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Genetic diversity of urinary bladder cancer and the risk of recurrence based on mutation analysis

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The aim of the study was to assess the genetic diversity of bladder cancer and determine the suitability of a proposed molecular marker panel to monitor the course of bladder cancer patients. The study involved 185 patients with diagnosed bladder cancer. The genetic diversity of the bladder cancer was evaluated by the prevalence of mutations in the *TP53*, *HRAS*, *FGFR3* and *WWOX* genes.

Mutations were detected in 62.2% of the tumor samples. The most frequently mutated genes were *FGFR3* (49.7%) and *TP53* (16.2%). No mutation was observed in the *WWOX* gene. *FGFR3* mutations, contrary to *TP53*, correlated with lower tumor stage and grade, and the presence of multiple tumors. The risk of death was significantly higher in patients with *TP53* mutant tumors (HR=3.12; 95%CI: 1.14-7.27; p=0.006) but lower in patients with *FGFR3* mutations (HR=0.36; 95%CI: 0.15-0.87; p=0.002). None of the investigated genes was an independent predictor of disease-specific survival, recurrence-free survival or progression-free survival.

The results confirm the existence of two alternative pathways of bladder cancer. However the presence of a high percentage of wild type variants in the higher stages of the disease suggest the existence of another pathway of molecular changes leading to the development of bladder cancer. Molecular analysis may have prognostic value and may facilitate the assignment of patients to appropriate forms of treatment – especially in the case of patients with a T1 tumor, where different mutational patterns were observed in each grade.

Key words: fibroblast growth factor 3, mutation, tumor suppressor protein p53, urinary bladder, urinary bladder neoplasms

The serious medical and social problem presented by bladder cancer (BC) is demonstrated by the unfavorable epidemiological data concerning its incidence and mortality. It is the second most common cancer of the urinary tract in men worldwide [1]. Although women are affected 3-4 times less than men, more frequently advanced lesions are observed at the time of diagnosis among this group, resulting in worse effects of treatment [2]. Apart from gender, the main risk factors include smoking, occupational exposure to chemical carcinogens (mainly benzene derivatives and arylamines) and an age at diagnosis of more than 60 years [3].

Over 90% of BC cases are urothelial cell carcinoma (UCC) [4]. It is characterized by a high heterogeneity, which may suggest that a variety of molecular changes underlie the development of this disease. Previous studies confirm the existence of two different phenotypes of UCC [4, 5]. Most UCCs belong to a low-grade non-muscle-invasive disease, characterized by *FGFR3* mutations, loss of chromosome 9, and an indolent clinical phenotype [6]. High-grade muscle-invasive carcinoma demonstrates numerous genetic and epigenetic alterations, such as loss of *TP53* function, which is associated with the high genetic instability of these tumors, and worse prognosis [4-6].

Although the value of genetic markers in the early diagnosis, prognosis and monitoring of UCC remains still rather limited,

Abbreviations: BC – bladder cancer; UCC – urothelial cell carcinoma

the understanding of the relationship between molecular findings and clinical outcomes is growing. However, it is important to choose appropriate molecular markers for any such analysis. In the case of UCC, research has focused on markers that might predict tumor recurrence, help identify patients in need of early and aggressive treatment, or might allow the most effective therapy to be to selected. The aim of our study was to determine the genetic diversity of bladder cancer based on molecular analysis of the *FGFR3, TP53, HRAS* and *WWOX* genes, and to determine the value of a proposed molecular marker panel to monitor the course of bladder cancer patients. Examined genes were selected based on previously published data [4, 5, 7, 8].

Materials and methods

The study included patients hospitalized at one of three Urology Departments in the Lodz region (Poland) during the period 2005 to 2012 with a suspicion of BC. The male/female ratio was 3:1, median age 68 years. Samples were obtained from urinary bladder by transurethral resection and evaluated by the trained pathologists to determine the presence of UCC, its grade and the invasion depth. Parallel sets of clinical data from patients were assembled. The study was approved by the Ethics Committee of the Medical University of Lodz (permission No: RNN/215/10/KE).

DNA examination. For the purposes of the study, samples of tumor tissue were taken from patients with suspicion of BC. The removed tumors were frozen in liquid nitrogen and stored at -70 °C until DNA isolation. After histopathological examination, 185 samples of tumor tissue (UCC) were enrolled to the study. The DNA isolation steps were carried out according to the manufacturer's protocol (Sherlock AX; A&A Biotechnology, Poland). The isolated DNA was stored at 4-8 °C.

