Exp Oncol 2014
36, 4, 246–251

Experimental ONCOLOGY

FGFR3 AND *TP53* MUTATIONS IN A PROSPECTIVE COHORT OF BELARUSIAN BLADDER CANCER PATIENTS

M.P. Smal[1], A.I. Rolevich[2], S.L. Polyakov[2], S.A. Krasny[2], R.I. Goncharova[1,]*
[1]Institute of Genetics and Cytology, National Academy of Sciences of Belarus, Minsk 20072, Republic of Belarus
[2]N.N. Alexandrov National Cancer Center of Belarus, Department of Urology, Minsk 223040, Republic of Belarus

Aim: **The aim of this study was to determine the frequencies of *FGFR3* and *TP53* mutations in a prospective cohort of 150 bladder cancer patients and to assess the relationship between their mutational status and clinicopathological variables.** ***Materials and Methods:*** **The *FGFR3* and *TP53* mutations were detected by the SNaPshot method and PCR-single-strand conformational polymorphism analysis followed by DNA sequencing.** ***Results:*** **The activating *FGFR3* mutations were found in 71 (47.3%) whereas *TP53* mutations were observed in 31 (20.7%) urothelial carcinomas. *FGFR3*-mutant tumors significantly correlated with lower tumor stage and grade, papillary form of bladder cancer and the absence of metastases while *TP53*-mutant tumors were strongly associated with higher tumor stage and grade as well as the presence of metastasis. We also found significant inverse correlation between *FGFR3* mutations and *TP53* alterations in urothelial carcinomas (p=0.03). Four possible genotypes were observed in the whole studied cohort, namely *FGFR3*mut/*TP53*wt (41.3%), *FGFR3*wt/*TP53*wt (38%), *FGFR3*wt/*TP53*mut (14.7%), and *FGFR3*mut/*TP53*mut (6%). Tumors with *FGFR3*wt/*TP53*wt genotype comprised the subgroup, in which all stages and grades were equally distributed.** ***Conclusions:*** **Our findings confirm the alternative role of *FGFR3* and *TP53* mutations in the development of bladder cancer. Together these two genetic markers are attributed to 62% of the tumors studied. Tumors with both wild type genes included urothelial carcinomas of all stages and grades and may develop through another genetic pathway. To elucidate complete molecular profile of bladder tumors further additional studies are needed.**

Key Words: **bladder cancer, *FGFR3* mutation, *TP53* mutation, tumor genotype.**

Bladder carcinoma is the most common malignancy of urinary tract. In Europe, as well as in Belarus, it is the 7th most frequent cancer in men [1, 2]. Urothelial cell carcinoma (UCC) comprises 90% of all bladder cancer cases. Bladder tumors are morphologically heterogeneous and can be categorized into two groups: non-muscle invasive and muscle invasive. At the time of diagnosis more than 75% of bladder tumors are non-muscle invasive Ta, CIS, and T1 neoplasms [1]. The remaining percentage of patients (i.e. 20–25%) is diagnosed with muscle invasive bladder cancer (MIBC) ⩾T2 who undergo cystectomy and have a poor prognosis [3]. After transurethral resection, most patients with non-muscle invasive bladder cancer (NMIBC) (70–80%) develop recurrences within 5 years, and some of them (10–20%) show progression into MIBC which is considered potentially lethal. It provides grounds to assume that NMIBC is a heterogeneous group of cancers with different prognosis. A number of researchers proposed the hypothesis that clinical and morphological heterogeneity of bladder tumors is determined by different molecular pathways of urothelial carcinoma pathogenesis [4–7]. Non-muscle invasive bladder tumors are mainly characterized by high frequency of activating *FGFR3* missense mutations, opposed to high frequency of inactivating *TP53* alterations in MIBC. The prevalence of *FGFR3* mutations in non-muscle invasive low grade urothelial carcinomas and their correlation with favourable prognosis suggested *FGFR3* mutation to be a potential molecular marker for prognosis of NMIBC [8, 9]. Further molecular studies of bladder cancer not only proved the existence of two alternative genetic pathways of urothelial carcinoma pathogenesis but also revealed a more complex picture of bladder tumor mutability and genome instability [10–12].

