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Review:

Nuclear magnetic resonance spectroscopy as a new approach for improvement of early diagnosis and risk stratification of prostate cancer*

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Abstract: Prostate cancer (PCa) is the second most common male cancer worldwide and the fifth leading cause of death from cancer in men. Early detection and risk stratification is the most effective way to improve the survival of PCa patients. Current PCa biomarkers lack sufficient sensitivity and specificity to cancer. Metabolite biomarkers are evolving as a new diagnostic tool. This review is aimed to evaluate the potential of metabolite biomarkers for early detection, risk assessment, and monitoring of PCa. Of the 154 identified publications, 27 and 38 were original papers on urine and serum metabolomics, respectively. Nuclear magnetic resonance (NMR) is a promising method for measuring concentrations of metabolites in complex samples with good reproducibility, high sensitivity, and simple sample processing. Especially urine-based NMR metabolomics has the potential to be a cost-efficient method for the early detection of PCa, risk stratification, and monitoring treatment efficacy.

Key words: Prostate cancer; Metabolomics; Nuclear magnetic resonance (NMR); Biomarker
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1 Introduction

Prostate cancer (PCa) is one of the major threats to men's health worldwide (Brawley, 2012; Center *et al.*, 2012; Jahn *et al.*, 2015; Siegel *et al.*, 2016). In the United States, PCa was estimated to make up roughly 20% of the new cancer cases in men in 2016. Deaths from PCa are expected to account for 8% of cancer-associated deaths (Siegel *et al.*, 2016). Epidemiological data from China are still rare and incomplete but were recently supplemented by high-quality data provided by the National Central Cancer Registry of China (NCCR) (Chen *et al.*, 2016).

The incidence rate of PCa in China from 1998 to 2008 increased by a factor of 3, from 35.2/100000 to 110.0/100000, and the average annual growth rate was as high as 12.6% reaching 60300 cases in 2015 (Coffey, 2001; Baade *et al.*, 2013; Zhu *et al.*, 2015; Chen *et al.*, 2016). While incidence rates in rural areas remained stable between 2006 and 2009, there was an

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increase in urban areas, especially documented in Hong Kong and Shanghai. The rapid rise of the incidence rate may be in part related to the aging of the population but there seems to be a strong link to Western-style diet (Lin *et al.*, 2015).

A comparison of the incidences of PCa in 2015 showed that although the total number of patients in the United States has reached 3.66 times that of China, the estimated death tolls in the two countries are almost similar (Table 1) (Chen *et al.*, 2016; Ervik *et al.*, 2016; Siegel *et al.*, 2016). Interestingly, the numbers in the European Union (EU, World Health Organization (WHO) region) are in between, which might reflect more regional variations in living conditions and diet. However, further investigations are required to come to valid conclusions.

Table 1 Comparison of the estimated new cases and deaths from prostate cancer in the United States, the EU (WHO region), and China, 2015

Country/ region	Estimated new cases	Estimated deaths	Ratio
US	220800	27540	0.12
EU	400364	92328	0.23
China	60300	26600	0.44

Data are from the studies of Chen *et al.* (2016), Ervik *et al.* (2016), and Siegel *et al.* (2016)

Effectivity of PCa treatment and cancer recurrence heavily depend on early detection and proper risk stratification (Moyer, 2012; Schroder *et al.*, 2012; Klotz *et al.*, 2015; Moller *et al.*, 2015). In the United States, the proportion of localized PCa accounts for more than 80% of all cases, which is also one of the major reasons why the mortality/morbidity rate in the United States is much lower than that in Asian countries, and continues to decrease (DeSantis *et al.*, 2014; Jemal *et al.*, 2015; Moller *et al.*, 2015). Therefore, early detection and diagnosis is the most effective way to improve the survival rate, and development of new biomarkers and/or reasonable combination of current diagnostic methods are hot spots in the field of PCa research (Felgueiras *et al.*, 2014).