Mutation analysis of the *FGFR3* (exons 7, 10, 15), *TP53* (exons 4-8), *HRAS* (exons 1-2) and *WWOX* (exon 6) genes was carried out using multi-temperature single strand conformational polymorphism (MSSCP) analysis and confirmed by sequencing, as detailed previously [9, 10, 11].

Characterization of study subjects. Complete clinical data was collected from 163 patients (88.1%). During follow-up (average 18.4 months), recurrence was noted in 101 patients (61.9%), of which 36 cases of disease progression were observed (22.1%). Death from BC was recorded in 26 cases (14%). The characteristics of the study group are shown in Table 1.

Statistical analysis. The statistical analyses were performed using STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA). In all conducted analyses, P-values less than 0.05 were reported as statistically significant. Categorical variables were compered by the χ^2 and Fisher's exact tests, the Kaplan-Meier method and log-rank test. Disease-specific survival was calculated from the time of enrollment into the study until the date of death due to BC. Event-free survival was calculated from the date of removal of the tumor until the date of disease relapse, progression or the date of the last visit. Recurrence was classified when the second UCC was of a better or similar stage/grade, and progression was associated with a more advanced stage/grade. The factors found to be statistically significant in univariate analyses were entered into the Cox model to determine the independent prognostic factors.

Results

Mutations frequency. Bladder cancer patients underwent molecular analysis of the *FGFR3*, *TP53*, *HRAS* and *WWOX* genes. In total, 62.2% (115/185) of all tumors were identified on the basis of these. Overall, 13.0% of samples (24/185) showed mutations in more than one testing gene. Among 144 detected mutations, 71.5% were localized in the *FGFR3*

Table 1. Clinicopathological characterization of 185 patients with diagnosed UCC.

Clinopathological parameters	Categorization	Number of patients n=185	Frequency [%]
	Primary tumour	121	65.4%
	Recurrent disease	64	34.6%
Sex			
	Female	35	18.9%
	Male	150	81.1%
Age at diagnosis			
	<60 years	50	27.0%
	≥60 years	135	73.0%
Smoking status			
	Yes	153	82.7%
	No	32	17.3%
Occupational exposure			
	Yes	89	48.1%
	No	96	51.9%
Tumor stage			
	Ta	106	57.3%
	T1	56	30.3%
	T2	18	9.7%
	T3	4	2.2%
	T4	1	0.5%
Tumor grade			
	G1	102	55.1%
	G2	54	29.2%
	G3	29	15.7%
Tumor size			
	<3 cm	101	54.6%
	≥3 cm	84	45.4%
Multiplicity			
	Solitary	116	62.7%
	Multiple	69	37.3%

gene, 26.4% in the *TP53* gene and 2.1% in the *HRAS* gene. None of the bladder tumors showed any *WWOX* alterations. All the detected mutations were point mutations: 142 missense mutations, one nonsense mutation and one frameshift mutation.

MSSCP analysis and DNA sequencing revealed changes in *FGFR3* in 49.7% (92/185) of cases. The most frequent *FGFR3* mutations were S249C (51.1%) and Y375C (39.1%). In the *TP53* gene, mutations were observed in 16.2 % (30/185) of cases, out of which 53.3% were detected in exon 8, 26.7% in exon 7, 13.3% in exon 5 and 6.7% in exon 6. Only three mutations were observed in the *HRAS* gene (Q61L). Due to the low prevalence of mutations found in this gene, they were excluded from further statistical analysis.

Association between FGFR3 and TP53 mutations and clinicopathological parameters. The relationship between the results of molecular analysis and clinical and histopathological characteristics are shown in Table 2. There was no significant association between mutational status of *FGFR3* gene and sex (p=0.820), age (p=0.710), smoking status (p=0.720), occupational exposure (p=0.610) or tumor size (p=0.090). *FGFR3* mutations were more frequent in Ta stage tumors (60.4%; p<0.001), well-differentiated tumors (60.8%; p <0.001) and multiple tumors (60.9%; p=0.020). Also, statistically significant relationships were found between the occurrence of the *TP53* mutation and tumor stage (p=0.040) and grade (p=0.003). Mutations in this gene were more frequent in the higher stages of disease (22.8%), and in G2 (20.4%) and G3 (34.5%) tumors. *TP53* mutations were not associated with sex (p=0.870), age (p=0.340), smoking status (p=0.67), occupational exposure (p=0.330), tumor multiplicity (0.080) or size (p=0.880).

