Review

Cancer Epidemiology, Biomarkers & Prevention

Metabolomic Biomarkers of Prostate Cancer: Prediction, Diagnosis, Progression, Prognosis, and Recurrence

Rachel S. Kelly[1,2], Matthew G. Vander Heiden[3,4,5], Edward Giovannucci[1,2,6], and Lorelei A. Mucci[1,2]

Abstract

Metabolite profiling is being increasing employed in the study of prostate cancer as a means of identifying predictive, diagnostic, and prognostic biomarkers. This review provides a summary and critique of the current literature. Thirty-three human case–control studies of prostate cancer exploring disease prediction, diagnosis, progression, or treatment response were identified. All but one demonstrated the ability of metabolite profiling to distinguish cancer from benign, tumor aggressiveness, cases who recurred, and those who responded well to therapy. In the subset of studies where biomarker discriminatory ability was quantified, high AUCs were reported that would potentially outperform the current gold standards in diagnosis, prognosis, and disease recurrence, including PSA testing. There were substantial similarities between the metabolites and the associated pathways reported as significant by independent studies, and important roles for abnormal cell growth, intensive cell proliferation, and dysregulation of lipid metabolism were highlighted. The weight of the evidence therefore suggests metabolic alterations specific to prostate carcinogenesis and progression that may represent potential metabolic biomarkers. However, replication and validation of the most promising biomarkers is currently lacking and a number of outstanding methodologic issues remain to be addressed to maximize the utility of metabolomics in the study of prostate cancer. *Cancer Epidemiol Biomarkers Prev; 25(6); 887–906. ©2016 AACR.*

Aims and Methods

The aim of this review is to summarize the existing literature where metabolomics has been used to evaluate prostate cancer, to explore potential metabolite biomarkers that could augment current diagnostic, prognostic, or screening strategies. The metabolites and pathways implicated by these studies are also discussed to consider what mechanistic information they may impart about prostate cancer.

PubMed was searched to identify studies of prostate cancer in humans that defined themselves as "metabolomics" studies, or which reported metabolic fingerprints, profiles, or signatures. Studies considering disease prediction, diagnosis, progression or treatment response, and studies using any biologic media were considered. The references of each identified study were screened for further qualifying manuscripts. Studies in animal models and in cell lines were not included. Where the full texts were not available the authors were contacted.

[1]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts. [2]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts. [3]Koch Institute for Integrative Cancer Research at Massachusetts Institute of Technology, Cambridge, Massachusetts. [4]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts. [5]Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, Massachusetts. [6]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Massachusetts.

Corresponding Author: Rachel S. Kelly, Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115. Phone: 617-525-0065; Fax: 617-432-1643; E-mail: hprke@channing.harvard.edu

doi: 10.1158/1055-9965.EPI-15-1223

Introduction

Prostate cancer represents the second leading cause of cancer mortality in many western countries and accounted for more than 28,000 deaths in the United States in 2013 (1). However, key questions remain on how best to diagnose and manage this cancer. In particular, the identification of the men at greatest risk from lethal prostate cancer, the prediction of treatment response, and the prediction of recurrence remain challenging.

Most prostate cancers are first found during screening with a prostate-specific antigen (PSA) blood test, alone or in combination with a digital rectal exam, followed by a diagnostic biopsy and potentially imaging if there is a suspicion of cancer spread. When prostate cancer is diagnosed, PSA levels are utilized in tumor staging and tracking cancer progression. Initial treatment may be in the form of a radical prostatectomy, radiotherapy, and/or androgen deprivation therapy (ADT), which is based on the fact that prostate cancer growth is dependent on the predominant male hormone testosterone. After treatment, PSA testing may again be employed to assess disease recurrence. However, there are well documented limitations at each of these stages. Only around 25% of men with an elevated PSA level, defined as > 4.0 ng/mL, are diagnosed with prostate cancer at biopsy, and conversely false-negatives are also common (2). Biopsies frequently miss cancer due to tumor heterogeneity, necessitating the need for multiple repeat biopsies which are potentially hazardous to patients and technically challenging (3). Radical prostatectomies are associated with frequent comorbidities, including erectile dysfunction and incontinence, but are associated with better survival outcomes that radiotherapy (4). Similarly, ADT has a number of adverse side effects and, on average, is only effective for two to three years before the emergence of castration-resistant prostate cancer (CRPC), an incurable and often fatal disease. There are currently no known methods to predict duration of treatment response or to identify those men who will respond most effectively (5, 6). Furthermore, due to the aforementioned issues with both screening and biopsy, overtreatment represents a significant problem in the management of prostate cancer; the majority of diagnoses will not prove fatal, but there is a subset of men with aggressive and lethal disease. Identifying these men at the earliest stage possible is of paramount importance to reduce to mortality from prostate cancer.

A number of additional biomarkers are now being explored to try and address these questions and challenges. This has been facilitated, to a large extent, by developments in high-throughput sequencing technologies. Gene expression signatures have been demonstrated to have the ability to group patients with CRPC into high- and low-risk group (7) and the quantification of circulating tumor DNA in the peripheral blood has been shown to determine prognosis and monitor treatment effects (8). However, the development of novel prostate cancer biomarkers is still in its infancy. Increasingly, metabolomics is being explored to address this need. This high-throughput methodology has the dual benefit of identifying biomarkers while also enabling a better understanding of the underlying disease mechanisms.

Metabolomics has been applied as an interdisciplinary "omics" science combining pattern recognition approaches and bioinformatics with epidemiology, analytical biochemistry, and biology in the study of the "metabolome" (9), which has been defined as "the quantitative complement of all of the low molecular weight molecules present in cells in a particular physiologic or developmental state" (10). Metabolomics provides a downstream measure of a whole system's activity that reflects the genome, epigenome, transcriptome and proteome, and their interactions with the environment (11). The metabolome, as a measure of systemic activity, has also been shown to be indicative of the disease state (12). In particular, neoplastic cells are known to possess unique metabolic signatures (13), including aerobic glycolysis (also known as the Warburg effect characterized by increased glucose uptake and lactate production) and production of choline-containing compounds (9, 14–16). Accordingly, a number of studies have attempted to capture metabolomic biomarkers of prostate cancer.

Prostate cancer represents a particularly attractive model for metabolite profiling. First, there is strong evidence to suggest that dysregulation of metabolism plays an important role in the development and progression of this malignancy. One of the most consistently cited risk factors is metabolic syndrome; a collection of pathophysiologic entities including visceral obesity, insulin resistance, low HDL cholesterol, high triglycerides, elevated C-reactive protein, and low adiponectin levels (17). This syndrome is associated with chronic inflammation and high concentrations of inflammation-related markers, which are thought to enhance tumor growth (18). Second, the healthy prostate is known to exhibit a unique metabolism to produce the components of prostatic fluid: PSA, spermine, myo-inositol, and citrate. In fact, the levels of citrate in the prostate are orders of magnitude higher than anywhere else in the body (19, 20). In addition to the metabolic features common to all malignancies, neoplastic prostate cells also lose the capacity to accumulate zinc which is thought to inhibit the ability to accumulate citrate. The metabolomic alterations reflecting this phenomenon, which is unique to prostate cancer cells, are hypothesized to result in a distinctive and specific metabolome that can be captured through metabolomic profiling.

Selected Studies

A search of the peer-reviewed literature identified a total of 33 qualifying studies (Table 1). The term "metabolomics" was not coined until 2002 by Fiehn and colleagues (21). However, researchers have been exploring the measurements of multiple metabolites as potentially useful biomarkers of prostate cancer for many years. Therefore, three studies predating this specific terminology which attempted to characterize a "metabolomic profile" are also included. Since 2002, there has been a steady increase in the number of metabolomics studies, with more than half of the selected studies published from 2011 onwards.

Study Designs and Methods

The included studies fell into five broad groups based on their stated aims; studies attempting to identify predictive biomarkers prior to diagnosis ($n = 2$; refs. 22, 23), studies aiming to distinguish malignant from benign ($n = 19$; refs. 24–42), studies considering biomarkers of tumor aggressiveness ($n = 1$; ref. 43), studies investigating the effect of therapy ($n = 3$; refs. 44–46), and studies considering multiple outcomes ($n = 8$; refs. 16, 47–53). In common with the majority of the existing metabolomics literature (54), all studies were case–control in design, including two nested within cohorts (22, 23). Twenty-two studies used external controls who were either healthy ($n = 13$ studies; refs. 22, 23, 34, 37–42, 49–52), suffering from benign prostatic hyperplasia (BPH; $n = 3$; refs. 26, 29, 53), non-recurrent cases ($n = 2$; refs. 45, 48), or they included multiple control groups ($n = 4$; refs. 25, 35, 36, 43). In the remainder, the prostate cancer cases acted as their own controls through the use of matched biologic samples or the measurement of metabolites pre-and post-therapy. The most commonly utilized biologic sample was prostate tissue ($n = 14$), followed by blood ($n = 10$), urine ($n = 5$), and expressed prostatic secretions ($n = 1$ study). In addition, three studies used a variety of media (Table 1).

The two most commonly used methods for performing metabolomics studies are mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (^{1}H-NMR). MS involves ionization of chromatographically separated chemical compounds and detection based on their mass-to-charge ratio and retention time during chromatography. Consequently, detection of metabolites by MS is biased towards those that ionize most efficiently. Nevertheless, MS it is the more sensitive of the two techniques and can be performed with smaller amounts of a biologic sample, an important consideration in large-scale human studies. The second method, NMR, subjects a sample to an electromagnetic field and measures the characteristic radio waves displayed by each compound in the sample in response to changes in the magnetic field. For a mixture of metabolites in a biologic sample the different patterns of energy release are represented as peaks in a chromatogram, and the area of the peaks is proportional to the concentration of each metabolite (19). NMR is a more analytically reproducible method, does not require sample separation, and provides

