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SHORT COMMUNICATIONS

Mutational Landscape of Prostate Tumors Revealed by Whole-Exome Sequencing

I. R. Gilyazova[a, b, *], M. A. Yankina[b], G. B. Kunsbaeva[a], E. A. Klimentova[b], A. A. Izmaylov[c], V. N. Pavlov[c], and E. K. Khusnutdinova[a, b, **]

[a] *Department of Genetics and Medicine, Bashkir State University, Ufa, 450074 Russia*
[b] *Institute of Biochemistry and Genetics, Ufa Research Center, Russian Academy of Sciences, Ufa, 450054 Russia*
[c] *Department of Urology, Bashkir State Medical University, Ufa, 450000 Russia*
*e-mail: *gilyasova_irina@mail.ru, **021gen@mail.ru*

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Abstract—The results of the whole-exome DNA sequencing of eight prostate adenocarcinoma patients are presented. DNA was isolated from the peripheral blood as well as healthy and tumor prostate tissue from each patient. Bioinformatics analysis was conducted and the most significant mutations in prostate cancer patients were revealed. The obtained data could be important for understanding of the molecular mechanisms of prostate cancer pathogenesis and facilitate development of new approaches for treatment of the disease.

Keywords: prostate cancer, whole-exome sequencing, somatic mutations, germinal mutations
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INTRODUCTION

Prostate cancer (PC) is one of the most common malignant tumors in the male population worldwide. In 2003, 13 881 subjects with the initial diagnosis of "prostate cancer" were registered in Russia. In 2013, this number increased to 31 569 [1]. PC was the second (12.9%) most common disease after tumors of bronchopulmonary system in the Russian male population [1]. Extremely rapid growth of PC morbidity reaching 3% per year, a severe course affecting the everyday life of patients, and high prevalence of the disease in the world require unraveling the basic mechanisms of PC development.

The most common histological form of PC is an adenocarcinoma. Numerous genetic studies have shown that, in prostate adenocarcinoma, a combination of genes *TMPRSS2* and *ERG* (*TMPRSS2*: *ERG*, or *T2*: *ERG*) occurs in about 50% of cases [2], and less often, a combination of some other genes from the ETS family, primarily *ETV1*, *ETV4*, *ELK4*, and *ETV5*, is encountered [3]. In addition, during PC, deletions in genes *NKX3.1* (8p21) [4–6] and *PTEN* (10q23) arise, and the number of copies of the *AR* gene increases [7].

Here, we present the results of the whole-exome sequencing of eight prostate adenocarcinoma patients. Specimens of the peripheral blood and healthy and tumor prostate tissues were obtained from each patient with informed consent. In view of intratumor heterogeneity, tumor tissue specimens from three regions of each tumor and adjacent specimens of normal tissue obtained after a radical prostatectomy were taken for analysis. Tumor tissues containing at least 85% of tumor cells were included in the study. Clinical and pathological features of patients are presented in Table 1.

DNA from prostate tissue and the peripheral blood of PC patients were isolated using standard phenol-chloroform extraction. The DNA concentration was measured using a Qubit 2.0 fluorimeter (Life Technologies, United States). DNA fragmentation, library preparation, and exome "capture" were conducted according to the manufacturer's recommendations. Selection of specific DNA fragments was conducted using the SureSelect system followed by concurrent sequencing of the obtained libraries using Illumina technology with a HiSeq 2000 device. All the sequences (reads) were aligned with the reference genome using Burrows-Wheeler Alignment (BWA) software [8]. We used the human genome sequence (Genome Reference Consortium Human Build 37 (GRCh37-hg19)) as a reference. Designation of variants was conducted using the Genome Analysis Tool Kit (GATK) [9]. The identified variants were annotated by ANNOVAR software using the scripts table_annovar.pl and annotate_variation.pl [10], which permits comparing single nucleotide substitutions obtained in the course of sequencing with the number of specialized databases and annotating prognostic functional significance of the revealed alterations using six in silico software programs (SIFT, PolyPhen-2, LRT, Mutation Assessor, Mutation-

Table 1. Clinical and pathological traits of prostate cancer patients

Specimen	Age at diagnosis	Glison index	TNM classification	PSA concentration, ng/mg
PCA758	75	3+4	T1c/Nx/M0	17.6
PCA759	61	3+4	T2/Nx/M0	19
PCA760	62	3+3	T2/Nx/M0	8.7
PCA761	63	4+4	T3/Nx/Mx	13.2
PCA762	66	3+4	T2/Nx/M0	10.3
PCA763	62	4+4	T3/Nx/M0	10.6
PCA764	70	3+3	T2/Nx/M0	10.4
PCA765	72	3+4	T2a/N0/Mx	15.5

According to TNM classification: T, tumor size; N, lymphogenic metastasis; M, hemetogenic metastasis. PSA, prostate specific antigen.