However, to date no serum or urine biomarker or biomarker panel meets the requirements for highly sensitive and specific detection of PCa and differentiation between indolent and significant PCa. We here explore the prospects of metabolomics to improve PCa detection, patient stratification, and treatment monitoring.

2 Metabolomics—a window into tumor pathology

2.1 Metabolomics in cancer diagnostics

Metabolites, typically less than 1000 Da, represent the end products of complex metabolic pathways. The metabolome closely reflects any changes in those pathways and therefore provides a reasonable basis for clinical diagnosis. Specific changes in the metabolome are thought to reflect pathological states of patients (Dunn *et al.*, 2013).

Depending on the grade of degeneration, tumor cells show alterations of basic biochemical processes. Therefore, defining the metabolic signature of malignancies and precursor cells is the current hot spot in cancer metabolic research.

Several cancer entities have been analyzed, aiming to better understand the pathological alterations in metabolic pathways and to uncover new diagnostic biomarkers.

2.1.1 Colon cancer

Chen *et al.* (2014) found altered glycolytic enzyme activity in the transcriptome of stem cell-like $CD133^+$ colon cancer initiating cells (CCICs) compared to $CD133^-$ colon cancer cells. Those alterations in metabolic enzyme expression correlated with metabolite production through the tricarboxylic acid (TCA) cycle and cysteine/methionine metabolism pathways. This suggests that the metabolic signature can be used as a starting point to determine the potential biological markers and the colorectal cancer therapeutic target.

2.1.2 Thyroid cancer

Clinical studies showed that there was a significant difference in the endogenous metabolism between patients with papillary thyroid cancer, benign thyroid tumors and healthy people. Compared to healthy people, in the serum samples of papillary thyroid cancer patients the valine, leucine, isoleucine, lactate, alanine, glutamine, and glycine levels were increased, while lipids, choline, and tyrosine levels were reduced. Interestingly, glycine levels were not different between benign thyroid lesions and healthy controls. Therefore, glycine could be a useful biomarker for early tumor detection (Zhao *et al.*, 2015).

2.1.3 Ovarian cancer

Jiang *et al*. (2015) found that the clinical staging of ovarian cancer patients was significantly correlated with the urine metabolites analyzed by ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS).

2.1.4 Renal cancer

In their research on the potential risk of the development of chronic liver disease to hepatocellular carcinoma, Trovato *et al.* (2015) proposed that in the future the periodic monitoring of specific metabolic markers for the urine of patients with chronic liver disease will make it possible to achieve clinical application as an effective method for cancer prevention or early diagnosis.

2.1.5 Bladder cancer

Various studies using mass spectrometric (MS) methods reported promising results for bladder cancer (BCa) detection and separation of invasive BCa from non-invasive BCa (Issaq *et al.*, 2008; Huang *et al.*, 2011; Pasikanti *et al.*, 2013; Chan *et al.*, 2015; Zhou *et al.*, 2016). However, cohort sizes were small in most studies and the effects of renal cancer and bladder inflammation were the major problem in BCa detection (Van *et al.*, 2011). In conclusion, MS-based metabolomics can improve BCa detection and risk stratification but the financial burden may hinder comprehensive routine implementation.

2.1.6 Prostate cancer

To date only a few studies have used nuclear magnetic resonance (NMR) to define metabolomic signatures of PCa for diagnosis and risk assessment (Kumar *et al.*, 2016a), while most studies used liquid chromatography-mass spectrometry (LC-MS) or capillary electrophoresis-mass spectrometry (CE-MS) (Roberts *et al.*, 2011; McDunn *et al.*, 2013; Thapar and Titus, 2014; Struck-Lewicka *et al.*, 2015).

2.2 Nuclear magnetic resonance spectroscopy in the study of cancer and biomarker development

NMR, LC-MS, and gas chromatography-mass spectrometry (GC-MS) are the most common techniques used in metabolomics. NMR technology stands out for the rapid detection and excellent reproducibility at high resolution, acceptable sensitivity (mmol), and quantitative accuracy.