Table 1. Metabolomics studies of prostate cancer

Biologic media	Authors	Sample	Primary aim/outcome	Technology	Comparison group	Population
Tissue	Halliday et al. (1988; ref. 24)	Surgically removed prostate tissue	Distinguish neoplastic from non-neoplastic tissue	13C-NMR spectroscopy	Adjacent hyperplastic tissue	7 prostate cancer patients, USA
	Schiebler et al. (1993; ref. 25)	Prostate tissue from prostatectomy	Distinguish adenocarcinoma, BPH and normal peripheral zone tissue	^{1}H-NMR spectroscopy	BPH and normal prostate tissue	13 prostate cancer patients, USA
	Hahn et al. (1997; ref. 26)	Prostate tissue from TURP or RP	Distinguish malignant from benign tissue	^{1}H-MRS	BPH patients	50 prostate cancer patients, Canada
	Menard et al. (2001; ref. 44)	Prostate biopsies	Distinguish malignant from benign tissue following radiotherapy	^{1}H-MRS	Benign prostate tissue	35 prostate cancer patients, Canada
	Swanson et al. (2003; ref. 27)	Postsurgical prostate tissue	Distinguish malignant from benign tissue	(^{1}H HR-MAS) NMR spectroscopy	Benign prostate tissue	26 prostate cancer patients, USA
	Cheng et al. (2005; ref. 28)	Prostate tissue from RP	Diagnostic biomarkers of prostate malignancy	^{1}H HR-MAS MRS	Benign prostate tissue	82 prostate cancer patients, USA
	Maxeiner et al. (2010; ref. 45)	Prostate tissue from needle biopsy	Biochemical recurrence after RP	^{1}H HR-MAS MRS	Tissue from cases that did not recur	16 recurrent prostate cancer cases and 32 non-recurrent cases, USA
	Komoroski et al. (2011; ref. 29)	Prostate tissue from TURP or RP	Distinguish malignant from benign tissue	31P NMR	BPH patients	8 prostate cancer patients and 13 BPH patients, USA
	Shuster et al. (2011; ref. 30)	Postoperative biopsy tissue	Diagnostic biomarkers of prostate malignancy	GC-MS and LC/MS-MS	Benign prostate tissue	8 prostate cancer patients, USA
	Brown et al. (2012; ref. 31)	Postoperative biopsy tissue	Diagnostic biomarkers of prostate malignancy	GC-MS and LC/MS	Benign prostate tissue	Reanalysis of Shuster et al. (2011; ref. 30) population
	Giskeødegård et al. (2013; ref. 47)	Prostate tissue from RP	Diagnostic biomarkers and biomarkers of tumor aggressiveness	HR-MAS MRS	Normal adjacent tissue	48 prostate cancer patients, Norway
	Kami et al. (2013; ref. 32)	Surgically removed prostate tissue	Distinguish malignant from normal prostate tissue	CE-TOF/MS	Normal prostate tissue	7 prostate cancer patients, Japan
	Li et al. (2013; ref. 33)	Prostate tissue from RP	Identification of prostate carcinogenesis relevant pathways	LC/GC-MS	Benign adjacent tissue	Reanalysis of Sreekumar et al (2009; ref. 52) population
	McDunn et al. (2013; ref. 16)	Prostate tissue from RP	Diagnostic biomarkers of prostate malignancy and biomarkers of biological recurrence	GC-MS and LC/MS	Benign prostate tissue	331 prostate cancer patients from two independent cohorts, USA
Blood	Osl et al. (2008; ref. 49)	Serum	Diagnostic biomarkers of prostate malignancy and biomarkers of tumor aggressiveness	FIAMS/MS or LC/MS-MS	Blood serum from healthy controls	206 prostate cancer patients and 114 healthy controls, Austria
	Lokhov et al. (2010; ref. 34)	Plasma	Diagnostic biomarkers of prostate malignancy	MicrOTOF-Q MS	Blood from healthy controls	40 prostate cancer patients and 30 healthy controls, Russia
	Fan et al. (2011; ref. 53)	Serum	Diagnostic biomarkers of prostate malignancy and biomarkers of tumor aggressiveness	13C NMR spectroscopy	Blood serum from BPH patients	42 Prostate cancer patients and 14 BPH patients, Ireland
	Miyagi et al. (2011; ref. 50)	Plasma	Diagnostic biomarkers of prostate malignancy and biomarkers of tumor aggressiveness	HPLC-ESI-MS	Blood from gender and age matched controls	134 prostate cancer patients and 666 cancer-free controls, Japan
	Saylor et al. (2012; ref. 46)	Plasma	Metabolic effects of Androgen deprivation therapy	GC-MS and LC/MS	Pre-therapy blood samples	36 prostate cancer patients treated with ADT, USA

(Continued on the following page)

Table 1. Metabolomics studies of prostate cancer (Cont'd)

Biologic media	Authors	Sample	Primary aim/outcome	Technology	Comparison group	Population
Blood	Zhou et al. (2012; ref. 40)	Plasma	Diagnostic biomarkers of prostate malignancy	ESI-MS/MS	Blood plasma from prostate-cancer free controls	105 prostate cancer patients and 36 male prostate-cancer free controls, USA
	Huang et al. (2014; ref. 51)	Serum	Prognostic biomarkers and biomarkers of therapeutic benefit	LC/MS	Blood from age-matched healthy controls	18 untreated prostate cancer patients, 36 prostate cancer patients receiving endocrine therapy, 18 healthy men, China
	Mondul et al. (2014; ref. 22)	Serum	Predictive biomarkers of prostate malignancy	LC/MS and GC-MS	Blood from age and date of baseline blood sample matched controls	74 prostate cancer cases and 74 controls selected from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort of male smokers, Finland
	Mondul et al. (2015; ref. 23)	Serum	Predictive biomarkers of prostate malignancy and aggressiveness	LC/MS and GC-MS	Blood from age and date of baseline blood sample matched controls	200 prostate cancer cases and 200 controls selected from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort of male smokers, Finland
	Zang et al. (2014; ref. 41)	Serum	Diagnostic biomarkers of prostate malignancy	LC/MS	Blood from age matched healthy controls	64 prostate cancer cases and 50 healthy individuals, USA
Urine	Cho et al. (2009; ref. 35)	Urine	Distinguish prostate cancer patients, BPH patients and healthy controls	LC/MS-MS	Urine from patients with benign prostatic hyperplasia and healthy controls	18 Prostate cancer patients, 16 BPH patients, and 18 healthy men, Korea.
	Wu et al. (2011; ref. 36)	Urine	Diagnostic biomarkers of prostate malignancy	GC-MS	Urine from patients with BPH and healthy controls	20 Prostate cancer patients, 8 BPH patients, 20 healthy controls, China
	Gamagedara et al. (2012; ref. 37)	Urine	Diagnostic biomarkers of prostate malignancy	LC/MS-MS	Urine from healthy controls	63 prostate cancer patients and 68 healthy controls, USA
	Zhang et al. (2013; ref. 38)	Urine	Diagnostic biomarkers of prostate malignancy	LC/HRMS	Urine from healthy controls	60 prostate cancer patients and 30 healthy controls, Hong Kong
	Struck-Lewicka et al. (2015; ref. 42)	Urine	Diagnostic biomarkers of prostate malignancy	GC-MS and LC/MS	Urine from age and BMI matched healthy controls	32 prostate cancer cases and 32 healthy volunteers, Poland
Prostatic secretions	Serkova et al. (2008; ref. 38)	Expressed prostatic secretions	Diagnostic biomarkers of prostate malignancy	^{1}H-NMR spectroscopy	Prostatic secretions from healthy controls	52 Prostate cancer patients and 26 healthy controls, USA
Multiple	Sreekumar et al. (2009; ref. 52)	Prostate tissue, urine and plasma	Diagnostic biomarkers of prostate malignancy and biomarkers of tumor aggressiveness	GC-MS and LC/MS	Benign adjacent prostate tissue, and urine/plasma from healthy controls	59 prostate cancer patients and 51 cancer-free controls, USA
	Thysell et al. (2010; ref. 43)	Prostate tissue, plasma and bone	Biomarkers of tumor aggressiveness	GC-MS	Non-malignant tissue and plasma samples from men with benign prostate disease and normal bone	13 prostate cancer patients with metastases, 13 prostate cancer cases without metastases and 30 men with benign prostate disease, Sweden
	Stabler et al. (2011; ref. 48)	Urine and serum	Biomarkers of tumor aggressiveness and biological recurrence	GC-MS	Urine and blood from non-recurrent cases	Urine: 25 recurrent prostate cancer cases and 29 non-recurrent cases. Blood: 28 recurrent prostate cancer cases and 30 non-recurrent cases, USA

Abbreviations: ADT, androgen deprivation therapy; CE-TOF, capillary electrophoresis-time of flight; ESI, electrospray ionization; FIA, flow injection analysis; GC, gas chromatography; HP, high performance; HR, high resolution; LC, liquid chromatography; MAS, magic angle spinning; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance; TURP, transurethral resection of the prostate; RP, radical prostatectomy.

both quantitative and structural information (55). However, because sensitivity is limited, it is only able to detect a smaller range of metabolites (2, 19). The majority (n = 21) of the selected studies performed metabolomic profiling using mass spectrometry (MS), with the remainder employing nuclear magnetic resonance (NMR)–based techniques.

Metabolite identification

Metabolomic studies generally fall into two classes; (1) targeted studies which focus on the, often quantitative, measure of specific metabolites, sometimes selected on the basis of biologic rationale, and (2) untargeted studies with no *a priori* hypothesis which measure a much larger number of metabolites, but the exact identity of many metabolites detected by untargeted MS is not apparent without further study. Seventeen studies took an untargeted approach to the search for discriminatory metabolites, with only 12 employing a targeted search. The targeted approach introduces an inherent bias as it is only able to detect the specific metabolites or metabolite classes, such as methionine metabolites (48), corticoids (35), or lipids (29, 40), which have been previously determined. However, it should be noted that the quantitative nature of the targeted studies offers the distinct advantage of easier translation into a clinical setting. Even with a "hypothesis-free" approach there is not a single analytical methodology that can, even closely, cover the whole metabolome, which is estimated to lie within the range of 10^4 to 10^5 metabolites (54). Thus, untargeted approaches also carry with them some bias based on their chosen methods such as the chromatography and detection techniques used to measure metabolites.

The number of metabolites identified in the selected studies varied by orders of magnitude and was dependent on the methods and technologies employed. Some studies reported the number of peaks, spectral regions, or metabolite features, which may not necessarily correspond to individual metabolites. Furthermore, while some studies included all metabolites in their analyses, others restricted analysis to those metabolites which could be annotated. This may complicate the interpretation and translation of the findings. In those studies including all metabolites, the unknowns cannot be biologically interpreted and potentially important information linking the metabolites with the disease process may be missed; furthermore, any pathway analysis will be inherently biased towards the known metabolites. Conversely, studies restricting to known metabolites may miss important components in metabolomic signatures reducing their discriminatory ability. When comparing between the two types of study studies, it is difficult to know whether replication was not achieved because it did not exist, or because of differences in the metabolites compared. To date, the definitive annotation of identified metabolites remains a bottleneck in untargeted metabolomics studies; absolute identification and quantification can only be made in the presence of an authentic standard. Only seven of the included studies were quantitative or semiquantitative in nature (16, 32, 35, 39, 47, 48, 50).

Statistical analyses

Regardless of the technologic methods used, complex analytical methods are required to deal with the multivariate, highly collinear noisy datasets produced (56, 57). Metabolomics studies often employ pattern recognition or clustering techniques as a means of reducing dimensionality including unsupervised methods, such as principal components analysis (PCA) which converts a set of observations into a set of linearly uncorrelated variables called principal components, and supervised methodologies, such as orthogonal partial least squares, discriminant analysis (OPLS-DA) a related method that is able to separate the components into predictive and uncorrelated information (9). These were employed by 15 of the selected studies, with the others using more classical statistical methods, including t tests and regression modeling. One study used a novel feature selection algorithm termed associative voting (49), which integrates class association rule data mining and classification, and one utilized an entirely pathway-based approach: subpathway-GM (33) which maps genes and metabolites of interest to metabolic pathways to identify biologically relevant subpathway regions [readers are referred to (49) and (33) for full methodological details]. In fact, the subpathway-GM method revealed novel associations beyond the original analyses of the same dataset using a more classical statistical approach (52). Thus pathway-based methods for analyzing metabolomics data may help to provide an improved mechanistic interpretation, but are limited by the fact that they are entirely reliant on the underlying databases utilized (58), and currently comprise only a small percentage of the metabolomics literature.

Results

The selected studies indicated that distinct clusters based on metabolite profiles could be identified in a variety of biologic media including blood (40, 41, 51), urine (36, 38, 42), tissue (16, 28, 31, 32, 45, 47, 52), and expressed prostatic secretions (39). These clusters were able to distinguish cancer from benign (28, 31, 32, 36, 38–42, 47), tumors by their degree of aggressiveness (16, 32, 47), cases who recurred (45), and those who responded well to therapy (51). The distinction between the groups of interest on the basis of their metabolic profiles suggested that the development of metabolomics-based biomarkers may be possible. Although all included studies were ultimately interested in the identification of discriminatory metabolites or profiles, 17 studies specifically aimed at the development and assessments of biomarkers either for diagnostic or prognostic purposes, and evaluated the utility of these biomarkers using ROC curve analyses and indices of specificity and sensitivity. In the first part of the results section, the findings of these 17 studies (Table 2) will be explored. In the second part, these findings will be placed in a wider biologic context using the results of the remaining 16 papers (Table 3), which did not explicitly search for, or assess biomarkers. The final part will detail the attempts at replicating and validating these findings.

