DOI: 10.17816/uroved8311-19

PROGNOSTIC SIGNIFICANCE OF THE EVALUATION OF EXPRESSION OF THE GENE *PCA3* IN URINE IN PATIENTS WITH LOCALIZED PROSTATE CANCER AND HISTOMORPHOLOGICAL CHANGES IN THE PERITUMORAL ZONE

© *Ph.S. Bova*

Rostov Research Oncological Institute of the Ministry of Health of Russia, Rostov-on-Don, Russia;
Regional Hospital No 2, Rostov-on-Don, Russia

For citation: Bova PhS. Prognostic significance of the evaluation of expression of the gene *PCA3* in urine in patients with localized prostate cancer and histomorphological changes in the peritumoral zone. *Urologicheskie vedomosti*. 2018;8(3):11-19. doi: 10.17816/uroved8311-19

Received: 02.08.2018 Accepted: 14.09.2018

Aim. To determine the prognostic significance *PCA3* gene expression in urine sediment and exosomes in patients with localized prostate cancer (PC) and associated histologic changes in the peritumoral zone as a predictor of biochemical recurrence after radical prostatectomy (RPE). **Materials and methods.** Of 148 patients with localized PC, 96 (65%) had high-grade prostatic intraepithelial neoplasia (PIN-2) in the peritumoral zone. The other 52 (35%) had no pathologic tissue of the peritumoral zone. PDA3 expression in urine sediment and exosomes was determined with real-time PCR with respect to the reference gene *KLK3*. **Results.** The *PCA3* gene expression level in urine exosomes in patients with PIN-2 in the prostatic peritumoral zone and synchronous pancreatic adenocarcinoma was higher among patients with subsequent disease recurrence. Increased *PCA3* gene expression in the urine sediment was also predictive of the risk of recurrence of a prostatic tumor with PIN-2 in the peritumoral zone, although to a lesser degree than the results with urine exosomes. When the *ΔCt PCA3-KLK3* was ≥1,86 in the urine sediment, biochemical recurrence of PC and PIN-2 developed more frequently in the peritumoral zone (84% versus 51%, p = 0,013). **Conclusions.** Increased *PCA3* gene expression in urine sediment and exosomes is a predictor of increased risk of biochemical recurrence after RPE in patients with localized PC and PIN-2 in the peritumoral zone.

Keywords: prostate cancer; biochemical relapse; peritumoral zone; prostatic intraepithelial neoplasia; prostate-specific antigen 3; urine.

ПРОГНОСТИЧЕСКАЯ ЗНАЧИМОСТЬ ОЦЕНКИ ЭКСПРЕССИИ ГЕНА *PCA3* В МОЧЕ У БОЛЬНЫХ ЛОКАЛИЗОВАННЫМ РАКОМ ПРЕДСТАТЕЛЬНОЙ ЖЕЛЕЗЫ И ГИСТОМОРФОЛОГИЧЕСКИМИ ИЗМЕНЕНИЯМИ ПЕРИТУМОРАЛЬНОЙ ЗОНЫ

© *Ф.С. Бова*

ФГБУ «Ростовский научно-исследовательский онкологический институт» Минздрава России, Ростов-на-Дону;
ГБУ Ростовской области «Областная больница № 2», Ростов-на-Дону

Для цитирования: Бова Ф.С. Прогностическая значимость оценки экспрессии гена *PCA3* в моче у больных локализованным раком предстательной железы и гистоморфологическими изменениями перитуморальной зоны // Урологические ведомости. – 2018. – Т. 8. – № 3. – С. 11–19. doi: 10.17816/uroved8311-19

Дата поступления: 02.08.2018 Статья принята к печати: 14.09.2018

Цель — определить прогностическую значимость оценки экспрессии гена *PCA3* в осадке и экзосомах мочи у больных с локализованным раком предстательной железы (РПЖ) и сочетанными гистоморфологическими изменениями в перитуморальной зоне при определении риска биохимического рецидива после радикальной простатэктомии (РПЭ). **Материалы и методы.** Обследованы 148 больных локализо-

