Prostate cancer (PCa) is a common tumor that poses a significant threat to men's health. Currently, serum prostate specific antigen (PSA) is the most important marker for screening patients for PCa. However, the sensitivity and specificity of PSA in the early detection of PCa are not satisfactory\(^1,2\), particularly when PSA falls within the range of 4–10 ng/mL. The detection rate of PCa is merely 25%, while the rate of negative biopsy was about 70%–80\(^2\). Some studies have demonstrated that negative biopsy results could not completely exclude the possibility of malignant tumors\(^3\). In addition, not only do inappropriate needle biopsies put patients at risk for prostate complications and increase their mental burden, but also it increases medical cost. Therefore, there is urgent demand for early diagnostic evidence of PCa, so that we can identify significant PCa to the extent possible, reduce the detection of latent and clinically insignificant tumors, and avoid inappropriate clinical treatment.

Recently, numerous molecular markers have been reported to be useful for early diagnosis of PCa or prognostic prediction in PCa patients.

**Biomarkers for the early diagnosis of prostate cancer**

Tumor biomarkers often signal the existence of tumors before other detection approaches, and thereby contribute to the diagnosis of tumors at early stages. That makes them an effective method to diagnose PCa earlier and is also a critical step in improving prognosis. At present, among studies that are exploring for more specific tumor biomarkers than PSA to improve the early diagnosis capacity of PCa, the most interesting tumor markers are PSA derivatives, hereditary prostate cancer 1 (HPC1), prostate cancer antigen 3 (PCA3), the TMPRSS2:ETS fusion gene, glutathione s-transferase \(\pi\) (GSTP1), \(\alpha\)-methylacyl-CoA racemase (AMACR), Golgi phosphoprotein 2 (GOLPH2), early prostate cancer antigen (EPCA), and sarcosine. 

**PSA derivatives**

The PSA gene is located on chromosome 19 (19q13.41) and encodes a 261-amino-acid preprotein. When a leader sequence at the end of the amino acid chain is cleaved, it becomes a pro-enzyme without catalyzing activity (ProPSA). When another 7-amino-acid leader sequence is cleaved from the terminal of ProPSA, it becomes a 237-amino-acid enzyme with catalyzing activity (PSA). There are various forms of PSA in the bloodstream, including free PSA (fPSA) and complex PSA (cPSA). Furthermore, fPSA includes nicked PSA, intact PSA, and ProPSA, while cPSA mostly refers to the PSA binding to \(\alpha\)\(_1\) antichymotrypsin (PSA-ACT) and less frequently the PSA binding to \(\alpha\)\(_2\) macroglobulin (PSA-A2M) and \(\alpha\)1 protease inhibitor.
A recent meta-analysis including 66 studies showed that fPSA [fPSA/total PSA (tPSA)] and cPSA had better diagnostic capacity than tPSA. However, Bratslavsky et al. expanded the biopsy scope in their study but failed to reveal any statistically significant difference between the diagnostic capacity of fPSA, tPSA, and cPSA. The reasons for this may be that fPSA in the bloodstream is unstable, that PSA is not specific to PCa, and that a prostate with a larger volume may dilute fPSA.

Benign PSA (BPSA) is formed when the internal peptide bonds between 145 and 146 amino acids and between 182 and 183 amino acids are ruptured. It is mainly related to the volume of the transition zone in the prostate. As cleaved by human kalikrein 2 (hk2), ProPSAs with leader peptides of 2, 4, 5, and 7 amino acids were named [-2]ProPSA, [-4]ProPSA, [-5]ProPSA, and [-7]ProPSA, respectively. Sokoll et al. illuminated the practical value of this change in a confirmative study in the Early Detection Research Network by the United States National Cancer Institute (NCI). When PSA fell within the range of 2 ng/mL-10 ng/mL, the areas under the curve (AUCs) of [-2] ProPSA (([-2])ProPSA/tPSA), a logistic regression model (with the combination of PSA, BPSA, % PSA, % [-2]ProPSA, [-2] ProPSA/BPSA, and testosterone), and %PSA were 0.73, 0.73, and 0.53, respectively. All these findings indicate that the substantially altered PSA metabolic pathway in the occurrence and development of PCa, as well as relevant PSA mathematical models, may aid in the early recognition of PCa.

Currently, the kinetic parameters of PSA, such as PSA velocity (PSAV), PSA doubling time (PSADT), and PSA half-life (PSAHLL), are mainly used in monitoring treatment response and disease progression and prognosis. Their significance in the early detection of PCa has yet to be developed. For the time being, serum PSA is still the most important parameter in PCa diagnosis and post-treatment follow-up. Therefore, it is extremely necessary to further study the metabolic pathway of PSA, relevant mathematical models for PSA, and PSA kinetics and their relationships with other tumor biomarkers, to optimize the early detection of PCa.