NMR spectroscopy is a well-established non-destructive analytical method based on quantum physical effects of atomic nuclei. It makes use of atomic nuclear spins being aligned when placed into a strong magnetic field and moving out of alignment by absorbance of isotope specific radio-frequencies. The tiny difference in realignment of the atomic spins with the magnetic field is then detected. Due to interference with nearby nuclei and electrons, information on the molecular makeup and structure of a probe can be deduced. While many nuclei can be detected, most commonly used isotopes are ^{1}H and ^{13}C. High-field NMR instruments (≥600 MHz) are needed to provide sufficient sensitivity and spectral resolution. Since sensitivity for ^{1}H is highest, one-dimensional ^{1}H analyses are preferred in most studies. NMR spectroscopy allows the direct identification, quantification, and structural analysis of small organic molecules, nucleic acids, proteins, and carbohydrates. Since most measured signals may come from aqueous solvents, deuterium (^{2}H) is often substituted. With a spin of 1, it does not show up in proton (^{1}H, spin 1/2) NMR. NMR signals are calibrated to known peaks, e.g. tetramethylsilane (TMS) for ^{1}H-NMR. The analysis includes identification of molecules by their specific chemical shift fingerprints and quantification may be done by comparison to peaks of pure standards in validation experiments. The application of NMR spectroscopy to biomarker detection involves extended multivariate statistical analyses, e.g. principal component analysis (PCA) or partial least square-discriminant analysis (PLS-DA).

In addition, the simplicity of sample preparation, low volume requirement (typically a few hundred microliters), non-destructive measurement, and last but not least cost efficiency led to rapid acceptance of NMR in noninvasive diagnostics (Motta *et al.*, 2012; Ibrahim *et al.*, 2013; Nagana Gowda and Raftery, 2015; Soininen *et al.*, 2015). The most outstanding point is that hundreds of metabolites can be analyzed in just one NMR measurement (Duarte *et al.*, 2014). One characteristic of cancer cells is the switch from aerobic oxygen-consuming energy production to glycolytic metabolism, known as the Warburg effect (Warburg, 1956). Changes in glycolytic metabolites and related amino acids are amongst the most promising for cancer detection: high lactate levels indicating enhanced anaerobic energy metabolism; enhanced

serine and glycine levels as result of de novo synthesis of serine via a side branch of glycolysis in highly proliferative cancer cells (Yang and Vousden, 2016). Serine is crucial for the growth and survival of many cancer cells and is closely related to the folate cycle as a donor of one-carbon units. Therefore, the enzymes involved in serine de novo synthesis, i.e. phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), and phosphoserine phosphatase (PSPH), may be good targets for therapeutic intervention (Yang and Vousden, 2016).

To significantly outperform current PCa biomarkers and to overcome the shortcomings of prostate specific antigen (PSA) screening, NMR-metabolomics must overcome several challenges. While liquid biopsy, especially urine, is well accepted by patients, standardization is a major issue for quality management concerning sample preparation, measurement, data processing, and statistical analysis. NMR data are highly comparable between different institutions, which is a great advantage and a prerequisite for comprehensive worldwide application (Ward *et al.*, 2010). New tools are under development for multivariate statistics of huge data volumes acquired by NMR and automatic classification of discriminatory from non-discriminatory metabolites (Motegi *et al.*, 2015; Zou *et al.*, 2016).

The workflow for implementation of urine NMR metabolomics into routine PCa diagnostics and treatment monitoring is shown in Fig. 1.

