Asian Journal of Andrology (2014) 16, 549–554

www.asiaandro.com; www.ajandrology.com

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INVITED REVIEW

Prostate Cancer

Current early diagnostic biomarkers of prostate cancer

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Prostate cancer (PCa) has become to have the highest incidence and the second mortality rate in western countries, affecting men's health to a large extent. Although prostate-specific antigen (PSA) was discovered to help diagnose the cancer in an early stage for decades, its specificity is relative low, resulting in unnecessary biopsy for healthy people and over-treatment for patients. Thus, it is imperative to identify more and more effective biomarkers for early diagnosis of PCa in order to distinguish patients from healthy populations, which helps guide an early treatment to lower disease-related mortality by noninvasive or minimal invasive approaches. This review generally describes the current early diagnostic biomarkers of PCa in addition to PSA and summarizes the advantages and disadvantages of these biomarkers.

Asian Journal of Andrology (2014) **16**, 549–554; doi: 10.4103/1008-682X.129211; published online: 09 May 2014

Keywords: early diagnostic biomarkers; MALAT1-derived miniRNA; PCA3; prostate cancer; PSA

INTRODUCTION

Prostate cancer (PCa) plagues male population and has become a major public health problem in most western countries. It is reported to be the first most frequently diagnosed cancer and the second leading cause of cancer death in the United States in 2013, accounting for 28% (238 590) of the total new cases and 10% (29 720) of the total cancer death.[1] With the vast increase of PCa population, early detection becomes one of the major approaches to solve the problem, which is supposed to be the key to an early intervention to reduce disease-related mortality.

Due to the clinical application of prostate-specific antigen (PSA) since 20 years ago, it has shown great of value in PCa detection, staging, and monitoring with high sensitivity. However, many other factors affect the usefulness of PSA as an early diagnostic biomarker. Researchers have found that benign prostate hyperplasia (BPH) and prostatitis may cause an elevation in PSA and there is no evidence to prove that BPH and prostatitis will develop into PCa. Further, it is confirmed that only 30% of patients with unusual PSA value (above 4 ng ml^{-1}) were finally diagnosed with PCa, leading to the over-treatment of low-risk patients,[2] unnecessary biopsies and nonessential radical prostatectomies.[3,4] In contrast, the prevention of such over-diagnosis and over-treatment may cause the progression of potential aggressive, life-threatening PCa.

Thus, novel early diagnostic biomarkers of PCa are urgently needed to be excavated and evaluated to distinguish patients with cancers from healthy population. The review aims to generally summarize the current diagnostic biomarkers of PCa, their status and future prospective.

PROSTATIC ACID PHOSPHATASE AND PROSTATE SPECIFIC ANTIGEN

Prostate acid phosphatase (PAP) was first reported to be elevated in the serum of patients with PCa metastasized to bone and could be regarded as a serum diagnostic biomarker in 1930s.[5] Unfortunately, PAP was proved to be not sensitive to detect localized lesion of PCa[6] and totally replaced by PSA that was discovered in 1970s. Recently, PAP was studied in deep because it was found that high levels of PAP expression were detected in high Gleason score PCa,[7] offering a new and interesting functional aspect of differentiating indolent and aggressive PCa. PAP can be also used as a target antigen for PCa therapy. A novel Food and Drug Administration (FDA)-approved therapy termed Provenge (Sipuleucel-T) aims at 95% PAP expressed PCa,[8] and a phase III clinical trial showed 4.1 months improvement in median overall survival compared with the control group.[9]

Prostate specific antigen, also known as kallikrein-3 (KLK3), is a glycoprotein enzyme encoded in humans by the *KLK3* gene. It was first discovered in late 1970s from prostate extracts.[10] PSA was later found increased in serum of patients with PCa resulting from the disruption of basal cell layer of prostate. It was initially used as a screening biomarker and officially approved for PCa screening by FDA in 1994. However, with the low specificity of PSA in indicating PCa, which may cause relative high false-positive rate in screening, the United States Prevention Services Task Force doesn't recommend PSA screening for early diagnosis and early treatment due to asymptomatic status of PCa for life and risks of complications originated by over-treatment. European randomized study of screening for PCa evaluated the effect of PSA screening and suggested that PSA-based screening could reduce the rate of death from PCa by 20%, but was associated with a high-risk of over-diagnosis.[2] Thus, scientists are focusing on the ways to improve PSA test in order to better distinguish patients with cancers from people with benign conditions. These advanced PSA tests include: (a) free PSA (fPSA) (unbound to other proteins in the serum), is usually used as the form of ratio of fPSA/total PSA (tPSA) which can exclude the suspect of having PCa if the