In our previous study of a prospective cohort of Belarusian patients with NMIBC and MIBC, we found a high frequency (45.5%) of activating *FGFR3* gene mutations that was significantly associated with low stage and low grade tumors [13]. To understand completely the genetic basis of urothelial carcinoma, there is a need to study the mutational status of other genes involved in bladder cancer development. The aim of the present study was to determine the frequencies of *FGFR3* and *TP53* mutations in a prospective cohort of bladder cancer patients and to find the relationship between clinicopathological parameters of the disease and tumor genotypes according to mutations in both genes.

MATERIALS AND METHODS

Patients and tissue samples. We analyzed the tumor tissues of 150 patients (115 men and 35 women) with histologically confirmed UCC who were treated at the Department of Urology of N.N. Alexandrov National Cancer Center of Belarus between 2010 and 2013. The median age of the patients was 67.5 years. Written informed consent was obtained from each patient. Fresh tissue samples and matched formalin-fixed paraffin-embedded (FFPE) tissue samples were used as biological material for analysis. Tumors were graded

Submitted: September 09, 2014.
*Correspondence: E-mail: R.Goncharova@igc.bas-net.by
Abbreviations used: MIBC – muscle invasive bladder cancer; NMIBC – non-muscle invasive bladder cancer; PCR – polymerase chain reaction; SSCP – single-strand conformation polymorphism; UCC – urothelial cell carcinoma; wt/mut – wild type/mutant.

according to the 1973 and 2004 WHO classification and staged according to the tumor-node-metastasis classification guidelines. Sociodemographic and clinical information is presented in Table 1.

Table 1. Characteristics of the patients and tumor samples

Parameter	Number of patients	Frequency, %
Gender		
male	115	76.7
female	35	23.3
Age, years		
< 50	13	8.7
51–60	28	18.7
61–70	44	29.3
⩾ 71	65	43.3
Mean age (m ± SD)		66.1 ± 10.2
Stage		
Ta	16	10.7
T1	81	54.0
T2	25	16.6
T3	13	8.7
T4	15	10.0
Grade (WHO 1973)		
G1	54	36.0
G2	65	43.3
G3	31	20.7
Grade (WHO 2004)		
low	84	56.0
high	66	44.0
Multiplicity		
solitary	52	34.7
multiple	97	64.7
missing	1	0.6
Shape		
papillary	110	73.3
non-papillary	40	26.7
Tumor size, cm		
⩽ 3	82	54.7
> 3	67	44.7
missing	1	0.6
Smoking		
yes	56	37.3
no	87	58.0
missing	7	4.7

DNA extraction. Genomic DNA was isolated from both fresh tissue samples and deparaffinized FFPE tissue sections (5–10 μm) by using a standard phenol-chlorophorm method.

FGFR3 mutation analysis. *FGFR3* mutational status was analyzed using a previously described highly sensitive SNaPshot assay [14] that allows screening for 11 point mutations (R248C, S249C, G372C, S373C, Y375C, G382R, A393E, K652E, K652M, K652Q, and K652T). Briefly, three regions comprising all known *FGFR3* mutations were amplified in one multiplex polymerase chain reaction (PCR), followed by extension of internal primers for each mutation with a labeled dideoxynucleotide. Extended primers were separated by capillary electrophoresis in an automatic sequencer ABI Prism 3500 (Applied Biosystems), and the presence or absence of a mutation was defined by the incorporated dideoxynucleotide.

TP53 mutation analysis. The regions comprising exons 5–8 of the *TP53* gene were amplified by PCR using the primers described by Thongsuksai et al. [15]. PCRs were performed in a total volume of 15 μl containing 100 ng genomic DNA, 1× buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 0.2 μM of each primer, 2 mM $MgCl_2$, 0.2 mM dNTPs, and 0.5 U Dream Taq DNA Polymerase (Thermo Scientific). The cycling conditions were as follows: denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 50 s, with a final extension at 72 °C for 10 min on C1000 thermal cycler (Bio-Rad).

For single-strand conformation polymorphism (SSCP) analysis, 7 μl of PCR product were added to 10 μl of a solution containing 95% formamide, 10 mM NaOH, 20 mM EDTA, 0.05% of xylene cyanol, and 0.05% of bromophenol blue. The mixture was denatured at 100 °C for 7 min then immediately cooled on ice and loaded onto a 10% acrylamide gel (20 x 20 cm plate) containing 5% glycerol. Electrophoresis was performed at 280 V constant power and run for 7 h at 8 °C. After electrophoresis the gel was stained with ethidium bromide.