The analysis of the relationship between the coexistence of *FGFR3* and *TP53* mutations and clinicopathological parameters in BC patients was based on four genotypes: *FGFR3*WT/*TP53*WT, *FGFR3*Mut/*TP53*WT, *FGFR3*WT/*TP53*Mut and *FGFR3*Mut/*TP53*Mut. A strong correlation was found between the existence of mutations in the examined genes and tumor stage (p=0.002) and grade (p=0.001). No correlation was observed between each genotype and

Table 2. Frequency of FGFR3 and TP53 mutations in BC patients compared with clinicopathological parameters.

Clinopathological parameters	Categorization	*FGFR3*		p	*TP53*		p
		WT	Mut (%)		WT	Mut (%)	
Sex							
	Female	17	18 (51.4%)	0.820	29	6 (17.1%)	0.870
	Male	76	74 (47.9%)		126	24 (16.0%)	
Age at diagnosis							
	<60 years	24	26 (52.0%)	0.710	44	6 (12.0%)	0.340
	≥60 years	69	66 (48.9%)		111	24 (17.8%)	
Smoking status							
	Yes	76	77 (50.3%)	0.720	129	24 (15.7%)	0.670
	No	17	15 (46.9%)		26	6 (18.8%)	
Occupational exposure							
	Yes	43	46 (51.7%)	0.610	77	12 (13.5%)	0.330
	No	50	46 (47.9%)		78	18 (18.8%)	
Tumor stage							
	Ta	42	64 (60.4%)	**<0.001***	94	12 (11.3%)	**0.040***
	>Ta	51	28 (35.4%)		61	18 (22.8%)	
Tumor grade							
	G1	40	62 (60.8%)	**<0.001***	93	9 (8.8%)	**0.003***
	G2	29	25 (46.3%)		43	11 (20.4%)	
	G3	24	5 (17.2%)		19	10 (34.5%)	
Multiplicity							
	Solitary	66	50 (43.1%)	**0.020***	93	23 (19.8%)	0.080
	Multiple	27	42 (60.9%)		62	7 (10.1%)	
Tumor size							
	<3 cm	45	56 (55.4%)	0.090	85	16 (15.8%)	0.880
	≥3 cm	48	36 (42.9%)		70	14 (16.7%)	

* bold face representing p-values <0.05
Mut – mutation; WT – wild type

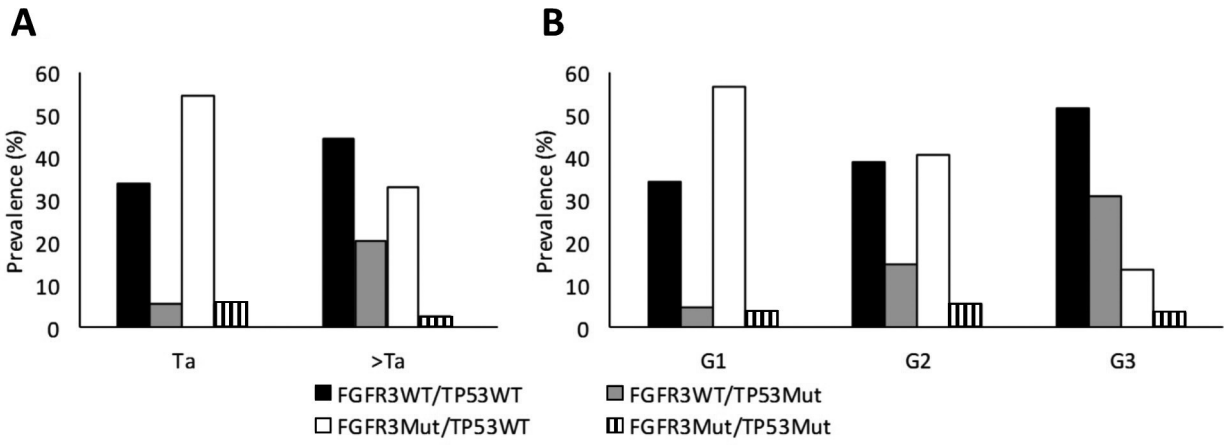

Figure 1. A comparison of the prevalence of different mutational patterns regarding (A) tumor stage and (B) grade in BC patients. Mut – mutation; WT – wild type

sex (p=0.970), age (p=0.490), smoking status (p=0.900), occupational exposure (p=0.770), multiplicity (p=0.070) or tumor size (p=0.400).

