Published in final edited form as:

Eur J Nucl Med Mol Imaging. 2013 July ; 40(0 1): 60–71. doi:10.1007/s00259-013-2379-x.

Metabolomic Imaging of Prostate Cancer with Magnetic Resonance Spectroscopy and Mass Spectrometry

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Abstract

Metabolomic imaging of prostate cancer (PCa) aims to improve in vivo imaging capability so that PCa tumors can be localized non-invasively to guide biopsy and evaluated for aggressiveness prior to prostatectomy, as well as to assess and monitor PCa growth for newly biopsy-diagnosed, asymptomatic PCa patients.

Metabolomics studies global variations of metabolites with which malignancy conditions can be evaluated by profiling the entire measurable metabolome, instead of focusing only on certain metabolites or isolated metabolic pathways. At present, the study of PCa metabolomics is mainly accomplished utilizing magnetic resonance spectroscopy (MRS) and mass spectrometry (MS). With MRS imaging, the anatomic image, obtained from magnetic resonance imaging, is mapped with values of disease condition-specific metabolomic profiles calculated from MRS of each location. For example, imaging of removed whole prostates demonstrated the ability of metabolomic profiles to differentiate cancerous foci from histologically benign regions. Additionally, MS metabolomic imaging of prostate biopsies uncovered metabolomic expression patterns that could discriminate between PCa and benign tissue.

Metabolomic imaging offers the potential to identify cancer lesions to guide prostate biopsy and evaluate PCa aggressiveness non-invasively in vivo, or ex vivo to increase the power of pathology analysis. Potentially, this imaging ability could be possible not only with PCa, but applied to different tissues and organs to evaluate other human malignancies or metabolic diseases.

Keywords

prostate cancer; metabolomics; metabolomic imaging; magnetic resonance spectroscopy; mass spectrometry

Introduction

Prostate cancer (PCa) is the second most frequently diagnosed malignancy worldwide, and according to the estimates of the National Cancer Institute, 1 in 4 males in the United States will be affected by PCa during his lifetime, where it also presents as the second leading

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Authors' Contributions: EAD, literature research and manuscript preparation; EMS, literature research and manuscript preparation; LLC, provide funding, and manuscript preparation and review.

Competing Interests: None

cause of cancer death for men [1, 2]. The serum prostate specific antigen (PSA) screening test greatly increased the incidence of PCa since its introduction to the clinic in the late 1980s, and shifted the diagnosed PCa population toward earlier disease stages [3, 4]. While PSA screening has saved lives, it has also created controversies for PCa management as the detection of PCa cannot currently be achieved through targeted biopsies, in general [5–7]. Thus, random, ineffective biopsies occur in the context of small, heterogeneously distributed, early-stage prostate tumors, commonly resulting in false negatives referred to as histological sampling errors [8]. In the current PSA screening era, biopsies from patients with early stage PCa very often appear to be histologically benign tissue samples.

PCa statistics strongly suggest that the vast majority of patients whose PCa has been discovered through PSA screening will likely follow a long and indolent course of development and progression. Many newly diagnosed PCa patients thus harbor a disease unlikely to ever evolve into a life-threatening, clinically significant condition. Current histology, however, is limited in characterizing asymptomatic PCa and distinguishing aggressive from indolent, biopsy-detected PCa. This leads to compromised clinical decisions between beneficial therapies and morbidities occasioned by unnecessary overtreatment [1, 9, 10]. A large population of newly diagnosed PCa patients, wary of the morbidities associated with radical treatments, elects to enter active surveillance, while the supporting empirical data remains very limited. Furthermore, the current inabilities of PCa imaging limit patient monitoring during active surveillance, where patients can only be subjected to periodic invasive prostate biopsies with known sampling errors [11]. At the same time, in order to ensure the few aggressive cases are not missed, another school of thought encourages radical procedures for a significant population, resulting in unnecessary overtreatments that cause adverse effects and impairs quality of life for many indolent PCa patients [12].