Samples that showed band shift on SSCP gel were subjected to direct sequencing, which was performed using the Big Dye Terminator v 3.1 cycle sequencing kit (Applied Biosystems) in accordance with the manufacturer's instructions. All mutations were confirmed by sequencing both DNA strands.

Statistical analysis. To assess the association between *FGFR3* and *TP53* genetic alterations and clinicopathological parameters of tumors Chi-square test, Fisher's exact test, or Mann — Whitney U test were used when appropriate, and values of $p < 0.05$ were considered statistically significant.

RESULTS

Frequency and spectrum of FGFR3 gene mutations. Somatic point *FGFR3* mutations were detected in 71 (47.3%) of 150 UCCs, double mutations being found in 6 tumors. Seven different missense mutations, namely S249C, R248C, Y375C, G372C, A393E, S373C, and K652M were identified. Among 6 cases with double mutation, three tumors harbored S249C/R248C alterations, while the other three had A393E/R248C, S249C/Y375C and K652M/A393E combinations. The frequency and distribution of all identified *FGFR3* mutations are shown in Table 2. The most common changes were S249C (68.8%) and Y375C (16.9%). The overall frequency of the mutations in codons 248, 249, and 375 — the major hot spots in bladder tumors — was 93.5% of total mutations in *FGFR3*.

Table 2. Frequencies and spectrum of *FGFR3* gene mutations

Exon	Nucleotide change	Amino acid change	Number of mutations, n	Frequency of mutations, %
7	742C > T	R248C	6	7.8
7	746C > G	S249C	53	68.8
10	1114G > T	G372C	1	1.3
10	1117A > T	S373C	1	1.3
10	1124A > G	Y375C	13	16.9
10	1178C > A	A393E	2	2.6
15	1955A > T	K652M	1	1.3

Frequency and spectrum of TP53 gene mutations. *TP53* alterations were found in 31 (20.7%) tumors, out of which 37.8% were detected in exon 8, followed by 35.2% in exon 7, 21.6% in exon 5 and 5.4% in exon 6. Twenty five patients had only one mutation, double *TP53* mutation was observed in 6 (4%) cases. Distribution of the *TP53* alterations over 27 distinct codons is shown in Table 3. All molecular changes

of the *TP53* gene were presented by missense point mutations except one patient, who had a silent mutation. 48.6% of the gene alterations were transitions and 51.4% were transversions. No mutation leading to a premature termination of the protein was found. The codons 248, 175, and 285 were mutational hot spots in bladder tumors.

Table 3. *TP53* gene mutations detected in bladder tumors

Sample	Stage	Grade	Exon	Codon	Mutation	Amino acid change
28	1	1 low	7	245	GGC-GTC	Gly-Val
39	1	1 low	8	273	CGT-TGT	Arg-Cys
73	1	1 low	7	246	ATG-ATA	Met-Ile
			8	280	AGA-ACA	Arg-Thr
105	1	1 low	7	241	TCC-TGC	Ser-Cys
101	1	2 low	5	175	CGC-CAC	Arg-His
64	1	2 high	6	195	ATC-ACC	Ile-Thr
1	1	3 high	5	175	CGC-CAC	Arg-His
128	1	3 high	8	274	GTT-TTT	Val-Phe
155	2	2 low	8	283	CGC-TGC	Arg-Cys
22	2	2 high	6	196	CGA-CCA	Arg-Pro
81	2	2 high	7	248	CGG-CAG	Arg-Gln
121	2	2 high	8	271	GAG-CAG	Glu-Gln
149	2	2 high	7	244	GGC-GAC	Gly-Asp
15	2	3 high	8	285	GAG-AAG	Glu-Lys
92	2	3 high	7	248	CGG-CAG	Arg-Gln
130	2	3 high	5	139	AAG-GAG	Lys-Glu
20	3	2 high	7	248	CGG-CGC	Arg-Arg
			8	280	AGA-ACA	Arg-Thr
70	3	2 high	8	271	GAG-CAG	Glu-Gln
			8	291	AAG-AAC	Lys-Asn
123	3	2 high	8	278	CCT-CTT	Pro-Leu
110	3	3 high	8	285	GAG-AAG	Glu-Lys
133	3	3 high	7	257	CTG-CCG	Leu-Pro
156	3	3 high	5	159	GCC-GTC	Ala-Val
			5	179	CAT-AAT	His-Asn
9	4	2 high	5	175	CGC-CAC	Arg-His
49	4	2 high	7	248	CGG-CAG	Arg-Gln
138	4	2 high	5	146	TGG-TGT	Trp-Cys
8	4	3 high	7	245	GGC-TGC	Gly-Cys
			7	246	ATG-ATC	Met-Ile
13	4	3 high	7	249	AGG-AGT	Arg-Ser
124	4	3 high	5	132	AAG-AAC	Lys-Asn
134	4	3 high	7	238	TGT-TTT	Cys-Phe
140	4	3 high	8	267	CGG-CAG	Arg-Gln
150	4	3 high	8	281	GAC-CAC	Asp-His
			8	285	GAG-CAG	Glu-Gln

