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Molecular Genetic Markers of Prostate Cancer

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Abstract

Prostate cancer is one of the most common types of cancer on the planet. The insufficient accuracy of standard research methods creates a need for the search for new, more convenient, and precise approaches. This article discusses recently discovered biomarkers for prostate cancer, the theoretical foundations of their application, and their practical significance. The necessity for further research on new markers and the implementation of existing ones into clinical practice is emphasized.

Key words: prostate cancer; biomarker; diagnosis

Introduction

Prostate cancer (PCa) is one of the most common oncological pathologies among men worldwide, representing a significant global public health problem. Understanding the scale and trends of PCa incidence is crucial for developing effective strategies for prevention, screening, and treatment.

According to the International Agency for Research on Cancer (IARC) and its GLOBOCAN database, prostate cancer ranks second in incidence among all types of cancer in men (37.5 per 100,000 population), surpassed only by lung cancer (39 per 100,000 population) [1]. More than 1.4 million new cases of PCa are registered annually worldwide, and this number continues to grow. In 112 countries, prostate cancer is the most prevalent cancer among men.

The geographical distribution of PCa incidence is highly uneven. Incidence rates in developed countries are 3 times higher than in developing countries (37.5 and 11.3 per 100,000, respectively) [1]. Mortality rates differ less significantly (8.1 and 5.9 per 100,000, respectively).

Despite the high incidence, PCa mortality rates are generally lower than those for many other types of cancer, which is due to its slow growth and frequent detection at early stages. Nevertheless, approximately 375,000 men worldwide die from prostate cancer annually. In 48 countries, PCa is the most common cause of cancer-related death.

Currently, the main diagnostic methods for PCa include: measuring PSA levels in urine, radiological diagnostic methods, digital rectal examination (DRE), and biopsy. However, all these methods are not reliably accurate. For example, the PSA test has a sensitivity of 60% and a specificity of 79%, which can lead to unreliable diagnoses [2]. Biopsy examination can also yield false-negative results due to insufficient volume of collected material, which may lack pathological tissue [3].

All of this highlights the need to search for new, more accurate and specific markers for PCa diagnosis. An ideal biomarker should meet several characteristics: safe and easy collection, preferably by non-invasive methods; reproducibility; relative affordability; high prognostic value; low false-negative rates, high sensitivity and specificity [4]. In recent years, many new PCa markers have been studied, many of which represent a complex of markers under a common name or formula. To a large extent, the study of these markers has become possible due to the development of genomic technologies.

The aim of this work is to investigate the possibility of using new biomarkers for PCa diagnosis.

Results

Micro-RNAs

The study of microRNAs (miRNAs) is a promising direction in oncology, with potential for application in the diagnosis and therapy of malignant tumors, including prostate cancer (PCa). As short, single-stranded RNA molecules (21–25 nucleotides in length), miRNAs play a key role in regulating gene expression. Their high stability in biological fluids and resistance to changing conditions make them particularly attractive as reliable PCa biomarkers [5]. It has been established that miRNAs are involved in fundamental processes of carcinogenesis, such as apoptosis, proliferation, invasion, and metastasis. Data from modern research confirm that the analysis of miRNA profiles in patient tissues, blood, and urine opens new opportunities for increasing the accuracy of PCa diagnosis, monitoring disease progression, and evaluating its dynamics [6].

Dysregulation of miRNA expression in oncological diseases is associated with a complex of molecular mechanisms, such as genomic mutations, epigenetic modifications, chromosomal rearrangements, and failures in the biogenesis process of miRNAs themselves [7].

ExoDx Prostate (IntelliScore) Test

The ExoDx Prostate (EPI) test is based on the analysis of exosomal microRNAs isolated from urine. The test evaluates the risk (in the range of 1–100) of high-grade PCa in men with PSA levels of 2–10 ng/mL who are scheduled for an initial biopsy [7]. Exosomes are vesicles secreted by cells that carry cellular material from their source cell. Functioning as key

mediators in intercellular communication, they transport signaling molecules, including proteins, nucleic acids, and metabolites, thereby regulating physiological and pathological processes in target cells [8].

In a study by E. Margolis et al., the impact of EPI on biopsy decisions was investigated. At a threshold of 15.6, the test prevents 23% of all biopsies and 30% of unnecessary interventions. The negative predictive value reaches 90%, and the risk of delayed diagnosis is 2.3%. The diagnostic efficacy of EPI (AUC 0.70) surpasses that of PSA (AUC 0.56) and the ERSPC (European Randomized Study of Prostate Cancer) risk calculator [9].

Prolaris

The Prolaris molecular test is a method for quantitative assessment of the expression of 31 Cell Cycle Proliferation (CCP) genes, reflecting the proliferative activity of the tumor. The analysis is performed on tissue obtained from prostate biopsy or after radical prostatectomy (RP) [10].

The prognostic significance of the CCP signature was initially established in a retrospective study by Nassir A.M. et al. in 2011, validated in two independent cohorts [11]. In the cohort of patients after PCa, CCP assessment demonstrated a statistically significant association with the risk of biochemical recurrence (HR = 1.77; 95% CI 1.40–2.22; $p < 0.001$). In a parallel cohort of patients with localized prostate cancer on conservative management, a high CCP score was associated with an increased risk of disease-specific mortality (HR = 2.57; 95% CI 1.93–3.43; $p < 0.001$).

