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Research Article

Development of a Multiplex PCR Test System for Diagnosis of Prostate Cancer

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Abstract

Prostate cancer (PC) is one of the most widespread diseases with high mortality rate. The main risk group is men over 50 years old. PSA determination is the main method of screening for PCa, but it is an organ-specific marker, and its increase can signal a variety of prostate diseases, not necessarily cancer, or previous manipulations. Recently, one of the tools of prostate cancer diagnostics is the analysis of mRNA expression of the PCA3 gene, the overexpression of which is found in 95% of cases of BPH. A genetic disorder involving the androgen-regulated gene TMPRSS2 and transcription factor genes ETS, ERG and ETV1 has been identified in prostate cancer. The ERG gene belonging to the ETS family belongs to the number of oncogenes that are reliably highly expressed in PCa (more than 70% of cases). In PCa ERG can be fused with TMRRSS2 gene, which encodes serine protease secreted by prostate epithelium in response to androgens. This gene rearrangement results in a fusion TMPRRSS2-ETS transcript through which ERG is expressed at elevated levels under androgen control. Determination of the chimeric gene TMPRSS22-ERG in tissue demonstrates high specificity and sensitivity in prostate cancer.

Keywords: Prostate Cancer; PSA; PCA3; TMPRSS2; ETS; TMPRSS2-ETS

Introduction

At present one of the most widespread oncological diseases in men is prostate cancer (PC). In the world it takes the 1st place by the morbidity, and in the Russian Federation it takes the 2nd place [1]. At the same time, it takes the second place by the mortality rate among the male cancer diseases in general. Every year more than 550,000 new cases of the disease are registered in the world. In the past 10 years in the Russian Federation the number of patients with newly diagnosed prostate cancer per 100,000 people has increased by 300 % [2]. The main risk group is men over 50 years old. Such a picture is caused not only by the natural growth of the disease, but also by the expansion of the list of basic diagnostic methods. At the moment they include finger rectal examination, determination of the level of prostate specific antigen (PSA) in blood serum, transrectal ultrasound examination followed by biopsy of the prostate [3].

For some time, the prostate specific antigen (PSA) level has been considered the most accurate indicator of prostate cancer, and its determination is the main method of screening. This test requires patients to carefully follow the recommendations prescribed by the doctor (diet, including alcohol consumption, lack of physical exercise, and so on), non-compliance with which, as well as the presence of a gray area (values of 4-10 ng/ml) can lead to misinterpretation of the results. Also, it should be noted that PSA is an organ-specific marker, and its increase can signal a variety of prostate diseases, and it is not necessarily cancer, or of previously performed manipulations. The final step for making the diagnosis is the histological examination of the biopsy material, which is the "gold standard" of diagnosis. A biopsy is a rather painful procedure with frequent complications. About 40% of biopsies are negative. Recently one of the tools for prostate cancer diagnostics is the analysis of PCA3 (Prostate Cancer Gene 3) mRNA expression, which is overexpression in 95% of BPH cases [4]. At the same time localization of the tumour in the prostate and presence of chronic prostatitis do not influence the level of PCA3 expression in urine [5].

According to various studies, the sensitivity of PCA3 expression analysis ranges from 46.9% to 82%, and the specificity ranges from 55% to 92% [6]. A genetic disorder involving androgen-regulated

gene TMPRSS2 and genes of transcription factors ETS, ERG and ETV1 has been established in prostate cancer. Gene ERG belonging to ETS family belongs to the number of oncogenes that are reliably highly expressed in prostate cancer (over 70% of cases) [7]. In prostate cancer ERG can be fused with TMRRSS2 gene, which encodes serine protease secreted by prostate epithelium in response to androgen action. This gene rearrangement results in a fusion TMPRRSS2-ETS transcript through which ERG is expressed at elevated levels under androgen control. Detection of chimeric gene TMPRSS22-ERG in tissue demonstrates high specificity (up to 99 %) and sensitivity (up to 86 %) in prostate cancer [8]. Fusion of the TMPRSS2 and ERG genes is observed in approximately 50-70% of prostate cancers and is undetectable in normal prostate tissue and in benign prostatic hyperplasia (BPH); specificity of detection of this newly formed gene product in tissue by immunohistochemical method is 85% and sensitivity - 100% [9].