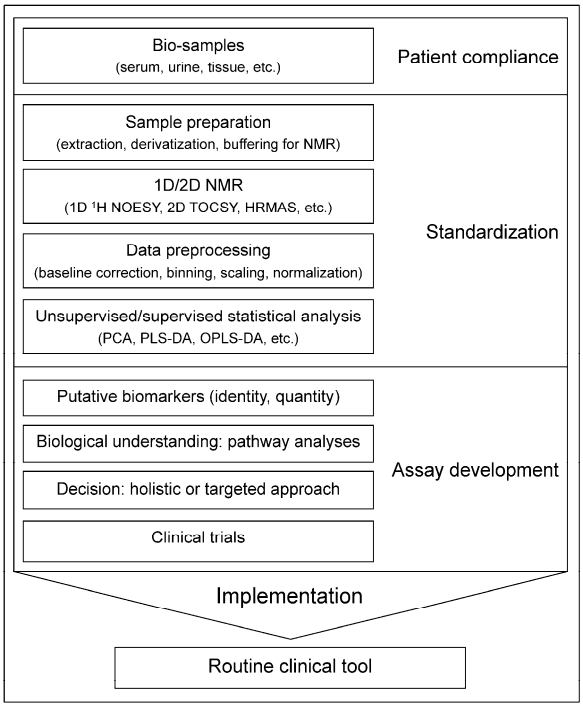

Fig. 1 NMR-based metabolomics workflow
Left hand: developmental steps; right hand: challenges. Patient compliance will be best when using urine as a non-invasive source. Standardization is the major challenge: development of a standard platform for comparability across laboratories and time. The major decision in assay development is between the holistic approach (as often preferred in MS/MS analyses or next generation sequencing (NGS)) and targeted metabolite quantification. Clinical trials in different countries with different socio-economic and genetic backgrounds are required for final adjustment of the assay. PCA: principal component analysis; PLS-DA: partial least square-discriminant analysis; OPLS-DA: orthogonal projection to latent structure discriminant analysis

NMR analytics have been used for biomarker detection in humans in several tumor entities other than PCa (Table 2): non-small-cell lung cancer (Doskocz *et al.*, 2015), oral squamous cell carcinoma (OSCC) (Gupta *et al.*, 2015), gastric cancer (Jung *et al.*, 2014), myeloma (Lodi *et al.*, 2013), pancreatic ductal adenocarcinoma (Davis *et al.*, 2013), lung cancer (Carrola *et al.*, 2011), and BCa (Bansal *et al.*, 2013).

2.2.1 Squamous cell carcinoma

Serum metabolomics successfully separated patients with oral leukoplakia (OLK; n=100) and OSCC (n=100) from healthy controls by the ^{1}H-NMR technique (Gupta *et al.*, 2015). OSCC and healthy control were accurately separated with high area under the curve (AUC; receiver operating characteristic (ROC): 0.97) according to the expression differences of four biomarkers, namely glutamine, propionic acid ester, acetone, and choline, while the comparative analyses of glutamine, acetone, ethyl acetate, and choline can accurately distinguish OLK and OSCC (ROC: 0.96). The two groups were separated with almost ideal sensitivity and specificity (Gupta *et al.*, 2015).

2.2.2 Gastric cancer

In a study on gastric cancer, Chan *et al*. (2016) analyzed 77 metabolites by ^{1}H-NMR in urine samples and found a clear gastric cancer specific metabolite profile. LASSO regularized logistic regression (LASSO-LR) identified three discriminatory metabolites: 2-hydroxyisobutyrate, 3-indoxylsulfate, and alanine, which clearly separated gastric cancer from healthy controls (AUC: 0.95). However, benign gastric disease showed large overlap with gastric

cancer, illustrating the major problem of current metabolomic biomarker studies, patient pre-selection (Chan *et al.*, 2016).

2.2.3 Lung cancer

Carrola *et al.* (2011) reported on urine analysis for lung cancer patients using ^{1}H-NMR combined with PCA, PLS-DA, and orthogonal partial minimum variance discriminant analysis. The results showed that the development process of lung cancer was closely related to potential biological markers, including hippurate, gourd trigonelline, β-hydroxyisovaleric acid, α-hydroxyisobutyric acid, *N*-aceglutamide, and creatine anhydride.