Therefore, improving in vivo PCa imaging ability will not only assist PCa detection through imaging-guided biopsies to overcome the currently observed histological sampling error, but also provide an urgently needed non-invasive tool to assess and monitor PCa growth for newly biopsy-diagnosed, asymptomatic PCa patients undergoing active surveillance. To address these needs, PCa metabolomic imaging has been developed as a unique way to probe the underlying biochemistry of malignancy development and progression. Metabolomics is the study of global variations of metabolites, the intermediates or end products from cellular biochemical reactions, with which malignancy conditions can be evaluated by profiling the entire measurable metabolome, instead of focusing only on certain metabolites or an isolated metabolic pathway [13].

The concept of metabolomics stems from the holistic approach of genomics and proteomics, from which studies of isolated genes and individual proteins evolved to correlative evaluations between the entire measurable genome and proteome, with physiological and pathological conditions. In these studies, and as the model for metabolomics studies, the biological system is analyzed as a whole, providing a more inclusive presentation of cancer progression to assist in disease diagnosis and detection [13]. In PCa research, while studies focusing on changes in individual metabolite levels have proven very valuable in advancing biological understanding of PCa, metabolomic profiles have shown even greater promise for improving PCa diagnosis and monitoring disease progression. Additionally, in comparison with genomics and proteomics, metabolomics has advantages in probing the status of disease conditions for it measures the downstream effects of changes in genes and proteins, thereby integrating the study of all alterations in genetic regulation, enzyme activity, and metabolic reactions into the reflection of the present disease status in terms of measurable biological activities [14]. In this review, we will address the role metabolomics plays in the study of PCa and the promise metabolomic imaging holds for improving PCa diagnoses and prognoses in the clinical setting.

PCa Metabolomics

At present, the study of PCa metabolomics is mainly accomplished utilizing one of two analytic platforms, nuclear magnetic resonance (NMR) spectroscopy, more commonly referred to as magnetic resonance spectroscopy (MRS), and mass spectrometry (MS). Although numerous studies applying these technologies to prostate cells lines [15, 16], animal prostate models [17–19], and biological fluids from PCa patients including urine, semen, and prostatic fluid [20–23], have been reported, in this review, we will focus on discussions centered around human prostate tissue samples to emphasize the potential and ultimate goal of immediate translation of PCa metabolomics into the clinical care of PCa patients, including in vivo imaging.

Currently, MRS presents as the most realistic option for implementation into the PCa clinic through an avenue known as magnetic resonance spectroscopy imaging (MRSI), achievable on human MR scanners. While MRSI based on measured cellular metabolites has been incorporated into practice in a number of clinical centers for human in vivo imaging, most studies of human PCa metabolomics at high spectral resolution (i.e. with optimal identification of individual metabolites) are researched ex vivo using the method of high resolution magic angle spinning (HRMAS) proton magnetic resonance spectroscopy (MRS), introduced for intact biological tissue analysis in 1996 [24, 25]. This method applies mechanical rotation of the sample at a 54.7 degree angle to the magnetic field, providing high resolution spectra and allowing for the observation of individual metabolites. A key advantage of the method is its preservation of tissue architectures, thus allowing for subsequent histopathology analysis of the same tissue specimen [26, 27].

Since the initial application of the HRMAS method, studies have found that quantifications of individual metabolite levels and metabolite ratios, as well as metabolomic profiles, show strong potential to improve accuracy in PCa detection, diagnosis and monitoring of disease progression. While metabolomic profiles have been shown to provide an enhanced capacity in the diagnosis and monitoring of PCa, there are many findings from studies focusing on individual metabolites in prostate tissue using HRMAS that have proven very valuable in advancing our understanding of PCa. The metabolites spermine, a polyamine abundant in the prostate and a proposed endogenous inhibitor of PCa growth [28], and citrate, a critical prostate metabolite related to zinc depletion in malignant prostate tissue [29], have both been found to correlate with the volume percentage of histologically benign prostatic epithelial cells, while the latter also correlated with PSA velocity, PSA density, and serum percent free PSA [30]. Other studies found phosphocholine, taurine, myo-inositol, scyllo-inositol, lactate, and alanine concentrations to be significantly higher, and ethanolamine concentrations, significantly lower, in PCa tissue compared with benign tissue [31–33].