ванным РПЖ. У 96 (65 %) пациентов основной группы в перитуморальной зоне предстательной железы имела место простатическая интраэпителиальная неоплазия высокой степени (ПИН-2). У 52 (35 %) пациентов группы сравнения гистопатологические процессы в перитуморальной зоне отсутствовали. В осадке и экзосомах мочи у пациентов двух групп методом ПЦР в реальном времени определяли экспрессию гена *PCA3* относительно референсного гена *KLK3*. **Результаты.** Уровень экспрессии гена *PCA3* в экзосомах мочи у больных при одновременном сочетании РПЖ и ПИН-2 в перитуморальной зоне был выше при последующем рецидивировании по сравнению с благоприятным течением заболевания. Для определения риска рецидивирования опухолевого заболевания при сопутствующих изменениях перитуморальной зоны эффективна также оценка экспрессии гена *PCA3* в осадке мочи, но в ограниченном диапазоне. При снижении в осадке мочи *ΔCt PCA3–KLK3* менее 1,86 включительно биохимический рецидив у пациентов с РПЖ и ПИН-2 в перитуморальной зоне развивался чаще (84 % против 51 %, p = 0,013). **Заключение.** Прогностическая значимость оценки экспрессии гена *PCA3* в осадке и экзосомах мочи для определения риска биохимического рецидива после РПЭ повышается у больных с локализованным РПЖ и ПИН-2 в перитуморальной зоне.

Ключевые слова: рак предстательной железы; биохимический рецидив; перитуморальная зона; простатическая интраэпителиальная неоплазия; простатспецифический антиген 3; моча.

INTRODUCTION

Gene expression analysis is a promising method for evaluating tumor tissue obtained through biopsy or surgery. Prostate cancer (PCa)specific gene expression measurement has been used for more than 10 years [1]. Gene expression analysis is intended to identify genetic signatures to more accurately diagnose malignant tumors, evaluate disease prognosis (indolent or aggressive disease), assess the metastatic and lethal potential of the tumor, and calculate the probability of relapse [2, 3]. Due to numerous obstacles, including multifocal lesions, interfocal tumor heterogeneity, and histological and morphological changes in the peritumoral area, genetic analysis of prostate tumor tissue is not part of routine clinical practice [4]. The assessment of gene expression in tumor tissue is often complemented by examination of other biological fluids or cells: blood serum, peripheral blood mononuclear cells, whole urine, urine sediment, and urine exosomes [4–6]. Therefore, highly sensitive, noninvasive diagnostic methods of evaluating the tumor and the peritumoral area are needed. The level of prostate-specific antigen (produced by the kallikrein-related peptidase 3 *[KLK3]* gene) can be used as a molecular marker of PCa [6]. Although the diagnostic value of prostate cancer antigen 3 *(PCA3)* gene expression in urine and exosomes in the differential diagnosis of benign and malignant prostate cancer has been established [4], its role in the subsequent course of the disease has not yet been studied.

This study was undertaken to estimate the prognostic value of *PCA3* gene expression in urine sediment and exosomes in patients with PCa and histological changes in the peritumoral area for predicting biochemical recurrence (BR) after radical prostatectomy (RPE).

MATERIALS AND METHODS

The study was conducted in the Centre of Urology, Nephrology, and Haemodialysis and the Department of Pathology of Rostov Regional Hospital No. 2 from 2015 to 2017. The study protocol was approved by a local Ethics Committee at the Rostov Cancer Research Institute, Ministry of Health of Russia. All involved patients gave their informed consent to participate in the study.

The inclusion criteria were as follows: (1) the presence of localized PCa (stages T_{1c} to T_{2c}), confirmed by both clinical and pathological examinations (histological analysis of biopsy samples or surgical specimens); (2) available results of histological examination of both tumor and peritumoral area; (3) no distant metastasis. Extracapsular tumor invasion confirmed by histological study was an exclusion criterion.

Surgical specimens from 148 patients with localized PCa (stages $T_{1c}N_0M_0$ to $T_{2c}N_0M_0$) were examined histologically. Patients' ages ranged from 54 to 79 years; the mean age was 65.6 ± 2.5 years. Of these patients, 15 (10.1%) had stage cT_{1c} disease; 14 (9,5%) had stage cT_{2a} disease; 43 (29.0%) had stage cT_{2b} disease; and 76 (51.4%) had stage cT_{2c} disease. Well-differentiated tumors (Gleason scores ≤6) were observed in 12 patients (8.1%), whereas moderately differentiated tumors (Gleason score of 7) and poorly differentiated tumors

(Gleason scores of 8 to 10) were found in 134 (90.5%) and 2 (1.4%) patients, respectively.

All study participants had adenocarcinomas. Ninety-six individuals (64.9%) also showed pathological changes in the peritumoral area and had a diagnosis of high-grade (grade 2) prostatic intraepithelial neoplasia (PIN-2); they were considered the experimental group. The remaining 52 patients (35.1%), considered the control group, showed no pathological changes in the peritumoral area.