Association of FGFR3 and TP53 mutations with clinicopathological parameters of tumors. Sociodemographical variables and morphologic features of tumors according to *FGFR3* and *TP53* mutational status are shown in Table 4. No correlation was found between *FGFR3* changes and various characteristics like age, gender, smoking, multifocality and tumor size. At the same time, statistically significant association of *FGFR3* mutations with papillary form of bladder cancer ($p < 0.001$) and non-metastatic disease ($p = 0.02$) was revealed.

The frequency of *FGFR3* mutations in non-muscle invasive tumors was 57.7% against only 28.3% in muscle invasive T2-T4 UCCs ($p < 0.001$). We found strong correlation between *FGFR3* mutations and tumor stage ($p < 0.001$). *FGFR3* changes had the highest frequency (62.5%) in Ta tumors and the lowest (13.3%) in T4 carcinomas. Mutational status of the *FGFR3* gene was also significantly associated with tumor grade ($p < 0.001$). The distribution of the frequency of *FGFR3* alterations according to tumor grade was as follows: 68.5% in G1, 47.7% in G2 and 9.7% in G3 tumors. Thus, non-muscle invasive papillary low grade UCCs are characterized by high frequency of *FGFR3* alterations.

As to *TP53* gene, no statistically significant difference was noted in the distribution of *TP53* mutations on the basis of gender, age and smoking. However, mutations were strongly related to non-papillary form of bladder cancer, large tumor size, solitarity and the presence of metastasis (Table 4). The frequency of molecular changes of this gene was substantially higher in MIBC (43.4%) than NMIBC (8.2%). A strong association was found between *TP53* mutations and high tumor stage ($p < 0.001$). No *TP53* mutation was detected in Ta tumors, the frequency of the gene changes increased from about 10% in T1 to 60% in T4 tumors. Mutational status of the *TP53* gene was significantly related to tumor grade: a higher rate of *TP53* alterations was observed in the group of G3 (45.2%) tumors compared to G2 (20%) and G1 (7.4%) tumors. Hence, high frequency of *TP53* genetic changes characterizes high grade muscle invasive tumors of large size.

Table 4. Clinicopathological variables according to *TP53* and *FGFR3* mutational status

Parameter	*FGFR3* mutation			*TP53* mutation		
	yes, n (%)	no, n	p	yes, n (%)	no, n	p
Gender			0.87			0.55
male	54 (47.0)	61		22 (19.1)	93	
female	17 (48.6)	18		9 (25.7)	26	
Age, years			0.86			0.59
< 50	6 (46.2)	7		5 (38.5)	8	
51–60	12 (42.9)	16		6 (21.4)	22	
61–70	19 (43.2)	25		7 (15.9)	37	
⩾ 71	34 (52.3)	31		13 (20.0)	52	
Stage			<0.001			<0.001
Ta	10 (62.5)	6		0 (0)	16	
T1	46 (56.8)	35		8 (9.9)	73	
T2	12 (48.0)	13		8 (32.0)	17	
T3	1 (7.7)	12		6 (46.2)	7	
T4	2 (13.3)	13		9 (60.0)	6	
Grade (WHO 1973)			<0.001			<0.001
G1	37 (68.5)	17		4 (7.4)	50	
G2	31 (47.7)	34		13 (20.0)	52	
G3	3 (9.7)	28		14 (45.2)	17	
Grade (WHO 2004)			<0.001			<0.001
low	53 (63.1)	31		6 (7.1)	78	
high	18 (27.3)	48		25 (37.9)	41	
Multiplicity			0.4			0.028
solitary	22 (42.3)	30		16 (30.8)	36	
multiple	48 (49.5)	49		15 (15.5)	82	
Shape			<0.001			<0.001
papillary	64 (58.2)	46		13 (11.8)	97	
non-papillary	7 (17.5)	33		18 (45.0)	22	
Tumor size, cm			0.33			0.001
⩽ 3	42 (51.2)	40		8 (9.8)	74	
> 3	29 (43.3)	38		22 (32.8)	45	
Metastasis			0.02			<0.001
yes	2 (15.4)	11		9 (69.2)	4	
no	69 (50.4)	68		22 (16.1)	115	
Smoking			0.66			0.41
> 20 years smoker	37 (50.0)	37		17 (23.0)	57	
non-smoker/ < 20 years smoker	32 (46.4)	37		12 (17.4)	57	