The application of the Prolaris biopsy test facilitates clinical decision-making in the early stages of the disease. Bishoff et al. showed that the CCP score obtained from biopsy material predicts not only biochemical recurrence after PCa (HR = 1.47; 95% CI 1.23–1.76; $p < 0.001$) but also subsequent metastasis (HR = 4.19; 95% CI 2.08–8.45; $p < 0.001$) [12].

In turn, Cuzick et al. demonstrated that in patients on active surveillance, the CCP score is an independent predictor of prostate cancer-specific mortality (HR = 1.76; 95% CI 1.44–2.14; $p < 0.001$) after adjusting for Gleason score, PSA level, and disease stage [13].

Based on accumulated evidence, the National Comprehensive Cancer Network (NCCN) guidelines recommend considering the use of the Prolaris biopsy test for men with very low, low, and favorable intermediate-risk prostate cancer with an expected life expectancy of ≥10 years [14].

Decipher

The Decipher test analyzes the expression of 22 genes (LASP1, IQGAP3, NFIB, S1PR4, THBS2, ANO7, PCDH7, MYBPC1, EPPK1, TSBP, PBX1, NUSAP1, ZWILCH, UBE2C, CAMK2N1, RABGAP1, PCAT-32, GLYATL1P4, PCAT-80, TNFRSF19) associated with cellular proliferation, differentiation, adhesion, cell cycle, and androgen signaling [15]. For analysis, RNA extracted from a tumor sample of at least 0.5 mm, taken from formalin-fixed, paraffin-embedded tissue, is required [16]. When biopsy material is analyzed, a biopsy score is determined; when prostatectomy material is analyzed, a prostatectomy score is determined. Both scores are a numerical value from 0 to 1. A value from 0 to 0.45 corresponds to low risk, from 0.46 to 0.6 to intermediate risk, and above 0.61 to high risk.

The biopsy report indicates the risk of adverse pathology, metastasis at 5 years, and cancer-specific mortality at 15 years. The prostatectomy report contains data on the risk of metastasis and mortality, which can be considered when deciding on radiation therapy

Previously, the original data for the test were derived from prostatectomy samples. However, in 2016, Knudsen et al. established the test's applicability to biopsy material. It was shown that 95% of transcriptomic information from prostatectomy samples could be obtained from biopsy with high correlation ($r = 0.96$) [17]. Further studies confirmed the clinical significance of the test. Klein et al. found that the Decipher biopsy score is a significant predictor of metastasis within 10 years with an AUC of 0.8 [18].

In a systematic review by Jairath et al., it was concluded that the Decipher test is an independent prognostic factor for adverse pathology, recurrence, metastasis, and survival [19]. The test is most important for managing intermediate-risk cancer and for making post-operative decisions.

According to NCCN guidelines, the Decipher test can be offered to patients with very low, low, and intermediate-risk cancer with an expected life expectancy of 10 years or more [14].

ConfirmMDx

ConfirmMDx is a tissue analysis for risk stratification in patients with prior negative biopsies. The test measures the methylation levels of the promoter regions of three tumor suppressor genes: RASSF1, GSTP1, and APC [20;21]. The analysis is performed on benign tissue from the biopsy. CpG island methylation in these genes increases the risk of cancer. The concept of the test is based on the idea that histologically normal tissue adjacent to a tumor may have epigenetic changes [22].

Key studies supporting the test are MATLOC and Cancer Detection Using Methylated Events in Negative Tissues [23;24]. The MATLOC study showed that ConfirmMDx has a sensitivity of 68% and a specificity of 64% for detecting occult cancer [23]. Cancer Detection Using Methylated Events showed that ConfirmMDx is an independent predictor for PCa compared to clinicopathological parameters and has a negative predictive value of almost 90% [24].

Occult cancer was defined as a subsequent positive biopsy within 30 months after an initial negative biopsy. It was also shown that the test can avoid up to 64% of repeat biopsies [23]. Van Neste et al. found that with a low methylation level, the negative predictive value for high-grade cancer is 96% [25].

Regarding the role of ConfirmMDx in clinical decision-making, Wojno et al. found that only 4.4% of patients with a negative test result underwent repeat biopsy. In the PLCO study, this rate was 43% [26]. All repeat biopsies in patients with a negative ConfirmMDx were also negative. Van Neste et al. also showed that by using a probability threshold of 15%, 30 unnecessary biopsies can be avoided per 100 patients [25;27].

Oncotype DX Genomic Prostate Score

Oncotype DX is a test that measures the expression of 12 cancer-related genes and 5 housekeeping genes. The cancer-related genes are involved in four cellular pathways: proliferation (TPX2), androgen receptor (AZGP1, KLK2, SRD5A2, FAM13C), cellular organization (FLNC, GSN, TPM2, GSTM2), and stromal response (BGN, COL1A1, SFRP4). Based on their combination, the Genomic Prostate Score (GPS) is calculated in a range from 0 to 100. The GPS score correlates with the probability of detecting aggressive pathology during subsequent prostatectomy [28].