Taster, phyloP, and GERP++) from dbNSFP v.3.0a. Additionally, we used CLINVAR and CADD (Combined Annotation Dependent Depletion) software [11]. After exome sequencing, in PC patients, 41542 changes per one specimen in healthy tissue and 45948 changes per specimen in tumor tissue were found on average. The analysis of exome DNA sequencing from healthy tissue revealed that 50.02% of changes are synonymous substitutions, 47.48% are nonsynonymous substitutions, 0.50% are mutations resulting in formation or abolition of stop codons (0.04% "stoploss"; 0.46% "stopgain"), 0.90% are open reading frame shift mutations, and 0.49% and 0.61% are insertions and deletions, respectively, which do not result in open reading frame shift. In the DNA from tumor tissue, the majority of the revealed disturbances are also nonsynonymous (47.45%) and synonymous (49.95%) substitutions; 0.70% are mutations resulting in formation or abolition of stop codons (0.07% "stoploss"; 0.62% "stopgain"); 0.80% are open reading frame shift mutations; 0.50% and 0.60% are insertions and deletions, respectively, which do not result in open reading frame shift.

All the examined specimens contained mutations in the recognized suppressor genes of tumor growth, *TP53* and *ATM*.

For somatic mutation search in the examined DNA specimens isolated from the tumor tissue, we used the most instructive database for mutations in oncological diseases, COSMIC (Catalog of Somatic Mutations In Cancer). In addition, for determination of reliability of the revealed somatic mutations, we used several bioinformatics approaches: for exclusion of germinal alterations, the data was filtered using databases dbSNP132 and Genome Project 1000. For identification of the events associated with tumors, we excluded the variants which are present in the databases dbSNP and 1000Genomes with the frequency exceeding 1%. The selection of the most important somatic mutations from all the pool of alterations was conducted by determination of the functional significance of the revealed changes in accordance with predicted pathogenicity described in the used databases. In addition, we primarily considered changes which result in the loss of function, namely, frameshift mutations and mutations producing formation of a premature stop codon. Such changes were validated by Sanger sequencing

Finally, in each specimen, 9418 somatic mutations were identified on average. The most pathogenic alterations revealed in PC patients are presented in Table 2.

One of the most interesting genes, whose mutations were revealed only in the tumor tissues in two PC patients, was a protein kinase A RIIα (*PRKAR2A*) gene. A pathogenic missense mutation p.Y282C was found in this gene. It is considered that PRKAR2A and its composing proteins are involved in response to chemotherapy. In the last decade, the important role of taxanes in therapy of hormone refractor cancer of the prostate was revealed. Nevertheless, many tumors do not noticeably respond to therapy, while other tumors develop resistance to treatment, resulting in relapse of the disease. In spite of the years of intensive studies, the mechanisms of taxane resistance during PC and other malignant tumors are not clear and are actively investigated. Karolczak-Bayatti et al. [12] showed that both the full and the shortened product at the N-terminus of the *PRKAR2A* gene significantly increased survival of prostate cancer cell lines treated with Taxol and Taxotere. In the *KAZALD1* gene, which is also known as *IGFBP-RP10*, from the peripheral blood and normal tissue of two patients, a pathogenic mutation at the second exon, c.T226G, was found, which produces amino acid substitutions in the protein (p.C76G). The *KAZALD1* gene belongs to the family of insulin-like growth factor IGFBP and contains the N-terminal domain, an inhibitor of serine protease type Kazal-1, follistatin-like domain, and C-terminal immunoglobulin-like domain. Serine proteases are known to be involved in various processes in the human body, which require exact and specific proteolysis, including the processes of cell neoplastic transformation and apoptosis. Specific proteinase inhibitors represent physiological regulators of prote-

