails from evaluation of tissue water to evaluation of cellular biology and chemistry; this ultimately could permit a routine, objective test able to facilitate personalized prostate cancer treatment (12–15).

Our recently developed capacity to assess molecular, physiological parameters at improved resolutions and to exploit statistical approaches derived from medical genomics and proteomics has made it possible to evaluate, for the purpose of clinical diagnosis, treatment strategy and prognosis, an entire ensemble of measured parameters, rather than analyzing these factors individually. Here, instead of investigating individual metabolites or their ratios, as would be done for medical MRS or spectroscopic imaging (MRSI), we have exploited the existence of relationships between cellular metabolism and specific pathological conditions to investigate the entire ensemble of measured prostate metabolites – metabolomics – so as to determine whether tumors can be localized by imaging prostate cancer-specific metabolomic profiles.

Although particular metabolic pathways may well be more directly linked with certain diseases, the collective evaluation of all measurable metabolic changes within these relationships through metabolomic profiles may more fully characterize individual pathological conditions (16–18). Based on this rationale, we previously hypothesized that simultaneous global evaluation of all measurable metabolites may reflect underlying pathological conditions more accurately than can the analysis of any single metabolite, a possibility assessed in a “bench-top” pilot study of intact human prostate cancer tissues, conducted on a 14 Tesla (T) MR spectrometer with a high-resolution magic angle spinning technique (HRMAS) (19). HRMAS exploits solid state NMR's magic angle spinning technology (20,21), generates detailed data of tissue metabolic compositions, and preserves the tissue pathological architectures needed to correlate metabolic changes with pathological alterations accurately (22). In our previous pilot study, we established parameters of

metabolomic profiles from intact-tissue analyses that identified the presence of prostate cancer and indicated tumor pathological stages. We also demonstrated a greater sensitivity and specificity in identifying pathology for these metabolomic profiles than for intensities of individual metabolites (12).

To assess and extend this earlier observation, we hypothesized that diagnostic radiology's capacity to locate malignancy in prostates could be improved through the imaging of prostate cancer-metabolomic profiles, instead of individual metabolites as now practiced in MRSI (23). We tested both our prior and extended hypotheses by examining excised human prostates with a 7T human, whole-body, MR scanner that could be integrated into the clinical armamentarium, with prostate cancer metabolomic profiles obtained from previous intact-tissue analyses on the 14T MR spectrometer.

Results

Construction of metabolomic profiles for phantom imaging with principal component analysis

Despite its conceptual simplicity, the translation of measured metabolite intensities from intact tissue MRS into profiles capable of characterizing specific pathologies presents a practical challenge. Even before considering pathologies, the complex metabolomic data matrix must be simplified through dimension reduction mechanisms; this was achieved statistically by principal component analysis (PCA). PCA defines orthogonally independent principal components (PCs) which present as linear combinations of measured metabolites, so that the first of a few PCs cover the majority of data variations of the entire matrix. Thus, the required evaluation of relationships between all metabolites and a pathology of interest reduces to an analysis of potential correlations between a much smaller set of PCs and this pathology.

To illustrate the PCA procedure and test the validity of prostate cancer metabolomic profiles obtained from intact-tissue MRS at 14T with HRMAS for prostate cancer imaging of the whole prostate at 7T with multi-voxel MRS, we conducted a phantom study that evaluated this transition between differing experimental conditions. Three solutions of common biological metabolites were prepared, including creatine, phosphocreatine, L-glutamic acid, sodium citrate, L-glutamine, taurine, myo-Inositol, N-acetylaspartate, choline, phosphorylcholine, glycerophosphorylcholine, lactic acid, acetate, spermine, spermidine, and putrescine. The concentrations of these metabolites were prepared by random variations of each chemical between 5 and 20mM of physiological levels. To imitate intact tissue analysis, we measured four independent spectra of 10 μ l with HRMAS technique at 14T for each of the three solutions (Fig. 1A). From these spectra, the ten most intense resonance peaks were selected and analyzed with PCA. Both principle components 1 (PC1) and 2 (PC2) showed statistical significance in discriminating between the three solutions, with coefficients representing each measured metabolite in the linear combination for PC2 (the so-called "loading factors" of PCA; cf. eq 1 in Method) presented as an illustration (Fig. 1B).