Klein et al. validated Oncotype DX using three patient cohorts: a prostatectomy discovery cohort, a prostate biopsy cohort, and an independent prostate biopsy validation cohort. In the prostatectomy cohort, 288 recurrence-related genes and 198 genes associated with aggressive disease were selected from 732 candidate genes. These genes were then tested in the biopsy cohort to identify a subgroup predicting adverse pathology [29].

The study by Cullen et al. showed that the GPS score predicts adverse pathology at prostatectomy and biochemical recurrence after treatment [30].

Regarding clinical application, a study by Badani et al. involving 158 patients showed that the use of the test changed treatment recommendations in 18% of cases. Active surveillance recommendations increased from 41% to 51%, while recommendations for prostatectomy and radiation therapy decreased by 33% [31].

According to NCCN guidelines, Oncotype DX may be offered to patients with very low, low, or favorable intermediate-risk cancer with a life expectancy of 10 years or more [14].

PCA3 Index

The PCA3 Index is one of the molecular-genetic biomarkers developed as an alternative to PSA [32]. PCA3 is a long, non-coding RNA whose expression

increases up to 66-fold in tumor tissue [33]. An important characteristic of PCA3 is that its expression does not increase in benign prostatic hyperplasia (BPH) or prostatitis, which gives it significantly higher specificity compared to PSA [34].

To determine the index, the ratio of PCA3 mRNA copies to KLK3 mRNA copies is measured. The KLK3 gene encodes prostate-specific antigen (PSA), and its expression in urine samples reflects the total number of prostatic epithelial cells present in the sample. Using KLK3 as a reference gene normalizes the results by accounting for variations in the number of shed cells, thereby increasing measurement accuracy and standardizing the analysis [35]. The higher this ratio, the greater the likelihood of clinically significant prostate cancer. The material for analysis is the patient's urine sediment, collected after a digital rectal examination (DRE). DRE promotes the shedding of tumor cells from the prostate gland into the urinary tract, making the test more accurate. The test is performed using reverse transcription polymerase chain reaction (RT-PCR). Some studies indicate that the PCA3 index surpasses PSA in diagnostic accuracy [36, 37].

The use of this marker has been approved in the USA since 2012 by the Food and Drug Administration (FDA).

PSMA marker

Prostate-specific membrane antigen (PSMA), also known as folate hydrolase 1 (FGCP-1) or N-acetylated alpha-linked acidic dipeptidase (NAALADase), is a type II transmembrane glycoprotein inherent to prostate gland cells [38]. This protein acts as a glutamate carboxypeptidase on various substrates, including nutritional folate and the neuropeptide N-acetyl-L-aspartyl-L-glutamate [38]. This protein consists of three main domains: a short intracellular portion, a transmembrane segment, and a bulky extracellular domain, which is the target for most therapeutic and diagnostic ligands [39].

PSMA is found in normal prostate cells as well as in epithelial cells of some other tissues (e.g., kidneys, small intestine, salivary glands, dorsal root ganglia of the spinal cord), but at significantly lower concentrations [40]. However, in prostate cancer, its expression on the cell surface sharply increases, often several hundred or even thousand-fold compared to normal tissue [41]. PSMA correlates with a decrease in tumor differentiation and is more frequently elevated in metastatic and hormone-refractory tumors [42].

In clinical diagnostics, isotope-labeled ligands of this antigen are used. The first low-molecular-weight PSMA inhibitors were clinically tested by Molecular Insight Pharmaceutical, Inc. (MIP) in 2008. 123I-MIP-1072 and 123I-MIP-1095 were used as ligands. In patients with metastatic PCa, single-photon emission computed tomography (SPECT) combined with anatomical detail from multislice CT (SPECT/CT scanning) demonstrated the ability to detect metastases in soft tissues, bones, and the prostatectomy bed [43]. More delayed images provided higher lesion contrast [43].

Beyond diagnosis, PSMA serves as a target for targeted radionuclide therapy. In this approach, PSMA ligands are labeled with therapeutic radionuclides, such as 177Lu (Lutetium-177), allowing for the delivery of high-dose radiation directly to PSMA-expressing tumor cells while minimizing exposure to healthy tissues. 177Lu-PSMA therapy (e.g., using 177Lu-PSMA-617) has shown significant efficacy in patients with metastatic castration-resistant PCa, demonstrating improved survival and quality of life [44].

Conclusion

The prostate cancer (PCa) biomarkers discussed in this article allow for more accurate diagnosis of prostate cancer without requiring uncomfortable and invasive diagnostic methods such as DRE (digital rectal examination) and biopsy. Furthermore, these biomarkers are primarily used as an additional diagnostic method when PSA levels are in the gray zone (4.0 - 10.0 ng/ml). This helps avoid both under- and overdiagnosis of PCa, preventing overtreatment of low-risk patients and allowing for adjusted treatment in high-risk patients. Wider adoption of these markers will enable personalized treatment for each patient through individualized prognostication. This, in turn, will improve the quality of care.

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