The *FGFR3*Mut/*TP53*WT mutational pattern dominated in both Ta and G1 tumors (54.7% and 56.9% respectively), while the percentages of *FGFR3*WT/*TP53*Mut and *FGFR3*Mut/*TP53*Mut genotypes varied from 3.9% to 5.7%. In the case of more advanced lesions, the percentage of *FGFR3*WT/*TP53*Mut tumors prevailed over *FGFR3*Mut/*TP53*WT tumors (Figure 1A, B). In these groups of tumors, a high prevalence of the *FGFR3*WT/*TP53*WT genotype was also observed (44.3% and 51.7% for tumors> Ta and G3 respectively).

An analysis of the *FGFR3* and *TP53* mutations based on combined stage and grade (Figure 2) indicated that *FGFR3*-Mut/*TP53*WT was the most prevalent genotype in the TaG1-2 and T1G1-2 tumors (54.7% and 46.5% respectively), whereas in T1G3 tumors, the *FGFR3*Mut/*TP53*WT variant was more common than the *FGFR3*WT/*TP53*Mut genotype (38.5% vs. 15.4%). In the more advanced lesions, the most common genotype was *FGFR3*WT/*TP53*WT (60.9%). These results were also statistically significant (p = 0.006).

Prognostic value of FGFR3 and TP53 mutations and their association with clinicopathological characteristics and patient outcome. The presence of a *TP53* mutation was found to be an unfavorable prognostic factor for disease-specific survival (p=0.016) (Figure 3A). Patients with a mutation in this gene had more than three times higher risk of death (HR=3.12; 95%CI: 1.14-7.27; p=0.006). Contrary to *TP53* mutations, alterations in *FGFR3* were connected with higher disease-specific survival (p=0.003) (Figure 3B). In this case, the risk of death was HR=0.36 (95%CI: 0.15-0.87; p=0.002). The 5-year survival rate was higher in patients with an *FGFR3* mutation than those with *TP53* alterations (85.5% vs. 66.6%). No statistically significant differences were found between the occurrence of mutations

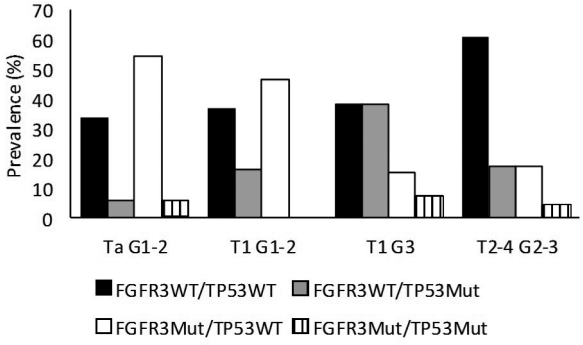

Figure 2. A comparison of the prevalence of different mutational patterns regarding tumor histopathological parameters in BC patients. Mut – mutation; WT- wild type.

in the examined genes, and recurrence-free survival and progression-free survival.

The Kaplan-Meier analysis for disease-specific survival with regard to four combinations of *FGFR3*/*TP53* Mut/WT genotypes revealed that patients with the *FGFR3*Mut/*TP53*Mut variant had significantly higher survival rates then *FGFR3*WT/*TP53*Mut patients (p<0.001) (Figure 3C). No significant differences were observed for recurrence-free survival rate or progression-free survival rate regardless of mutational pattern of the *FGFR3*/*TP53* genes.