All patients underwent open RPE. Tissue samples collected by a surgeon were fixed in 4% buffered formalin and delivered to the Department of Pathology, where they were stored for no longer than 24 hours. Specimens of tumor tissue (at least 3 cm in diameter) and the peritumoral area (at least 2 cm from the tumor border; samples were taken from both lobes) were cut into 1.0 cm × 0.5 cm conical pieces, which were then transversely cut into 15 to 20 slices 0.3 mm thick. A pathologist evaluated histological tumor type and differentiation grade.

For histological examination, the tissues were fixed in 10% buffered formalin (pH of 7.4) and embedded into paraffin through a standard method. Prepared serial sections (3 to 5 μm) were deparaffinized according to a standard technique, stained with hematoxylin and eosin, and evaluated with the TOPIC-T CETI light microscope (The Netherlands).

In all patients, serum prostate-specific antigen (PSA) level was measured every 3 months after RPE. Enzyme-linked immunosorbent assay was used to measure PSA levels. The primary endpoint was BR, defined as an increase in PSA level by more than 0.2 ng/mL on three consecutive measurements at least 2 weeks apart.

Urine specimens (70 mL) were collected into special containers after prostate massage, in which each lobe was pressed three times. Each specimen was divided into two portions: The first portion (20 mL) was used for evaluating urine sedimentation, and the second portion (50 mL) was used for exosome extraction.

To evaluate urine sedimentation, the 20-mL portions were centrifuged for 15 minutes at 3000 rpm. Afterwards, the supernatant was removed, the pellet was resuspended, and a 1.5-mL aliquot of it was placed into a fresh tube. To preserve urine sediment, 1 mL of RNA medium (InterLabService LLC, Moscow) was added, and the tube was closed and sealed with laboratory film.

To obtain exosomes, 50-mL portions were centrifuged for 15 min at 10,000 rpm. The supernatant was then centrifuged for 3 h at 100,000 rpm. After that, the pellet was rinsed with 3 mL of phosphate-buffered saline, centrifuged for a short time, and resuspended in 200 μL of phosphate-buffered saline.

Total RNA was isolated from urine samples by a sorbent method with the AmpliPrime Ribo-Sorb kit (NextBio, Russia) following the manufacturer's instructions. Specimens were treated with DNase (6 Units) for 40 minutes at room temperature to remove genomic DNA (reagents from Applied Biosystems, USA).

The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California) was used for reverse transcription. Random primers were used to obtain complementary DNA (cDNA) from RNA, and 40 μL was taken from the kit, per the manufacturer's recommendations.

The expression of the *PCA3* gene in urine sediment and exosomes was evaluated with real-time polymerase chain reaction (RT-PCR). Cycle threshold (*Ct*) values of the test and the reference genes were compared. The *KLK3* gene was used as a reference gene because it has prostate-specific expression. The reaction mix contained 1.0 μL of cDNA from urine sediment or exosomes, 8.0 μL of deionized water, 1.0 μL of primer mix and TaqMan probe, and 10.0 μL of concentrated buffer solution with polymerase, per the manufacturer's instructions. The PCR program started with initial denaturation at 95 °C for 10 min, followed by 47 cycles of denaturation at 95 °C for 15 s and 60 °C for 1 min (detection).

RT-PCR involved the use of predesigned primers for the *KLK3* gene (assay ID Hs02576345_mL; Applied Biosystems, FAM) and the *PCA3* gene (assay ID Hs01371939_g1, Applied Biosystems, FAM), TaqMan probes with dyes, and minor groove binders. Only urine specimens in which the *KLK3* gene expression was detected were used (*Ct* value <45). Each sample was tested three times, and the mean Ct value was then calculated.

The CFX96 thermal cycler (Bio-Rad, Hercules, California) and special software, Bio-Rad CFX Manager (version 2.1), were used to run RT-PCR. To analyze *PCA3* gene expression, Δ*Ct* was calculated as *Ct* of *PCA3*–*Ct* of *KLK3*.

Data analysis was performed with Statistica 12 software. The modules of descriptive statistics, frequency

analysis, and cross-tabulation were used. Medians and 25th and 75th percentiles were calculated. The continuous variables were compared in the Mann-Whitney test with a significance level of $p \leq 0.05$. The categorical variables were compared in Pearson's χ^2 test. The receiver operating characteristic (ROC) curve analysis was also performed.