While the study of individual metabolites has uncovered much about the biochemical changes taking place during PCa development and progression, they do not provide a holistic picture of the malignant status, and therefore, to better evaluate the overall status, a metabolomic approach has been tested as a superior method for assisting in PCa detection and predicting pathological stage and recurrence potential. A critical study that illuminated the strength of metabolomic profiles over individual metabolites, as well as histopathology evaluations alone, was published in 2005[34]. Metabolomic profiles obtained from principal component analysis and canonical analysis of HRMAS proton MRS of 199 samples from 82 PCa patients were correlated with pathological status. These profiles could differentiate cancerous and histologically benign samples from the same patients with 98.2% accuracy (Figure 1). Most importantly, due to the advantage of the preservation of tissue pathology with HRMAS, 179 of these 199 samples were uncovered to be histologically benign tissue samples from PCa patients. However, metabolomic profiles calculated from these

histologically benign tissue samples were able to differentiate pathological cancer stage T2ab cancer (prostate-confined in one of the two lobes) from T2c (prostate-confined but extended to both lobes, $p < 0.002$) and T3 cancer (invading extraprostatic tissue, $p < 0.00002$) (Figure 2). Furthermore, metabolomic profiles calculated from these samples were able to delineate a subset of less aggressive (GS6 and T2ab) tumors and predict tumor perineural invasion, an indicator of tumor aggressiveness, within the subset [34]. The discovery of these metabolomic field effects, where histologically benign prostate tissue from a cancerous prostate can reveal PCa status, presents a real promise for rectifying the current issue of histological sampling error. In a study to be discussed later, metabolomic profiles obtained from relative spectral intensities (normalized by the spectral region of 0.5–4.5 ppm) of the same patient population could also differentiate cancerous samples from histologically benign ones with 93% accuracy [35].

Metabolomic profiles calculated from intact tissue HRMAS proton MRS can also be used to assess PCa biochemical recurrence potential, defined as the detection of serum PSA elevations after radical prostatectomy. A retrospective study in 2010 analyzed 16 cases of PCa recurrence with clinically and pathologically stage-matched cases without recurrence. From the MRS results for these 48 individuals, the 27 most common and intense spectral metabolic regions were identified to calculate the metabolomic profiles. Applying the metabolomic profiles obtained from the clinical stage-matched group (training cohort) to the pathological stage-matched group (testing cohort) revealed an ability to predict cancer recurrence potential with an overall accuracy of 78% [36]. Since empirical clinical evaluations cannot further sub-characterize these matched groups, an accuracy of greater than 50% has the possibility to improve ability of the current PCa clinic for predicting recurrence potential.

The significant advantage of MRS-based metabolomic studies of human PCa is its potential for in vivo translation. The ultimate goal of PCa metabolomics is to improve in vivo imaging capability so that PCa tumors can be localized before biopsy to reduce the occurrence of histological sampling error and the number of false negative biopsies, and also evaluated for aggressiveness and malignant potential so as to better guide treatment. For ex vivo studies, HRMAS has important characteristics that make it a vital method for PCa analysis. The technology provides high resolution spectra without sacrificing tissue architectures, as is seen in the tissue chemical extract procedures used for solution NMR analyses, and also requires less sample volume and shorter preparation time than solution extraction methods [26]. In addition, the innovation of slow sample spinning rates, followed by various rotor-synchronized spinning sideband suppression methods, allows for subsequent uncompromised histopathology analysis, the current gold standard for PCa diagnosis and monitoring, of the same tissue specimen [37, 38].

Another significant area of PCa metabolomics has been developed with mass spectrometry (MS). While MS requires an initial separation of metabolites by gas or liquid chromatography (GC, LC), and therefore destruction of the tissue histology structures, it has much greater sensitivity and can detect metabolites at much lower concentrations than MRS [14]. Although MS can currently only be used in ex vivo PCa studies and the translation possibility of the technology into in vivo clinic imaging is not as likely as with MRS, significant results of PCa metabolomics have been revealed with MS analyses.

Using a combination of high-throughput GC-and-LC-based MS, Sreekumar et al. detected 626 metabolites from 42 prostate tissue samples, of which 16 were histologically benign, 12 were from patients with clinically localized PCa, and 14 were from metastatic PCa patients. Out of the 626 metabolites, 60 were found in localized and/or metastatic PCa but not in the benign samples. Also, 516 differential metabolites between localized PCa and benign

samples were determined, where 50 were increased and 37 downregulated in PCa, when compared to benign samples and 124 were increased and 107 downregulated in metastatic samples, when compared to localized PCa samples (Figure 3)[39]. As a benefit of evaluating altered biochemical pathways from the analysis of the overall differential metabolomic profiles, instead of measuring individual metabolites, the study revealed an increase in amino acid metabolism and nitrogen breakdown pathways during PCa metastatic progression. These findings provide a more robust view of the changes taking place during PCa development and will therefore provide stronger parameters for detection and monitoring.