Univariate analysis identified histopathological criteria, tumor size, age at diagnosis and occurrence of mutations in *FGFR3* and *TP53* genes as factors associated with BC outcomes. Since these parameters are not necessarily independent, multivariate analysis was used to determine the impact of each factor. None of the investigated genes was an independent prognostic factor for disease-specific survival, recurrence-free survival or progression-free survival (Table 3). Multivariate Cox regression analysis showed that for disease-

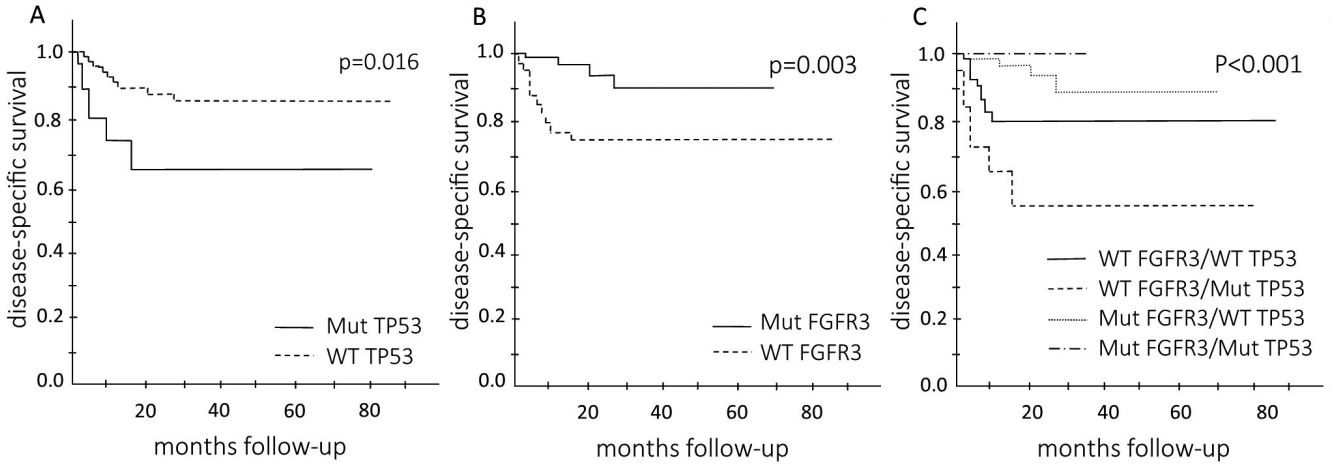

Figure 3. Kaplan-Meier plots for disease-specific survival in relation to (A) *TP53* and (B) *FGFR3* mutations and (C) four combinations of genotypes. (A) Mutations in *TP53* gene were significantly associated with poor prognosis (p[log-rank]=0.016) as opposed to (B) *FGFR3* mutations, which significantly correlated with longer survival (p[log-rank]=0.003) in BC patients (n=185). (C) Good prognosis was also associated with the genotype *FGFR3*-Mut/*TP53*Mut (p[log-rank]<0.001). A dashed vertical line on survival curves mark censored cases; circle-indicate uncensored cases. Mut – mutation; WT – wild type.

specific survival, the only independent prognostic factor was a tumor size more than 3 cm (HR=6.53; 95%CI: 1.46-29.18; p=0.014). Grade G3 turned out to be a negative prognostic factor for recurrence-free survival (HR=3.08; 95%CI: 1.40-6.80; p=0.005). Statistically significant variables predicting progression-free survival were stage >Ta (HR=2.82; 95%CI: 1.07-7.42; p=0.040) and G3 grade (HR=3.64; 95%CI: 1.13-11.78; p=0.030).

Discussion

By analyzing the mutational status of *TP53*, *HRAS*, *FGFR3* and *WWOX* genes, it was possible to identify 62.2% of all examined tumors. Bladder cancer development is clearly associated with the mutations in the *FGFR3* and *TP53* genes [4, 12]. Our observations indicate that *FGFR3* mutations are characteristic of multiple, well-differentiated Ta tumors, whereas *TP53* mutations are more common in clinically and histologically-advanced disease. In addition, our results confirm that *TP53* mutations are an unfavorable prognostic factor. Alterations in this gene are associated with poor differentiation, advanced UCC and poor prognosis [12, 13]. Contrary to our present study, previous conclusions concerning the link between *TP53* mutational status and prognosis were drawn mostly based on the expression of nuclear p53 protein as a surrogate marker of mutations [12, 14, 15]. However, p53 nuclear overexpression is not always caused by mutations [13, 15], it may also be the result of physiological processes occurring in response to DNA damage. Hence, other studies demonstrate many discrepancies based on the method of analysis of *TP53* as a prognostic marker in BC patients, as well as differences in the composition of the study group [16]. The greatest differences concern recurrence, insofar that while our studies indicate that *TP53* mutations are not associated with the recurrence of BC, Shariat et al. [17] claim the opposite. It remains unclear whether changes in *TP53* act as markers of outcome in patients with BC [15].