Table 2 Urine NMR metabolomics in biomarker research

Publication year	Cancer entity	Topic	Method	Findings	Reference
2015	Non-small-cell lung cancer	Assessment of toxic kidney's injury	J-RES NMR; focused on amino acids and organic acids (lactid acid and pyruvic acid) profiles	Increase of alanine, leucine, isoleucine, and valine concentrations after the application of cisplatin	Doskocz *et al.*, 2015
2015	Oral squamous cell carcinoma	Early stage detection of oral cancer (OC) and oral leukoplakia (OLK)	^{1}H-NMR; PCA; OPLS-DA	Accurate separation of OC by glutamine, propionate, acetone, and choline (AUC 0.97; sensitivity 92.7%, specificity 93.8%)	Gupta *et al.*, 2015
2014	Gastric cancer	Development of non-invasive screening method	^{1}H-NMR; pre- and post-surgery urine; PCA; OPLS-DA	GC predicted with high accuracy (AUC 0.9731); 86% of sensitivity and 92% of specificity; 17 urinary metabolites identified as potential biomarkers	Jung *et al.*, 2014
2013	Myeloma	Prediction of risk of disease state	^{1}H-NMR; paired blood and urine samples; PCA; PSL-DA	Carnitine and acetylcarnitine potential biomarkers; diagnosis and relapse; pathways may be involved in pathology	Lodi *et al.*, 2013
2013	Gastric adenocarcinoma; mouse model	Tumor biomarkers; monitoring of treatment effect (adriamycin)	^{1}H-NMR; PCA; PSL-DA	Significantly altered metabolites in tumor model: trimethylamine oxide, hippurate, taurine, 3-indoxylsulfate, trigonelline citrate, trimethylamine, and 2-oxoglutarate	Kim *et al.*, 2013
2013	Pancreatic ductal adenocarcinoma	Tumor biomarkers; screening	^{1}H-NMR; targeted profiling; PCA; OPLS-DA	Sixty-six metabolites quantified; AUROC (0.988); 21 "key" metabolites	Davis *et al.*, 2013
2012	Bladder cancer; canine model	Tumor detection and grading	Spontaneous canine transitional cell carcinoma; ^{1}H-NMR	Statistical model (PLS-DA) based on 6 metabolites: urea, choline, methylguanidine, citrate, acetone, β-hydroxybutyrate; TCC detection: AUC (0.85), sensitivity (86%), specificity (78%)	Zhang *et al.*, 2012
2011	Lung cancer	Tumor detection	^{1}H-NMR; PLS-DA; OPLS-DA; Monte Carlo Cross Validation	AUC (0.935); sensitivity (93%); specificity (94%)	Carrola *et al.*, 2011
2010	Bladder cancer	Tumor detection and grading	400 MHz ^{1}H-NMR; OPLS-DA	Citrate lowered, hippurate elevated in BCa; taurine exclusively detected in BCa; no discrimination of tumor grades	Srivastava *et al.*, 2010
2010	Gastric cancer; mouse model	Toxico-metabolomics	^{1}H-NMR; PLS-DA; OPLS-DA	Altered in gastric cancer: trimethylamine oxide (TMAO), 3-indoxylsulfate, hippurate, citrate levels, and 3-indoxylsulfate	Kim *et al.*, 2010