It has been established in researches that gene TMPRSS2-ERG is highly specific and is found in 50% of cases in Europeans with prostate cancer, but its expression among tumor foci is heterogeneous and in presence of malignant growths in the prostate the necessary amount of TMPRSS2-ERG gene for positive results may not get into the urine [10], that reduces the sensitivity of this investigation, but this problem can be solved by using the TMPRSS2-ERG gene in combination with other biomarkers. The combination of PCA3 and TMPRSS2-ERG genes proposed in 2007 allows increasing the diagnostic potential of both markers and predicting with more accuracy the presence of clinically significant prostate cancer [11]. Thus, the aim of our study was to determine the diagnostic significance of the complex study of PCA3 and TMPRSS2-ERG gene expression by real-time RT-PCR in urine samples for the diagnosis of prostate cancer.

Materials and Methods

Samples of cellular urine sediment obtained without prostate massage in patients with the results of histological examination of prostate biopsy were the object of the study. Samples of cellular urine sediment with the results of histological examination of biopsy material were provided by the Republican Clinical Oncologic Dispensary of the Ministry of Health of the RT. (Kazan). A total of 37 samples were examined. To obtain cell sludge, urine was centrifuged for 20 minutes at 5,000 rpm, after which the supernatant was removed using sterile tips, and a solution for RNA stabilization and storage (Eurogen, ZAO) was added to the sludge. Intact RNA fixative for RNA stabilization (Eurogen, ZAO) in an amount of 1 ml was added for storage and transportation of urine sediments for RNA stabilization. Before RNA isolation, the sediments were washed of the fixative and insoluble salts using sterile physiological solution, and an internal control sample was added.

RNA was isolated from sediments using the Real Best Extraction kit according to the instructions. The reverse transcription reaction was performed using a reagent kit for detection of PCA3 gene mRNA expression and determination of its level of expression using Prosta-Test kit (TestGen LLC.) according to the manufacturer's instructions. The obtained cDNA was used to perform a PCR reaction to determine the relative expression level of the PCA3 gene and the expression level of the T2E gene using the developed multiplex test system and Prosta-Test reagent kit (TestGen LLC). The developed multiplex system includes several pairs of primers and fluorescence-labeled probes that provide simultaneous detection of the expression of the T2E and PCA3 oncomarker gene complex. Control of material collection, RNA isolation, and reverse transcription reactions were performed by PCR with the addition of specific primers for the KLK3 gene in the same tubes.

The following reagents were used to prepare the reaction mixture: 5x PCR buffer (Eurogen, ZAO) and sterile water. Concentrations of primers and fluorescent probes were selected experimentally. A DTprime real-time detection device (DNA-Technology Ltd.) was used for the amplification reaction. The relative level of PCA3 gene expression was assessed based on PCA3/KLK3 ratio calculations according to the formula specified in the Prostate Test kit instructions. The expression of the TMPRSS2-ERG chimeric gene was assessed qualitatively based on the fluorescence signal accumulation plot for a given target with a threshold cycle (Cp) of at least 40. The prognostic significance of the multiplex system was assessed by comparing the results of PCA3 gene relative level and TMPRSS2-ERG gene expression level with the results of histological examination of biopsy material.

Results

Thirty-seven urine samples were analyzed, of which 20 were confirmed to have prostate cancer by histological examination, and 17 samples received a negative histological report. Of the 20 samples with a positive histological conclusion, 16 samples (43%) showed a high level of relative expression when using the Prostate test kit and 17 samples (45%) when using the multiplex system, while chimeric gene expression was detected in 1 sample (3%). Out of 17 negative samples, a high level of PCA3 gene expression was detected in 9 samples (24 %) using the Prostate test kit, and in 4 samples (11 %) using the multiplex system. At the same time, the sample with chronic inflammation in the aggravation stage in the histological report showed high expression of PCA3 gene and TMPRSS2-ERG gene. Thus, the developed multiplex system to determine the expression of prostate cancer markers has the following diagnostic parameters: sensitivity - 85 %, specificity - 71 %, positive prognostic value - 77 %, negative prognostic value - 80 %, diagnostic accuracy - 78 %.

