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Review

Urinary biomarkers in prostate cancer detection and monitoring progression

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ABSTRACT

Prostate cancer (CaP) is the most common cancer in men and the second leading cause of cancer deaths in males in Australia. Although serum prostate-specific antigen (PSA) has been the most widely used biomarker in CaP detection for decades, PSA screening has limitations such as low specificity and potential association with over-diagnosis. Current biomarkers used in the clinic are not useful for the early detection of CaP, or monitoring its progression, and have limited value in predicting response to treatment. Urine is an ideal body fluid for the detection of protein markers of CaP and is emerging as a potential source for biomarker discovery. Gene-based biomarkers in urine such as prostate cancer antigen-3 (PCA3), and genes for transmembrane protease serine-2 (*TMPRSS2*), and glutathione S-transferase P (*GSTP1*) have been developed and evaluated in the past decades. Among these biomarkers, urinary PCA3 is the only one approved by the FDA in the USA for clinical use. The study of urine microRNAs (miRNAs) is another burgeoning area for investigating biomarkers to achieve a pre-biopsy prediction of CaP to contribute to early detection. The development of mass spectrometry (MS)-based proteomic techniques has sparked new searches for novel protein markers for many diseases including CaP.

Urinary biomarkers for CaP represent a promising alternative or an addition to traditional biomarkers. Future success in biomarker discovery will rely on collaboration between clinics and laboratories. In addition, research efforts need to be moved from biomarker discovery to validation in a large cohort or separate population of patients and translation of these findings to clinical practice. In this review, we discuss urine as a potential source for CaP biomarker discovery, summarise important genetic urine biomarkers in CaP and focus on MS-based proteomic approaches as well as other recent developments in quantitative techniques for CaP urine biomarker discovery.

1. Introduction

Prostate cancer (CaP) is the most common cancer diagnosed in men, with an estimated incidence of 16,665 new cases in Australia in 2017 (AIWH, 2017) and 161,360 new cases in the USA in 2017 (Siegel et al., 2017). In 2017, CaP is the second leading cause of male cancer death in Australia with more than 3000 men dying every year (AIWH, 2017). There were an estimated 26,120 deaths from CaP in the USA during 2016 and approximately 12.9% of men will be diagnosed with CaP during their lifetime, based on 2011–2013 data (Insitute, 2015; Siegel et al., 2016). The incidence of CaP increases with age and the risk of a male being diagnosed with CaP by their 85th birthday is 1 in 5 (AIWH, 2014).

There may be no symptoms in the early stages of CaP. In the later stages, symptoms include frequent urination, particularly at night (nocturia), pain on urination (dysuria), blood in the urine (hematuria) or a weak stream and pain in the lower back, upper thighs or hips. More widespread disease often spreads to the bones and gives pain or unexplained weight loss and fatigue. Early detection and treatment can significantly improve CaP survival (Obirieze et al., 2015). The traditional tests used to aid early detection of CaP are digital rectal examination (DRE) and the blood test for prostate specific antigen (PSA).

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A transrectal ultrasound (TRUS) guided biopsy is used to determine its aggressiveness by histopathology.

The serum PSA (PSA) test has been widely used as a screening test for CaP diagnosis for several decades. However, elevated PSA is not specific to CaP as PSA levels often increase in benign prostatic hyperplasia (BPH) and prostatitis, and the false-positive rate (negative prostate biopsy in patients with PSA > 4 ng/mL) of the PSA test is very high (Ferro et al., 1987; Gann et al., 1995; Stamey et al., 1987; Vickers et al., 2008). Furthermore, men with low PSA levels can also develop CaP (Thompson et al., 2004). There are no tests available with sufficient accuracy to screen populations of men for early signs of CaP. Also, a normal DRE result does not rule out CaP (Catalona et al., 1994). Neither test used separately or in tandem, is accurate enough to distinguish potentially fatal cancers from benign tumours (Catalona et al., 1994; Gann et al., 1995). The introduction of PSA testing and DRE leads to a significant increase in the discovery of the disease and has been criticised for contributing to over-diagnosis of CaP (Sandhu and Andriole, 2012). Such over-diagnosis can lead to unnecessary treatments and associated adverse effects such as sexual impotence, urinary incontinence and bowel problems (Gann et al., 2010; Guessous et al., 2016; Ilic et al., 2013; Moyer, 2012). A biopsy is the only way in which a definitive diagnosis of CaP can be made.

Current diagnostic approaches used in the clinic are not useful for the early detection of CaP, or monitoring its progression, and have limited value in predicting response to treatment. Thus, novel non-invasive CaP-specific biomarkers that can assist in the accurate detection of CaP and progression monitoring are in need. In this review, we discuss urine as a potential source for CaP biomarker discovery, summarise several important urine biomarkers identified in CaP and focus on mass spectrometry (MS)-based proteomic approaches for CaP urinary biomarker detection and future exploration.

2. Urine as a potential source for biomarker discovery

Urine is a liquid waste produced from the kidneys, containing inorganic and organic compounds (proteins, hormones and metabolites). The urethra runs through the prostate gland and merges with ejaculatory ducts through which prostate fluid is propelled into the urethra. Studies on urine provide an opportunity to evaluate the well-being of the prostate, and potentially allow early diagnosis of CaP. Urine's anatomic proximity to the prostate gland and the presence of tumour cells in the urine sediment (Dijkstra et al., 2014; Fujita et al., 2009), particularly enriched after a slight prostate massage (Haese et al., 2008), make it possible to develop potential non-invasive diagnoses of CaP using urine based markers.