Part 1: Assessing Biomarkers

Biomarkers of prostate cancer

Nine of the studies comparing malignant and nonmalignant prostate cancer samples reported AUCs for diagnostic accuracy ranging from 0.67 for urinary sarcosine levels in a U.S.-based case–control study (52) to 0.982 for a biomarker profile including phosphocholine and choline, which was developed by comparing tumor to benign prostate tissue from the same patients (28). The second highest reported AUC was 0.973 from Zhou and colleagues' case–control study. This blood-based profile included 15 phosophocholine-containing species, and although an AUC

Table 2. Assessment of the utility of proposed metabolomics biomarkers

Authors	No. of metabolites	Biomarker	ROC AUC	Sensitivity	Specificity	Other results	Conclusions	Validation
Aim: malignant vs. benign								
Cheng et al. (2005; ref. 28)	36 metabolite groups	The 13th principal component, phosphocholine and choline	0.982			The classification accuracy of the profile was 92.3%. The profile was highly correlated with PSA levels, was able to identify less aggressive tumors and to predict perineural invasion	Metabolite profiles can differentiate malignant from benign samples, identify aggressive tumors and predict tumor perineural invasion	Comparison to histopathology findings
Osl et al. (2008; ref. 49)	112 metabolites	Biomarker profile including lysophosphatidylcholines (LPC) with saturated fatty acid chains, serotonin, a monoamine, aspartic acid (Asp) and ornithine	0.716–0.864				Metabolic profiling of blood samples can distinguish healthy cases and controls	Cross validation
Serkova et al. (2008; ref. 39)	10 metabolites	Citrate Myo-inositol Spermine	0.89 0.87 0.79				Citrate, myo-inositol and spermine concentrations are inversely associated with prostate cancer risk, and represent potentially important age-dependent markers of prostate cancer in human EPS.	Prospective validation is ongoing
Sreekumar et al. (2009; ref. 52)	Plasma: 478 metabolites, Urine: 583 metabolites, Plasma: 626 metabolites	Sarcosine in urine sediment Sarcosine in urine supernatant	0.71 0.67			Sarcosine levels were increased in the tissue of prostate cancer patients compared with healthy controls, but no differences were detected in plasma. Sarcosine levels in tissue and the levels of five other metabolites; uracil, kynurenine, glycerol-3-phosphate, leucine and proline were also elevated in the progression from benign to metastatic prostate cancer	Sarcosine may have potential as a diagnostic biomarker	Sarcosine findings were validated in an independent population, and through cell line studies
Lokhov et al. (2010; ref. 34)	25 of the most intense peaks of 1900 detected ions	acylcarnitine arachidonoyl amine	0.97 0.86			Four other cancer associated metabolites were also identified Isolithocholic acid, Testosterone sulfate/Dehydroepiandrosterone sulfate, Androsterone sulfate/5α-hydrotestosterone sulfate	Six potential diagnostic markers for prostate cancer, two of which were determined to have higher AUCs than the PSA test in this population	Cross validation and comparison to two other statistical methods

(Continued on the following page)

Table 2. Assessment of the utility of proposed metabolomics biomarkers (Cont'd)

Authors	No. of metabolites	Biomarker	ROC AUC	Sensitivity	Specificity	Other results	Conclusions	Validation
Aim: malignant vs. benign								
Miyagi et al. (2011; ref. 50)	19 amino acids and related molecules	Ala, Ile, Orn, Lys (downregulated), Gln, Val, Trp and Arg (upregulated)	Linear discrimination analysis model: 0.786 Conditional logistic regression model: 0.783				Metabolic profiles can be used to distinguish prostate cancer cases from controls	Cross validation
Wu et al. (2011; ref. 36)	81 (of which 59 could be identified)	First three components of the prostate cancer model based on nine discriminatory metabolites: Pyrimidine, Creatinine, Purine, Glucopyranoside, Xylopyranose and Ribofuranoside (downregulated), Propenoic acid, Dihyroxybutanoic acid and Xylonic acid (upregulated) (discriminatory metabolites	0.943			Sarcosine levels did not significantly differ between prostate cancer cases and healthy controls	Urinary metabolomic profiles may have potential diagnostic ability	No
Zhou et al. (2012; ref. 40)	390 lipid species	Top 15 species, all of which contained phosphocholine	0.973	93.60%	90.1%	LPC(22:6) demonstrated the most significant difference in plasma concentrations between cases and control.	Three lipid classes, phosphatidyle-thanolamine (PE), ether-linked phosphatidyle-thanolamine (ePE) and ether-linked phosphatidylcholine (ePC) could be considered as biomarkers in diagnosis of prostate cancer	No
Zhang et al. (2013; ref. 38)	5200 features	ureido isobutyric acid, indolylacryloylglycine, acetylvanilalinine and 2-oxoglutarate	0.896	83.3%	83.3%	No significant difference in sarcosine levels between cases and controls	The combined biomarker has diagnostic potential in prostate cancer that is comparable to PSA	yes-findings were validated in a geographically independent cohort
Giskeødegård et al. (2013; ref. 47)	23 metabolites	Levels of citrate, taurine and creatine (downregulated) GPC, PCho, Cho, and glycine (upregulated)		86.9%	85.2%	Metabolic profiles were significantly correlated with Gleason score	Metabolic profiles were able to distinguish normal and tumor tissue, and indolent from aggressive prostate cancer	No
Zang et al. (2014; ref. 41)	Biomarker panel containing top 40 features	Biomarker panel containing top 40 features		92.1%	94.4%	Biomarker panel displayed 93% accuracy for discriminating prostate cancer cases from healthy controls	Levels of fatty acids, amino acids, lysophospholipids, and bile acids ae dysregulated in prostate cancer patients, proving further insight into the metabolic alterations associated with carcinogenesis.	Internal cross-validation

(Continued on the following page)

Table 2. Assessment of the utility of proposed metabolomics biomarkers (Cont'd)

Authors	No. of metabolites	Biomarker	ROC AUC	Sensitivity	Specificity	Other results	Conclusions	Validation
Aim: malignant vs. benign								
Struck-Lewicka et al. (2015; ref. 42)	1132 features	235 features selected from LC-TOF/MS analyses in positive ionization mode 248 features selected from LC-TOF/MS analyses in negative ionization mode 28 features selected from GC-MS analyses				$R2 = 0.756$, $Q2 = 0.579$ $R2 = 0.763$, $Q2 = 0.508$ $R2 = 0.789$, $Q2 = 0.711$	Metabolites involved in amino acid, organic acid, sphingolipid, fatty acid and carbohydrate pathways showed significant differences in the urine of prostate cancer patients compared to healthy controls. Results suggest prostate carcinogenesis is associated with a metabolic phenotype promoting abnormal cell growth and intensive cell proliferation.	Internal cross-validation
AIM: Normal vs. BHP								
Hahn et al. (1997; ref. 26)	450 point spectral region	6 discriminatory spectral regions including those corresponding to citrate, taurine and glutamate		100.0%	95.5%	Overall classification accuracy of 96.6% (Training set: 100%, Test set: 92.7%)	1H HMRS can be used to reliably distinguish between benign and malignant prostatic tissue	Data was partitioned into a training and test set
Fan et al. (2011; ref. 53)	9 metabolites	Acetoacetate, cystine, formate, glutamate, lysine, tyrosine, lipids	0.876				Metabolomics was out performed by proteomics at distinguishing BPH from prostate cancer patients	No
Wu et al. (2011; ref. 36)	81 (of which 59 could be identified)	First three components of a prostate cancer model based on five discriminatory metabolites: dihyroxybutanoic acid and xylonic acid (upregulated), pyrimidine, xylopyranose, and ribofuranoside (downregulated)	0.825			Sarcosine levels did not significantly differ between prostate cancer cases and BPH patients	Urinary metabolomic profiles may have potential diagnostic ability, but sarcosine had no diagnostic potential in this population.	No
Aim: Tumor aggressiveness								
Osl et al. (2008; ref. 49)	112 metabolites	Profile included: Phsophatidyl choline, serotonin, aspartate, ornithine, threonine, arachidonic acid, fatty acids, sphingomyelins, tyrosine and leucine	0.522-0.673 (dependent on feature selection method)				Metabolite profiles displayed little ability to discriminate by Gleason grade	Cross validation
Fan et al. (2011; ref. 53)	9 metabolites	Acetoacetate, cystine, formate, glutamate, lysine, tyrosine, lipids	Gleason5 vs. Gleason 7: AUC: 0.532, Organ confined vs. non-organ confined: AUC 0.311				Metabolomics does not perform as well as proteomics in classifying tumor types	No

(Continued on the following page)

Table 2. Assessment of the utility of proposed metabolomics biomarkers (Cont'd)

Authors	No. of metabolites	Biomarker	ROC AUC	Sensitivity	Specificity	Other results	Conclusions	Validation
Miyagi et al. (2011; ref. 50)	19 amino acids and related molecules	Alanine, isoleucine, ornithine, lysine (downregulated), glycine, valine, tryptophan, and arginine (upregulated)	Controls vs. Stage II (B) patients: 0.764 Controls vs. Stage III (C) patients: 0.777 Controls vs. Stage IV (D) patients: 0.873				Metabolic profiles can be used to classify prostate cancer stage	Cross validation
Aim: Normal vs. BHP								
McDunn et al. (2013; ref. 16)	326 compounds identified in both cohorts	5,6-dihydrouracil, choline phosphate, glycerol, and methylpalmitate combined with Gleason score, serum PSA, and clinical stage	0.62			Sarcosine was significantly elevated only in those tissue sections with Gleason pattern 8 or worse prostate cancer	The inclusion of metabolic markers improved the prediction of tumor aggressiveness compared to clinical indices alone	No, but reanalysis of Sreekumar's data
Aim: Recurrence								
Menard et al. (2001; ref. 44)	450 point spectral region	Biomarker profile including choline, creatine, glutamine, and lipids		88.9%	92%	Overall classification accuracy of 91.4%	The choline, creatine, glutamine, and lipid spectral regions demonstrate diagnostic ability	Cross validation
Maxiener et al. (2010; ref. 45)	27 most common and intense spectral metabolic regions	First nine principal components: major contributors to the profile were glutamine, glutamate, myo-inositol, myo-inositol, scylloinositol, phosphoryl choline, spermine/ polyamines	0.78			Recurrence was predicted with an accuracy of 78%.	The identified tissue metabolomic profiles represent potential biomarkers for the prediction of recurrence	No
Stabler et al. (2011; ref. 48)	8 metabolites	Homocysteine Homocysteine + PSA Cystathionine Cystathionine + PSA Cysteine Cysteine + PSA Homocysteine + cystathionine + cysteine + PSA + Gleason grade	0.74 0.78 0.7 0.79 0.8 0.82 0.86			Addition of serum homocysteine provided the greatest improvement of the logistic regression models compared to the base model with PSA and biopsy Gleason (P = 0.0007), followed by cysteine (P = 0.0017), and cystathionine (P = 0.0037)	Methionine metabolites combined with serum PSA could act as biomarkers to increase the ability to predict aggressive prostate cancer features and early biochemical recurrence over and above existent clinical variables	No
McDunn et al. (2013; ref. 16)	326 compounds identified in both cohorts	7-a-hydroxy-3-oxo-4-cholestenoate, pregnen-diol disulfate, and mannosyl tryptophan combined with Gleason score, serum PSA, and clinical stage	0.64			Sarcosine was significantly elevated only in those tissue sections with Gleason pattern 8 or worse prostate cancer	The inclusion of metabolic markers improved the prediction of biological recurrence compared to clinical indices alone	No, but reanalysis of Sreekumar's data

Table 3. Results from the metabolomics studies that did not assess biomarker utility

Authors	No. of metabolites	Results	Validated?	Conclusions
Halliday et al. (1988; ref. 24)	49 metabolites	Lactate levels and lipid signals are higher in tumor tissue, citrate levels are decreased.	No	13C NMR spectroscopy can be used to differentiate neoplastic and non-neoplastic prostate tissue
Schiebler et al. (1993)	13 metabolite peaks	Citrate, Acetate, inositol and lactate differed significantly between adenocarcinoma and normal peripheral zone tissue, however there were a number of similarities between the spectra of BPH and adenocarcinoma	No	The citrate standardized peak area alone cannot be used to diagnose prostate adenocarcinoma
Swanson et al. (2003)	8 metabolites	Glandular tissue: healthy tissue had significantly higher levels of citrate, Taurine, myo-inositol, and scyllo-inositol and polyamines, and lower levels of choline, phosphocholine (PC), and glycerophosphocholine (GPC) relative to tumor tissue. Stromal tissue: healthy tissue had lower levels of choline compounds and higher levels of Taurine, myo-inositol, and scyllo-inositol.	Cross validation	Distinctive metabolic patterns were identified for tumor and healthy tissues and for cancers of varying grade; however, tissue type may affect the findings.
Cho et al. (2009)	21 corticosteroids	Urinary cortisol levels were significantly higher in prostate cancer patients than in healthy controls and BHP patients	Currently ongoing in a larger population	The results indicate dysregulated cortisol metabolism in prostate cancer patients and suggest that metabolic profiling may be the optimal way to measure this.
Thysell et al. (2010; ref. 43)	Bone 123 metabolites of which 49 could be identified. Tissue: 157 metabolites of which 59 could be identified. Plasma: 179 metabolites of which 50 could be identified	Bone: 58% of metabolites differed between normal and metastatic bone. Amino acid synthesis and metabolism were upregulated in metastatic bone, and high levels of cholesterol, myo-inositol-1-phosphate, citric acid, fumarate, glycerol-3-phosphate, and fatty acids detected. Tissue: 8% of metabolites differed between tissue from patients with and without metastases including four of those significantly increased in metastatic bone: asparagine, threonine, fumaric acid, and linoleic acid. Plasma: 15% of metabolites differed in the plasma of patients with and without bone metastases including four identified in metastatic bone; glutamic acid, taurine, and phenylalanine and stearic acid. Sarcosine was found to be increased in the bone of men with metastatic disease but not in their tumor tissue or plasma	No	Cholesterol is a possible therapeutic target for advanced prostate cancer.
Komoroski et al. (2011)	4 phospholipid metabolites	Phosphoethanolamine and glycerophosphoethanolamine levels differed significantly between the cancer and BPH groups. None of the four metabolites were associated with Gleason score	No	These metabolites may be useful in the diagnosis of prostate cancer and may help to explain the high choline resonance identified in other studies.