The strong associations between *FGFR3* and *TP53* mutational status and tumor stage and grade are clearly depicted in Fig. 1. An inverse relation was found between *FGFR3* mutations and *TP53* alterations ($p = 0.037$). Stage and grade information taken together showed a high proportion of *FGFR3* mutations and *TP53* alterations in tumors with low and high malignant potential, respectively.

Analysis of tumor genotypes revealed that genotype *FGFR3*mut/*TP53*wt was the most prevalent, accounting for 41.3% of UCCs; tumors *FGFR3*wt/*TP53*wt lacking alteration in both genes were observed in 38% of cases, and frequencies of genotypes *FGFR3*wt/

*TP53*mut and *FGFR3*mut/*TP53*mut were 14.7% and 6%, respectively. Interestingly, *FGFR3* and *TP53* alterations were almost mutually exclusive, and they coincided in only 9 tumors of 150 (6%).

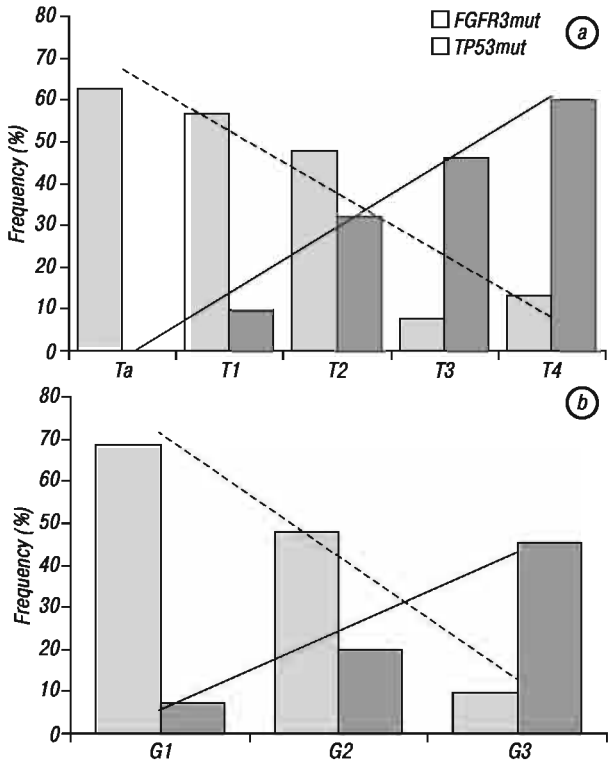

Fig. 1 *FGFR3* and *TP53* mutations according to tumor stage (*a*) and grade (*b*)

Tumor genotypes at various stages and grades are shown in Fig. 2.