We then situated three solution-filled spherical phantoms (ϕ =19mm) in one inclusive gel phantom (ϕ =48mm) of a size comparable to that of a human prostate, positioned it within a 7T human whole-body MR scanner, and measured localized, multi-voxel MRS (Fig. 1C). The resulting voxel spectra of corresponding solutions were compared with the measurements at 14T (Fig. 1A). PC2 values for these voxels calculated with metabolite coefficients (Fig. 1B) could differentiate the three spherical phantom solutions with statistical significance (p <0.0001) (Fig. 1D). The PC2 color map provides a visual presentation of this metabolomic imaging differentiation (Fig. 1E). These phantom PCA

results strongly supported the applicability, as a first step of approximation, of prostate cancer metabolomic profiles obtained with intact-tissue at 14T to the imaging of whole prostates at 7T.

Identification of prostate cancer metabolomic profiles through canonical analysis

As no external concentration references can be effectively established for the entire measured whole prostate, we had to re-analyze our previously established, concentration-based, prostate cancer metabolomic profiles (19) according to their relative metabolite intensities, with each individual metabolite intensity normalized by intensity of the total metabolite region of 0.5~4.5ppm measured from the same spectrum. Similar to published protocols, these re-analyses employed both PCA to reduce data dimensions and canonical analysis to reorient the spatial distributions of principal components (PCs), chosen for their significant correlation with tissue pathology. This reorientation extends beyond the PCA requirement for orthogonality among PCs, and recognizes simultaneous and independent relationships of multiple PCs with a particular pathological event. The detailed equation representing scores from canonical analysis are presented in Methods.

Following a re-analyses of PCA accomplished by using the relative intensities of all 36 metabolite regions identified in our previous report (19), a new metabolomic profile, canonical score 1, was calculated by selecting PC1, 3, 6 and 10, each of which, with statistical significance, was either linearly correlate with volume percentage of prostate pathology features or could differentiate cancer from histologically benign samples (Table 1). Overall loading factors (f in eq. 2, Methods; this variable combines coefficients from Both PCA and canonical analysis) for the evaluated 36 metabolite regions were obtained for standardized relative intensities (Fig. 2A).

The metabolomic profile calculated from relative intensities was slightly less significant (overall accuracy, 93%, as presented by the Area Under Curve, AUC, calculated from the Receiver Operation Characteristic, ROC, curve) than the one previously reported for metabolite concentrations (overall accuracy, 98%) (19). However, the new profile was able to differentiate cancerous samples (15) from histologically benign ones (27) collected from 13 patients (Gleason score, GS, 5 [1 case], 6 [7], 7 [4], and 9 [1]). The median (M, black square) and the standard deviation (SD, vertical bar) from the 42 tested samples were determined (Fig. 2B). The values of all histo-benign samples fell below the level of the empirical and arbitrary threshold $M+SD$. Of note, 12 of the 13 patients whose data are included in this figure had pathological stage II (T2) tumors (disease confined in the organ), as defined by the American Joint Commission on Cancer (AJCC) TNM classification(5); one was a T3 (tumor extended beyond the prostate) patient of GS 7. Thus, this relative-resonance intensity-based metabolomic profile, while able to differentiate cancer from histo-benign tissue, may be tumor-stage sensitive and heavily skewed toward T2 tumors.

Metabolomic imaging of excised human prostates

Five prostates, freshly removed by prostatectomy, were measured with the 7T MR scanner. To avoid any interference with clinical pathology after MRS measurements, we acquired spectra with these prostates placed in air, without immersing them in fluids of magnetic susceptibility compensation. A single-loop coil was placed axially around the middle of the prostate, and multi-voxel MR spectra were acquired for three prostate cross-sectional planes, with the center-plane situated approximately at coil level, and two parallel off-center planes 6mm above and below the center-plane. Each plane (3mm thickness) consisted of 16×16 voxels, each about $(3 \times 3 \text{ mm}^2)$, as prescribed on a T2-weighted axial image (Fig. 3A).

Spectral data from voxels within the white border, excluding the three voxels of the urethra (H8, I7, and I8), were processed individually. Spectra from the voxels surrounding the prostate edges were too featureless to be included for further analysis, likely due to susceptibility effects at the tissue-air interface, and were excluded. For each remaining voxel, we applied the overall loading factors of each metabolite region obtained from 14T data (Fig. 2A), on metabolic intensities normalized by the spectral intensity between 0.5~4.5ppm from the same voxel, to calculate the value of a prostate cancer metabolomic profile for the voxel. We individually examined the calculated values for voxels adjacent to the excluded voxels, to prevent any “out-of-voxel” interference from affecting the spectra of voxels adjacent to the excluded, featureless edge-voxels.