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Received: 03 December 2013; Revised: 25 February 2014; Accepted: 11 March 2014

ratio level is lower than normal when PSA is 2.6–10 ng ml^{-1}.[11,12] (b) PSA velocity (rate of change in a man's PSA level per year), is reported that people with PSA velocity >2 ng ml^{-1} year^{-1} may have a relative high-risk of death from PCa before diagnosis, demonstrating its role in predicting cancerous state that will lead to death. Moreover, FDA approved the availability of prostate health index (PHI) as a newly developed test combination in 2012. It composes of the blood measurements of %fPSA (fPSA/tPSA), PSA and proPSA, to calculate a score. A recent meta-analysis has proved PHI's diagnostic ability of increasing the sensitivity to 90% and specificity to 32% particularly in the group of patients with PSA between 2 ng ml^{-1} and 10 ng ml^{-1}.[13] Further, Perdona *et al.*[14] tested PHI score in 160 suspected patients followed by biopsies and found that PHI score was significantly higher in PCa group than PCa-negative group. These results reconfirmed that PHI could be used as a diagnostic tool in suspected patients before the first time biopsy.

Current diagnostic biomarkers

Based on the low specificity of PSA in detecting PCa, numerous novel diagnostic biomarkers found to be valuable in researches are currently applied or going to be applied in clinical uses to detect patients with a disease or abnormal condition. In general, these biomarkers can be roughly divided into three groups including deoxyribonucleic acid-based biomarkers, ribonucleic acid (RNA)-based biomarkers and protein biomarkers. Further, based on approaches to find the biomarkers, they can be divided into tissue, serum, urine, and semen diagnostic biomarkers (**Table 1**).

Prostate specific antigen isoforms

Serum PSA originates from proPSA synthesized in prostate cells. On an average 70%–90% of serum PSA is complexed with serum protease inhibitors and is termed complexed PSA (cPSA), a kind of PSA isoform. In the process of proPSA degradation, different forms of proPSA are developed, including (−2), (−4), and (−5) proPSA, of which (−2) proPSA (p2PSA) plays the most important role and accounts for the most concentration in PCa.[15]

Complexed PSA is the sum of immunodetectable forms of PSA with serum protease inhibitors. Several studies with a large testing population have proved cPSA to have a better diagnostic efficiency, especially in specificity with a larger area under the curve (AUC) value than tPSA alone.[16–18]

(−2) proPSA, also known as p2PSA, is tested in its derivatives, %p2PSA (p2PSA/tPSA) which may improve both discrimination between male patients with PCa-positive and those with PCa-negative.[19,20] In a recent study, Lazzeri *et al.*[21] testified clinical performance of serum p2PSA and its derivatives, %p2PSA in 1026 men with a family history of PCa, demonstrating that both p2PSA value and %p2PSA were significantly higher in patients than healthy men, and indicating that a better threshold of %p2PSA as 1.66 with sensitivity and specificity of 70.4% and 70.1%, respectively. The results showed great improvement of p2PSA as an isoform of PSA in early detection of PCa. In addition to the diagnostic value, p2PSA also presents characteristics in the prediction of pathologic outcomes before operation,[22] which needs more researches to confirm in the future.