RESULTS

Baseline and postoperative clinical characteristics of patients from the experimental and control groups are shown in Table 1.

The study included only the patients in whom the clinical and pathomorphological stages of PCa were identical, according to the results of histological examination of surgical specimens. Patients with extracapsular tumor invasion were thus excluded, because it is a key risk factor for disease recurrence. There were no significant differences in the distribution of clinical and pathomorphological stages and Gleason scores between the two groups ($p > 0.05$), which allowed analysis of PIN-2 in the peritumoral area on the postoperative disease course without consideration of the main recognized risk factors for progression.

BR was observed in 5 of 52 patients (9.6%) in the control group and in 25 of the 96 patients (26.0%) in the experimental group (PCa + PIN-2). Therefore, BR was more common among patients in the experimental group ($p = 0.03$).

Both median preoperative serum PSA level and its interquartile range did not vary significantly between the groups ($p = 0.96$; Table 2).

The median *ΔCt PCA3–KLK3* in the urine sediment and its interquartile range for the experimental group did not significantly differ from that of the control group ($p = 0.89$; see Table 2). Negative *ΔCt* values indicated that the *PCA3* gene demonstrated higher expression than the *KLK3* gene. The level of *PCA3* expression in the urine sediment did not vary between the groups ($p = 0.89$).

Table 1

Baseline clinical and postoperative characteristics of patients with localized prostate cancer

Parameter	Experimental group (PCa + PIN-2) ($n = 96$)	Control group PCa ($n = 52$)	p
Age	64.5 ± 2.7	66.1 ± 2.3	>0.05
Clinical stage, abs. (%):			
cT_{1c}	10 (10.4%)	5 (9.6%)	>0.05
cT_{2a}	9 (9.4%)	5 (9.6%)	>0.05
cT_{2b}	26 (27.1%)	17 (32.7%)	>0.05
cT_{2c}	51 (53.1%)	25 (48.1%)	>0.05
Pathomorphological stage (surgical specimens), abs. (%):			
pT_{2a}	9 (9.4%)	5 (9.6%)	>0.05
pT_{2b}	26 (27.1%)	17 (32.7%)	>0.05
pT_{2c}	51 (53.1%)	25 (48.1%)	>0.05
PCa was diagnosed by needle biopsy	10 (10.4%)	5 (9.6%)	>0.05
Tumor differentiation assessed by Gleason score (surgical specimens), abs. (%):			
• Well-differentiated (Gleason score ≤6)	8 (8.4%)	4 (7.7%)	>0.05
• Moderately differentiated (Gleason score 7)	87 (90.6%)	47 (90.4%)	>0.05
• Poorly differentiated (Gleason score 8–10)	1 (1.0%)	1 (1.9%)	>0.05
Preoperative serum PSA, ng/mL ($M \pm m$)	12.1 ± 1.3	12.5 ± 1.6	>0.05
Serum PSA at the moment of BR diagnosis, ng/mL ($M \pm m$)	3.4 ± 0.5	2.0 ± 0.3	<0.05

Note. PCa, prostate cancer; PIN-2, high-grade prostatic intraepithelial neoplasia; PSA, prostate-specific antigen; BR, biochemical recurrence.

Patients in the experimental group had higher PCA3 mRNA levels in urine exosomes than did controls ($p = 0.04$). The median *ΔCt PCA3–KLK3* in urine exosomes was –2.57 in the experimental group and –1.13 in the control group (see Table 2). Lower *ΔCt* values indicated higher *PCA3* mRNA concentrations compared to *KLK3* mRNA in urine exosomes.

The results of preoperative genetic examinations were subjected to retrospective analysis in view of the 2-year data on recurrence (Table 3).

There were no significant differences in mean preoperative serum PSA levels and preoperative levels of *PCA3* expression in urine sediment between patients with and without BR in both the experimental and control groups ($p > 0.05$). The level of *PCA3* expression in urine exosomes was not associated with BR in control patients ($p = 0.32$). However, in the experimental group, patients with a combination of prostate adenocarcinoma, PIN-2 in the peritumoral area, and with BR demonstrated higher levels of *PCA3* expression in urine exosomes than did those with no BR (median *ΔCt PCA3–KLK3,* –3.17 vs –1.14, $p = 0.02$).