(Continued on the following page)

Table 3. Results from the metabolomics studies that did not assess biomarker utility (Cont'd)

Authors	No. of metabolites	Results	Validated?	Conclusions
Shuster et al. (2011; ref. 30)	260 metabolites	83 (32%) of metabolites were significantly different between cancer and benign tissue, with 82 at higher levels in cancer tissue. Common amino acids, long chain fatty acids and phospholipids were increased. Higher levels of uracil, kynurenine, glycerol-3-phosphate, leucine and proline were reported in agreement with Sreekumar's study but sarcosine was below the limit of detection.	No	Molecular biomarkers could augment histology in the characterization of disease.
Brown et al. (2012; ref. 31)	260 metabolites	Ala, Ile, Orn and Lys were downregulated in prostate cancer patients compared to healthy controls and Gln, Val, Trp and Arg were upregulated	No	It is possible to determine a metabolomic signature of prostate cancer
Gamagedara et al. (2012; ref. 37)	4 metabolites	No difference in the mean levels of proline, kynurenine, uracil or Glycerol-3-phosphate between prostate cancer cases and healthy controls	Validating Sreekumar's findings	The explored biomarkers had no diagnostic or prognostic potential in prostate cancer, and could not distinguish prostate cancer from other malignancies
Saylor et al. (2012)	292 identified metabolites	56 metabolites changed significantly from baseline to three months: Multiple steroids, Markers of lipid beta-oxidation, markers of omega-oxidation and markers of insulin resistance (2-hydroxybutyrate, branch chain keto-acid dehydrogenase complex products) were lower. Most bile acids and their metabolites were higher.	Cross validation	Identified novel and clinically important ADT-induced metabolic changes
Kami et al. (2013; ref. 32)	86 (of which 39 could be absolutely quantified)	TCA cycle intermediates, Succinate, fumarate, malate, pyruvate and lactate levels were higher, and ADP and phosphoenolypyruvate lower in tumor than in normal tissue.	Cross validation	Tumor metabolic profiles can help to distinguish normal from tumor tissue, and tumor stage
Li et al. (2013)	626 metabolites	53 metabolites associated with metastatic prostate cancer, 16 significant pathways including amino acid metabolism, tryptophan metabolism, cysteine and methionine metabolism, arachidonic acid metabolism and histidine metabolism	No	Identified novel disease relevant pathways using an alternative statistical method on Sreekumar et al.'s data
Huang et al. (2014; ref. 51)	8,232 signals	In patients who did not develop castration-resistant prostate cancer (CRPC) for at least 2 years, serum deoxycholic acid (DCA), glycochenodeoxycholate (GCDC), L -tryptophan, docosapentaenoic acid (DPA), arachidonic acid, deoxycytidine triphosphate, and pyridinoline levels reverted to near healthy control levels during endocrine therapy. In contrast, the metabolite levels remained abnormal in patients who developed CRPC within 1 year	Validation currently ongoing	DCA, GCDC, L -tryptophan, DPA, arachidonic acid, deoxycytidine tri-phosphate, and pyridinoline represent potential biomarkers for evaluating patient response to endocrine therapy. These results suggest a role for cholesterol in prostate cancer progression

(Continued on the following page)

Table 3. Results from the metabolomics studies that did not assess biomarker utility (Cont'd)

Authors	No. of metabolites	Results	Validated?	Conclusions
Mondul et al. (2014; ref. 22)	420 metabolites	Circulating 1-stearoylglycerol was inversely associated with the risk of developing prostate cancer up to 23 years after blood collection. The magnitude of this association did not differ by disease aggressiveness. There were also suggestive inverse associations for glycerol and alpha-ketoglutarate.	Only the association between alpha-ketoglutarate and aggressive prostate cancer was replicated in a subsequent study including different participants from the same population.	The results support a role for dysregulation of lipid metabolism in the development of prostate cancer
Mondul et al. (2015; ref. 23)	626 metabolites	Strong inverse associations between energy and lipid metabolites particularly glycerophospholipids and fatty acids and aggressive cancer were observed with aggressive disease risk. Thyroxine and trimethylamine oxide were associated with aggressive disease risk while Alpha-ketoglutarate and citrate were inversely associated. Metabolites associated with nonaggressive cancers included 2′-deoxyuridine, adenosine 50 -monophosphate (AMP), 11-dehydrocorticosterone, 21-hydroxypregnenolone monosulfate, cotinine, and hydroxycotinine.	Meta-analyses with the findings of a previous study confirmed a role for glycerophos-pholipids and long chain fatty acids	Prospective study data indicate that several circulating glycerophospholipid, fatty acid, energy and related metabolites are inversely associated with aggressive prostate cancer up to 20 years prior to diagnosis. Metabolite associations vary by cancer aggressiveness.

was not computed, Giskeødegård and colleagues also included phosphocholine in their tissue-based biomarker profile, which had a sensitivity and specificity >85% (47). Three of the articles (34, 39, 52) reported the AUC for individual metabolites, with acylcarnitine ranking the highest (AUC: 0.97 in plasma; ref. 34). Carnitines were also identified in Struck-Lewicka and colleagues' diagnostic signature in urine. Similarly, another metabolite with a high individual AUC, citrate, which had an AUC of 0.89 in Serekova's study (39), was a component of Giskeødegård and colleagues' signature (47). The remaining studies reported on profiles comprising multiple markers. Interestingly, there was some crossover between the profiles developed in the case–control studies of Osl and colleagues (49), Miyagi and colleagues (50), Zang and colleagues (41), and Struck-Lewicka and colleagues (42), with all including multiple amino acids such as lysine, glutamine, and ornithine in their profiles. Osl and colleagues (49) also included arachidonic acid in their signature, a compound related to arachidonoyl amine, which was reported to have an AUC of 0.86 in Lokhov and colleagues' study (34). Furthermore, Osl and colleagues (49) included a number of phosphorylcholines, in agreement with the findings of Zhou and colleagues' targeted lipidomics analysis (40), while Zang and colleagues (41) identified multiple lysophospholipids. The sensitivity and specificity of Zang and colleagues' diagnostic profile, which also contained metabolites of the steroid hormone biosynthesis pathway and bile acids, was 92%, and 94% respectively. Struck-Lewicka and colleagues also did not report AUC's but rather, the R^2 and Q^2 values for their partial least squares-discriminant analysis model which were 0.789, 0.711 respectively, under the best performing GC-MS–derived model, indicating a robust signature with good predictive ability (42).

The studies by Osl (49), Miyagi (50), Zhou (40), Zang (41), and Lokhov (34) and colleagues developed their signatures in blood samples, and it is of note that there was little crossover in terms of the specific constituent metabolites with the urine-based diagnostic signatures proposed by Zhang and colleagues (38), Struck-Lewicka and colleagues (42), or Wu and colleagues (36). All but two of the studies (28, 47), compared prostate cancer cases with healthy controls. However, the results of Giskeødegård and colleagues' and Cheng and colleagues' inter-individual comparisons among prostate cases were markedly concordant with these healthy versus diseased comparisons, both in terms of the constituents of the signatures they developed including phosphocholines, and citrate, and in the predictive ability of these signatures. Similarly, study population size which ranged from 40 (20 prostate cancer cases and 20 healthy controls) in Wu and colleagues' study (36) to 800 (134 cases and 666 controls) in Miyagi and colleagues' study (50), did not appear to confer any advantages in terms of the predictive ability of the developed signature.

In summary, the most promising candidate biomarkers for distinguishing prostate cancer cases from healthy controls include sarcosine, choline, phosphocholines, phosphorylcholines, carnitines, citrate, amino acids, arachidonoyl amine, and lysophospholipids (Table 2).

Biomarkers of benign prostatic hyperplasia and tumor aggressiveness

Wu and colleagues also reported on a biomarker profile with an AUC of 0.825 to distinguish benign prostatic hyperplasia (BPH) and prostate cancer patients constituting five metabolites (36). All five (dihyroxybutanoic acid, xylonic acid, pyrimdine, xylopyranose, and ribofuranoside) were also constituents of the healthy versus diseased profile. Hahn and colleagues reported that MRS spectra could be used to classify benign and malignant prostate tissue with a sensitivity of 100%, a specificity of 96%, and an overall classification accuracy of 97%, with citrate, glutamate, and taurine playing important discriminatory roles (26). Fan and colleagues' 9-feature profile had an AUC of 0.876 for distinguishing prostate cancer and BPH patients. Similar to their malignant versus benign analyses, this serum-based signature included glutamate, lysine, and lipid species, suggesting a possible dose-dependent relationship with the progression from normal to BPH to cancer. However Fan and colleagues' found that the signature did not perform well at distinguishing Gleason 5 from Gleason 7 (AUC, 0.532) or organ-confined from non-organ–confined cancers (AUC, 0.311; ref. 53).

Three other articles also developed biomarker profiles of tumor aggressiveness. Osl and colleagues compared Gleason 6 with Gleason 8–10 cancers, and reported that the discriminatory metabolites, which included a number of sphingomyelin lipids, were distinct from those that differed between normal and tumor tissue (49). However, the AUCs were much lower in the tumor aggressiveness analyses suggesting discriminatory power is poor. Conversely, Miyagi and colleagues reported increasing AUCs when comparing healthy controls to stage II patients (AUC, 0.764), stage III patients (AUC, 0.777), and stage IV patients (AUC: 0.873) using a profile comprising 8 amino acids: alanine, isoleucine, ornithine, lysine, glutamine, valine, tryptophan, and arginine (50). McDunn and colleagues' study the AUC for a combination of four different biomarkers: 5,6-dihydrouracil, glycerol, methylpalmitate, and choline phosphate, was 0.62 for discriminating organ-confined from non-organ–confined prostate cancer (16).

In summary, the most promising candidate biomarkers for identifying BPH and tumor aggressiveness include dihyroxybutanoic acid, xylonic acid, pyrimdine, xylopyranose, ribofuranoside citrate, glutamate, sphingomyelin lipids, amino acids, 5,6-dihydrouracil, glycerol, methylpalmitate, and choline phosphate (Table 2). Again, there was little difference in the results between those studies that compared healthy individuals to prostate cancer cases and those investigating intertumor differences within controls, or any differences due to study population size.