However, one of the limitations of MS, similar to chemical extractions of tissue samples for solution MRS measurements, is the destruction of the studied specimens in order to extract metabolites for quantification. To address this issue, a recent study has proposed a new protocol for MS analysis, the Molecular Preservation by Extraction and Fixation (mPREF) method. Using this method, prostate needle biopsies were fixed in methanol to both extract small molecules and fix the tissue. With this procedure, both histological and immunohistochemical analyses were successful following fixation, and 260 metabolites were detected in the alcohol extracts using MS. Of these compounds, 82 showed increased levels in extracts from cancer containing biopsies, when compared to extracts from histologically benign biopsies [40]. Although not nearly as many metabolites were quantified as in the study reviewed earlier, the preservation of prostate tissue for subsequent histopathology analysis is an important development in the progress of PCa metabolomics using MS. While MS may never be compatible with in vivo imaging, it does provide a realistic option for an additional pathological measure for clinical prostate biopsy samples.

Metabolomic Imaging

The proposal of the concept of PCa metabolomics is inspired by the developments of genomics and proteomics. However, the development of MRS-based PCa metabolomic imaging was only feasible with the technical advancements of MR technologies seen in human MR scanners. Metabolomic imaging utilizes the capacity of MR scanners in acquiring localized multi-voxel spectroscopy, and maps the anatomic image obtained from MRI with values of disease condition-specific metabolomic profiles calculated from MRS of each voxel. Such a strategy is fundamentally different from the commonly practiced MRS imaging (MRSI), where mapping was achieved with intensities of single metabolites to produce a collection of maps reflecting spatial distributions of metabolic concentrations. However, with a single set of multivoxel MRS, metabolomic imaging can generate a series of distribution maps of disease conditions, rather than distributions of individual metabolites as seen in MRSI.

In vivo MRSI is a non-invasive method that captures spatial metabolic intensities measurable from MRS localized by MRI. However, since metabolites occur in much lower concentrations than water, measurable with MRI, the signal-to-noise ratio (SNR) of metabolites may be improved by applying lipid and water suppression techniques and using larger MRS voxel sizes, at about 0.3 cm^3 on 1.5T scanners, than those for MRI. The voxel size can be further reduced at higher field strengths, such as to 0.1 cm^3 at 3T [33, 41, 42]. Close fitting coils, or endorectal coils in the case of prostate imaging [43], can also contribute to improved MRS quality.

The most active topic, with the longest investigation history, is MRSI of the brain. Due to the low SNR with metabolites, signal averages are always required for MRS measurements. Thus, in addition to the intrinsic advantage of the absence of adipose connective tissue and the lack of respiratory motions in the brain, the head can be conveniently stabilized with

tight fitting coils during MRSI examination to produce sufficient SNR and high spectral resolution. Overlaying these high resolution MRS results with images of MRI measured at the same location, can produce metabolite maps that reflect disease related metabolite activities, as illustrated by results from a human brain tumor study (Figure 4). In this study, spatial metabolite maps of choline/N-acetylaspartate (NAA) ratio acquired with a 3T MR scanner, as well as lipid and reference water scans, are presented from a patient with an oligodendroglioma. Tumor lesions are characterized by increases in metabolite intensities of choline and decreases in NAA, while the localized metabolite changes coincide with the lesions reflected by hyperintensities in T₂-weighted MRI [44].

MRSI studies of the brain can be traced back to early 1980's when the proof-of-concept of MRSI was reported with studies of intracellular cerebral metabolism of newborn infants by phosphorus MRS. By investigating and comparing cerebral metabolism of healthy and birth-asphyxiated newborns, low values of the ratio phosphocreatine/inorganic orthophosphate were correlated with poor prognosis for survival and early neuro developmental onset [45, 46]. The methodology has continually contributed to neurological studies of brain tumors [47], neurodegenerative diseases [48, 49], psychiatric disorders [50–52], trauma [53], ischemia [54], epilepsy [55], etc., for diagnostics, therapy monitoring or patient follow-up.