To be continued

Table 2

Publication year	Cancer entity	Topic	Method	Findings	Reference
2015	None	New multivariate statistical approach to NMR data analysis	^{1}H-NMR spectral dataset analysis of known standard mixtures	Cluster-aided MCR-ALS	Motegi *et al.*, 2015
2015	None; hypertensive disorders of pregnancy	Screening for prediction of preeclampsia	Prospective study; ^{1}H-NMR analysis of urine and serum	Prediction of preeclampsia from urine metabolomic profiles (51.3% sensitivity); hippurate most important metabolite	Austdal *et al.*, 2015
2015	None	Monitoring changes of metabolites in urine during diurnal rhythm	^{1}H-NMR	Thirty-two metabolites identified; diurnal rhythm (wake-sleep) has significant influence on urine metabolites	Giskeodegard *et al.*, 2015
2014	None	Technological optimization	^{1}H-NMR	Optimized workflow	Dona *et al.*, 2014
2014	None; acute pancreatitis	Phenotyping	^{1}H-NMR	Pancreatitis: high levels of urinary ketone bodies, glucose, plasma choline, and lipid, and relatively low levels of urinary hippurate, creatine, and plasma-branched chain amino acids; able to distinguish between cholelithiasis and colonic inflammation	Villaseñor *et al.*, 2014
2012	None	Metabolic phenotyping; effects of cadmium exposure	^{1}H-NMR; PLS-DA; OPLS-DA	Six metabolites associated with cadmium exposure: citrate, 3-hydroxyisovalerate, 4-deoxyerythronic acid, dimethylglycine, creatinine, and creatine	Ellis *et al.*, 2012
2011	None	Diet effects (cruciferous vegetables) on urine metabolomics; cancerogens	^{1}H-NMR; J-RES NMR; PLS-DA; OPLS-DA	Four single peaks identified as *S*-methyl-L-cysteine sulfoxide (SMCSO) and 3 other peaks related to SMCSO can serve as biomarkers for putatively cancerogenic cruciferous vegetable diet	Edmands *et al.*, 2011
2010	None; autism	Phenotyping; autistic subjects; their siblings, unrelated healthy subjects	^{1}H-NMR; J-RES NMR; PLS-DA; OPLS-DA	Creatine, creatinine, glycine, hippurate, NMNA, NMND, PAG, 4-cresol sulfate, succinate, and taurine; indication of differences in microbiota	Yap *et al.*, 2010a
2010	None; heart disease; stroke	Population metabolic phenotyping: northern vs. southern Chinese population	^{1}H-NMR; J-RES NMR; PLS-DA; OPLS-DA; 24 h urine samples	Higher in the north: dimethylglycine, alanine, lactate, branched-chain amino acids (isoleucine, leucine, valine), *N*-acetyls of glycoprotein fragments (including uromodulin), *N*-acetyl neuraminic acid, pentanoic/heptanoic acid, and methylguanidine; higher in the south: hippurate, 4-cresyl sulfate, phenylacetylglutamine, 2-hydroxyisobutyrate, succinate, creatine, scyllo-inositol, proline betaine, and trans-aconitate	Yap *et al.*, 2010b

PubMed search (access: Aug. 26, 2016) revealed 154 publications of which 29 review articles were identified. Twenty-seven and 38 were original research papers filtered by the additional keywords “urine” and “serum”, respectively. Those 65 papers were evaluated and relevant papers were summarized. J-RES: J-resolved; NMR: nuclear magnetic resonance; PCA: principal component analysis; OPLS-DA: orthogonal projection to latent structure discriminant analysis; PSL-DA: partial least-square discriminant analysis; AUC: area under the curve; AUROC: area under receiver operating characteristic curve; TCC: transitional cell carcinoma; MCR-ALS: multivariate curve resolution-alternating least squares; NMNA: *N*-methylnicotinic acid; NMND: *N*-methylnicotinamide; PAG: phenylacetylglycine

2.2.4 Bladder cancer

Only two NMR studies are reported in the literature. One study on serum ^{1}H-NMR revealed good separation of low- and high-grade BCa from healthy controls (AUC 0.95, sensitivity 96% and specificity 94%) (Bansal *et al.*, 2013). A panel of six metabolites (dimethylamine (DMA), malonate, lactate, glutamine, histidine, and valine) was derived from the orthogonal projection to latent structure discriminant analysis (OPLS-DA) model. Low- and high-grade BCa could be best separated (AUC 0.97) by using a panel of three metabolites (DMA, malonate, and lactate) (Bansal *et al.*, 2013). Another ^{1}H-NMR study showed altered levels of citrate, DMA, taurine, phenylalanine, and hippurate in BCa compared to healthy controls in urine (Srivastava *et al.*, 2010). However, the authors were not able to differentiate between carcinoma in situ (CIS) and stage Ta or T1 tumors.