The results of our research show that patients with mutations in both *FGFR3* and *TP53* genes have significantly longer overall survival than patients with the *FGFR3*WT/*TP53*Mut genotype. This confirms that *FGFR3* and *TP53* mutations stimulate the development of distinct types of BC. Mutations in the *FGFR3* gene are being considered as surrogate markers for the detection of genome stable bladder tumors [18]. Mutations in this gene correlate with a low frequency of chromosome alterations, which explains its "protective effect" on patient survival, the fact that these alterations are characteristic for tumors in the Ta stage, and are associated with good prognosis [19]. However, as bladder cancer development is a complex process which is not clearly understood, evaluation of *FGFR3* mutational status is not a sufficient prognostic marker: The study of single determinants does not accurately reflect the cascade of molecular aberrations. Ploussard et al. found that the prognostic value of *FGFR3* mutational status for disease recurrence and progression depends on allelic losses at 9p22 [20]. Likewise, Rebouissou et al. suggest that the homozygous deletion of CDKN2A (9p21) leads to the progression of *FGFR3*Mut non-muscle-invasive disease [21]. However, van Rhijn et al. showed that the combination of *FGFR3* with MIB-1 (Ki67) seemed to confer a more accurate prediction of progression and disease-specific survival than each of these markers alone [22].

The molecular diversity of bladder cancer is even more apparent when analyzing the frequencies of four different genotypes, *FGFR3*WT/*TP53*WT, *FGFR3*Mut/*TP53*WT, *FGFR3*WT/*TP53*Mut and *FGFR3*Mut/*TP53*Mut, with regard to tumor stage and grade. A high percentage of *FGFR3*Mut/*TP53*WT genotypes were observed in Ta and G1 tumors, while *FGFR3*WT/*TP53*Mut variants were more common in higher stages/grades of tumors, which is consistent with previous studies [23, 24]. The combination of the T and G characteristics, and the mutations in the two genes, revealed the presence of *FGFR3* mutations in more than half of the Ta G1-G2 tumors. The most common variant in the advanced tumors (T2-4 G2-3) was *FGFR3*WT/*TP53*WT (60.9%), while mutations in *FGFR3* and *TP53* occurred with equally low incidence (17.4%). However, the most interesting observations concern stage T1. Tumors in T1 G1-2

Table 3. Multivariate Cox regression analysis for the prediction of disease-specific survival, recurrence and progression in 185 BC patients. The table shows variables that were found to be significant prognostic factors for disease-specific survival, recurrence- free survival and progression – free survival (log-rank test).

Clinopathological parameters	Categorization	p†	Univariate			Multivariate		
			HR	95% CI	p‡	HR	95% CI	p‡
DISEASE-SPECIFIC SURVIVAL								
Age at diagnosis								
	<60 years	**0.024***	1		**0.050***	1		0.283
	≥60 years		4.36	1.02-18.76		2.32	0.50-10.77	
Tumor stage								
	Ta	**<0.001***	1		**<0.001***	1		0.095
	>Ta		16.48	3.82-70.97		4.66	0.77-28.37	
Tumor grade								
	G1		1			1		
	G2	**<0.001***	5.52	1.46-20.83	**0.012***	1.95	0.41-9.29	0.402
	G3		26.67	7.19-99.01	**<0.001***	3.67	0.69-19.53	0.127
Tumor size								
	<3 cm	**<0.001***	1		**0.001***	1		**0.014***
	≥3 cm		11.32	2.64-48.64		6.53	1.46-29.18	
FGFR3								
	Mut	**0.003***	1		**0.002***	1		0.089
	WT		2.77	1.16-6.63		2.73	0.86-8.69	
TP53								
	Mut		3.12	1.14-7.27		1.24	0.46-3.37	0.670
	WT	**0.016***	1		**0.006***	1		
RECURRENCE-FREE SURVIVAL								
Tumor stage								
	Ta	**0.013***	1			1		
	>Ta		1.68	1.12-2.50	**0.010***	1.28	0.73-2.27	0.390
Tumor grade								
	G1	**<0.001***	1			1		
	G2		1.25	0.80-1.94	0.320	1.07	0.61-1.89	0.810
	G3		3.84	2.07-7.12	**<0.001****	3.08	1.40-6.80	**0.005***
PROGRESSION-FREE SURVIVAL								
Tumor stage								
	Ta	**<0.001***	1			1		
	>Ta		3.89	1.92-7-87	**<0.001****	2.82	1.07-7.42	**0.040***
Tumor grade								
	G1	**<0.001****	1			1		
	G2		2.05	0.97-4.35	0.061	1.09	0.42-2.83	0.860
	G3		8.52	3.37-21.53	**<0.001****	3.64	1.13-11.78	**0.030***