Table 1: Current early diagnostic biomarkers of prostate cancer

Marker	*Product*	*Detection methods*	*References*
PAP	Enzyme	Serum	5,7
PSA	Glycoprotein enzyme	Serum	2,10
cPSA	Glycoprotein enzyme	Serum	15–17
p2PSA	Glycoprotein enzyme	Serum	18–20
AMACR	Enzyme	Tissue biopsy, serum, urine	21–24
PCA3	Non-coding RNA	Urine	25–31
TMPRSS2-ERG gene fusion rearrangement	Fusion genes	Tissue biopsy, urine	32–36
MALAT1 derived miniRNA	miniRNA	Serum	37,38
Genetic score by SNPs	DNA	Serum, saliva	39,40
LOH test	DNA	Urine	41
KLK2	Protein	Serum	42
PSMA	Membrane glycoprotein	Tissue biopsy	43–45
GSTP1	DNA	Urine	46–48
GOLPH 2	mRNA	Tissue biopsy	49–51
Sarcosine	Protein	Tissue biopsy, serum, urine	52,53
CTCs	Circulating cells	Plasma	54

PAP: prostate acid phosphatase; PSA: prostate specific antigen; cPSA: complexed prostate specific antigen; p2PSA: (−2) proPSA; AMACR: alpha-methylacyl-coA racemase; *PCA3*: prostate cancer antigen 3; *MALAT1*: metastasis associated lung adenocarcinoma transcript 1; SNP: single nucleotide polymorphism; LOH: loss of heterozygosity; KLK2: kallikrein and kallikrein-related peptidase 2; PSMA: prostate-specific membrane antigen; *GSTP1*: glutathione s-transferase pi 1; *GOLPH 2*: golgi phosphoprotein 2; CTC: circulating tumor cells; RNA: ribonucleic acid; DNA: deoxyribonucleic acid; mRNA: messenger RNA

Alpha-methylacyl-coa racemase

Alpha-methylacyl-coa racemase (AMACR) belongs to the family of isomerase, which specifies racemases and epimerases acting on other compounds. It has been shown to be associated with human cancers including neuroendocrine neoplasms of the stomach,[23] hepatocellular carcinoma,[24] and colorectal adenomas[25] with higher expression in cancerous tissues than normal. In prostate, AMACR was proved to be overexpressed in cancer epithelium, hence becoming a potential diagnostic biomarker for cancer cells within PCa.[26] In addition, epidemiologics, genetics, and laboratory studies have all pointed out the importance of AMACR in PCa.[27] Although, it has been recognized that AMACR may play a key role in PCa genesis, detection approaches in the prostate tissue still perplex whether to have a biopsy for early diagnosis because of potential severe complications brought by invasive examinations. Sreekumar *et al.*[28] screened sera for AMACR in both patients with PCa and controls. They found AMACR immunoreactivity to be statistically significantly higher in the sera from cancer case subjects than that from control subjects. In urine samples, Rogers *et al.*[29] detected AMACR elevation in 100% patients with adenocarcinoma of prostate confirmed by biopsy. All these results show the potential application of AMACR as an early diagnostic biomarker, replacing invasive biopsy. However, AMACR still has some limitations that affect its improvement. It can cause humoral responses and production of endogenous antibody, which might lower the serum AMACR detection rate.[30] Nevertheless, more testing is under way to evaluate the possibility of using AMACR as an early diagnostic biomarker for PCa

Prostate cancer antigen 3

Prostate cancer antigen 3 (*PCA3* or *DD3*) is a non-coding RNA, which is only detected in human prostate tissue and highly overexpressed in PCa.[31] *PCA3* can be detected in urine and has a relative lower sensitivity, but much higher specificity in diagnosing PCa when compared with PSA.[32] In the first two clinical trials from Canada[33] and Austria,[34] *PCA3* was evaluated for the potential diagnostic value in urine. More than 700 men undergoing prostate biopsy donated urine after digital rectal examination in order to make sure that there were sufficient prostate epithelial materials in the urine to be detected. The sensitivity and

specificity of *PCA3* were 66%–82% and 76%–89%, respectively. The *PCA3* score has been considered as a new genetic test that determines whether products of genes associated with PCa are present in the urine, which can be calculated by the ratio of *PCA3* to PSA messenger RNA (mRNA). It represents the expression of *PCA3* corrected for the background of normal or BPH epithelial cells present in the specimen. A strong correlation was observed between *PCA3* score and the probability of PCa, ranging from 14% when *PCA3* score lower than 5 to 69% when higher than 100. The authors also concluded that *PCA3* score is useful in patients with a previous negative biopsy or without any biopsy.[35,36] It can also be used to predict tumor aggressiveness and treatment options.[37] In addition, a recent prospective study suggested that urinary *PCA3* testing with increased PSA value can decrease the number of unnecessary prostate biopsy.[38] However, the limitation of *PCA3* includes lacking most appropriate cutoff level of *PCA3* score and false negative results that some PCa, especially aggressive tumors, may present with a low *PCA3* score, affecting the determination.