Table 2. The most pathogenic variants revealed in normal and tumor prostate tissues on the basis of exome sequencing

Chromosome	Gene name	rs	Alteration in the coding part	Alteration in protein	Frequency according to Databas 1000 Genomes (EUR)	Assessment of functional importance according to several databases				
						CADD[1]	SIFT[2]	MutationTaster[3]	Mutation Assessor[4]	PolyPhen2[5]
Pathogenic variants revealed in the tumor tissue of prostate										
5	TRAPPC13	rs201305773	c.C52T	p.R18W	0.001	33	0,D	1,1.0,D	2.325,0.683,M	1.0,D
3	TRANK1	rs76739135	c.G3451T	p.V1151L	0.001	26.9	0.04,D	0.998,0.998,D	2.395,0.689,M	0.775,P
8	KLHL38	rs201098575	c.G331A	p.A111T	0.001	29.6	0,D	1,1.0,D	3.57,0.791,H	1.0,D
8	CSMD1	rs190894161	c.C6781G	p.P2261A	0.002	23.6	0,D	1,1.0,D	3.255,0.764,M	1.0,D
17	ABCA6	rs149025377	c.G4135A	p.A1379T	0.002	22.9	0,D	1,1.0,D	2.985,0.740,M	0.994,D
3	SSUH2	rs150785183	c.C158T	p.T53M	0.003	25.7	0,D	0.956,0.956,D	2.51,0.699,M	1.0,D
9	DMRT1	rs140506267	c.A671G	p.N224S	0.003	25.4	0.04,D	0.999,0.999,D	2.9,0.733,M	0.995,D
12	MUC19	rs4768264	c.G3886A	p.G1296S	0.003	26.1	0.05,D	1,1.0,D	2.92,0.735,M	
3	PRKAR2A	rs2230509	c.A845G	p.Y282C	0.004	26.4	0,D	1.000,1.000,D	2.67,0.713,M	1.0,D
1	DCDC2B	rs144804850	c.T233C	p.V78A	0.005	26.2	0,D	1.000,1.000,D	3.045,0.746,M	1.0,D
12	TAS2R8	rs142540719	c.G164T	p.R55I	0.009	26.1	0,D	0.988,0.988,D	3.535,0.788,H	1.0,D
Pathogenic variants revealed in the normal tissue and in the peripheral blood										
19	MUC16	–	c.C40696T	p.Q13566X	–	58	0,64	–	–	–
19	MUC16	–	c.C37048T	p.Q12350X	–	52	0, D	–	–	–
19	MUC16	rs76869876	c.T27619C	p.S9207P	–	2	0,D	–	0.695,0.542,M	0.963,D
19	MUC16	–	c.A40204C	p.K13402Q	0.001	8	0,158	–	1.79,0.637,L	0.975,D
19	MUC16	–	c.37139_37144del	p.12380_12382del	–	60	–	–	–	–
17	LASP1	rs141320621	c.C13T	p.R5X	0.002	18.4	1	1,1,A	–	–
1	MYBPHL	rs146641385	c.C193T	p.R65W	0.001	33	0,D	0.869,0.869,D	3.04,0.745,M	1.0,D
4	PDE6B	rs79826315	c.G145T	p.D49Y	0.001	22.4	0,D	1,1.0,D	0.975,0.566,L	0.619,P
6	CD2AP	rs143297472	c.G1488A	p.M496I	0.001	22.6	0.03,D	1.000,1.000,D	2.455,0.694,M	0.997,D
5	SLC27A6	rs144670577	c.C458T	p.P153L	0.002	24.7	0,D	1,1.0,D	2.635,0.710,M	0.797,P
9	OR13C4	rs75361513	c.A905G	p.D302G	0.002	23.4	0,D	0.803,0.803,D	3.205,0.760,M	0.991,D
10	KAZALD1	rs11547671	c.T226G	p.C76G	0.002	28.5	0.01,D	1,1.0,D	3.63,0.796,H	1.0,D
17	EME1	rs77309724	c.G1435C	p.A479P	0.003	31	0.02,D	1,1.0,D	2.645,0.711,M	1.0,D
17	MYH1	rs117616137	c.C3814T	p.R1272W	0.004	34	0,D	1.000,1.000,D	4.085,0.836,H	1.0,D
20	CYP24A1	rs35873579	c.C469T	p.R157W	0.004	29.6	0,D	1.000,1.000,D	2.725,0.718,M	1.0,D
4	SULT1B1	rs4149416	c.A612C	p.E204D	0.005	24.5	0,D	0.964,0.964,D	4.04,0.832,H	0.64,P
20	CHD6	rs61752057	c.C7165T	p.R2389C	0.005	35	0,D	1,1.0,D	2.005,0.655,M	1.0,D