Discussion

Previous studies have established that non-invasive detection of TMPRSS2 and ERG or ETV1 gene fusion product is possible in urine by PCR-RV, the specificity is 94% for PCA, the sensitivity is 37%, but when combined with PCA3 determination, the sensitivity increases to 73%. At the same time the product TMPRSS2-ERG also has a prognostic value and predicts the Gleason index and

the clinical stage of prostate cancer, which the PCA3 assessment does not have [12]. Our results correlate with the literature data. TMPRSS2-ERG correlates with Gleason index and tumor volume, and in combination with PCA3, the predictive ability of the presence of PCA at biopsy is increased and allows the use of the combination of these markers for prostate cancer stratification by risk groups [13-15]. It is expected that the predictive marker, the fusion gene TMPRSS2-ERG, will make the prediction of the response of patients with castration-resistant prostate cancer to new drugs realistic [16].

Conclusion

The results show that the developed multiplex system can detect the expression of PCA3 and TMPRSS2-ERG genes with rather high accuracy (78 %). Despite the fact that chimeric gene expression was detected only in 3% of cases, the combination of these genes shows greater sensitivity and specificity compared to the Prostate-Test kit, which is confirmed by histological report.

References

1. Clinical guidelines (2018) Prostate Cancer. Approved by the Russian Ministry of Health, Russia.
2. Pushkar D Yu, Govorov AV, Sidorenkov AV, Prilepskaya EA, Kovylina MV (2015) Early diagnosis of prostate cancer: guidelines, Moscow, Russia. 19: 56.
3. Babaian RJ, Mettlin C, Kane R, Murphy GP, Lee F, et al. (1992) The relationship of prostate-specific antigen to digital rectal examination and transrectal ultrasonography: findings of the American Cancer Society National Prostate Cancer Detection Project. Cancer 69(5): 1195-1200.
4. Toropovsky AN, Nikitin AG, Pavlova ON, Viktorov DA (2019) Prospects for improving the diagnosis of prostate cancer based on the analysis of the PCA3 gene. Urology 2: 82-87.
5. Vlaeminck-Guillem V, Bandel M, Cottancin M, Rodriguez-Lafrasse C, Bohbot J M, et al. (2012) Chronic prostatitis does not influence urinary PCA3 score. Prostate 72(5): 549-554.
6. Luo Y, Gou X, Huang P, Mou C (2014) Prostate cancer antigen 3 test for prostate biopsy decision: a systematic review and meta-analysis. Chin Med J 127(9): 1768-1774.
7. Petrovics G, Liu A, Shaheduzzaman S, Furusato B, Sun C, et al. (2005) Frequent overexpression of ETS-related gene-1 [ERG1] in prostate cancer transcriptome. Oncogene 24(23): 3847-3852.
8. Clark J, Merson S, Jhavar S, Flohr P, Edwards S, et al. (2007) Diversity of TMPRSS2-ERG fusion transcripts in the human prostate. Oncogene 26(18): 2667-2673.
9. Van Leenders GJ, Boormans JL, Vissers CJ, Hoogland AM, Bressers AM, et al. (2011) Antibody EPR3864 is specific for ERG genomic fusions in prostate cancer: implications for pathological practice. Mod Pathol 24(8): 1128-1138.
10. Zheng Yang, Lu Yu, Zhe Wang (2016) PCA3 and TMPRSS2-ERG gene fusions as diagnostic biomarkers for prostate cancer. Chin J Cancer Res 28(1): 65-71.
11. Sivkov AV, Efremov GD, Mikhailenko MV (2014) Grigorieva combination of PCA3 and TMPRSS2-ERG markers in the early diagnosis of prostate cancer (literature review). Experimental and Clinical Urology 3: 20-23.
12. Leyten GH, Hessels D, Jannink SA, Smit FP, Jong H, et al. (2014) Prospective multi centre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. Eur Urol 65(3): 534-542.
13. Apolikhin OI, Sivkov AV, Efremov GD (2015) PCA3 and TMPRSS2-ERG in the diagnosis of prostate cancer: the first experience of using a combination of markers in Russia. Experimental and Clinical Urology 2: 30-36.
14. Hessels D, Smit FP, Verhaegh GW, Witjes JA, Cornel EB, et al. (2007) Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. Clinical Cancer Res 13(17): 5103-5108.
15. Lin DW, Newcomb LF, Brown EC, Brooks JD, Carroll PR, et al. (2013) Urinary TMPRSS2: ERG and PCA3 in an active surveillance cohort: results from a baseline analysis in the canary prostate active surveillance study. Clin Cancer Res 19(9): 2442-2450.
16. Arlen PM, Bianco F, Dahut WL, Amico AD, Figg WD, et al. (2008) Prostate Specific Antigen Working Group guidelines on prostate specific antigen doubling time. J Urol 179(6): 2181-2185.

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