**Hereditary prostate cancer 1 (HPC1)**

Hereditary prostate cancer 1 (HPC1), an important and susceptible gene in PCa, is located on chromosome 1 (1q24-25). The RNASEL (2-5A-dependent ribonuclease) gene is located at the 1q25 site. RNASEL interferes with the antiviral and antiproiferative activities mediated by the 2-5A pathway, which, alternately, is regulated by interferon. The E265X mutation in RNASEL results in significantly reduced activity of RNASEL. Therefore, based on the linkage and segregation phenomena identified between PCa and the deletion mutation (E265X) and the mutation in the initiation code (M11) in two families carrying HPC1, RNASEL is considered a candidate allele for HPC1. In addition, Rokman et al. revealed in their study that deletion mutation E265X and missense mutation R462Q in RNASEL were associated with an increased risk for PCa. HPC1 is probably involved in the initiation of hereditary PCa. Yet, Rennet et al. failed to identify the association between the mutations of the RNASEL gene and PCa risk in Asian (Indian) patients with PCa. Such inconsistency may derive from the heterogeneity of hereditary factors. Despite all this, the significance of these studies is not limited to illustrating the important role of genetic factors in hereditary PCa; they also provide evidence for revealing the complicated biologic features of PCa and for exploring new diagnostic and treatment strategies.

**Prostate cancer antigen 3 (PCA3)**

The DD3PCA3 encoding gene is located on chromosome 9 (9q21-22). The gene includes four exons and three introns. In PCa, the most frequent mutation is the selective splicing of the second exon. At present, there is a vast body of ongoing studies on PCA3. Hopefully they can further confirm the role of PCA3 in the occurrence and the development of PCa and provide new treatment targets for patients with PCa. Hessels et al. suggested that using quantitative reverse transcriptase polymerase chain reaction (RT-PCR) for the detection of urine DD3PCA3 was a valuable molecular detection method in patients with PCa and could help reduce unnecessary biopsies. In a multicenter study designed to examine the diagnostic capacity of urine PCA3 detection, the AUC of urine PCA3 detection was 0.66, while the AUC of serum PCA3 detection was merely 0.57. The sensitivity and specificity of PCA3 detection were 65% and 66%, respectively. Recently, researchers have suggested that serum PSA level plus PCA3 detection was the most promising diagnostic method for PCa. All these studies show that PCA3 is probably an important urine marker for PCa. It also provides a new clue for developing noninvasive detection methods for PCa. Hence, PCA3 may have considerable significance in multiple tumor-marker screening of patients for PCa in the future.

**TMPRSS2:ETS fusion gene**

TMPRSS2 encodes an androgen-dependent transmembrane serine protease. The ETS transcription factor regulates those genes related to cancerous biologic processes (such as cell growth, differentiation, and transformation). Numerous published studies have already revealed the fusion of the TMPRSS2 gene (which is located on 21q22.3) and the ETS transcription factor family (such as ERG [21q22.2], ETV1 [7p21.2], ETV4 [17q21], and ETV5 [3q28]) in PCa. The TMPRSS2:ETS fusion gene enables the ETS gene to be activated by the promoter of the TMPRSS2 gene, and thus launches the effects of the ETS transcription factor in cancerous biologic processes. The latest research also revealed that the TMPRSS2:ETS fusion gene is in 50% or more of early- or middle-stage localized PCAs and hormone-resistant metastatic PCAs, while in high-grade prostate intraepithelial neoplasia, such gene fusion was rarely seen. Furusato et al. used RT-PCR and found the TMPRSS2:ETS gene fusion in at least one tumor site in 30 out of 45 patients. In 80 tumor sites, 39 patients presented such gene fusion. More importantly, the sensitivity, specificity, negative predictive value, and positive predictive value of the detection of the TMPRSS2: ETS fusion gene in urine samples were 37%, 93%, 36%, and 94%, respectively. This provides evidence for developing and optimizing urine detection for PCa in the future. Gene fusion is one of the mechanisms that initiates tumor occurrence. It is
membrane protein. Studies have demonstrated elevated levels of cancerous molecular mechanisms and common anticancer approaches for PCa will certainly be impaired. It is possible that AMACR is a common molecular basis for cancer occurrence, expressed both in other normal tissues and in malignant tumors. As a result, the specificity of AMACR as a screening marker for early PCa detection is that AMACR sensitivity and specificity were 62% and 72%, respectively. However, the main shortcoming of AMACR as a biomarker for early PCa detection is that AMACR is also expressed both in other normal tissue and in malignant tumor tissue. As a result, the specificity of AMACR as a screening approach for PCa will certainly be impaired. It is possible that AMACR is a common molecular basis for cancer occurrence, therefore it may have an important role in revealing common cancerous molecular mechanisms and common anticancer targets.

