

Urinary tumor markers for diagnosis of prostate cancer

Mine Ün¹, Mustafa Sel²

¹Istanbul Aydın University Medical Faculty, Istanbul, Turkey

²Biruni University Medical Faculty, Istanbul, Turkey

ABSTRACT

Prostate cancer is currently one of the most common health problems in the male population. It holds place as the most commonly diagnosed cancer in the United States and most regions worldwide and is the second most common cause of cancer-related deaths in males. High incidence of this cancer has brought developments in diagnostic and treatment methods. The most important screening method in diagnosis is measurement of serum prostate specific antigen (PSA). Digital rectal examination is also an important component of diagnosis. While PSA is the most important diagnostic method, it is specific to the prostate but not specific to prostate cancer. Elevated PSA levels may also occur in benign prostate hyperplasia (BPH) and inflammation of the prostate gland. In other words, PSA is a method with low specificity that does not only increase in prostate cancer and may result in false positives and unnecessary biopsies. Therefore, new markers for the diagnosis of prostate cancer are being researched. For this purpose, it has been observed that many proteins, molecules, DNA and RNA markers in urine can be used in the diagnosis of prostate cancer.

Keywords: MicroRNA, non-PSA biomarkers, prostate cancer.

Prostate cancer is the most common solid tissue cancer in Western populations. Its high incidence has increased the importance given to diagnosis. The most commonly used method in diagnosis is measurement of prostate specific antigen (PSA).^[1]

The widespread use of PSA has significantly reduced metastases and deaths due to prostate cancer.^[1] Although PSA is specific to the prostate organ, it is not specific to prostate cancer. In other words, serum PSA levels may increase in some diseases other than prostate cancer (such as benign prostate enlargement, and inflammation of the prostate gland). This leads to the diagnosis and unnecessary treatment of clinically insignificant prostate cancers.^[2,3]

For these reasons, serum PSA measurement alone does not carry the ideal specificity for detecting prostate cancer. This limitation of serum PSA measurement has increased the search for markers with higher specificity that can detect

high risk prostate cancers, which are the main target for treatment.^[2,3]

NON-PSA TUMOR MARKERS

In the last decade there have been promising developments in the clinical use of new markers that can be measured in urine and are more specific to prostate cancer.^[4]

To date, many molecules found in urine have been studied on this subject (Table 1).^[4]

During the period of molecular studies for the diagnosis of prostate cancer, micro-ribonucleic acid (microRNAs) were found to be involved in the regulation of many basic cellular functions, however, abnormal miRNA levels in the cell were found to be associated with cancer development in humans. Therefore, it was established that microRNAs have a function as oncogenes or tumor suppressors in tumor development.^[4]

İletişim adresi: Mine Ün. İstanbul Aydın Üniversitesi Tıp Fakültesi, 34295 Sefaköy, Küçükçekmece, İstanbul, Türkiye.

e-posta: unmine13@gmail.com

Cite this article as:

Ün M, Sel M. Urinary tumor markers for diagnosis of prostate cancer. D J Tx Sci 2019;4(1-2):67-72.

Considering the localization and expression profiles of most miRNAs, they were predicted to have a key role in cancer formation.^[5,6]

The expression profile of many genes in a certain tumor can be extracted by robotic technologies (microarray), with the prediction of miRNome members, and even the fingerprint of the tumor being investigated can be determined.^[7]

MARKERS FOUND IN URINE

Markers found in urine are used in screening, diagnosis, and treatment of prostate cancer. Easy obtainment of urine has increased the interest in these markers.^[8,9] Urine-based markers are evaluated in three groups: protein-based, deoxyribonucleic acid (DNA)-based, and RNA-based (Table 1).^[10-12]

Protein based markers

Protein based biomarkers in urine (Annexin A3, matrix metalloproteinases, and urine/serum PSA ratio) have conflicting results. Detailed evaluation of human urine has shown that there are over 1,500 types of protein and that use of these proteins as biomarkers is relatively difficult.^[11]

Annexin A3

Annexin A3 is responsible for tumor formation and migration. It has been found to be negatively correlated with ovarian cancer and colon cancer.^[13]

Annexin A3 has been shown in urine in prostate cancer with the Western-blot method.^[14] Annexin A3 is tested in addition to serum PSA and is negatively correlated with prostate cancer. A combination of both Annexin A3 and PSA tests compared to PSA alone has been proven more effective in diagnosing prostate cancer.^[14]

Matrix metalloproteinases

Matrix metalloproteinases have an important role in the growth, invasion, and metastasis of some tumors.^[15] Roy et al.^[16] reported that 82% specificity and 74% sensitivity of matrix metalloproteinases in urine.