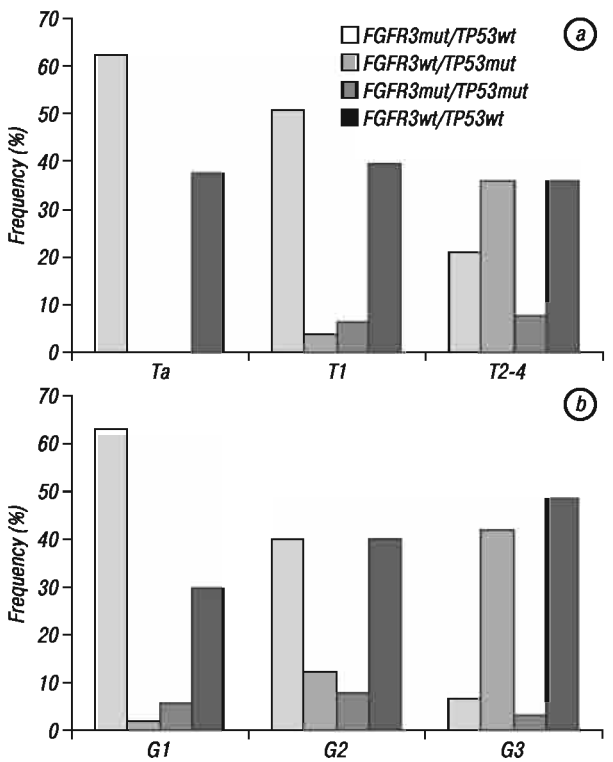

Fig. 2 Tumor genotypes according to mutations in both *FGFR3* and *TP53* at various stages (*a*) and grades (*b*)

In Ta tumors, more commonly observed genotypes were *FGFR3*mut/*TP53*wt (62.5%) and *FGFR3*wt/*TP53*wt (37.5%). In the group of T1 carcinomas, the frequencies of various genotypes were as under: *FGFR3*mut/*TP53*wt — 50.6%, *FGFR3*wt/*TP53*wt — 39.5%, *FGFR3*mut/*TP53*mut — 6.2% and *FGFR3*wt/*TP53*mut — 3.7%. In invasive T2–4 tumors, genotypes *FGFR3*wt/*TP53*mut (35.8%) and *FGFR3*wt/*TP53*wt (35.8%) were the most prevalent, followed by genotypes *FGFR3*mut/*TP53*wt (20.8%) and *FGFR3*mut/*TP53*mut (7.5%).

Distribution of four genotypes according to tumor grade showed that the majority of low grade UCCs had *FGFR3*mut/*TP53*wt and *FGFR3*wt/*TP53*wt genotypes, while in high grade carcinomas, genotypes *FGFR3*wt/*TP53*wt and *FGFR3*wt/*TP53*mut were more common. On the whole the data indicate that there is a strong association of *FGFR3* mutations with favorable disease parameters (low grade, non-muscle invasive), and *TP53* alterations with unfavorable tumor features (high grade, muscle invasive). Patients carrying either *FGFR3* or *TP53* mutation accounted for 62% of the whole cohort studied. The group of UCCs that were wild type for both genes represented a considerable part (more than 35%) of tumors of all stages and grades.

DISCUSSION

To our knowledge, the first two pathway model of urothelial carcinoma pathogenesis was proposed by Spruck et al. [4]. Stratification of tumors into groups was based on the differences in the frequencies of *TP53* mutations and chromosome 9 deletions. Later, this model was modified by van Rhijn et al. [6] by adding *FGFR3* marker. The authors found an inverse relationship between *FGFR3* and *TP53* mutability and clinical morphological parameters of tumors and/or disease-specific survival (a higher frequency of *FGFR3* mutations is indicative of high disease-specific survival while elevated rate of *TP53* alterations indicates low disease-specific survival). This finding suggested that mutations of corresponding genes may serve as the molecular markers of good and poor prognosis in bladder cancer patients.

We also have demonstrated an inverse relation between *FGFR3* and *TP53* mutations in Belarusian bladder cancer patients. In non-muscle invasive papillary low grade tumors, we found high percentage of *FGFR3* mutations which is in accordance with previous studies in European [16, 17] and Belarusian populations [13]. In the present investigation, like in other reports [18, 19], high frequency of *TP53* alterations was observed in advanced tumors.

Moreover, we analyzed the frequencies of genotypes according to *FGFR3* and *TP53* mutational status in the groups of tumors of different stages and grades. In Ta group, no alteration of the *TP53* gene was found. However, a number of studies reported that *TP53* mutations may occur at this tumor stage as well, though quite rarely [19, 20, 21].

In non-muscle invasive T1 tumors, we found the high frequency of *FGFR3*mut/*TP53*wt genotype accounting for more than 50%, while UCCs with *FGFR3*wt/*TP53*mut

genotype were observed in 3.7%. Conversely, the previous studies reported that <27% and >20% of T1 tumors had *FGFR3*mut/*TP53*wt and *FGFR3*wt/*TP53*mut genotypes, respectively [5, 19]. Such differences in the frequencies of T1 tumor genotypes in Belarus compared to Western Europe may be explained by high percentage of muscle invasive disease at first diagnosis in Belarus and further by prevalence of T1 UCCs among the majority of patients with NMIBC. T1 group comprises tumors with different genotypes suggesting their genetic heterogeneity which is reflected in the clinical picture of the disease.