Golgi phosphoprotein 2 (GOLPH2)
GOLPH2, also known as GOLM1 or GP73, is a type II Golgi membrane protein. Studies have demonstrated elevated levels of GOLPH2 mRNA expression in PCa tissue. Since proteins and lipids synthesized in the endoplasmic reticulum will be further processed, modified, and classified in the Golgi apparatus and then partially excreted out of the cells and partially transferred into the cytomembrane and the endosome, changes in the structure and function of the Golgi apparatus may impact the structures, functions, and characteristics of the cells. Wei et al. used real-time RT-PCR, Western blot, and tissue microarray techniques and further confirmed that the expression level of GOLPH2 was elevated in PCa. It was also demonstrated by the semi-quantitative evaluation system for staining intensity that the expression level of GOLPH2 was higher in PCa than in normal tissue (P < 0.001). The GOLPH2 expression level was upregulated in 567 out of 614 tumor tissue specimens; elevated GOLPH2 expression was seen in 26 out of 31 AMACR-negative PCa specimens. These findings suggest that changes in the structures and functions of subcellular structure (Golgi apparatus, nucleus, mitochondria, and so forth) may also have an important role in the occurrence of cancer.

Early prostate cancer antigen (EPCA)
EPCA is a nuclear matrix protein. Using immunohistochemical staining, the staining intensity of EPCA was significantly different between patients with PCa and controls (P < 0.001), with sensitivity and specificity of 84% and 85%, respectively. It was also found that men with negative results on pathology but positive results for anti-EPCA antibody staining in biopsy tissue would be diagnosed with PCa within or after 5 years. Fundamental research showed that some nuclear matrix proteins could be seen in all types of cells and physiologic status and others are tissue-specific or vary with cell status. In various cells, different nuclear matrix proteins maintain diverse cell nuclear shape, function, and elements. Changes in nuclear matrix proteins may be an early event in tumor occurrence. Paul et al. used ELISA (with a prespecified absorbance threshold of 1.7 at 540 nm) to compare study subjects, including patients with PCa, other tumors, or spinal cord injury, and healthy individuals, and found that only those with PCa had serum EPCA levels above the threshold. The EPCA level was significantly different between patients with PCa and other groups, particularly healthy individuals (P < 0.001), patients with bladder cancer (P < 0.003), and patients with spinal cord injury (P < 0.001), with the sensitivity and specificity of serum EPCA-2 in recognizing PCa were 92% and 94%, respectively. An recent study suggested that the sensitivity and specificity of serum EPCA-2 in recognizing PCa were 94% and 92%, respectively. Moreover, it could distinguish localized and metastatic PCa (AUC = 0.89, 95% CI 0.82-0.97; P < 0.001). EPCA probably precedes microscopic pathologic changes and is thus a potential tumor marker that can actually detect early signs of cancer. Hence, further investigation is needed to reveal its role in disease development and thereby provide evidence for designing highly sensitive and specific PCa screening methods in the future.

Sarcosine
In 2009, Sreekumar et al. reported the value of sarcosine in PCa. In an independent subset containing 89 tissue specimens,
the sarcosine level was significantly elevated in localized PCs as compared to adjacent benign prostate specimens. When compared to localized PCs, the sarcosine level in metastatic specimens was even higher. When compared to a control group with negative biopsy results, the sarcosine level was significantly higher in urine sediments and urine supernatants obtained from patients with positive biopsy. The sarcosine level was markedly increased in PCs cell lines as compared to benign cell lines. Since a large number of current studies are exploring changes in genetic and protein profiles of tumors, changes in the metabolic profiles of tumors is poorly understood. Highlighting the metabolic features of tumors may help us understand tumors in a more comprehensive way and discover new diagnostic strategies and treatment targets for tumors. Therefore, this study is valuable in that it provides new directions for future studies.

Others

Currently, studies regarding prostate-specific membrane antigen (PSMA) are mainly focusing on investigating the value of PSMA as a treatment target by using anti-PSMA dendritic cells and anti-PSMA antibodies carrying radioactive isotopes or toxins, while studies with prostate stem cell antigen (PSCA) are mainly concentrating on the correlations between PSCA and the risk factors, high Gleason scores, later stage, frequent metastasis, and treatment targets of PCs. Whether they can become diagnostic or treatment tools has yet to be explored. Other tumor markers of interest include kallikrein-related peptidase 2 (KLK2), urokinase plasminogen activator and receptor (uPA and uPAR), Hepsin, Annexin A3 (ANAX3), insulin-like growth factors and binding proteins (IGFs and IGFBPs), transforming growth factor β1 (TGFβ1), enhancer of zeste homolog 2 (EZH2), prostate secretory protein 94 (PSP94), and cysteine-rich secretory protein 3 (CRISP-3).