Our prostate cancer metabolomic profiles were structured to produce a higher value wherever voxels appeared more cancer-suspicious (cf. Fig. 2B). For this reason, if the profile value of an adjacent voxel was higher than its immediate inner voxel, the value of the particular adjacent voxel was excluded and the whole data set was recalculated to avoid potential false positives caused by “out-of-voxel” susceptibility effects. After exclusion of all potentially contaminated voxels, the final calculated profile value for each voxel was weighted by the values of surrounding voxels through a deconvolution process. Finally, all resulting values of the metabolomic profile were overlaid onto the MR anatomic image (Fig. 3B), with excluded voxel areas marked between solid and dashed lines. These analyses and exclusions were conducted prior to reviewing the pathology.

Correlation between metabolomic images with cancer histology

The results of whole-mounted pathology obtained at multiple levels of the five prostates revealed that the center-planes of two prostates contained no histologically-detectable cancerous lesions. For each of these two prostates, both “above and below” off-center planes were included in the analysis to reduce any potential bias that might have been introduced because of the distance between an off-center plane and the coil level. Thus, for these five prostates, seven 16×16 voxel cross-sectional planes were analyzed.

The histopathological image of the prostate at approximately the same level as the cross-sectional plane examined by the MRS revealed irregular cancer lesions in the top left and right corners (Fig. 3C). By calculating the value of median plus one standard deviation ($M+SD$), established as a testing threshold (Fig. 1A), we identified voxel areas that had at least two connected voxels each possessing a profile value (PV, i.e. the calculated canonical score 1 obtained based on Fig 2A) higher than $M+SD$, as determined for the particular cross-sectional plane. Representative spectra from elevated and non-elevated voxels are shown (Fig. 3D).

Independently from the results of histopathology, we identified, from the seven analyzed cross-sectional planes, 13 profile-elevated regions, each of which had at least two consecutive voxels. All these voxels possessed values of metabolomic profiles above the threshold of $M+SD$, as determined for their corresponding planes. However, since $M+SD$ values are calculated within each examined cross-sectional plane, and certain voxels in any given plane will possess values higher than their own $M+SD$, whether or not prostate cancer is present, the $M+SD$ values are relative and difficult to cross-examine among different planes and for different cases. To circumvent this relativity, we defined a malignancy index for these cross-section planes (MI_p), here shown as:

$$MI_p = \sum_{\substack{PV > M + SD \\ i=1}}^n PV_i \times VS_i \quad (1)$$

where the sum represents the addition of all connected voxels that possess prostate cancer profile values higher than the threshold, with PV representing profile value, and VS, voxel size.

Overall this index measures the absolute elevation of the metabolomic profile and may be compared among different cross-sections from different cases. The seven cross-sections analyzed for the five prostates yielded seven histologically identifiable tumor regions in five cross-sections. Of the seven histologically identified tumor regions, two were located entirely in the excluded voxels and thus could not be included for further analysis. For the remaining five tumors (all GS 7, 4 T2 and 1 T3), we estimated two-dimensional size (mm²) from each histological image, as well as partial size by excluding the areas overlapped with the excluded voxels. We then identified one metabolomic region from the above-mentioned 13 profile-elevated regions that potentially corresponded with a histological region of the five tumors. The identified region, on the same plane as the tumor, either overlapped with the histological tumor region or was located at less than the length of one voxel between the edges of histological and metabolomic regions. The remainder of the eight regions, which were either situated within prostate imaging planes that had no histologically identifiable tumor, or more than two voxels away from the tumor on the same plane, were considered as histo-benign. We next defined a weighted distance (WD_{C-C}) by normalizing distances between the centers of the estimated, partial sizes of tumors, and their potentially corresponding elevated metabolomic profile regions, with the number of involved voxels.