TMPRSS2-ERG gene fusion rearrangement

Gene fusion between *TMPRSS2* (*21q22*) and *ERG* (*21q22*), *ETV1* (*7q21*), *ETV4* (*17q21*) or *ETV5* (*3q27*) are commonly identified in PCa.[39] Among them, *TMPRSS2-ERG* fusions-positive is detected in approximately 50% of Caucasian with PCa.[40] *TMPRSS2* refers to transmembrane protease serine two which is androgen responsive. *ERG* encodes for a protein that functions as a transcriptional regulator, the overexpression of which may contribute to development of androgen-independence in PCa. Essentially, the fusion between *TMPRSS2* and *ERG* may disrupt the ability of cells to differentiate into proper and normal prostate cells, resulting in forming unorganized tissue.[41] The fusion can be detected in patients' urine without any invasive approaches, and it was shown that the fusion could be detected in 42% of patients with localized PCa, indicating the ability for noninvasive early diagnosis.[42] Moreover, with the deepening of studies, researchers found that *TMPRSS2-ERG* gene fusion was associated with disease progression. A cohort tracking study was performed by Pettersson *et al.*[43] for 12.6 years after 1180 men being treated with radical prostatectomy, and a meta-analysis including 5074 men, both found the relationship between *TMPRSS2-ERG* gene fusion and the stage of diagnosis. Hessels *et al.*[44] intriguingly, discovered the combination of *TMPRSS2-ERG* and *PCA3* could increase the sensitivity from 37% to 73% and give a better indication for patients to have repeat biopsies. In the latest research, combining urinary detection of *TMPRSS2-ERG* and *PCA3* with serum PSA can greatly improve the prediction (AUC = 0.88; specificity = 90% at 80% sensitivity).[45] However, the limitation of using *TMPRSS2-ERG* gene fusion rearrangement as the early diagnostic biomarker lies in its inconsistence between different populations, as we found the detection rate of *TMPRSS2-ERG* was only 18.5% in Chinese population, which was obviously lower than that in Caucasians.[46]

Metastasis associated lung adenocarcinoma transcript 1 derived mini ribonucleic acid

Metastasis associated lung adenocarcinoma transcript 1 (*MALAT1*) was first discovered to be a long noncoding RNA that can predict metastasis and survival in early-stage non-small cell lung cancer in 2003.[47] Further studies have confirmed that the overexpression of *MALAT1* is related to human hepatocellular carcinoma, breast cancer, pancreatic cancer, colon cancer, and PCa.[48] We performed an RNA-sequencing in 14 paired Chinese PCa samples and identified significant *MALAT*1 overexpression in PCa tissue.[46] Further, to decide whether *MALAT1* can be applied to early diagnosis of PCa in serum, we discovered that *MALAT1* had the form of miniRNA fragment in the sera of patients with PCa and thus examined the diagnostic ability in 87 cancer, 82 BHP and 23 healthy controls sera samples. We found that *MALAT1*-derived miniRNA was more effective to distinguish PCa from non-PCa than PSA.[49] The results showed a great potential of *MALAT*1-derived miniRNA applied in PCa early diagnosis in the future.

Genetic score based on single nucleotide polymorphisms

The fact that PCa is linked to genetic factors was verified by twin studies which suggested that over 42% of etiologic factors result from genes.[50] Scientists have discovered more than 50 risk single nucleotide polymorphisms (SNPs) associated with the tumorigenesis of PCa in Caucasian population,[51] and more than twenty ones in Chinese population, including two novel ones in our previous findings.[52] Genetic score is a recently proposed measurement of inherited risk for PCa, calculated by genotypes of the panel of PCa-related risk SNPs that are weighted by their relative risk (RR) to PCa.[53] In the REduction by DUtasteride of PCa events study, genetic score was presented to increase PCa risk prediction in patients with PSA > 2.5 ng ml^{-1} who needed rebiopsy operation.[54] We selected 33 PCa-related risk SNPs as a panel to be tested in Chinese population and found the score was significantly higher in patients with cancer than in controls. This confirmed the improved prediction ability of PCa by genetic score.[55] However, genetic score has the plateau effect on discriminating PCa. We evaluated the performance of 29 risk SNPs related to PCa and compared with the previous 24 ones. Consequently, genetic scores and AUC were both similar between them, leading to the indication that PCa-related risk SNPs with small RR was unlikely to further improve the predictive effect.[56]