Urine PSA

Prostate specific antigen was first detected in urine in the 1980s.^[17] Despite intensive research on serum PSA, there have not been enough studies on PSA in urine.^[18]

Prostate specific antigen detected in initial urine stream has been found to better represent local PSA production compared to PSA obtained from mid- and end-stream urine.^[18]

Studies have shown that urinary PSA is more valuable than serum PSA in prostate cancer diagnosis.^[19,20]

Deoxyribonucleic acid based markers

Studies on DNA based markers in urine have especially focused on methylation and hypermethylation of Glutathione S-transferase π (GSTP1).^[12]

Glutathione S-transferase π

Enzymes of the Glutathione S-transferase family have important effects on cell metabolism. One of the most important effects is the removal of harmful substrates from the body.^[21]

Lee et al.^[22] showed hypermethylation of the regulatory sequences of the GSTP1 gene in all prostate cancer tissue samples. In addition, while increased GSTP1 production is observed in normal prostate epithelium, decreased GSTP1 production is observed in prostate cancer epithelium.^[22] These studies suggested that evaluation of methylation

Table 1. Prostate cancer markers in urine

| Protein based | DNA based | RNA based |
|---------------------------|------------------|--|
| Annexin A3 | GSTP1 | PCA-3 test |
| Matrix metalloproteinases | Hypermethylation | TMPRSS2-ERG gene fusion |
| Urine/serum PSA ratio | | GOLPH2 transcript expression levels SPINK 1 |

DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; GSTP1: Glutathione S-transferase π ; PCA-3: Prostate cancer antigen-3; TMPRSS2: Transmembrane protease, serine 2; ERG: v-ets erythroblastosis virus E26 oncogene homolog; PSA: Prostate specific antigen; GOLPH2: Golgi phosphoprotein 2; SPINK1: Serine protease inhibitor Kazal type 1.

of GSTP1 may be a useful tumor marker in screening prostate cancer.^[23-26]

Ribonucleic acid based markers

Ribonucleic acid based markers are the most commonly researched group. Among them, the PCA-3 test, transmembrane protease, serine 2-v-ets erythroblastosis virus E26 oncogene homolog (TMPRSS2-ERG) gene fusion, Golgi phosphoprotein 2 (GOLPH2) transcription expression levels, and serine protease inhibitor Kazal type 1 (SPINK1) and combinations are the most notable.^[10] However, the PCA-3 test stands out the most in most studies.^[8,9]

Prostate cancer antigen-3 (PCA-3)

Prostate Cancer Antigen-3, or PCA-3, was first isolated from prostate cancer tissue in the 1990s. Its use as a potential biomarker for prostate cancer came into question in 1999, with findings of increased PCA-3 mRNA production in the prostate compared to normal tissue. Prostate cancer antigen (PCA-3) is a non-coding RNA on chromosome 9 and is the most specific marker for prostate cancer in clinical use today. The production of PCA-3 RNA occurs only in prostate tissue and is not found in any other organs or tumors in humans.^[27]

Numerous studies have shown that PCA-3 is produced 66 times more than normal tissue in prostate cancer tissue, and 11-fold increase in prostate cancer specificity even in prostates with less than 10% cancer cells has drawn attention.^[28]

In coming years, due to its high specificity for prostate cancer, PCA-3 in conjunction with other biomarkers is a candidate for becoming an important guide in the treatment of prostate cancer patients.^[28-34]

TMPRSS2-ERG gene transfection

There is evidence that TMPRSS2 plays an important role in the rearrangement of genes in the initial stages of cancer development. In recent years, presence of fusion between androgen-dependent transmembrane serine 2 (TMPRSS2) gene and ERG gene of the ETS family has been shown in prostate cancer.^[35]

ERG is an oncogene that plays a key role in the development of prostate cancer, and gene fusion between TMPRSS2 and ERG is the most commonly encountered genetic disorder. These

two genes are located on chromosome 21 and fusion occurs when there is DNA loss.^[36] As fusion of these genes has been shown to have an impact on the development of prostate cancer, their use as a biomarker has become a current issue.^[37]

TMPRSS2-ERG gene fusion, as in PCA-3, can be detected in urine following prostate massage. One study showed TMPRSS2-ERG gene fusion in urine samples after prostate massage in eight of 19 prostate cancer patients.^[37-40]

While TMPRSS2-ERG is promising in differentiating patients with prostate cancer from those without prostate cancer due to its high specificity, there is need for more comprehensive studies.^[41]

miRNA in prostate cancer

Another interesting and complex subject in prostate cancer is micro-RNA or non-coding RNAs.^[42] Micro-RNAs (miR) are small, non-coding RNAs that regulate production of protein-coding genes. They are a potential marker for tumor formation and metastasis.^[43]

MiR21, miR125b, miR221, and miR222 belong to the oncogenic microRNA family and are associated with aggressive prostate cancer.^[44]

MiR21 suppresses tumor suppressor genes, regulating tumor growth in prostate cancer.^[45] Sun et al.^[46] showed increased tendency of general expression of miRNAs in prostate cancer. MiR21 expression was found to be significantly increased in all tumor tissues, especially prostate cancer.^[47-49] Galardi et al.^[50] found increased expression of miR221/222 in prostate cancer, similar to miR21.^[51-55] Micro-RNAs are predicted to be used as both biomarkers and a tool for target-specific treatment individualized to the patient. Combination of miRNA and PSA can be useful in diagnosing prostate cancer. However, it is difficult to obtain nucleic acid and it is difficult to make miRNA studies available in clinical practice due to limited use.^[50-55]

In conclusion, considering the changes in miRNA expression in tumor tissues and the mRNAs they target, evidence of significant benefit of these small, non-coding RNAs in early diagnosis and development of therapeutic agents has emerged. However, in order to benefit from

miRNAs as a biomarker of cancer development or as therapeutic agents, the findings must be brought to certain standards. As the ongoing search for new biomarkers continues, cost analyses must also be considered.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

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