In addition to the above discussions, our assumptions and approximations also extended to other confounding factors, such as magnetic field inhomogeneity, and the possibility of a specimen shrinkage during histology (an average reduction of $27.3 \pm 7.7\%$ was found in this study). It would thus be unlikely for any locations of voxels with suspicious metabolomic profiles of prostate cancer and any malignant regions identified by histology to overlap precisely. However, a corresponding relationship between the two approaches (metabolomic profiles vs. histology) was illustrated by the positive linear correlation between tumor size, as determined by histology, and the value of the Malignancy Index (MI_p) (Fig. 4A). It is interesting to note that this apparently significant linear relationship only existed among T2 tumors, probably because the current MI_p was heavily skewed towards T2 tumors, as previously discussed. An inverse linear correlation was also found between the histologically and metabolomically identified regions by comparing the average intensities of metabolomic profiles for each voxel in the suspicious regions and the WD_{C-C} (Fig. 4B). This inverse linear correlation supports our expectation that the closer the two centers, the higher the per-voxel value of the metabolomic profile. Further, although our findings are quite preliminarily, due to the limited number of studied samples, the MI_p's capacity to differentiate between prostate cancer positive and negative regions is still significant (Fig. 4C), with overall accuracies estimated for all five tumors (93%), or for four T2 tumors (97%, Fig. 4D).

Discussion

The fundamental hypothesis underlying the understanding of cancer metabolomics evaluated in our current study involves three interlocking concepts. First, mechanisms that permit the

growth of malignant tumors are unlikely to affect only one, or a few, metabolites. Next, alterations occur in overall measurable metabolites (i.e., metabolomic profiles) in the presence of tumor growth. Finally, metabolomic profiles are more sensitive than any single metabolite in identifying and characterizing pathological disease (24–27).

All of these concepts are based on the well established view that cellular metabolic status at the time of tissue excision is preserved within intact tissues. Our previous study thus proposed the hypothesis of prostate cancer metabolomics, discussed earlier (19); the current study, which assesses this hypothesis, was inspired by the potential of metabolomic imaging to detect sites of human prostate cancer, a task that no existing radiological protocol currently has the capacity to accomplish reliably.

By applying prostate cancer metabolomic information obtained from intact tissue analyses to evaluations of removed, whole prostates obtained at human, whole-body MR imaging, the current study linked metabolomics with their clinical potential to direct prostate cancer biopsy. Metabolomic analysis of the sizes of suspicious regions and distances (WD_{C-C}) renders it possible to mark areas and locations of biopsy interest. For instance, using the MI_p , cancer lesions with an overall size of more than twice the MRS voxel size ($\sim 18\text{mm}^2$, used for this study) may be detected from the center of a metabolomically-identified suspicious region within a $5.7 \pm 1.5\text{mm}$ perimeter, with an overall accuracy of 97% (T2 only) or 93% (including T3). This illustration is likely to overestimate accuracy, given the small sample sizes examined. It nevertheless points toward the utility of metabolomic mapping to an important and vexing clinical dilemma.

Of further note, the prostate cancer MRS imaging (MRSI) literature supports the ratio of spectral regions for Choline-to-Creatine over Citrate (CC/Ci) in evaluating clinical in vivo MRSI results (12,13,28). Using data from the present study, we analyzed the CC/Ci maps in the same manner as the metabolomic profile presented here. None of the CC/Ci results in the analyzed voxels were able to indicate tumor regions with statistical significance. This is likely due to our sample size, which was much smaller than the reported MRSI studies. However, these results serve to illustrate the strength of metabolomic profiling in diagnosing individual patients, a matter of greater clinical interest than retrospective group comparison.

This study was limited by technical factors, as previously discussed. Importantly, because the current ex vivo study of removed prostates was conducted on a human 7T MR scanner, confounding technical issues might be better addressed, at least hypothetically, by future in vivo implementation of the demonstrated metabolomic imaging concept. For instance, magnetic susceptibility complications caused by tissue-air interface may result in potential exclusions of true cancer positive voxels, as seen in (Fig. 3C), but be of less concern at the in vivo MRS level. Thus, MR spectra from prostate edge voxels may permit the complete inspection of the entire prostate. More interestingly, results in (Fig. 4B) may also suggest that a metabolomic profile can also be sensitive to histologically benign prostate regions adjacent to a histologically defined cancer region, and so further assist other radiological approaches in detecting malignancy at the edge of the prostate.

Challenges already experienced in adapting in vivo prostate MRS from 1.5T to 3T scanners indicate that implementing in vivo prostate metabolomic imaging on a 7T platform will not be straightforward (29–34). Nevertheless, metabolomic imaging has the potential to detect lesions, guide biopsy, and eventually to identify other conditions of malignancy, such as tumor aggressiveness; it can be applied to evaluate other human malignancies and can extend beyond a mapping of tissue metabolites to include other disease-sensitive radiological parameters.