Biomarkers of disease recurrence

Interestingly, choline phosphate (phosphorylcholine) was also identified as a major contributor to the metabolic profile predicting recurrence by Maxeiner and colleagues (45), which had an AUC of 0.78. This profile was based on the first nine principal components of all the measured metabolites and the loadings plots determined that myoinositol and spermine, both of which were identified in Serkova and colleagues' (39) diagnostic signature, and glutamate, which was a component of Fan and colleagues' (53) aggressiveness signature, were major contributors to the recurrence profile.

Similarly cysteine, a further component of Fan and colleagues's aggressiveness signature, was investigated as a marker of recurrence by Stabler and colleagues (48). This metabolite was found to have an AUC of 0.82 when combined with PSA, outperforming two other methionine metabolites: homocysteine (AUC, 0.78) and cystathionine (AUC, 0.79). When these three metabolites were combined, the AUC was 0.86, providing an increased ability to detect recurrence over clinical indices alone.

Although Menard and colleagues (44) did not perform ROC analysis their profile, including choline, creatine, glutamine, and lipids, but they were able to identify a malignant biopsy following radiotherapy with a sensitivity of 89%, a specificity of 92%, and an overall classification accuracy of 91%.

Despite the differences in study designs, Menard and colleagues (44) and McDunn and colleagues (16) considered intertumor differences, while Maxiener and colleagues (45) and Stabler and colleagues (48) compared the recurrent and nonrecurrent cases in tissue and in blood and urine, respectively; the results were largely concordant between the studies. It is also of note that the smallest predictive ability was reported for the largest study (16). In summary, the most promising candidate biomarkers for predicting disease recurrence included phosphorylcholine, myoinositol, spermine, glutamate, cysteine, choline, creatine, glutamine, and lipids (Table 2).

PSA testing and current gold standards

Prostate cancer is a relative rarity among malignancies in that it has been shown to be amenable to population-wide screening programs utilizing prostate-specific antigen (PSA), which is also monitored as a biomarker of biochemical recurrence (6). Therefore, novel biomarkers must perform better than this current gold standard to be useful. Seven of the studies discussed above explicitly compared their biomarkers to the use of PSA (34, 39–41, 48, 52, 53). An often cited study reports an AUC for PSA testing of 0.682 [95% confidence interval (CI) 0.67–0.69] for the diagnosis of prostate cancer, and that a PSA cut-off value of 4.1 ng/mL has a specificity of 93.8% but a sensitivity of only 20.5% (59). Zhou and colleagues' multi-marker plasma metabolite profiles outperformed these metrics in diagnosis (40), although parsimony must also be taken into account when considering clinical translation. Similarly, Serkova and colleagues reported AUCs for three metabolites that would outperform PSA, and added that unlike PSA, they are not associated with age, suggesting improved specificity (39). Although it should be noted that Serkova was comparing the discriminatory ability of these metabolites in expressed prostatic secretions to a blood-based PSA test. Lokhov (34), Zang (41), and Stabler and colleagues (48) computed the utility of PSA in their respective study populations for the diagnosis of prostate cancer [Lokhov (34) and Zang and colleagues (41)] and the prediction of recurrence (Stabler and colleagues; ref. 48), and all reported that their blood-based biomarkers outperformed PSA. Although the serum biomarker profile developed by Fan and colleagues outperformed PSA at distinguishing BPH from prostate cancer in their population, it was comparable or inferior to PSA testing in discriminating tumors by their degree of aggressiveness (53). Finally, Sreekumar and colleagues reported that the measurement of sarcosine in urine was superior to PSA at predicting a positive prostate cancer biopsy within the clinical PSA gray zone of 2–10 ng/mL (52).

For the tissue-based diagnostic studies (26, 28, 47), the current gold diagnostic standard is histopathology, but as histopathologic analysis is used to determine the presence of prostate cancer, it cannot be compared with metabolomics profiling. Nevertheless in all the studies, good correlation between the metabolic findings and the histopathologic findings was demonstrated. Furthermore for disease recurrence, McDunn and colleagues reported that the inclusion of a metabolomics profile afforded increased prediction compared with clinical indices alone (16).

Part 2: Hypothesis-Generating Studies

Metabolites and pathways implicated in prostate cancer tumorigenesis, progression, and recurrence

In the remaining metabolomics studies, the primary aim was not the identification of biomarkers or indices of biomarker utility were not reported upon. However, among the results, there was substantial crossover with the metabolites and pathways discussed in Part 1, in particular with those thought to be involved in pathogenesis. In studies comparing prostate cancer patients with healthy controls, differences in metabolites and pathways relating to energy metabolism were reported including TCA cycle intermediates (24, 27, 32), lactate (24, 32), citrate (23), phosphoenolypyruvate, and adenosine diphosphate (32). Metabolites vital to cell growth and proliferation were also identified; these included common amino acids (15, 30) bile acids (60), polyamines (27), glycerol-3-phosphate (30), and a number of constituents of cells membranes including long chain fatty acids (22, 23, 51), phospholipids (30), phosphocholines (61), and choline (27, 55). Steroid hormones (23) which help regulate the growth and function of the prostate were also implicated (62), as were inositol and its isomers (27) which are involved in osmoregulation, and have been shown to be dysregulated in several other cancers (55) and cortisol (35) which is thought to be related to cancer development via the mechanism of chronic stress (63). A number of these metabolites, citrate, inositol, lactate (25), and cortisol (35, 63), were additionally observed to differ between BPH and prostate cancer patients, along with phosphoethanolamine, and glycerophosphoethanolamine (29) which are also components of membranes and acetate (25) which is thought to support cell proliferation through *de novo* lipid biosynthesis (64).

Levels of metabolites including sarcosine, uracil, kynurenine, leucine, and proline (43) were shown to be increased during the progression to metastatic disease (32, 33, 43), as were pathways involved in nitrogen breakdown (43). Nitrogen metabolism is known to be altered in tumors to accommodate their enhanced glutamine requirements and the increase in nucleotide and protein synthesis (65). Arachidonic acid metabolism was also altered which is in line with the suspected association between dietary fat and prostate cancer (33, 66). Metabolites from the pathways of energy and lipid metabolism were again demonstrated to be of importance (23, 43) in the degree of disease aggressiveness. Taken together with the results of the biomarkers studies, these findings suggest a particularly important role for pathways involved in abnormal cell growth and intensive cell proliferation in prostate carcinogenesis and progression. Dysregulation of lipid and fatty acid metabolism may be particularly crucial to the disease process.

Multiple steroids, markers of lipid beta-oxidation, markers of omega-oxidation, and markers of insulin resistance (67) were observed to decrease following therapy in the study by Saylor and colleagues (46), while bile acids (60), steroids, and their metabolites increased. This was in line with the findings of Huang and colleagues (51), who reported that the metabolite profiles of patients successfully treated with endocrine therapy closely resembled those of healthy controls. Among all the included studies, one did not report any significant findings (37). This study, by Gamagedara and colleagues was targeted to only four metabolites that had previously been reported as significant in tissue (37). Discussed further in the following section, it is perhaps not surprising that they were unable to replicate the findings in a different biologic media. Furthermore, they were unable to reliably measure the strongest candidate, sarcosine. Nevertheless, it must be taken into consideration that the very small number of null findings may reflect more on the bias towards publishing studies with positive findings, than on the application of metabolomics profiling.

Part 3: Replication and Validation

To validate their findings, 11 studies used internal cross-validation methods (16, 26, 40–44, 47, 49, 50, 53), while one compared the tissue metabolomics findings to those obtained by histopathology (27). Two further studies reported that validation in independent cohorts was ongoing at the time of publication (39, 51) but these data are not yet publically available. Two studies (22, 23) were based within the same parent cohort, but there was no overlap between the populations included. The same metabolite classes were identified as potentially predictive biomarkers in the both studies, and a meta-analyses of the findings provided robust results, particularly for aggressive disease.

Only two studies attempted replication of their findings in an entirely independent cohort (38, 52). Zhang and colleagues (38) compared an additional 30 prostate cancer patients from a different geographic region to their original control population, reporting that 14 of 33 (42%) putative diagnostic biomarkers retained statistical significance. These included four novel metabolites; ureido isobutyric acid, indolylacryloylglycine, acetylanilalinine, and 2-oxoglutarate. After isolating sarcosine as a differential metabolite between benign and prostate cancer tissue samples, Sreekumar and colleagues replicated the experiment in 89 independent samples and reported that not only were sarcosine levels in tissue significantly increased in the cancer specimens there was a further increase among patients with metastatic disease, thereby both confirming and extending upon their original findings (52).

Sreekumar and colleagues (52) further explored the potential of urinary sarcosine, observing significantly increased levels in biopsy positive patients. Two other studies (43, 48) also used multiple biologic media. Stabler and colleagues (48) only replicated one of their markers of recurrence, cysteine, between urine and serum blood samples. Thysell and colleagues looked at bone, tissue, and plasma, and again only replicated a small number of metabolites between media, and none were common to all three. Finally, Brown (31) and Shuster and colleagues. (30) analyzed the same eight prostate samples using different statistical techniques. They reported similar results and both concluded that a metabolic approach could reliably distinguish between benign and cancerous samples and indicate tumor aggressiveness. In the remaining studies, no attempt at replication was made.

The sarcosine debate

Following the publication of Sreekumar and colleagues' findings on sarcosine, an intermediate and byproduct in glycine synthesis and degradation, a number of studies attempted to replicate these promising results (52). Li and colleagues' (33) pathway-based analysis of Sreekumar and colleagues' dataset reported an association between metastatic prostate cancer and methionine metabolism pathways, which can be involved in the formation of sarcosine. McDunn and colleagues (16) observed significantly elevated sarcosine levels in Gleason grade 8 tumors or higher compared with benign tissue, while Thysell and colleagues (43) found a significant increase in sarcosine in the bone of men with metastatic disease. Although recurrence was not a focus of Sreekumar and colleagues' original study (52), Stabler and colleagues (48) observed that urinary Sarcosine levels at the time of surgery were significantly higher among those men whose cancer subsequently recurred.

Conversely, Wu (36) and Zhang and colleagues (38), reported no significant differences in urinary sarcosine levels between prostate cancer cases, BPH cases and healthy controls, and sarcosine was not significant in Mondul and colleagues' (2014) blood-based study (22). Contrary to their findings in bone, Thysell and colleagues found no association between prostate cancer and sarcosine levels in blood or tissue (43). Sarcosine could not be reliably measured in two further studies (30, 37); however, Shuster and colleagues (30) replicated Sreekumar and colleagues' (52) positive findings for uracil, kynurenine, glycerol-3-phosphate, leucine, and proline levels in tissue (30), while Gamegadara and colleagues (37) observed no diagnostic potential for these metabolites in urine.

It has been suggested that these differences in findings may be the result of technical differences between the studies. It is it notable that is it analytically challenging to precisely and accurately measure sarcosine, particularly at low concentrations (68), as is evidenced by the two studies that were unable to do so (30, 37). It has been suggested that liquid chromatography for sample separation prior to MS, may be preferable to gas chromatography for the measurement of sarcosine and that this may explain the conflicting findings (68). However, within the studies reported here, a combination of liquid- and gas-based chromatography was used by both the studies reporting positive findings (16, 43, 48, 52) and those reporting null findings (22, 36, 43, 69). The studies were also coherent with regards to the statistical analyses performed (comparisons of means, OPLS-DA, and ROC curves). With the exception of McDunn and colleagues, all compared to prostate cancer cases to controls and there was no striking differences in population sizes between the positive and null studies (16). It has also been suggested that population differences in sarcosine levels may lead to false-negative or false-positive findings. Among the studies reporting positive findings, three were based in the United States (16, 48, 52) and one in Sweden (43), while among the null studies, two were based in Asia (36, 38) and two in Europe (22, 43). However, the explanation for the differences in findings most likely relates to the multitude of technical challenges and that too, in particular, in the urine-based studies, the difficulty in accurately determining the sarcosine/creatine ratio is likely to be playing a role (36, 68).