The success of MRSI in studies of the brain encouraged its explorations for imaging of human prostate cancer. Most noticeably, Kurhanewicz and colleagues applied MRSI to PCa evaluation on a 1.5T scanner with an endorectal coil in 1996. Using the intensities from single metabolites and metabolite ratios, the existence and spatial extent of PCa was defined [56]. Since then MRSI has become a tool of growing interest in PCa studies. At 1.5T, MRSI was shown to discriminate healthy from cancerous prostate tissue with an overall accuracy of 81% when tissue samples were selected from a group of biopsy-proven PCa patients for postsurgical HRMAS proton MRS measurements [33]. MRSI has further demonstrated its potential to guide biopsy including directing biopsy towards the most aggressive part of the tumor, as well as detecting residual or recurrent cancer lesions [57–60].

Additionally, MRSI has shown potential in identifying tumor extracapsular expansion and invasion in surrounding tissue, both crucial for staging and further treatment planning [61]. PCa aggressiveness, as classified by Gleason score, can also be differentiated in vivo by MRSI [62]. In 2012, Nagarajan and colleagues showed statistically significant positive correlations between metabolite ratios and surgical Gleason scores, as well as tumor volume, from results of MRSI obtained at 1.5T [41]. Another in vivo MRSI study of biopsy-proven PCa patients on a 3T scanner, followed by ex vivo HRMAS proton MRS of spatially matched tissue samples, showed strong correlations between metabolic ratios measured from in vivo and ex vivo matched spectra, as well as a correlation between Gleason score and metabolic ratios [63].

While the potential utilities of MRSI for the PCa clinic have been demonstrated by a large number of research publications, the routine clinical adaption of MRSI has been hampered by complications in the interpretations of the MRSI results. These complications can be seen from Figure 5, where, unlike the presentations of metabolite maps previously shown for brain tumors, a spectroscopist or trained radiologist has to evaluate not only where the spectra were measured from the prostate but also the relative ratios between different metabolic regions, such as evaluations of the proposed indicator, (C+C)/C (spectral intensities of regions between creatine and choline over citrate) [41, 59, 64].

The achievements of PCa metabolomics from intact tissue studies have presented an opportunity for PCa in vivo imaging, with the reconstruction of MRSI data according to disease specific profiles. If successful, imaging results would not only be presented as

simplified maps of values of disease specific profiles, but would also provide more accurate assessments of the disease. This metabolomic imaging concept was demonstrated by a report in 2010 [35]. Five whole prostates removed from biopsy-proven cancer patients were obtained after prostatectomy and measured with multi-voxel MRS on a 7T human whole-body MR scanner before whole-mount histology. Due to the lack of PCa parameters for profile constructions determined specifically for 7T measurements, the authors tested these multi-voxel MRS results with PCa metabolomic profiles obtained with intact tissue specimens measured at 14T under HRMAS condition, and compared the metabolomic imaging results with the whole-mount prostate histology (Figure 6). By using the threshold value of the median plus one standard deviation ($M+SD$), suggested by the intact tissue results, the report identified voxel areas that had at least two connected voxels possessing profile values higher than the $M+SD$ determined for the particular cross-section. For the measured five prostates, 13 regions of metabolomic profiles on the analyzed cross-sections were identified as possessing values above the threshold determined for their corresponding planes. However, since $M+SD$ values are calculated within each examined cross-section and certain voxels will possess values higher than the $M+SD$ calculated from its given plane, disregarding the statute of PCa, the $M+SD$ values are relative and difficult to cross-examine among different planes and cases. To address this relativity, the authors defined a Malignancy Index (M_i) as the product of the sum of the profile values of the connected suspicious voxels and voxel size (mm^2). The M_i represents a value that measures the absolute elevation of the metabolomic profile and may be compared among different cross-sections from different cases. The M_i is able to discriminate PCa-positive and PCa-negative regions significantly with an overall accuracy of 93% to 97% (Figure 7). Of an interesting note, the report indicated that the analysis of the $(C+C)/C$ ratios suggested by in vivo MRSI studies were not able to detect tumor regions with statistical significance, which the authors attributed to the smaller sample size used in the study than those seen with in vivo MRSI [35].