The genetic nature of tumors which are wild type for both *FGFR3* and *TP53* genes (38% of UCCs in our study) remains unclear. The tumors of this subgroup can develop either through the third pathway of bladder cancer pathogenesis, supposed by van Rhijn et al. [6], or due to hyperexpression of the normal *FGFR3* gene observed by Tomlinson et al. in 42% of UCCs with wild type *FGFR3* [22]. Recent investigations on bladder cancer revealed that its formation and development may not be referred to just two main pathways based on *FGFR3* and *TP53* alterations, respectively [23].

Whole-genome studies of different cancers found a considerable number of somatic mutations in the tumor genome [12] and it raised a problem of defining the key drivers. The key mutations rather than accompanying (passenger) ones could be used in bladder cancer diagnosis and prognosis. Detailed molecular genetic analyses conducted by Lindgren et al. and Guo et al. [10, 11] validated the role of *FGFR3* and *TP53* mutations in UCC pathogenesis. Besides, these analyses along with other data established the pathogenetic role of mutations in several other genes, particularly *RB1*, *PIK3CA, KRAS, HRAS, NRAS, CDKN2A,* and *TSC1*. However, the distribution of *RAS* [10, 24, 25], *CDKN2A* and *TSC1* [10] mutations is independent of tumor stage and grade, and thus, they are not of high prognostic value.

According to whole-genome analysis, UCC was divided into two molecular subtypes (MS1 and MS2), differentiated by the mutational status and patterns of gene expression [10]. MS1 tumors are characterized by high frequency of activating *FGFR3* mutations which often coincide with *PIK3CA* alterations, whereas *TP53* and *RB1* changes are more common in MS2 tumors. When comparing mutational profiles of MS1 and MS2 carcinomas with clinicopathological parameters, the authors distinguished the subgroups of UCCs according to the level of genome instability. Tumors with *FGFR3* mutations were found genetically more stable than those with *TP53* alterations, characterized by high proportion of chromosome aberrations [10, 17].

Whole-genome and whole-exome sequencing of 99 bladder tumors [11] showed alterations in 37 genes including well known *FGFR3*, *TP53, RB1*, *PIK3CA, KRAS, HRAS,* and *TSC1*. Moreover, the authors identified the essential role of mutations in the genes involved in sister chromatid cohesion and segregation process. The frequency of genetic alterations affecting this process was 32% and related to more aggressive tumor phenotype. Besides, the high frequency of mutations in the genes involved in cell cycle control, DNA repair and chromatin post-translational modifications was found. The role of these genes in determination of molecular subtypes of bladder tumors will be elucidated by studying the association of mutations with genome instability and clinicopathological parameters.

The data mentioned indicate a complex genetic nature of UCCs. Our findings confirm the alternative role of *FGFR3* and *TP53* mutations in non-muscle and muscle invasive bladder tumors as well as their genetic heterogeneity. Our data support the idea that tumors with *FGFR3*wt/*TP53*wt genotype may represent the third pathway of bladder cancer pathogenesis. Further study of the molecular profile of UCC is needed to define molecular subtypes of bladder tumors, which are important to improve bladder cancer diagnosis and prognosis.

REFERENCES

1. **Babjuk M, Burger M, Zigeuner R, *et al.*** EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. Eur Urol J 2013; **64**: 639–53.

2. **Okeanov AE, Moiseyev PI, Levin LF.** Statistics of cancer diseases in the Republic of Belarus (2003–2012). Minsk: N.N. Alexandrov National Cancer Centre of Belarus, 2013. 373 p.

3. **Stenzl A, Cowan NC, De Santis M, *et al.*** Guidelines on bladder cancer: muscle-invasive and metastatic. Arnhem (The Netherlands): European Association of Urology (EAU), 2010. 64 p.

4. **Spruck CH, Ohneseit PF, Gonzalez-Zulueta M, *et al.*** Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res 1994; **54**: 784–8.