The *PCA3* expression in urine exosomes in patients with progressive PCa increased in the case of peritumoral area compromise. Both PIN-2 in the peritumoral area and elevated *PCA3* expression in urine exosomes were associated with an increased

Table 2

Serum prostate-specific antigen and *PCA3* expression levels in urine sediment and exosomes

Parameter	Control group (PCa) ($n = 52$)		Experimental group (PCa + PIN-2) ($n = 96$)		p
	Me	[25%; 75%]	Me	[25%; 75%]	
Serum PSA level, ng/mL	12.6	[9.3; 17.8]	12.0	[8.5; 18.6]	0.96
Δ*Ct PCA3–KLK3* in urine sediment	–0.02	[–0.27; 0.79]	–0.49	[–0.35; 0.86]	0.89
Δ*Ct PCA3–KLK3* in urine exosomes	–1.13	[–1.96; 1.58]	–2.57	[–3.02; 0.12]	**0.04**

Note. Δ*Ct*, cycle threshold; PCa, prostate cancer; PIN-2, high-grade prostatic intraepithelial neoplasia; PSA, prostate-specific antigen.

Table 3

Serum prostate-specific antigen and *PCA3* expression levels in urine sediment and exosomes in patients with prostate cancer according to recurrence

Parameter	Control group (PCa) ($n = 52$)			Experimental group (PCa + PIN-2) ($n = 96$)		
	BR ($n = 5$)	No BR ($n = 47$)	p	BR ($n = 25$)	No BR ($n = 71$)	p
Serum PSA level, ng/mL, Me [25%; 75%]	13.1 [9.7; 18.3]	12.2 [9.0; 16.2]	0.64	15.8 [10.6; 19.1]	11.2 [8.3; 17.5]	0.37
Δ*Ct PCA3–KLK3* in urine sediment Me [25%; 75%]	–0.21 [–0.36; 0.55]	0.03 [–0.22; 0.94]	0.58	–0.68 [–0.92; 0.63]	–0.15 [–0.31; 0.88]	0.41
Δ*Ct PCA3–KLK3* in urine exosomes Me [25%; 75%]	–1.24 [–1.53; 1.42]	-0.86 [–0.91; 1.63]	0.32	–3.17 [–4.13; 0.04]	–1.14 [–2.08; 0.36]	**0.02**

Note. PCa, prostate cancer; PIN-2, high-grade prostatic intraepithelial neoplasia; PSA, prostate-specific antigen; BR, biochemical recurrence; Δ*Ct*, cycle threshold.

Table 4

Number of patients in the study group and control group according to the level of serum prostate-specific antigen and *PCA3* expression in urine sediment and exosomes in patients with prostate cancer

Parameter	Control group (PCa) (n = 52) abs. (%)	Experimental group (PCa + PIN-2) (n = 96) abs. (%)	p^*	p^{**}
Serum PSA level, ng/mL:				
<10	2 (4%)	1 (1%)	0.59	0.07
10–20	49 (94%)	84 (88%)	0.31	
>20	1 (2%)	11 (11%)	0.087	
Δ*Ct PCA3–KLK3* in urine sediment:				
>3.3	3 (6%)	2 (2%)	0.48	0.065
[1.86–3.3]	28 (54%)	37 (39%)	0.10	
< 1.86	21 (40%)	57 (59%)	**0.04**	
Δ*Ct PCA3–KLK3* in urine exosomes:				
>1.48	2 (4%)	4 (4%)	0.73	0.73
≤1.48	50 (96%)	92 (96%)	0.73	

Note. *Probability for χ^2 values in pairwise comparison. **Probability for χ^2 values in multiple comparison. PCa, prostate cancer; PIN-2, high-grade prostatic intraepithelial neoplasia; PSA, prostate-specific antigen; Δ*Ct*, cycle threshold.

risk of BR. To analyze this association in detail, a frequency analysis was conducted with cross-tabulation (Table 4).

The analysis revealed that patients with adenocarcinoma and PIN-2 in the peritumoral area were more likely to have *ΔCt PCA3–KLK3* values of less than 1.86 (cutoff value for distinguishing PCa and benign prostatic hyperplasia according to Apolinkhin et al. [6]) than were controls (40% vs. 59%, $p = 0.04$). Therefore, comparative analysis of *PCA3* expression in urine sediment can provide valuable information for assessing the risk of recurrence in patients with pathological changes in the peritumoral area and *ΔCt PCA3–KLK3* values lower than 1.86.

The results of frequency analysis of serum PSA levels and *PCA3* expression levels in urine sediment and exosomes and their association with BR are shown in Table 5.

Increased expression of the *PCA3* gene in urine sediment in patients with changes in the peritumoral area was associated with BR after surgery ($p = 0.013$; Table 5).