Loss of heterozygosity test

Loss of heterozygosity (LOH) occurs in PCa as a form of copy number changes, which locates in *7q*, *8p*, *10q*, *12p*, *13p*, *16p*, *17p,* and *18q,* respectively.[57] Urinary LOH test was performed and discriminated between patients with PCa and healthy ones, obtaining sensitivity as high as 87% through a PCR based assay. However, the cases for analysis were limited to 19 specimens.[57] Although LOH test is widely used in detecting bladder cancer, it has little been applied to PCa detection.

Tissue kallikrein and kallikrein-related peptidase 2

Kallikrein comprise a family of 15 homologous secreted trypsin-or chymotrypsin-like serine protease.[58] Kallikrein-related peptidase 2 (KLK2) has the most organ restricted expression in the luminal epithelium of human prostate with KLK3 PSA. Unlike PSA, most of KLK2 have an unbound form in patients' sera. The ratio of KLK2 to PSA was reported to increase PCa detection rate in patients with tPSA ranging from 2 to 10 ng ml^{-1}.[59] Further studies are required to evaluate the diagnostic effect of KLK2.

Prostate-specific membrane antigen

Prostate-specific membrane antigen (PSMA), also known as glutamate carboxypeptidase II, is a kind of membrane glycoprotein that is strongly expressed in epithelia cells of prostate, encoded by folate hydrolase 1 gene.[60] PSMA was proved to be overexpressed 8- to 12-fold in PCa tissues rather than in noncancerous tissues.[61] Although PSMA has been regarded as a targeted biomarker for therapy and prognosis prediction for a long time due to its high concentration in PCa,[62,63] it showed much greater effect on PCa detection when combined with PET imaging recently.[64] Moreover, researchers in University of California have developed a test by using modified phage viruses to detect PSMA for PCa early diagnosis, which is supposed to be operated at home.[65]

Glutathione s-transferase pi 1

Glutathione s-transferase pi 1 (*GSTP1*) is a polymorphic gene encoding active, functionally different *GSTP1* variant proteins that are thought to function in xenobiotic metabolism and play a role in susceptibility to cancer, and other diseases.[66] Many studies have detected and confirmed that *GSTP1* methylation in urine can help enhance the sensitivity and specificity of PCa diagnosis in a noninvasive manner than PSA.[67–69] Due to several specific characteristics, including its prevalent status in the cancer condition and noninvasive detection through urine, *GSTP1* is regarded as a good and promising diagnostic biomarker.

Golgi phosphoprotein 2

Golgi phosphoprotein 2 (*GOLPH 2*) mRNA was first found to have upregulated expression in PCa tissue in 2002.[70] Further study confirmed the overexpression of *GOLPH 2* mRNA in PCa tissues rather than normal and adjacent tissues, and 91.4% of PCa cases showed an upregulation of *GOLPH*, which could serve as a novel diagnostic biomarker in the condition of AMACR-negative tissues.[71] In addition to detecting *GOLPH 2* in PCa tissue, *GOLPH 2* mRNA was described to be one of the complex biomarker panels in urine to diagnose PCa even regardless of serum PSA.[72] All these results greatly manifest the extraordinary talent of *GOLPH 2* in early diagnosis of PCa, especially in AMACR-negative ones.