Future Directions

Demonstration of the feasibility of PCa metabolomic imaging with removed prostates requires further verification and tests with larger patient populations. Moreover, translation from ex vivo to in vivo is required before metabolomic imaging can be applied by radiologists as part of clinical exam. To achieve this, studies have to overcome the technical challenges of scanning the prostate in living human patients at 7T [65]. Finally, testing at lower field strengths, such as 3T and 1.5 T, should also be considered for general clinical applicability since 7T scanners may never be universally available to most of the clinical facilities.

In addition to MRS-based PCa metabolomic imaging, mass spectrometric imaging can also present as a powerful tool in interrogating molecular details, according to cellular morphology, to discover biomarkers for PCa diagnosis. With recent technical advancements, it has become possible to record localized mass spectrometry, even at the single-cell resolution, to investigate molecular spatial distributions. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry imaging was reported to allow for the visualization of two-dimensional spatial distributions of diverse biomolecules such as proteins, peptides, lipids and sugars, which can then be compared to the histology evaluations of the same tissue. Findings of a protein expression pattern that discriminates between PCa and benign tissue presented potential for future discovery of PCa biomarkers as well as elucidation of PCa pathogenesis through MALDI imaging (Figure 8) [66].

In conclusion, the implementation of metabolomic imaging, either with MRS or MS, offers the potential to either identify cancer lesions to guide biopsy and evaluate cancer

aggressiveness non-invasively in vivo, or increase the power of ex vivo pathology analysis. Metabolomics, in general, can obtain the most representative and complete depiction of the bimolecular alterations during PCa development and progression. It, thus, exists as one of the most promising tools to improve PCa detection and monitoring through clinical imaging. Most importantly, paradigms created for the metabolomic imaging of PCa may ultimately be applied to different tissues and organs to evaluate other human malignancies or metabolic diseases.

Acknowledgments

This work was supported by PHS/NIH grants CA115746, CA162959, and CA141139 (LLC), and the MGH A.A. Martinos Center for Biomedical Imaging.

Abbreviations

(C+C)/C	creatine and choline over citrate
GS	Gleason score
HRMAS	high resolution magic angle spinning
M+SD	median plus standard deviation
MALDI	Matrix-assisted laser desorption ionization
MRS	magnetic resonance spectroscopy
MRSI	magnetic resonance spectroscopy imaging
MS	mass spectrometry
NAA	N-acetylaspartate
PCa	prostate cancer
PSA	prostate specific antigen
SNR	signal-to-noise ratio
T	Tesla

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Figure 1. Metabolomic profiles of human PCa from HRMAS proton MRS

(A) high-resolution magic angle spinning ^1H MR spectrum of intact tissue obtained from the removed prostate of a 61-year-old patient with GS 6 T2b tumors. Histopathology analysis of the tissue sample (insert) after its spectroscopy measurement revealed that the sample contained 40% histopathologically defined benign epithelium and 60% stromal structures, with no identifiable cancerous glands. Cellular metabolites mentioned in the text are labeled on the spectrum. The 36 most intense resonance peaks or metabolite groups above the horizontal bars were selected for analyses, whereas the other regions were excluded from calculation, partly due to surgery-related alcohol contamination. (B) three-dimensional plot of principal component 13 (PC13 correlates linearly with percent volume of cancer cells in tissue samples) versus phosphocholine versus choline. Cancerous and histologically benign (histo-benign) tissue samples from 13 patients can be visually separated in observation plane. The paired Student's *t* test results (cancer versus histo-benign from the same patients)

for principal component 13, phosphocholine, and choline are 0.012, 0.004, and 0.001. Only results from these 13 patients could be evaluated with paired tests for other cancer positive samples were collected from patients from whom no histo-benign samples were analyzed.