The combination of multiple tumor biomarkers

Environmental factors, genetic factors, or the interaction between the two initiate the molecular mechanisms underlying PCa occurrence and development. So far, numerous molecules related to PCa have been found, which might derive from different stages and different molecular pathways in PCa occurrence and development. This explains why the combination of multiple tumor markers may improve the accuracy of PCa diagnosis.

Hessels et al. demonstrated in their study that the concomitant detection of urine TMPRSS2:ERG fusion gene and PCA3 could improve the sensitivity in diagnosing PCs. Combined detections of GOLPH2, serine peptide inhibitor, Kazal type 1 (SPINK1), PCA3, and TMPRSS2:ERG fusion gene transcript in urine sediments was also better at identifying PCs than detecting PSA or PCA3 alone. Vener et al. used quantitative methylation specific PCR to measure urine GSTP1, retinoic acid receptor beta (RARB), and adenomatous polyposis coli (APC) as early detectors for PCs. They reported that the sensitivity, specificity, and AUC of the combined detections of these three markers in the first group of 121 patients were 55%, 80%, and 0.69, respectively, and in the second group of 113 patients, the sensitivity, specificity, and AUC were 53%, 76%, and 0.65, respectively. Schostak et al. also reported that PSA plus urine Annexin A3 (ANAX3) detection was better than the detection of either parameter alone. AUC was 0.82 when tPSA fell within the range of 2–6 ng/mL and was 0.83 when tPSA was 4–10 ng/mL. For all patients, AUC was 0.81. In addition, they also found excellent detection capacity for the combination in the subgroups of patients with negative results on digital rectal exam (DRE) and low tPSA levels. Apparently, the combination of multiple tumor markers can significantly improve diagnostic accuracy, representing an important future direction in the screening of patients for PCs.

Conclusions and prospects

The studies mentioned above explored the screening of PCs on different levels (metabolic pathway, genetic, transcriptional, protein, subcellular, and metabolic), but the clinical value of the tumor biomarkers selected in these studies has not been fully confirmed yet, so multi-center and large-scale studies are needed.

With the progress in high throughput techniques including PCR, gene microarray, protein microarray, and the combination of chromatogram/mass spectrogram, our ability to detect tumor biomarkers has been greatly improved. If we can achieve the detection of multiple tumor markers in urine to screen patients for PCs in the future, we will enjoy the convenience in just one single sample while both reducing detection costs and avoiding the damage from invasive procedures for patients.

Negative markers (those with absent or decreased expression in tumor tissue) may improve the specificity of detection. The combination of positive (those with increased expression in tumor tissue) and negative markers may help optimize the multiple tumor-marker screening of PCs in the future. However, the problem with the combination of multiple tumor markers is that, with a parallel combination of multiple tumor markers, the sensitivity will definitely be impaired, and when they are combined serially, the specificity will be decreased. Therefore, in future studies on tumor screening and early detection, it is very important to optimize a group of tumor markers with high sensitivity and specificity and to establish a perfectly sensitive and specific detection model that can effectively integrate (rather than simple parallel or serial combinations) selected tumor markers.

At present, a number of studies are still centering on the changes in one gene or one protein. However, tumor occurrence and development is a dynamic and interrelated process. It is therefore extremely significant to establish genomic, transcriptomic, proteomic, metabolomic, and subcellular (Golgi apparatus, mitochondria, nucleus, ribosome, and so forth) information that covers the dynamic transformation from normal to abnormal cells on genetic, transcriptional, protein, metabolic, and subcellular levels. Since those information take into consideration the relationships among the various components and the interactions between the various components and cells are capable of revealing relevant biologic networks and
relationships between abnormal expression and abnormal function, they will definitely be helpful in fully understanding the complicated biologic features of PCAs and in establishing and optimizing the multiple tumor-marker screening models for PCAs.

So far, most studies have selected patients with high-risk PCa as rated by existing clinical standards. Therefore, the sensitivity and specificity of tumor markers for screening the general population may be impacted and those tumor markers that can actually recognize early signs of cancer are probably neglected. On the other hand, the gold standard for diagnosis of PCa in existing clinical settings is that cancerous cells have been detected in the tissue samples, which may lag behind the progress in tumor markers. Using this criteria in future studies may thus decrease the sensitivity of some tumor markers. Hence, study methods need to be further optimized.

References