Materials and Methods

This study of human prostates was approved by the IRB at Massachusetts General Hospital in accordance with an assurance filed with and approved by the US DHHS. Five prostates removed from prostatectomy surgeries of patients with biopsy-proven prostate cancer were transferred on ice to a Siemens 7T 90cm human MR scanner (Erlangen, Germany). MR imaging and spectroscopy were conducted at room temperature, with scanner body-coil as transmitter and a circular 7cm diameter surface coil as receiver. For accurate MRS voxel placement and co-registration with the pathology, T2-weighted images of 1mm thickness were collected using a Turbo Spin Echo sequence on approximately 30 slices with field of view (FOV) approximately 40×40mm² (depending on prostate size).

Two-dimensional multi-voxel proton MRS was acquired on three different levels of the prostate. The volume of interest (VOI) was selectively excited by using TE/TR=30/1700 PRESS with CHESS water suppression. The FOV of approximately 40×40mm² was partitioned into 16×16 phase-encoding steps. The individual voxel size was approximately 3×3×3mm³. A spectral width of 4.0kHz, water suppression bandwidth of 130Hz, and 16 acquisitions were used. Each experiment required ~23 minutes of a time frame that can be accommodated for future in vivo implementation. MRS data, processed using NMR Software Nuts (Acorn NMR Inc., Livermore, CA), determined the intensities of metabolites. After MRI/S measurements, the prostates were fixed, and whole-mounted histopathology slides produced and examined.

The value of the metabolomic profile for each voxel was calculated by using modified parameters determined from proton MR spectroscopy of intact human prostate tissues (19). The point spread function of the multi-voxel MRS is not an ideal impulse function, so the value of the metabolomic profile for each voxel may have included contributions from neighboring voxels. A deconvolution process was employed by filtering profile values with a 3×3 two-dimensional finite impulse response filter of the following coefficients: 1/4 for the center voxel of interest, 1/8 for the adjacent four voxels sharing one edge with the center voxel, and 1/16 for the four diagonally adjacent voxels. For edge voxels, the values were adjusted to compensate for the lack of data outside the image. To visualize the processed metabolomic profiles, the image data was scaled to the full range of a 64-color map and overlaid onto the anatomical image.

Statistical methods

Following principal component analysis, principal component j for a hypothetical sample X can be written as:

$$PC_{j,X} = A_j - (c_{j,1} * p_{1,X} + c_{j,2} * p_{2,X} + c_{j,3} * p_{3,X} + \dots + c_{j,i} * p_{i,X}) = A_j - \sum_i c_{j,i} * p_{i,X}; \quad (2)$$

wherein A_j is an arbitrary constant that determines the mean of the PC to 0, p represents the standardized peak intensity of resonance i , and $c_{j,i}$ is the PCA coefficient (aka. “loading factor”) for PC_j and p_i determined by the eigenvectors of the correlation matrix of p .

By contrast, after canonical analysis, the canonical score k involving selected PCs ($L, M, \dots, N$) for sample X would be represented as:

$$\begin{aligned}
& B_k - (e_{L,k} * PC_L \\
& \quad + e_{M,k} * PC_M \\
& \quad + \dots + e_{N,k} * PC_N) \\
& = B_k - \sum_j e_{j,k} * PC_j \\
& = B_k - \sum_j e_{j,k} * (A_j \\
& \quad - \sum_i c_{j,i} * p_{i,X}) = B_k - (\sum_j e_{j,k} * A_j) \\
& \quad - \sum_i (\sum_j e_{j,k} * c_{j,i}) p_{i,X} \\
& = C_k - \sum_i f_{i,k} p_{i,X}.
\end{aligned} \tag{3}$$

in which, B_k , functioning similarly as A_j for PC, is a constant from canonical analysis, and C_k is an overall constant involving constants A and B, $e_{j,k}$ the canonical coefficient for PC_j and score k, and $f_{i,k} = \sum_j e_{j,k} * c_{j,i}$; the “overall loading factor” that for p_i combines coefficients from PCA and canonical analysis.

All statistical analyses were conducted with JMP 7.0 software (SAS Institute Inc. Cary, NC). All measures of statistical significance were tested with two-sided p values.

Summary

Localized, multi-cross-sectional, multi-voxel magnetic resonance spectroscopy was used to produce metabolomic imaging maps of prostate structures in the context of cancer. Prostate cancer specific biomolecular profile values, with the potential to detect prostate cancer sites, guide biopsy, and assist in prognostication, were calculated.