(C) the canonical plot resulting from discriminant analysis of the three variables in B presents the maximum separation between the two groups. (D) the resulting receiver operating characteristic curves indicates the accuracy of using the three variables in B to positively identify cancer samples. (Figure adapted with permission from Cheng et al. 2005, Figure 1)

Figure 2. Principal components 2 and 5 as predictors of tumor stage

Principal component 2 (A) can differentiate T2c stage tumors from T2ab and T3 tumors, whereas principal component 5 (B) can differentiate T2ab from T2c and T3 stages, as defined by AJCC/TNM staging system with histo-benign samples, and with histo-benign GS 6 and 7 samples (C and D). In the latter, principal components 2 and 5 can differentiate among three tumor groups: GS 6 T2ab, GS 6 T2c, and GS 6 T3 plus GS 7 tumors. (Figure adapted with permission from Cheng et al. 2005, Figure 3)

Figure 3. Metabolomic alterations of PCa progression

(a) Heat map showing 87 differential metabolites in PCA relative to benign samples (Wilcoxon $P < 0.05$). Localized PCA samples are grouped as i., low grade (Gleason < 6) and ii., high grade (Gleason ≥ 7). Metastatic samples are grouped by the site of tissue procurement namely iii., soft tissue, iv., rib/diaphragm or v., liver. (b) Benign-based z-score plot of named metabolites from (a). Each point represents one metabolite in one sample, colored by tissue type (jade = benign, yellow = PCA). (c) As in (b) except for the comparison between Mets (red) and PCA (yellow), with data represented relative to the mean of the PCA samples. For clarity, the plots in (b) and (c) have been truncated at 15

standard deviations above the mean of the benign and PCA samples, respectively. (Figure adapted with permission from Sreekumar et al. 2005, Figure 2)

Figure 4. The 3D metabolite mapping in a patient with a brain tumor at 3 Tesla
 T2-weighted MRI, metabolite maps and water reference scan demonstrate spatial heterogeneity of the lesion. Data were acquired in 10 min including water reference scan using $32 \times 32 \times 8$ spatial matrix and 7 mm isotropic voxel dimensions (0.34 cc voxel size). (Figure adapted with permission from Posse et al. 2012, Figure 10)

Figure 5. Data from MRSI and MRI of prostate directly aligned and overlaid

(a) A T2-weighted FSE axial image taken from a volume data set demonstrating a large tumor in the right midgland to base. The selected volume for spectroscopy (bold white box) and a portion of the $16 \times 8 \times 8$ spectral phase-encode grid from one of eight axial spectroscopic slices is shown overlaid (fine white line) on the T2-weighted image. Spectra in (d, red box) regions of cancer demonstrate dramatically elevated choline, and a reduction or absence of citrate and polyamines relative to (c, green box) regions of healthy peripheral zone tissue. In this fashion, metabolic abnormalities can be correlated with anatomic abnormalities from throughout the prostate. The strength of the combined MRI/ MRSI exam is demonstrated when changes in all three metabolic markers (choline, polyamines, and citrate) and imaging findings are concordant for cancer. (Figure adapted with permission from Kurhanewicz et al. 2002, Figure 2)

Figure 6. Metabolomic imaging of cancer from excised human prostate

(A) MRI of a prostate axial cross section (from a 47-year-old TNM stage pT2cNxMx patient) overlaid with a grid to indicate the locations of 16×16 voxels for which multivoxel MR spectra were acquired. The outer white border delineates the outline of the prostate cross section; 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) Because of the magnetic susceptibility interference at the 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 for all remaining voxels; the values were then mapped onto the MR image with a color range calibrated to -100 and 100. (C) Histologically identified cancer regions are circled in red and plotted onto a whole mount prostate histology image at approximately the same prostate cross-sectional level as in (B). 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, whereas 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 nonelevated [b in (B)] profile values are plotted. (Figure adapted with permission from Wu et al. 2010, Figure 3)

Figure 7. Correlations of metabolomic profiles with histology

(A) 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 (B) for all tumors and for T2 tumors only. AUC, area under curve. (Figure adapted with permission from Wu et al. 2010, Figure 4)

Figure 8. Direct tissue mass spectrometric analysis of human prostate tissue reveals cell-specific profiles

Frozen prostate tissue was subjected to MALDI-IMS. MALDI-IMS of a single prostate tissue containing tumor and uninvolved regions. **(a)** H&E image of a tissue specimen containing a defined area of PCa glands and benign glands. Insets, magnified ($\times 10$) views of each cell type. **(b)** resulting two-dimensional ion density map showing high expression of a peak at m/z 4,355 (red) in the PCa area (inset is a scan of the tissue after matrix deposition). (Figure adapted with permission from Cazares et al. 2009, Figure 1)