2.2.5 Prostate cancer

Kline *et al.* (2006) reported that citrate measured by high-field ^{1}H-NMR in seminal fluid outperformed PSA in detection of PCa in PCa patients when compared to healthy controls. Recently, Kumar *et al*. (2016b) showed that panels of metabolites from serum can separate benign prostatic hyperplasia (BPH)+ PCa from healthy controls and PCa from BPH. Interestingly, [^{68}Ga] citrate was recently successfully used as a radiotracer in positron emission tomography for imaging of PCa in a small study group of castration-resistant prostate cancer (CRPC) (n=8), demonstrating the diagnostic potential of newly detected metabolites (Behr *et al.*, 2016).

Giskeødegård *et al.* (2015a; 2015b) analyzed blood plasma and serum samples from 29 PCa patients and 21 controls with BPH by a combination of magnetic resonance spectroscopy, MS, and GC. They could separate PCa from BPH patients with good sensitivity (81.5%) and specificity (75.2%), demonstrating that fatty acids (acyl carnitines), choline (glycerophospholipids), and amino acids (arginine) can be used as metabolic markers for the diagnostic differentiation between PCa and BPH.

Clinical studies have demonstrated that using the NMR metabolic proteomics method to describe the metabolic signature of potential cancer patients and identifying cancer-associated characteristics of early biological markers can help early diagnosis of PCa, while also providing a reference indicator for prognosis and therapeutic effect evaluation (Bertini *et al.*, 2012; Smolinska *et al.*, 2012; Beger, 2013; Emwas *et al.*, 2013; James and Parkinson, 2015).

2.3 What is the best sample for NMR cancer metabolomics?

2.3.1 Serum

Serum is the most versatile body fluid and can be used for quantitative NMR metabolic analysis of many different malignancies (Bertini *et al.*, 2012; Zhang *et al.*, 2013; Wang *et al.*, 2013; Armitage and Barbas, 2014; Kumar *et al.*, 2015; Jobard *et al.*, 2015). However, the technical challenges are higher in serum than in urine, since serum has the prospect of possible interference of high abundance metabolites with low abundance target metabolites requiring special fractionation procedures (Ferreiro-Vera *et al.*, 2012).

2.3.2 Seminal fluid

In the case of PCa, seminal fluid has been appreciated as a direct reflection of prostate consistence by the use of ejaculate or expressed prostatic secretions in PCa biomarker research mostly based on proteomics (Drake *et al.*, 2010; Kim *et al.*, 2012; Neuhaus *et al.*, 2013; Principe *et al.*, 2013; Trock, 2014).

However, it is difficult to establish the ejaculate analysis as a routine clinical test due to critical acceptance by the patients. Thus, in consideration of patient compliance, clinical work flow, and technical feasibility, urine is the most promising body fluid for NMR metabolic studies.

2.3.3 Urine

Urine is outstanding in reflecting the health of a patient, since being composed of renal draining urine contains a wealth of biomarkers derived from all organs. Therefore, urine analyses can detect diseases as different as inflammatory bowel disease (Stephens *et al.*, 2013) and Alzheimer's disease (Fukuhara *et al.*, 2013). PCA3 (prostate cancer gene 3) in urine is the only Food and Drug Administration (FDA)-approved urinary biomarker of clinical PCa, while the fusion gene *TMPRSS2 ERG*, α-formyl coenzyme A racemic enzyme (*AMACR*), single nucleotide polymorphism (*SNP*), and others have also been reported and confirmed to have correlations with PCa. Therefore, they

could be rated as potential PCa biomarkers in urine and may find their way into clinical diagnosis and assessment of therapeutic efficacy (Prensner *et al.*, 2012; Salagierski and Schalken, 2012; Salami *et al.*, 2013; Shipitsin *et al.*, 2014; Wei *et al.*, 2014; Bansal *et al.*, 2015; Frantzi *et al.*, 2015). Recently, prostate born exosomes in urine—called prostasomes—have been used as a promising source of biomarkers (Ronquist and Brody, 1985; Duijvesz *et al.*, 2011; Zijlstra and Stoorvogel, 2016). Surprisingly, only one original study on urine metabolomics in the PCa context is available (Öman *et al.*, 2014). As the ultimate product, the metabolite is more stable than DNA and proteins (Patel and Ahmed, 2015). Further standardization of sampling, analysis, and statistical methods will improve the reliability and outcome of NMR-based urine metabolomics (Emwas *et al.*, 2016).