Receiver operating characteristic (ROC) curve analysis was performed to determine the cutoff value for Ct in urine exosomes in order to identify patients with PIN-2 in the peritumoral area who were at high risk for BR The calculated cutoff point for *ΔCt PCA3–KLK3* was –2.6. Therefore, patients with prostate adenocarcinoma and PIN-2 in the peritumoral area and *ΔCt CA3–KLK3* in urine exosomes of 2.6 or lower are at high risk for BRwithin 24 months after RPE. The diagnostic sensitivity of this method reached 88%, whereas the sensitivity was 86%. The proportions of false-negative and false-positive results were 12% and 14%, respectively. The overall diagnostic efficiency of *PCA3* gene expression in urine exosomes for predicting BR after RPE was 86%. The area under the ROC curve was 0.812 ± 0.0003 ($p < 0.0001$), which suggests high diagnostic value of the method.

DISCUSSION

Patients with diagnoses of prostate adenocarcinoma and PIN-2 in the peritumoral area are more likely to develop BR than were those with PCa alone (26% vs. 10%). Earlier studies of histological characteristics of surgical specimens taken from patients with verified PCa have also revealed the combination of adenocarcinoma and PIN in 40% to 73% of patients [7]. Yurmazov et al. (2010) described more aggressive disease in patients with PCa and PIN: they observed higher serum PSA levels, multifocal lesions, more frequent perineural invasion, lower differentiation grade, and higher density of HER2/neu receptors on the surface of cancer cells [8].

In routine clinical practice, the possibility of PCa recurrence is monitored by regular measurement of serum PSA. However, the assessment of serum PSA alone leads to overdiagnosis of BR in 1.7% to 67.0% of cases [9]. Despite the established tactics and additional strategies for serum PSA monitoring in patients with PCA after RPE (in view of patient age, PSA rate of increase, its doubling time, isoforms, and den-

Table 5

Recurrence-specific number of patients in the study group and control group according to the level of serum prostate-specific antigen and *PCA3* expression in urine sediment and exosomes in patients with prostate cancer

Parameter	Control group (PCa) (n = 52) abs. (%)			Experimental group (PCa + PIN-2) (n = 96) abs. (%)		
	BR (n = 5)	No BR (n = 47)	p*	BR (n = 25)	No BR (n = 71)	p*
Serum PSA level. ng/mL:						
<10	0	2 (4%)		0	1 (1%)	0.064
10–20	5 (100%)	44 (94%)		19 (76%)	65 (91.5%)	
>20	0	1 (2%)	0.84	6 (24%)	5 (8.5%)	
Δ*Ct PCA3–KLK3* in urine sediment:						
>3.3	0	3 (6%)		0	2 (3%)	**0.013**
[1.86–3.3]	1 (20%)	27 (58%)		4 (16%)	33 (46%)	
<1.86	4 (80%)	17 (36%)	0.16	21 (84%)	36 (51%)	
Δ*Ct PCA3–KLK3* in urine exosomes:						
>1.48	0	2 (4%)		1 (4%)	3 (4%)	0.59
≤1.48	5 (100%)	45 (96%)	0.45	24 (96%)	68 (96%)	0.59

Note. *Probability for χ^2 values in pairwise comparison. **Probability for χ^2 values in multiple comparison. PCa, prostate cancer; PIN-2, high-grade prostatic intraepithelial neoplasia; Δ*Ct*, cycle threshold.

sity) [9, 10], a wide range of biochemical and molecular markers in various biological fluids and tissues is being studied to improve the reliability of disease prognosis after surgery. Apolikhin et al. proposed the evaluation of *PCA3* expression in urine sediment and exosomes as a promising method of PCa diagnosis [6]. Researchers started using exosomes as an object for genetic research in 2007. Exosomes contain messenger RNA and are involved in cell communications [11]. Tumor cells produce more exosomes than do nontumor cells. Moreover, exosomes were found to be involved in forming premetastatic niches and remodeling of the tumor microenvironment [12]. Tumor cells prepare a premetastatic niche through remote information transmission, including transmission via exosomes. Before cancer cell arrival, this niche is considered premetastatic; when cancer cells get into this microenvironment, they begin proliferating. The measurement of exosomes improved early detection of PCa and reduced the number of unnecessary biopsies.