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Figure 1. Metabolomic imaging of solution phantoms

(A) Spectra of three solutions made from common biological metabolites at varying concentrations (5~20mM, see Text for details). The bottom traces are spectra acquired at 14T by using the same HRMAS procedure as that used for intact tissue analysis, while the top traces are spectra measured from voxels indicated in (C), the image for the placement of multi-voxel MRS at 7T. (B) Coefficients for principal component 2, as a metabolomic profile calculated from the ten most intense metabolic resonances measured at 14T. (D) Values of metabolomic profiles measured from voxels can differentiate between three solution phantoms with statistical significance (ANOVA, $p < 0.0001$). (E) The intensities of voxel profile values are color plotted according to the color coding bar at right.

Figure 2. Prostate cancer metabolomic profiles of relative metabolic intensities at 14T

To compensate for the lack of an established in vivo concentration reference standard, we re-analyzed tissue metabolomic profiles according to relative metabolite intensities (normalized by the metabolite spectral region 0.5~4.5ppm) for 42 samples from 13 patients (19). (A) The overall loading factors (combined coefficients from PCA and canonical analysis) for the 36 metabolites and regions included, which provide examples of phosphorylcholine (PCh, 3.22ppm), spermine (Spm, 3.05–3.15ppm) and creatine (Cr, 3.03ppm), are labeled. (B) Metabolomic profile – Canonical Score 1 – identifies cancer (filled dots) from histo-benign (open dots) samples (overall accuracy 93%, indicated by ROC curve) with statistical significance ($p < 0.0001$). Median (M) and standard deviation (SD) values were calculated for all samples.

Figure 3. Metabolomic imaging of cancer from excised human prostate

(A) MRI of a prostate axial cross-section (from a 47y.o. TNM stage pT2cNxMx patient) overlaid with a grid to indicate the locations of 16×16 voxels for which multi-voxel MR spectra were acquired. The outer white border delineates the outline of the prostate cross-section, while the inner white border circles the urethra. Spectra in voxels outside the outer border and inside the inner border were not included in the analyses. (B) Due to the magnetic susceptibility interference at tissue-air interface, spectra in voxels between solid and dashed lines were eliminated from further analysis. The values of the metabolomic profile for the remaining voxels were calculated by using coefficients presented in (Fig. 2A) (cf. eq. 3) for all remaining voxels; the values were then mapped onto the MR image with a color range calibrated to (−100, 100). (C) Histologically-identified cancer regions are circled in red and plotted onto the whole-mounted prostate histology image at approximately the same prostate cross-sectional level as in 3B. Metabolomic profile regions having at least two connected voxels with profile values greater than $M+SD$ are plotted in shaded red. Partial tumor sizes are estimated by excluding their overlaps with purple voxels; here, the “partial tumor size” for the right lesion on the top-right of the histological slide equals the total tumor size, while for the cancer region on the top-left of the histological slide it is less than half the tumor size. Geometrical centers are used to calculate the center of profile-elevated regions, and to estimate the center of partial tumor size, excluding the discarded voxels. (D) Representative spectra from voxels with elevated (a in B) and non-elevated (b in B) profile values are plotted.

Figure 4. 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 shown in Fig. 3 are presented in ■, other T2 tumors are in ●, and the T3 tumor in ◆. (B) A significant inverse linear relationship 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 histological and metabolomically represented voxel centers, the stronger the level of cancer profile in the metabolomic map. (C) The Malignancy Index provides a threshold indication of malignant potential for a profile-elevated region ($p<0.008$,

for all tumors; and $p < 0.004$, for T2 tumors); overall accuracies are presented by the ROC curves (**D**) for all tumors and for T2 tumors only.

Table 1
Characteristics of the four principal components (PCs) involved in canonical analysis

The first ten PCs were evaluated against tissue pathologies based on the criterion of Eigenvalue ≥ 1.0 . V_{Eigen} : Eigenvalue; Cum%: cumulated percentage; PCa: prostate cancer; and HB: histologically-benign.

	V_{Eigen}	%	Cum%	PCa vs. HB	HB Epi	PCa Epi	Stroma
PC1	9.2	25.5	25.5	p<0.050	p<0.001		
PC3	3.4	9.4	49.7			p<0.001	
PC6	1.9	5.3	69.0	p<0.017		p<0.002	p<0.003
PC10	1.0	2.8	83.3	p<0.026			