In summary, individual metabolic signatures are likely to develop into valuable tools for the non-invasive detection of diseases, which are characterized by massively altered local or systemic metabolism. NMR technology has evolved into a precise, generally applicable and, at least in high throughput, cost-efficient method (Sokolenko *et al.*, 2013; Mathé *et al.*, 2014). The idea of using a complex mixture of small urine compounds to assay for PCa is the basis of metabolomics in its holistic approach. Interestingly, even organic compounds evaporated from urine can be used for PCa discrimination from BPH by using unspecific multisensor ion mobility spectrometry, supporting the notion of disease-specific urine metabolite composition (Roine *et al.*, 2014). We performed a PubMed search focusing on urine NMR metabolomics and summarized the most relevant papers in Table 2.

3 Concluding remarks

Metabolomics, recognized as a new diagnostic technology, has made rapid progress following genomics, proteomics, and transcriptome studies. In a short time span of 20 years, it has achieved broad development and application prospects in the fields of disease diagnosis, research, and development of new drugs, drug mechanisms of action research, and food science. To date NMR-based metabolomics of PCa has focused on serum in a small number of studies. However, the results are encouraging and especially urine metabolomics has the potential to create a new path for the clinical diagnosis and risk stratification of PCa.

In its holistic approach, unlabeled NMR will be able to detect small changes in urine metabolite composition, reflecting disease-specific alterations in cancer-bearing organs or body metabolism. While even at this early stage NMR technology will be able to be used in clinical laboratories, the definition of disease-specific biomarker panels will increase the acceptance and might open new horizons for the development of novel and easy to use detection equipment. There is good support for the notion that NMR technology will be a cost-efficient tool to be also used in PCa risk assessment, prognosis and monitoring of PCa progression and treatment efficacy. Because of its feasibility in large patient cohorts, NMR-based urine analysis bears the promise of uncovering population-specific metabolic peculiarities in Western and Asian populations, thereby helping to understand the differences in PCa prevalence in those countries.

Given the special Chinese situation with growing PCa detection rates and increasing morbidity of the detected PCa, early non-invasive NMR urine diagnostics could completely change the situation of PCa in China, saving tens of thousands of lives each year, resulting in fundamental changes of the diagnosis and treatment of PCa. In contrast, risk assessment, monitoring, and treatment control will be the most promising avenues in Western countries.

Compliance with ethics guidelines

Bo YANG, Guo-qiang LIAO, Xiao-fei WEN, Wei-hua CHEN, Sheng CHENG, Jens-Uwe STOLZENBURG, Roman GANZER, and Jochen NEUHAUS declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：核磁共振波谱作为提高前列腺癌早期诊断和危险度分级的新方法

概　要：前列腺癌（PCA）是全球第二个最常见的男性癌症，同时也是男性癌症死亡的第五大原因。早期发现和危险度分级是提高前列腺癌患者生存率最有效的方法。目前前列腺癌的生物标志物缺乏足够的敏感性和特异性，而代谢产物作为生物标志物可以作为一种新的提高早期诊断的工具。我们检索了 154 篇出版物，其中 27 篇和 38 篇是分别关于尿液和血清代谢组学分析的原研论文，提示了核磁共振波谱分析是一种很有前景的检测方法，可用于测量复杂的样本中代谢物的浓度，具有良好的重现性、高灵敏度和样本处理的便捷性。特别是基于核磁共振的代谢组学检测尿液已成为检测前列腺癌的早期潜在的危险度分级和监测治疗效果的有效的方法。

关键词：前列腺癌；代谢组学；核磁共振（NMR）；生物标志物