To continue the research on noninvasive monitoring of patients with PCa, the preoperative expression of the *PCA3* gene in urine sediment and exosomes was examined in patients with PCa with and without BR. This approach allowed assessing the prognostic value of *PCA3* gene expression in urine sediment and exosomes to estimate the risk of early recurrence before surgery.

Because the cutoff value of 1.48 for *ΔCt PCA3–KLK3* in urine exosomes has already been used for deferential diagnosis between benign and malignant prostate diseases, ROC analysis was performed to determine the cutoff value for *ΔCt PCA3–KLK3* that can be used for identifying patients with PIN-2 in the peritumoral area who are at high risk for BR.

The level of *PCA3* expression in urine exosomes in patients with prostate adenocarcinoma and PIN-2 in the peritumoral area was found to be higher in patients with recurrence than in those with a more favorable disease course. Thus, the presence of PIN-2 in the peritumoral area and elevated *PCA3* gene expression in urine exosomes were associated with an increased risk of BR. Patients with prostate adenocarcinoma and PIN-2 in the peritumoral area who demonstrated *ΔCt PCA3–KLK3* values of 2.6 or lower were at high risk for BR in the next 24 months (diagnostic sensitivity of 88% and specificity of 86%).

To determine the risk of cancer recurrence in patients with pathological changes in the peritumoral area, assessment of *PCA3* gene expression in urine sediment (in a limited range only) is recommended. Patients with PCa and PIN-2 in the peritumoral area and *ΔCt PCA3–KLK3* values of 1.86 or lower developed BR more frequently than did those with PCa alone (84% vs. 51%, p = 0.013).

The results of this study are consistent with other studies of *PCA3* gene expression, as well as the expres-

sion of other genes with prostate-specific expression in whole urine, urine sediment, and exosomes [6, 13]. The authors emphasized high diagnostic value of such tests for differential diagnosis of malignant and benign prostate tumors.

This study demonstrated that the assessment of *PCA3* expression in urine sediment and exosomes can provide valuable information; however, it also has several limitations in predicting BR in the next 2 years.

The findings are highly important for clinical practice because they can be very helpful in identifying patients with localized PCa at high risk for BR after RPE via noninvasive measurement of *PCA3* expression in urine sediment and exosomes and histological examination of surgical specimens from the peritumoral area. The existing strategies used for PCa progression monitoring have recently been changed as a result of evidence suggesting that tumor marker expression in urine sediment and exosomes is highly important before surgery and that histological examination of the peritumoral area after surgery is important for estimating the risk of early recurrence and identifying patients at high risk for more careful postoperative monitoring.

CONCLUSION

1. The prognostic value of *PCA3* gene expression in urine sediment and exosomes for evaluating the risk of BR after RPE is higher in patients with localized PCa and PIN-2 in the peritumoral area than in patients with PCa alone.

2. Elevated levels of *PCA3* gene expression in urine sediment and exosomes in patients with localized PCa and PIN-2 in the peritumoral area are associated with an increased risk of BR within 2 years after RPE.

Funding. The study had no additional sources of funding

The author declares no conflicts of interest related to the current manuscript.