Methodological Considerations

Comparisons with other cancers

One of the most common applications of metabolomics is in the study of cancer (54), and a number of metabolomics biomarkers with discriminatory abilities comparable with or even better than those for prostate cancer have been proposed, particularly in cancers of the gastrointestinal system (70–72) and pancreas (73, 74). Malignant cells are known to possess metabolic phenotypes that differ from many normal tissues, characterized by a shift toward aerobic glycolysis and pathway alterations that support biomass accumulation for cell proliferation (14, 15, 75, 76). As such, these reported findings, particularly in the non-tissue–based studies, may identify signatures that reflect malignancy in general and which are not specific to prostate cancer. With this possibility in mind, seven studies investigated additional malignancies (24, 31–33, 37, 43, 50).

Halliday and colleagues (24) compared the metabolic profiles of prostate cancer, lung cancer, and colon adenocarcinoma, and reported tumor-specific differences. Similarly, Kami and colleagues (32) detected a clear distinction between lung and prostate cancer samples based on their metabolic profiles, and reported that lung versus prostate differences were greater than normal versus tumor differences within the same organ. However, they did note that compared with their normal counterparts both tumor types shared a number of features such as higher levels of amino acids, lactate, succinate, fumarate, and malate. Intriguingly, these TCA intermediates have also been shown to be increased in both colon and stomach cancer (77).

Conversely, Gamegedara and colleagues (37), who investigated the biomarkers identified by Sreekumar and colleagues (52) in urine, reported that in their population these biomarkers could not distinguish between prostate cancer cases and controls, nor could they distinguish prostate from breast cancer. While Thysell and colleagues (43), who considered breast, esophageal, lung, and kidney cancer, observed that the significant differences in sarcosine levels between normal bone and the bone metastases of prostate cancer patients were also evident in these other cancers, indicating such differences may not be prostate cancer specific.

Three other studies investigated additional cancers, including kidney (31), colorectal adenoma (33), lung, colorectal, breast, and gastric cancer (50), although they did not specifically compare the metabolome of these malignancies to prostate cancer. A common theme between all cancers was a significant alteration in amino acid levels. A large number of differential metabolites were identified for all the investigated cancers, including prostate, particularly among the amino acids. Nevertheless, Miyagi and colleagues (50) reported that the greatest proportion of differential metabolites were tumor site–specific.

In summary, in these studies, a prostate cancer–specific metabolome was demonstrated both in tissue (24), and in plasma (50), although these metabolomes are characterized more on the basis of the patterns and behaviors of grouped metabolites including lipids and TCA cycle intermediates, rather than on individual metabolites. A prostate cancer–specific metabolome was not demonstrated in urine (37).Within the wider cancer metabolomic literature, a number of the putative "prostate biomarkers" discussed in this review have also been proposed as biomarkers of other malignancies. Aspartic acid, 2-hydroxybutyrate, and kynurenine have been suggested as metabolomics biomarkers of colorectal cancer; the malignancy in which the field of metabolomics is perhaps most advanced (78). Lactate, threonine, acetate, uracil, succinate, lysine and tyrosine, myoinositol, taurine and creatine have been shown to be associated with the presence of rectal cancer, and correlated with its progression (79). Taurine is also increased in squamous cell carcinoma (80), while lactate has additionally been shown to be associated with esophagogastric cancer (81), along with fumarate, valine, glutamine, glutamate (81), xylonic acid (81, 82), tyrosine, phenylalanine, and tryptophan (83). Together, these metabolites indicate a general dysregulation in the metabolism of cellular respiration, energy, amino acids, ketone body, and choline metabolism which, as discussed, could be applicable to all cancers. Similarly, choline, phosphocholine, phosphatidylcholine, lysophosphocholine, and glycerophosphocholine, which are necessary for cell membrane synthesis and intercellular signaling (55), have been identified in metabolomic profiling studies of multiple cancers including brain, breast, lung, and liver (84). One of the prostate diagnostic biomarkers with the highest reported AUCs, acylcarnitine in blood, has also been shown to distinguish kidney cancer patients from controls, and by their degree of severity when measured in urine (85); this is hypothesized to reflect alterations in immune surveillance and again may point to the overall phenotype required to support the growth and proliferation of malignant cells. Even citrate, which could have been hypothesized to be prostate cancer–specific given its importance in the prostate has been shown to be increased in bile samples of patients with biliary tract cancers (86).

In fact, the vast majority of the metabolites reported here have also been identified in other studies supporting the concept of a carcinogenesis metabolome. However, tumors still retain much of their unique organ-specific metabolism (32, 75), and, to date, neither spermine nor sarcosine have been proposed as metabolomic biomarkers of any other malignancies. However whether this denotes these metabolites as prostate specific, or it merely reflects the nature of metabolomics and the possibility these metabolites have not been measured in the profiling of other cancers, remains to be seen.

Technological and analytical issues

In addition to the "epidemiologic validation" of findings through replication, is it also vital to show that a proposed biomarker displays `technical validation' in terms of intrinsic measurement error and analytic sensitivity (87). Much of the between-study heterogeneity and inconsistency can be attributed to the use of differing technological, experimental, and analytical methods, which can affect the measurements of metabolites in an as yet to be determined way (88). Only seven studies reported on their quality control (QC) procedures, to allow the consideration of system stability, with varying degrees of detail (22, 23, 35, 38, 42, 46, 51), while only eight (22, 23, 30, 35, 38, 42, 46, 51) reported on the relative SD (RSD%) threshold for the included features (38).

Bias and confounding

To fully understand the impact of disease, the composition of the "normal" metabolome in healthy individuals must also be established. The metabolome will be dependent on the originating tissue or biologic media, but will additionally vary by age, BMI, diet, and other lifestyle factors (89). This is further complicated by the fact that a genetic component to the metabolome has been demonstrated (90). There is also a wide range of stability among metabolites and those metabolites that are more stable, including amino acids, are more likely to show differences if they exist between cases and controls. The temporal fluctuation of the metabolome also introduces novel challenges particularly in those studies assessing recurrence and treatment response, and it is of note that none of the diagnostic or aggressiveness studies utilized repeat measurements to address this (87). Similarly none of the studies addressed the temporal fluctuation in the metabolome through the use of repeat samples.

Consequently, as well as technologically induced variation, confounding represents an important issue in any metabolomics study (91). In epidemiologic studies of prostate cancer, a number of potential confounders are commonly included in statistical models including age, race, BMI, and often Gleason grade, due to their reported associations with prostate cancer independence, progression, and lethality (92, 93). Importantly, these variables may also affect the metabolome (94, 95). However they were rarely taken into account in the selected studies.

The impact of interindividual variation represents a strong argument for cases acting as their own controls (96), and in 10 of the studies intratumor differences within the same patients were compared (16, 24, 27, 28, 30–33, 44, 47). Similarly, Saylor and colleagues compared blood samples pre- and post- therapy (46). The remainder utilized external controls. Although the use of external controls ensures that the comparison group is "healthy" and not subject to underlying or latent disease pathology, it also introduces the potential for false positives to arise through batch effects or confounding. Among the studies in this review, the majority of blood- and urine-based studies utilized external controls, so it is difficult to assess whether this resulted in an excess of positive findings. Eight studies matched on age (22, 23, 41, 42, 45, 50, 51, 97), 4 on sampling time (22, 23, 43, 45), one on Gleason grade (45) and one on BMI (42). However, neither age (39, 40), smoking status (22, 50), clinical variables (48), BMI, serum cholesterol, educational level, nor various dietary factors (22, 23) were found to act as confounders in those studies where they were considered. Confounding was not addressed in the remainder of the studies, and none considered other potentially important factors such as subtype heterogeneity (98). Treatment was shown to be a further modifier of the metabolome in the studies investigating its effect (46, 51), and although six studies stated that the biologic samples were collected prior to any radiation or hormonal therapy (32, 36, 47–50), one study included metabolic profiles ascertained after therapy (43), and the remaining case–control studies did not report on this covariate.

The majority of the studies were conducted in predominantly Caucasian populations with the exception of six Asia-based studies (32, 35, 36, 38, 50, 51), and only two studies (40, 41) reported on the ratio of races within their study. This is of particular importance given the largely unexplained disparities in prostate cancer incidence between ethnicities (99); however, in Zhou and colleagues' (40) study, race was not found to act as a confounder. Of the remaining studies, 19 were based in North America and eight were based in Europe, therefore given the suspected impact of environmental and dietary factors on the metabolome, the wider generalizability of the reported findings must be considered.

Multiple testing

Despite the high-dimensional nature of metabolomics, only five of the selected studies controlled for multiple testing (16, 22, 23, 47, 52). Although the significant findings reported in these three studies were robust to such correction, many applied a nominal significance level of 0.05 regardless of the number of metabolites under investigation. There was additional possibility of false positives arising in those studies comparing multiple biologic media. Only four studies (16, 40, 50, 53) reported on the statistical power of their approach in the search for discriminatory metabolites.

Biologic samples

The media in which a biomarker can be reliably measured is of importance in the consideration of its forward translation. The tissue-based biomarkers would have no utility in predicting incident disease, and even for diagnostic or prognostic purposes, blood and urine can be obtained in a less invasive and more cost-effective fashion. Furthermore, tissue is a limited resource, and it may be prudent to preserve it for other uses including subsequent histopathologic analysis. Disease-associated metabolites tend to be more concentrated the closer in proximity they are to the organ of interest (30); however, only one study considered prostatic secretions (39), possibly due to challenges related to its collection. Interestingly, the use of tissue did not appear to confer any advantages over the other biologic specimens, with some of the strongest results reported in blood samples.

Discussion

Because widely used PSA testing remains somewhat controversial (36, 100, 101), additional biomarkers that could help refine practices would be a welcome addition to the management of prostate cancer. With the advancement of high-throughput technologies, metabolomics is emerging as a promising tool in biomarker development (9, 12, 69, 91, 102–106). Its downstream nature provides a holistic picture of the malignant state and consequently insight into dysregulated metabolic pathways and inherent disease development. The selected literature provides encouraging results in the field of prostate cancer; however, it also demonstrates the novel challenges faced by metabolomics, which are only just beginning to be addressed.

The metabolomics of prostate cancer remains a small field with the majority of studies focused on the identification of biomarkers to distinguish malignant and benign prostate tissue, with few studies investigating disease progression and treatment response. Only two studies to date have employed a prospective design to look for predictive biomarkers (22, 23). Therefore, important issues of cause and effect must be considered when evaluating the utility of the diagnostic biomarkers and the role of BPH as a disease continuum-intermediate may be particularly important in this respect.

The majority of the included studies reported distinct clustering by metabolome profiles, with differentiation status playing an important role in the determination of the profiles (32). In those studies which developed biomarkers with the potential for clinical translation, the reported AUCs associated with the biomarkers were high. In many cases these AUCs were higher than for PSA, suggesting these new biomarkers would outperform the current gold standard in many cases. This is in line with the generally accepted consensus that the metabolome represents a rich source for biomarker identification. However, replication, particularly between biologic media, and independent validation was lacking, multiple testing was rarely accounted for, and the extent to which the reported findings may represent false positives is difficult to assess.

This was true even of one of the most commonly cited "metabolomics successes" sarcosine, and the importance of technical issues in metabolomics studies is perhaps best exemplified by the debate over the potential use of this biomarker. Nevertheless a number of metabolites and pathways were repeatedly implicated by the studies with amino acid and lipid metabolism appearing to play a predominant role in carcinogenesis, progression, and recurrence. Caution must be taken to ensure such findings do not merely reflect the most abundant, easy to measure or stable metabolites, although encouragingly, a number of the studies indicate that the "metabolome" of prostate cancer is distinct from that of other malignancies.

These challenges inherent to metabolomics extend even beyond those facing researchers when they first tried to characterize the human genome, due to the additional temporal component as well as issues regarding the stability, variation, and plasticity of the metabolites (107). Therefore, collaboration between groups conducting metabolomics-based studies is vital, both in terms of standardizing the optimal methods and analytical strategies, to maximize reproducibility, reliability, and sensitivity and also for replication or the accrual of sufficient sample sizes for these highly dimensional studies (108).