5. **Bakkar AA, Wallerand H, Radvanyi F, *et al.*** FGFR3 and TP53 gene mutations define two distinct pathways in urothelial cell carcinoma of the bladder. Cancer Res 2003; **63**: 8108–12.

6. **van Rhijn BW, van der Kwast TH, Vis AN, *et al.*** FGFR3 and p53 characterize alternative genetic pathways in the pathogenesis of urothelial cell carcinoma. Cancer Res 2004; **64**: 1911–4.

7. **Knowles MA.** Molecular pathogenesis of bladder cancer. Int J Clin Oncol 2008; **13**: 287–97.

8. **Pandith AA, Shah ZA, Siddiqi MA.** Oncogenic role of fibroblast growth factor receptor 3 in tumorigenesis of urinary bladder cancer. Urol Oncol 2013; **31**: 398–406.

9. **Knowles MA.** Role of FGFR3 in urothelial cell carcinoma: biomarker and potential therapeutic target. World J Urol 2007; **25**: 581–93.

10. **Lindgren D, Frigyesi A, Gudjonsson S, *et al.*** Combined gene expression and genomic profiling define two intrinsic molecular subtypes of urothelial carcinoma and gene signatures for molecular grading and outcome. Cancer Res 2010; **70**: 3463–72.

11. **Guo G, Sun X, Chen C, *et al.*** Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved in sister chromatid cohesion and segregation. Nat Genet 2013; **45**: 1459–63.

12. **Alexandrov LB, Nik-Zainal S, Wedge DC, *et al.*** Signatures of mutational processes in human cancer. Nature 2013; **500**: 415–21.

13. **Smal MP, Kuzhir TD, Rolevich AI, *et al.*** FGFR3 gene mutation status in a prospective cohort of bladder cancer patients. Doklady of the NASB 2013; **57**: 96–101.

14. **van Oers JM, Lurkin I, van Exsel AJ, *et al.*** A simple and fast method for the simultaneous detection of nine fibroblast growth factor receptor 3 mutations in bladder cancer and voided urine. Clin Cancer Res 2005; **11**: 7743–8.

15. **Thongsuksai P, Boonyaphiphat P, Sriplung H, Sudhikaran W.** P53 mutations in betel-associated oral cancer from Thailand. Cancer Lett 2003; **201**: 1–7.

16. **van Rhijn BW, Vis AN, van der Kwast TH, *et al.*** Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. J Clin Oncol 2003; **21**: 1912–21.

17. **Junker K, van Oers JM, Zwarthoff EC, *et al.*** Fibroblast growth factor receptor 3 mutations in bladder tumors correlate with low frequency of chromosome alterations. Neoplasia 2008; **10**: 1–7.

18. **Fujimoto K, Yamada Y, Okajima E, *et al.*** Frequent association of p53 gene mutation in invasive bladder cancer. Cancer Res 1992; **52**: 1393–8.

19. **Neuzillet Y, Paoletti X, Ouerhani S, *et al.*** A meta-analysis of the relationship between FGFR3 and TP53 mutations in bladder cancer. PLoS One 2012; 7: e48993.

20. **Berggren P, Steineck G, Adolfsson J.** p53 mutations in urinary bladder cancer. Br J Cancer 2001; **84**: 1505–11.

21. **Borkowska E, Binka-Kowalska A, Constantinou M, *et al.*** P53 mutations in urinary bladder cancer patients from Central Poland. J Appl Genet 2007; **48**: 177–83.

22. **Tomlinson DC, Baldo O, Harnden P, Knowles MA.** FGFR3 protein expression and its relationship to mutation status and prognostic variables in bladder cancer. J Pathol 2007; **213**: 91–8.

23. **Menashe I, Figueroa JD, Garcia-Closas M, *et al.*** Large-scale pathway-based analysis of bladder cancer genome-wide association data from five studies of European background. PLoS One 2012; 7: e29396.

24. **Jebar AH, Hurst CD, Tomlinson DC, *et al.*** FGFR3 and Ras gene mutations are mutually exclusive genetic events in urothelial cell carcinoma. Oncogene 2005; **24**: 5218–25.

25. **Kompier LC, Lurkin I, van der Aa MN, *et al.*** FGFR3, HRAS, KRAS, NRAS and PIK3CA mutations in bladder cancer and their potential as biomarkers for surveillance and therapy. PLoS One 2010; **5**: e13821.