† log-rank test; ‡Cox regression; * bold face representing p-values <0.05; Mut – mutation; WT – wild type

were characterized by high prevalence of *FGFR3* mutations (46.5%), while mutations in *TP53* were more common in the T1 G3 tumors (38.5%). The presence of two different frequencies of *TP53* mutations may indicate the presence of two different molecular pathways for T1 tumor development. The molecular analysis of *FGFR3* and *TP53* genes in this group of BC patients may allow patients to be directed towards appropriate forms of treatment.

Our findings indicate that the prevalence of tumors with wild-type *FGFR3* and *TP53* genes increased with malignancy. Similar observations were reported by Neuzillet et al. [24]. This phenomenon may be explained by the high expression of cell-cycle genes for the cases with the *FGFR3*WT/*TP53*WT genotype, even more pronounced than in cases with the *FGFR3*WT/*TP53*Mut variant [25]. Also, *ARID1A* mutations were most frequently observed in the *FGFR3*WT tumors, associated with poor prognosis [26], while methylation of *RASSF1A* in this kind of BC correlated with higher stage and grade [27]. Furthermore, data from our previous loss of heterozygosity (LOH) study indicates that LOH of the *TP53*, *RB1* and *CDKN2A/ARF* genes is more often observed in >Ta and G3 tumors [28].

To understand how this cancer develops, it is necessary to identify other genetic markers associated with BC. Therefore, we attempted to verify whether *WWOX* gene mutations are linked to the development of more clinically and histopathologically advanced tumors. *WWOX* is one example of a tumor suppressor gene. In human tumors, *WWOX* is inactivated by LOH, homozygous deletions or epigenetic methylation. Little is known about these alterations in bladder cancer, but they may possibly lead to loss of *WWOX* expression in higher stage and grade tumors [7, 8]. The literature gives no reports concerning the prevalence of *WWOX* mutations in BC, only a few papers concerning the incidence of these gene mutations have been published regarding other neoplasms [10, 29, 30]. Unfortunately, no *WWOX* mutations were identified in the examined DNA samples.

Low mutation prevalence were found in *HRAS* gene. Previously published data showed large discrepancies in *HRAS* mutations occurrence in BC patients (vary between 0-30%) [31]. Beukers et al. indicated higher number of mutations in this gene in young BC patients (<20 years) compared with older age group [32]. They hypothesized that some of the younger patients might be mosaic for the *HRAS* germinal mutation and therefore could express some of the clinical features of Costello Syndrome, like tumor predisposition. Their results suggest that mosaicism for oncogenic *HRAS* mutations may increase the risk for developing BC at a young age.

Most molecular markers show a relation to tumor grade and stage, and may be used to predict survival, but not recurrence [16], which has been confirmed by our research. While univariate Cox regression analysis found *FGFR3* and *TP53* mutations to be connected with patient survival, they were not found to be independent prognostic factors in multivariate Cox regression analysis (age, stage, grade, tumor size and mutations in *FGFR3* and *TP53* genes). The only independent prognostic factor for disease-specific survival was tumor size greater than 3cm. Grade G3 turned out to be an independent prognostic factor for recurrence, and two factors were found to be statistically independent for progression: stage > Ta and grade G3.

In conclusion, the high prevalence of wild variants in BC, particularly in the advanced form, suggests the existence of another pathway of molecular changes underlying the development of this disease. Our observations confirm the high heterogeneity of BC, and the fact that it is impossible to find one molecular marker to predict the course of the disease. It is clearly connected with the differences in the pathways of BC development and progression. The molecular background of BC development should be considered with regard to aspects which are different but closely related and interdependent: chromosomal aberrations, gene mutations, abnormalities in protein functions. Only a comprehensive approach to these molecular changes can allow the design of standards for molecular diagnostics in routine clinical practice.

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