Clinical translation remains the end goal, but a number of important factors remain to be considered before this is feasible for the current studies, including issues of bias, confounding, and generalizability. Beyond the efficacy of a biomarker, the feasibility of clinical translocation must also be considered. More than two decades since PSA testing was introduced no such biomarkers have been clinically approved (109). In fact it may be that the utility of the metabolites and metabolite profiles identified here lies not in their clinical usage but in the insights they provide into the mechanisms of carcinogenesis. For example McDunn and colleagues (16) postulated that there may be a variety of pathways that lead to the development and progression of prostate cancer, and therefore multiple metabolite models that are able to predict the outcome of interest in a certain subpopulations. The differing results and lack of replication in the included studies may support this theory.

In conclusion, the study of the metabolome of prostate cancer remains in the early phases, but could yet represent an important tool both in the understanding of prostate cancer development and progression, and in the development of biomarkers to aid its management.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work is supported by the DF/HCC Specialized Programs in Research Excellence (SPORE) in Prostate Cancer funded by the NCI/NIH (grant number: 2P50CA090381-11A1; Principal Investigator: Philip Kantoff), P01 CA055075, CA133891, CA141298, CA136578, and UM1 CA167552 (to M.G. Vander Heiden, E. Giovannucci, and L.A Mucci). L.A. Mucci is a Young Investigator of the Prostate Cancer Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 2, 2015; revised March 8, 2016; accepted March 23, 2016; published OnlineFirst April 6, 2016.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10–29.
2. Crawford ED, Ventii K, Shore ND. New biomarkers in prostate cancer. Oncology 2014;28:135–42.
3. Nicholson A, Mahon J, Boland A, Beale S, Dwan K, Fleeman N, et al. The clinical effectiveness and cost-effectiveness of the PROGENSA® prostate cancer antigen 3 assay and the prostate health index in the diagnosis of prostate cancer: a systematic review and economic evaluation. Health Technol Assess 2015;19:1–13.
4. Sooriakumaran P, Nyberg T, Akre O, Haendler L, Heus I, Olsson M, et al. Comparative effectiveness of radical prostatectomy and radiotherapy in prostate cancer: observational study of mortality outcomes. BMJ 2014; 348.
5. Xia S, Kohli M, Du M, Dittmar RL, Lee A, Nandy D, et al. Plasma genetic and genomic abnormalities predict treatment response and clinical outcome in advanced prostate cancer. Oncotarget 2015;6: 16411–21.
6. Kohli M, Tindall DJ. New developments in the medical management of prostate cancer. Mayo Clin Proc 2010;85:77–86.
7. Olmos D, Brewer D, Clark J, Danila DC, Parker C, Attard G, et al. Prognostic value of blood mRNA expression signatures in castration-resistant prostate cancer: a prospective, two-stage study. Lancet Oncol 2012;13:1114–24.
8. Danila DC, Fleisher M, Scher HI. Circulating tumor cells as biomarkers in prostate cancer. Clin Cancer Res 2011;17:3903–12.
9. Spratlin JL, Serkova NJ, Eckhardt SG. Clinical applications of metabolomics in oncology: a review. Clin Cancer Res 2009;15:431–40.
10. Goodacre R, Vaidyanathan S, Dunn WB, Harrigan GG, Kell DB. Metabolomics by numbers: acquiring and understanding global metabolite data. Trends Biotechnol 2004;22:245–52.
11. Holmes E, Loo RL, Stamler J, Bictash M, Yap IKS, Chan Q, et al. Human metabolic phenotype diversity and its association with diet and blood pressure. Nature 2008;453:396–400.
12. Denkert C, Bucher E, Hilvo M, Salek R, Oresic M, Griffin J, et al. Metabolomics of human breast cancer: new approaches for tumor typing and biomarker discovery. Genome Med 2012;4:37.
13. Cantor JR, Sabatini DM. Cancer cell metabolism: one hallmark, many faces. Cancer Discov 2012;2:881–98.
14. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol 1927;8:519–30.
15. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the warburg effect: the metabolic requirements of cell proliferation. Science 2009;324:1029–33.
16. McDunn JE, Li Z, Adam K-P, Neri BP, Wolfert RL, Milburn MV, et al. Metabolomic signatures of aggressive prostate cancer. Prostate 2013;73: 1547–60.
17. McGrowder DA, Jackson LA, Crawford TV. Prostate cancer and metabolic syndrome: is there a link? Asian Pac J Cancer Prev 2012; 13:1–13.
18. Hsing AW, Sakoda LC, Chua SC. Obesity, metabolic syndrome, and prostate cancer. Am J Clin Nutr 2007;86:843S–57S.
19. Trock BJ. Application of metabolomics to prostate cancer. Urol Oncol 2011;29:572–81.
20. Costello LC, Franklin RB. Concepts of citrate production and secretion by prostate. 1. Metabolic relationships. Prostate 1991;18: 25–46.
21. Fiehn O.Metabolomics–the link between genotypes and phenotypes. Plant Mol Biol 2002;48:155–71.
22. Mondul A, Moore S, Weinstein S, Männistö S, Sampson J, Albanes D. 1-Stearoylglycerol is associated with risk of prostate cancer: results from a serum metabolomic profiling analysis. Metabolomics 2014; 10:1036–41.
23. Mondul A, Moore SC, Weinstein SJ, Karoly ED, Sampson JN, Albanes D. Metabolomic analysis of prostate cancer risk in a prospective cohort: the alpha-tocolpherol, beta-carotene cancer prevention (ATBC) study. Int J Cancer 2015;137:2124–32.

24. Halliday KR, Fenoglio-Preiser C, Sillerud LO. Differentiation of human tumors from nonmalignant tissue by natural-abundance 13C NMR spectroscopy. Magn Reson Med 1988;7:384–411.
25. Schiebler ML, Miyamoto KK, White M, Maygarden SJ, Mohler JL. In vitro high resolution 1H-spectroscopy of the human prostate: benign prostatic hyperplasia, normal peripheral zone and adenocarcinoma. Magn Reson Med 1993;29:285–91.
26. Hahn P, Smith ICP, Leboldus L, Littman C, Somorjai RL, Bezabeh T. The classification of benign and malignant human prostate tissue by multivariate analysis of 1H magnetic resonance spectra. Cancer Res 1997; 57:3398–401.
27. Swanson MG, Vigneron DB, Tabatabai ZL, Males RG, Schmitt L, Carroll PR, et al. Proton HR-MAS spectroscopy and quantitative pathologic analysis of MRI/3D-MRSI-targeted postsurgical prostate tissues. Magn Reson Med 2003;50:944–54.
28. Cheng LL, Burns MA, Taylor JL, He W, Halpern EF, McDougal WS, et al. Metabolic characterization of human prostate cancer with tissue magnetic resonance spectroscopy. Cancer Res 2005;65:3030–4.
29. Komoroski RA, Holder JC, Pappas AA, Finkbeiner AE. 31P NMR of phospholipid metabolites in prostate cancer and benign prostatic hyperplasia. Magn Reson Med 2011;65:911–3.
30. Shuster JR, Lance RS, Troyer DA. Molecular preservation by extraction and fixation, mPREF: a method for small molecule biomarker analysis and histology on exactly the same tissue. BMC Clin Pathol 2011;11:14-.
31. Brown M, McDunn J, Gunst P, Smith E, Milburn M, Troyer D, et al. Cancer detection and biopsy classification using concurrent histopathological and metabolomic analysis of core biopsies. Genome Med 2012;4:33.
32. Kami K, Fujimori T, Sato H, Sato M, Yamamoto H, Ohashi Y, et al. Metabolomic profiling of lung and prostate tumor tissues by capillary electrophoresis time-of-flight mass spectrometry. Metabolomics 2013;9: 444–53.
33. Li C, Han J, Yao Q, Zou C, Xu Y, Zhang C, et al. Subpathway-GM: identification of metabolic subpathways via joint power of interesting genes and metabolites and their topologies within pathways. Nucleic Acids Res 2013;41:e101–e.
34. Lokhov P, Dashtiev M, Moshkovskii S, Archakov A. Metabolite profiling of blood plasma of patients with prostate cancer. Metabolomics 2010;6: 156–63.
35. Cho H-J, Kim JD, Lee W-Y, Chung BC, Choi MH. Quantitative metabolic profiling of 21 endogenous corticosteroids in urine by liquid chromatography–triple quadrupole-mass spectrometry. Analytica Chimica Acta 2009;632:101–8.
36. Wu H, Liu T, Ma C, Xue R, Deng C, Zeng H, et al. GC/MS-based metabolomic approach to validate the role of urinary sarcosine and target biomarkers for human prostate cancer by microwave-assisted derivatization. Anal Bioanal Chem 2011;401:635–46.
37. Gamagedara S, Kaczmarek AT, Jiang Y, Cheng X, Rupasinghe M, Ma Y. Validation study of urinary metabolites as potential biomarkers for prostate cancer detection. Bioanalysis 2012;4:1175–83.
38. Zhang T, Watson DG, Wang L, Abbas M, Murdoch L, Bashford L, et al. Application of holistic liquid chromatography-high resolution mass spectrometry based urinary metabolomics for prostate cancer detection and biomarker discovery. PLoS One 2013;8:e65880.
39. Serkova NJ, Gamito EJ, Jones RH, O'Donnell C, Brown JL, Green S, et al. The metabolites citrate, myo-inositol, and spermine are potential age-independent markers of prostate cancer in human expressed prostatic secretions. Prostate 2008;68:620–8.
40. Zhou X, Mao J, Ai J, Deng Y, Roth MR, Pound C, et al. Identification of plasma lipid biomarkers for prostate cancer by lipidomics and bioinformatics. PLoS One 2012;7:e48889.
41. Zang X, Jones CM, Long TQ, Monge ME, Zhou M, Walker LD, et al. Feasibility of detecting prostate cancer by ultraperformance liquid chromatography–mass spectrometry serum metabolomics. J Proteome Res 2014;13:3444–54.
42. Struck-Lewicka W, Kordalewska M, Bujak R, Yumba Mpanga A, Markuszewski M, Jacyna J, et al. Urine metabolic fingerprinting using LC–MS and GC–MS reveals metabolite changes in prostate cancer: a pilot study. J Pharm Biomed Anal 2015;111:351–61.
43. Thysell E, Surowiec I, Hörnberg E, Crnalic S, Widmark A, Johansson AI, et al. Metabolomic characterization of human prostate cancer bone metastases reveals increased levels of cholesterol. PLoS One 2010;5: e14175.
44. Menard C, Smith ICP, Somorjai RL, Leboldus L, Patel R, Littman C, et al. Magnetic resonance spectroscopy of the malignant prostate gland after radiotherapy: a histopathologic study of diagnostic validity. Int J Radiat Oncol Biol Phys 2001;50:317–23.
45. Maxeiner A, Adkins CB, Zhang Y, Taupitz M, Halpern EF, McDougal WS, et al. Retrospective analysis of prostate cancer recurrence potential with tissue metabolomic profiles. Prostate 2010;70:710–7.
46. Saylor PJ, Karoly ED, Smith MR. Prospective study of changes in the metabolomic profiles of men during their first three months of androgen deprivation therapy for prostate cancer. Clin Cancer Res 2012;18:3677–85.
47. Giskeodegard GF, Bertilsson H, Selnaes KM, Wright AJ, Bathen TF, Viset T, et al. Spermine and citrate as metabolic biomarkers for assessing prostate cancer aggressiveness. PLoS One 2013;8:e62375.
48. Stabler S, Koyama T, Zhao Z, Martinez-Ferrer M, Allen RH, Luka Z, et al. Serum Methionine Metabolites Are Risk Factors for Metastatic Prostate Cancer Progression. PLoS One 2011;6:e22486.
49. Osl M, Dreiseitl S, Pfeifer B, Weinberger K, Klocker H, Bartsch G, et al. A new rule-based algorithm for identifying metabolic markers in prostate cancer using tandem mass spectrometry. Bioinformatics 2008;24:2908–14.
50. Miyagi Y, Higashiyama M, Gochi A, Akaike M, Ishikawa T, Miura T, et al. Plasma free amino acid profiling of five types of cancer patients and its application for early detection. PLoS One 2011;6:e24143.
51. Huang G, Liu X, Jiao L, Xu C, Zhang Z, Wang L, et al. Metabolomic evaluation of the response to endocrine therapy in patients with prostate cancer. Eur J Pharmacol 2014;729:132–7.
52. Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature 2009;457:910–4.
53. Fan Y, Murphy TB, Byrne JC, Brennan L, Fitzpatrick JM, Watson RWG. Applying random forests to identify biomarker panels in serum 2D-DIGE data for the detection and staging of prostate cancer. J Proteome Res 2011;10:1361–73.
54. Liesenfeld DB, Habermann N, Owen RW, Scalbert A, Ulrich CM. Review of mass spectrometry–based metabolomics in cancer research. Cancer Epidemiol Biomarkers Prev 2013;22:2182–201.
55. Griffin JL, Shockcor JP. Metabolic profiles of cancer cells. Nat Rev Cancer 2004;4:551–61.
56. Chadeau-Hyam M, Athersuch TJ, Keun HC, De Iorio M, Ebbels TM, Jenab M, et al. Meeting-in-the-middle using metabolic profiling - a strategy for the identification of intermediate biomarkers in cohort studies. Biomarkers 2011;16:83–8.
57. Nicholson G, Rantalainen M, Maher AD, Li JV, Malmodin D, Ahmadi KR, et al. Human metabolic profiles are stably controlled by genetic and environmental variation. Mol Syst Biol 2011;7:525.
58. Xia J, Broadhurst D, Wilson M, Wishart D. Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics 2013;9:280–99.
59. Thompson IM, Ankerst DP. Prostate-specific antigen in the early detection of prostate cancer. Canadian Med Assoc J 2007;176:1853–8.
60. Li T, Apte U. Bile acid metabolism and signaling in cholestasis, inflammation, and cancer. In: James PH, editor. Advances in pharmacology : Academic Press; 2015. p. 263–302.
61. Currie E, Schulze A, Zechner R, Walther Tobias C, Farese Jr Robert V. Cellular fatty acid metabolism and cancer. Cell Metab 2013;18: 153–61.
62. Soronen P, Laiti M, Törn S, Härkönen P, Patrikainen L, Li Y, et al. Sex steroid hormone metabolism and prostate cancer. J Steroid Biochem Mol Biol 2004;92:281–6.
63. Moreno-Smith M, Lutgendorf SK, Sood AK. Impact of stress on cancer metastasis. Fut Oncol 2010;6:1863–81.
64. Hosios AM, Vander Heiden MG. Acetate metabolism in cancer cells. Cancer Metab 2014;2:27.
65. Medina MÁ. Glutamine and cancer. TJ Nutr 2001;131:S2539–S42.
66. Yang P, Cartwright CA, Li JIN, Wen S, Prokhorova IN, Shureiqi I, et al. Arachidonic acid metabolism in human prostate cancer. Int J Oncol 2012;41:1495–503.