[1] CADD: the higher is the value, the higher the pathogenicity of the variant.
[2] SIFT: D, disease causing.
[3] MutationTaster: A, disease causing automatic; D, disease causing.
[4] MutationAssessor: H, high; M, medium; L, low.
[5] PolyPhen2: D, probably damaging (≥0.957); P, possibly damaging (0.447 ≤ pp2_hdiv ≤ 0.909).

olysis. Disturbance of the balance between proteolytic enzymes and their inhibitors can produce an excessive activation of proteolysis, which is an important pathogenetic element in the development of destructive and malignant processes [13]. It was shown that the *KAZALD1* gene is hypomethylated in glioma, while its decreased expression inhibits cellular proliferation and invasion both in vitro and in vivo. The role of the *KAZALD1* gene in the pathogenesis of PC is not clear at present.

It is worth noting that all patients possessed various mutations in the genes of the mucin family (Table 2). Mucins are high-molecular-weight glycoproteins, which are synthesized in a wide range of epithelial tissues. It is considered that mucins play an important role in pathobiology of malignant tumors in various locations. MUC16, also known as CA-125, is the largest transmembrane mucin, which demonstrates prooncogenic and pro-metastatic traits [14] and plays an important role in formation of metastases by defending tumor cells from cytotoxic reactions, which occur in response to natural killer cells. The *MUC16* gene is shown to be involved in pancreatic cancer progression and proliferative processes in breast cancer [15, 16]. In pancreatic cells, MUC16 interacts with GPI-associated protein mesothelin and thus activates MMP-7, an extracellular matrix-remodeling enzyme. This results in the increased motility of the malignant cells and invasion [17].

In two patients with the third stage of the disease, a mutation c.C13T in the second exon of the *LASP1* gene, which produces synthesis of a shortened protein (p.R5X), was discovered. The *LASP1* gene encodes a protein of the LIM family. LASP1 is a cAMP- and cGMP-dependent protein, which is important in regulation of actin fibril activity in the cytoskeleton. Its involvement in the development of metastatic breast cancer, B-cell lymphoma, and colon cancer is demonstrated. It is also well established that PDEF, which is involved in the pathogenesis of prostate cancer as a potential tumor suppressor, might serve as a regulator of LASP1 expression [18].

In 2012, exome sequencing of European patients with PC showed that 5.4% of tumors contained mutations in the *MED12* gene. Moreover, more than 70% of tumors in the 26th exon of the *MED12* gene possessed the mutation p.L1224F [7]. We did not observe this mutation in our patients. The *MED12* gene of three patients examined by us carried two missense mutations in the heterozygous state: in exon 16, c.C2344A (p.L782M), and in exon 2, c.C176A (p.A59D). Moreover, one of the patients carried both mutations, while others carried only one of the mutation variants in the *MED12* gene. Interestingly, according to the data reported in the previous study [19], sequencing of 61 specimens of predominantly castration-resistant PC demonstrated that mutations in the *MED12* gene were observed only in 3.3% cases and the spectrum of mutations in the *MED12* gene differed from that revealed in another study [7]. In [19], no p.L1224F mutation was found in prostate cancer tumors. This variability might be associated with the type of examined disease. For example, in [19], lethal cases of PC were examined. In 2013, sequencing of the exome and RNA of 64 tumor specimens obtained from 55 PC patients with aggressive, relapsing, and nonrelapsing disease forms was conducted. However, not a single tumor carried a mutation in the *MED12* gene [20].

Therefore, full exome sequencing of PC patients made it possible to reveal several genes whose role in PC was not described before. However, further in-depth analysis of the results for identification of the functional importance of the revealed variants and the involvement of the revealed genes in PC pathogenesis is required.

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