REFERENCES

1. Толкач Ю.В., Рева С.А., Носов А.К., и др. Клиническая значимость генетической характеризации рака предстательной железы: обзор литературы // Онкоурология. – 2015. – № 2. – С. 99–106. [Tolkach YuV, Reva SA, Nosov AK, et al. Clinical Significance of Genetic Characterization of Prostate Cancer: A Review of Literature. *Onkourologiya.* 2015;(2):99-106. (In Russ.)]. doi: 10.17650/1726-9776-2015-11-2-99-106.
2. Сергеева Н.С., Скачкова Т.Е., Маршутина Н.В., и др. Клиническая значимость ПСА-ассоциированных тестов в диагностике и стадировании рака предстательной железы // Онкология. – 2018. – № 1. – С. 55–67. [Sergeeva NS, Skachkova TE, Marshutina NV, et al. Clinical significance of PSA-associated tests in the diagnosis and staging of prostate cancer. *Onkologija.* 2018;(1):55-67. (In Russ.)]. doi: 10.17116/onkolog20187155-67.
3. Шкурников М.Ю., Алексеев Б.Я. Ассоциация экспрессии генов рецепторов фактора роста тромбоцитов альфа и бета (*PDGFRA* и *PDGFRB*) с биохимическим рецидивом рака предстательной железы после радикальной простатэктомии // Онкоурология. – 2017. – Т. 13. – № 4. – С. 45–50. [Shkurnikov MYu, Alekseev BYa. Association of gene expression of platelet-alpha-beta-beta receptor (*PDGFRA* and *PDGFRB*) receptors with biochemical recurrence of prostate cancer after radical prostatectomy. *Onkourologiya.* 2017;13(4):45-50. (In Russ.)]. doi: 10.17650/1726-9776-2017-13-4-45-50.
4. Михайленко Д.С., Новиков А.А., Григорьева М.В., и др. Сравнение экспрессии гена *PCA3* в осадках и экзосомах мочи при раке предстательной железы // Онкоурология. – 2017. – Т. 13. – № 3. – С. 54–60. [Mihajlenko DS, Novikov AA, Grigor'eva MV, et al. Comparison of the expression of the *PCA3* gene in precipitates and urine exosomes in prostate cancer. *Onkourologiya.* 2017;13(3):54-60. (In Russ.)]. doi: 10.17650/1726-9776-2017-13-3-54-60.
5. Коган М.И., Чибичян М.Б., Водолажский Д.И. Изменение экспрессии генетических локусов в мононуклеарной фракции периферической крови больных раком предстательной железы // Клиническая онкология. – 2012. – № 5. – С. 59–60. [Kogan MI, Chibichjan MB, Vodolazhskij DI. Change in the expression of genetic loci in the mononuclear fraction of peripheral blood of patients with prostate cancer. *Klinicheskaja onkologija.* 2012;(5):59-60. (In Russ.)]
6. Аполихин О.И., Сивков А.В., Ефремов Г.Д., и др. *PCA3* и *TMPRSS2: ERG* в диагностике рака предстательной железы: первый опыт применения комбинации маркеров в России // Экспериментальная клиническая урология. – 2015. – № 2. – С. 30–35. [Apolikhin OI, Sivkov AV, Efremov GD, et al. The first Russian experience of using *PCA3* and *TMPRSS2-ERG* for prostate cancer diagnosis. *Experimental'naya klinicheskaya urologiya.* 2015;(2):30-5. (In Russ.)]
7. Mc Neal JE. Origin and development of carcinoma in the prostate. *Cancer*. 1969;23:24-34.
8. Юрмазов З.А., Васильев Н.В. Клинические и морфологические особенности рака предстательной железы в сочетании с ПИН // Сибирский онкологический журнал. – 2010. – Приложение № 1. – С. 118. [Jurmazov ZA, Vasil'ev NV. Clinical and morphological features of prostate cancer in combination with PIN. *Sibirskij onkologicheskij zhurnal.* 2010;(Suppl. 1):118. (In Russ.)]
9. Loeb S, Bjurlin MA, Nicholson J, et al. Overdiagnosis and overtreatment of prostate cancer. *Eur Urol.* 2014;65(6):1046-1055. doi: 10.1016/j.eururo.2013.12.062.

10. Adhyam M, Gupta AK. A review on the clinical utility of PSA in cancer prostate. *Indian J Surg Oncol.* 2012;3(2):120-129. doi: 10.1007/s13193-012-0142-6.
11. Valadi H, Ekstrom K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol.* 2007;9:654-659. doi: 10.1038/ ncb1596.
12. Молекулярный канцерогенез / Под ред. М.А. Красильникова, И.Б. Зборовской. – М.: АБВ-пресс, 2016. – 418 с. [Molecular carcinogenesis. Ed by M.A. Krasil'nikov, I.B. Zborovskaya. Moscow: ABV-press; 2016. 418 p. (In Russ.)]
13. Louie KS, Seigneurin A, Cathcart P, Sasieni P. Do prostate cancer risk models improve the predictive accuracy of PSA screening? A meta-analysis. *Ann Oncol.* 2015;26(5):848-64. doi: 10.1093/annonc/mdu525.

Information about the author:

Philip S. Bova – Candidate of Medical Sciences, Doctorant, Rostov State Scientific Research Institute of Oncology of the Ministry of Health of Russia, Rostov-on-Don, Russia; Head, Center of Urology, Nephrology and Hemodialysis of the Rostov Region State Regional Hospital "Regional Hospital No 2". Rostov-on-Don, Russia. E-mail: alald@inbox.ru.

Сведения об авторе:

Филипп Сергеевич Бова — канд. мед. наук, докторант, ФГБУ «Ростовский научно-исследовательский онкологический институт» Минздрава России, Ростов-на-Дону; руководитель центра урологии, нефрологии и гемодиализа, ГБУ Ростовской области «Областная больница № 2», Ростов-на-Дону. E-mail: alald@inbox.ru.