Sarcosine

Sarcosine is known as an intermediate and byproduct in glycine synthesis and degradation. Sreekumar *et al*.[73] profiled more than 1126 metabolites across 262 clinical samples including tissue, serum and urine and found that sarcosine was highly increased in urine in group of PCa, indicating its vital role in prostate tumorigenesis. Another study investigated serum samples from 290 patients with PCa and 310 healthy men, and concluded that serum sarcosine could increase the accuracy of PCa detection, especially in patients with tPSA <4.0 ng ml^{-1}.[74] However, several studies discovered that sarcosine was not good enough to be detected and considered it as a promising diagnostic and prognostic prediction biomarker in urine and serum.[75,76] More detailed investigations are required to decide whether or not sarcosine can be a diagnostic biomarker of PCa.

Circulating tumor cells

Circulating tumor cells (CTCs) are cells that shed from the origin part of the cancer and circulate into the bloodstream, forming seeds for cancer growth of additional cancers in vital distant organs and triggering a mechanism responsible for cancer-related deaths.[77] CTCs were first observed in 1869 by Thomas Ashworth and validated the existence in various cancers. In Schulich School of Medicine and Dentistry, Dr. Hong Leong ran a blood test of CTCs in 50 noncancer and cancer patients. The results showed 90% accuracy in predicting patients with actual PCa and no false positives (no data shown). Since the application of single-cell sequencing is widespread recently,[78] the test of CTCs will be easily carried out and the effectiveness of cancer related events will be largely improved, especially in prognostic prediction of overall survival in patients with metastatic PCa,[79] and in therapy target decision in personalized medicine era.[80] Taking more efforts, such as generation of enormous data and comprehensive studies, are necessary and promising to contribute to the development of CTCs applied to PCa diagnosis and treatment.

CONCLUSIONS AND PROSPECTIVE

Prostate cancer is a kind of heterogeneous cancer with different phenotypes and outcomes.[81] Most PCa present to be indolent with mild and atypical clinical manifestations, which may confuse and mislead clinicians and patients at the point of disease diagnosis. The screening of PCa by PSA in the last century has greatly promoted early diagnosis and intervention, thus lowering the disease related mortality. However, PSA has the shortage of low specificity, which leads to over-diagnosis and over-treatment for the patients. Thus, extensive efforts have been made to identify better biomarkers in order to guide early diagnosis and prevent the disease from progression. Numerous emerging biomarkers for PCa have been discovered and been applied to clinical uses recently, bringing new insight of PCa to researchers and clinicians as well as producing plenty of novel screening tests for potential patients. However, it is a challenge to substitute for PSA because of its minimally invasive characteristic and relatively low cost, accompanying its high sensitivity.

High-throughput technology methods and advances in molecular biology are helping and accelerating the exploration to useful biomarkers. Next generation sequencing (NGS), as a newly developed technology, has been widely applied to detect biomarkers in the area of PCa and other diseases, as it produces thousands or millions of sequences concurrently with lower cost.[82] The methods of NGS include single-molecule real-time sequencing, ion semiconductor, pyrosequencing, sequencing by synthesis and sequencing by ligation with different mechanisms in genetic sequence mensuration.

Although an increasing number of biomarkers are discovered to contribute to the early diagnosis of PCa, the consideration of both their sensitivity and specificity is still challenging. The analysis of a panel of multiple biomarkers may better indicate the presence and progression of the disease. Besides, testing methods such as via patients' serum, or urine, or biopsy is critical to the application of a diagnostic biomarker for it should follow the rules that noninvasive testing is the most suitable approach to the patients without any traumatic complications.

In summary, as a significant public health threat worldwide, especially in countries where patients can have life expectancies long enough to a clinical manifestation of the disease, the early detection of PCa is critical and necessary to reduce the burden of men's health problem. In the future, more studies are needed to reconfirm the features of the existing biomarkers and further discover novel potential ones to better predict the presence of the disease.

AUTHOR CONTRIBUTIONS

YHS and SCR conceived the idea and made up the structure. MQ drafted and revised the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ACKNOWLEDGMENT

This study was supported by the National Basic Research Program of China (No. 2012CB518306), the National Natural Science Foundation of China (No. 81101946), the Prostate Cancer Foundation Young Investigator Award, and the Shanghai Pujiang Program (No. 12PJD008).

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How to cite this article: Qu M, Ren SC, Sun Y. Current early diagnostic biomarkers of prostate cancer. *Asian J Androl* 09 May 2014. doi: 10.4103/1008-682X.129211. [Epub ahead of print]