Urine has become one of the most attractive bio-fluids in clinical proteomics. Compared with other clinical biological specimens, urine has many advantages for determination of both diagnostic and prognostic biomarkers (Fernandez-Serra et al., 2015). It is easy to collect, non-invasive and harmless to the human body. Urine can be obtained in large quantities and there is no significant proteolytic degradation compared with other bio-fluids (Thomas et al., 2010). In addition, urine has a less complex composition compared to serum or plasma, which reduces interferences in isolation and facilitates the evaluation of new biomarkers. A workflow from urine sample collection to biomarker discovery is shown in Fig. 1. The potential urinary biomarkers of CaP, outlined in Fig. 1, will be discussed in the following sections.

3. Genetic biomarkers identified in CaP urine

CaP specific biomarkers can be identified through a urine diagnostic test based on the fact that prostate cells can be detected in urine (Fujita et al., 2009). With the development of molecular biology, massive profiling studies of genes associated with CaP have recently been made possible. The most promising genetic and epigenetic biomarkers including specifically overexpressed genes in CaP cells were identified. These important urine biomarkers in CaP include long non-coding RNA (lncRNA) biomarkers such as prostate cancer antigen-3 (PCA3), CaP-specific fusion gene biomarkers such as transmembrane protease serine-2 (*TMPRSS2*), and CaP specific methylation biomarkers such as glutathione S-transferase P (*GSTP1*). The study of urine microRNAs (miRNAs) is another burgeoning area for investigating biomarkers to achieve a pre-biopsy prediction of CaP to contribute to early detection. The genetic biomarkers identified in CaP urine are shown in Table 1.

3.1. Long non-coding RNA biomarkers

PCA3, also known as DD3, a prostate-specific lncRNA, was first identified in 1999 by Bussemakers et al. and the gene is located on chromosome 9q21-22 and consists of 4 exons (Bussemakers et al., 1999). The *PCA3* gene is dramatically overexpressed in human CaP tissue relative to normal prostate tissue (Bussemakers et al., 1999; de Kok et al., 2002; Hessels et al., 2003) as the total RNA level ($p < 0.0001$) and as a PCA3/PSA ratio ($p < 0.0001$) (Kulda et al., 2016). However, significant differences in the expression of PSA mRNA in tumour tissue relative to normal prostate tissue were not found (Kulda et al., 2016). De Kok et al. found PCA3 in urine and prostate fluid from CaP patients and suggested using it as a possible urinary biomarker (de Kok et al., 2002). Based on the quantitative real time polymerase chain reaction (qRT-PCR) analysis, the PCA3 test received European Conformity in 2006 and obtained approval by the FDA in the USA in 2012 for clinical use. The PCA3 score is calculated as the ratio of PCA3 to PSA mRNA (PCA3 mRNA/PSA mRNA x 1000) (Luo et al., 2014) in a post-DRE urine sample and was found to be associated with the probability of diagnosing CaP in the prostate biopsy (Crawford et al., 2012).

PCA3 was more accurate in predicting clinically significant CaP than the widely used PSA, and could be used as a basis to decide upon the repetition frequency of biopsy in patients with a previous negative result, to improve the accuracy of CaP detection. However, the definition of the best discriminating value is controversial (Leyten et al., 2014). A recent meta-analysis of 11 clinical studies with 3373 CaP patients found that a cut-off PCA3 of 20 (sensitivity 72%, specificity 53%) was preferable to cut-off of 35 (sensitivity 49%, specificity 74%) (Luo et al., 2014), as unnecessary biopsies can be reduced by more than half at a PCA3 cut-off score of 20. These findings are consistent with a prior study that concluded that a PCA3-based nomogram was more accurate than clinical models without PCA3 and up to 55% of men would avoid biopsy by setting the PCA3 cut-off at 21 and only a few cases of high-grade CaP ($\leq 2\%$) would be missed (Hansen et al., 2013). In another study, a PCA3 cut-off score of 20 was also suggested in ruling out a repeat prostate biopsy (Wei et al., 2014). In this trial from 11 centres, urine specimens were collected after an attentive DRE and before undergoing prostate biopsy in 859 cases of CaP. Forty-six percent of the men with PCA3 less than 20 would have avoided a biopsy, however, 12% would have had undiagnosed CaP and 3% would have had undiagnosed high-grade CaP. A discriminating value of 35 was suggested in the 2015 clinical guide of the National Comprehensive Cancer Network to decide on a repeat biopsy in patients with a previous negative result (Carroll et al., 2016). However, PCA3's prognostic value is most likely limited as PCA3 was not correlated with biopsy Gleason score and clinical tumour stage (Leyten et al., 2014). It has not found wide use in the medical community due to these factors.

In 2 recent studies, other urinary lncRNAs such as metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1) (Wang et al., 2014), a multiple cancer-associated lncRNA, and FR0348383 (Zhang et al., 2015), a CaP-associated lncRNA, also demonstrated significant correlations with CaP, especially in the "diagnostic grey zone" (PSA 4–10 ng/mL). By setting the MALAT-1 threshold at 25%, or the FR0348383 threshold at 30%, 30–47% or 52% biopsies could be avoided respectively without missing any high-grade CaP (Wang et al., 2014; Zhang et al., 2015). MALAT-1 or FR0348383 have great potential