67. Djiogue S, Nwabo Kamdje AH, Vecchio L, Kipanyula MJ, Farahna M, Aldebasi Y, et al. Insulin resistance and cancer: the role of insulin and IGFs. Endocr Rel Cancer 2013;20:R1–R17.
68. Cernei N, Heger Z, Gumulec J, Zitka O, Masarik M, Babula P, et al. Sarcosine as a potential prostate cancer biomarker—a review. Int J Mol Sci 2013;14:13893–908.
69. Zhang A, Sun H, Yan G, Wang P, Han Y, Wang X. Metabolomics in diagnosis and biomarker discovery of colorectal cancer. Cancer Lett 2014;345:17–20.
70. Ni Y, Xie G, Jia W. Metabonomics of human colorectal cancer: new approaches for early diagnosis and biomarker discovery. J Proteome Res 2014;13:3857–70.
71. Jayavelu ND, Bar NS. Metabolomic studies of human gastric cancer: review. World J Gastroenterol 2014;20:8092–101.
72. Antonowicz S, Kumar S, Wiggins T, Markar SR, Hanna GB. Diagnostic metabolomic blood tests for endoluminal gastrointestinal cancer—a systematic review and assessment of quality. Cancer Epidemiol Biomarkers Prev 2016;25:6–15.
73. Xie G, Lu L, Qiu Y, Ni Q, Zhang W, Gao Y-T, et al. Plasma metabolite biomarkers for the detection of pancreatic cancer. J Proteome Res 2015;14:1195–202.
74. Mayers JR, Wu C, Clish CB, Kraft P, Torrence ME, Fiske BP, et al. Elevated circulating branched chain amino acids are an early event in pancreatic adenocarcinoma development. Nat Med 2014;20: 1193–8.
75. Hu J, Locasale JW, Bielas JH, O'Sullivan J, Sheahan K, Cantley LC, et al. Heterogeneity of tumor-induced gene expression changes in the human metabolic network. Nat Biotechnol 2013;31:522–9.
76. Vander Heiden MG.Targeting cancer metabolism: a therapeutic window opens. Nat Rev Drug Discov 2011;10:671–84.
77. Hirayama A, Kami K, Sugimoto M, Sugawara M, Toki N, Onozuka H, et al. Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. Cancer Res 2009;69:4918–25.
78. Nishiumi S, Kobayashi T, Ikeda A, Yoshie T, Kibi M, Izumi Y, et al. A novel serum metabolomics-based diagnostic approach for colorectal cancer. PLoS ONE 2012;7:e40459.
79. Wang H, Wang L, Zhang H, Deng P, Chen J, Zhou B, et al. ^{1}H NMR-based metabolic profiling of human rectal cancer tissue. Mol Cancer 2013; 12:121-.
80. Rocha CM, Barros AS, Goodfellow BJ, Carreira IM, Gomes A, Sousa V, et al. NMR metabolomics of human lung tumours reveals distinct metabolic signatures for adenocarcinoma and squamous cell carcinoma. Carcinogenesis 2015;36:68–75.
81. Abbassi-Ghadi N, Kumar S, Huang J, Goldin R, Takats Z, Hanna GB. Metabolomic profiling of oesophago-gastric cancer: a systematic review. Eur J Cancer 2013;49:3625–37.
82. Song H, Wang L, Liu HL, Wu XB, Wang HS, Liu ZH, et al. Tissue metabolomic fingerprinting reveals metabolic disorders associated with human gastric cancer morbidity. Oncol Rep 2011;26:431–8.
83. Wiggins T, Kumar S, Markar SR, Antonowicz S, Hanna GB. Tyrosine, phenylalanine, and tryptophan in gastroesophageal malignancy: a systematic review. Cancer Epidemiol Biomarkers Prev 2015;24:32–8.
84. Beger R. A review of applications of metabolomics in cancer. Metabolites 2013;3:552.
85. Ganti S, Taylor SL, Kim K, Hoppel CL, Guo L, Yang J, et al. Urinary acylcarnitines are altered in human kidney cancer. Int J Cancer 2012; 130:2791–800.
86. Wen H, Yoo SS, Kang J, Kim HG, Park J-S, Jeong S, et al. A new NMR-based metabolomics approach for the diagnosis of biliary tract cancer. J Hepatol 2010;52:228–33.
87. Vineis P, Perera F. Molecular epidemiology and biomarkers in etiologic cancer research: the new in light of the old. Cancer Epidemiol Biomarkers Prev 2007;16:1954–65.
88. Garcia-Segura JM, Sanchez-Chapado M, Ibarburen C, Viano J, Angulo JC, Gonzalez J, et al. *Invivo* proton magnetic resonance spectroscopy of diseased prostate: spectroscopic features of malignant versus benign pathology. Magn Reson Imaging 1999;17:755–65.
89. Krug S, Kastenmuller G, Stuckler F, Rist MJ, Skurk T, Sailer M, et al. The dynamic range of the human metabolome revealed by challenges. FASEB J 2012;26:2607–19.
90. Gieger C, Geistlinger L, Altmaier E, Hrabé de Angelis M, Kronenberg F, Meitinger T, et al. Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. PLoS Genet 2008;4: e1000282.
91. Nordström A, Lewensohn R. Metabolomics: moving to the clinic. J Neuroimmune Pharmacol 2010;5:4–17.
92. Ma J, Li H, Giovannucci E, Mucci L, Qiu W, Nguyen PL, et al. Prediagnostic body-mass index, plasma C-peptide concentration, and prostate cancer-specific mortality in men with prostate cancer: a long-term survival analysis. Lancet Oncol 2008;9:1039–47.
93. Wilson KM, Giovannucci EL, Mucci LA. Lifestyle and dietary factors in the prevention of lethal prostate cancer. Asian J Androl 2012;14:365–74.
94. Morris C, O'Grada C, Ryan M, Roche HM, Gibney MJ, Gibney ER, et al. The relationship between BMI and metabolomic profiles: a focus on amino acids. Proc Nutr Soc 2012;71:634–8.
95. Menni C, Kastenmüller G, Petersen AK, Bell JT, Psatha MTsai P-C, , et al. et al.Metabolomic markers reveal novel pathways of ageing and early development in human populations. Int J Epidemiol 2013; 42:111–9.
96. Jentzmik F, Stephan C, Miller K, Schrader M, Erbersdobler A, Kristiansen G, et al. Sarcosine in urine after digital rectal examination fails as a marker in prostate cancer detection and identification of aggressive tumours. Eur Urol 2010;58:12–8.
97. Touvier M, Fezeu L, Ahluwalia N, Julia C, Charnaux N, Sutton A, et al. Association between prediagnostic biomarkers of inflammation and endothelial function and cancer risk: a nested case-control study. Am J Epidemiol 2013;177:3–13.
98. Wyatt AW, Mo F, Wang Y, Collins CC. The diverse heterogeneity of molecular alterations in prostate cancer identified through next-generation sequencing. Asian J Androl 2013;15:301–8.
99. Centers for Disease Control and Prevention. Prostate cancer rates by race and ethnicity ; 2014[cited 2015 Feb 27. Available from: http://www.cdc.gov/cancer/prostate/statistics/race.htm.
100. Lehrer S, Diamond EJ, Mamkine B, Droller MJ, Stone NN, Stock RG. C-reactive protein is significantly associated with prostate-specific antigen and metastatic disease in prostate cancer. BJU Int 2005;95:961–2.
101. Chou R, Croswell JM, Dana T, Bougatsos C, Blazina I, Fu R, et al. Screening for prostate cancer: a review of the evidence for the U.S. preventive services task force. Ann Intern Med 2011;155:762–71.
102. Bictash M, Ebbels TM, Chan Q, Loo RL, Yap IKS, Brown IJ, et al. Opening up the "Black Box": metabolic phenotyping and metabolome-wide association studies in epidemiology. J Clin Epidemiol 2010;63:970–9.
103. Ritchie S, Ahiahonu P, Jayasinghe D, Heath D, Liu J, Lu Y, et al. Reduced levels of hydroxylated, polyunsaturated ultra long-chain fatty acids in the serum of colorectal cancer patients: implications for early screening and detection. BMC Med 2010;8:13.
104. Hilvo M, Denkert C, Lehtinen L, Müller B, Brockmöller S, Seppänen-Laakso T, et al. Novel theranostic opportunities offered by characterization of altered membrane lipid metabolism in breast cancer progression. Cancer Res 2011;71:3236–45.
105. Ye G, Zhu B, Yao Z, Yin P, Lu X, Kong H, et al. Analysis of urinary metabolic signatures of early hepatocellular carcinoma recurrence after surgical removal using gas chromatography–mass spectrometry. J Proteome Res 2012;11:4361–72.
106. Asiago VM, Alvarado LZ, Shanaiah N, Gowda GAN, Owusu-Sarfo K, Ballas RA, et al. Early detection of recurrent breast cancer using metabolite profiling. Cancer Res 2010;70:8309–18.
107. Kyrtopoulos SA.Making sense of OMICS data in population-based environmental health studies. Environ Mol Mutagen 2013;54:468–79.
108. Schalken JA.Is urinary sarcosine useful to identify patients with significant prostate cancer? The trials and tribulations of biomarker development. Eur Urol 2010;58:19–20.
109. Wu C-L, Jordan KW, Ratai EM, Sheng J, Adkins CB, DeFeo EM, et al. Metabolomic imaging for human prostate cancer detection. Sci Translat Med 2010;2:16ra8–ra8.