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FGFR3 Germline Mutations Identified in Skeletal Dysplasia Significantly Cause Low-Grade and Low-Stage Bladder Cancer by Somatic Mutations

Arshad A Pandith,[1] Zaffar A Shah,[1] Nighat P Khan,[2] Mohammad S Wani,[3] Adfar Yousuf,[2] Roohi Rasool,[1] Dil Afroze,[1] Mushtaq A Siddiqi[1]

[1]Department of Immunology and Molecular Medicine, [2]Department of Biochemistry, [3]Department of Urology, Sher-I-Kashmir Institute of Medical Sciences, Srinagar, Kashmir, India

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ABSTRACT

INTRODUCTION: Germline mutations identified in the *FGFR3* gene cause skeletal disorders. The same point mutations have been reported to cause bladder carcinomas, but more research is needed into the various signal pathways of this gene. The purpose of the present study was to analyze the frequency and distribution of *FGFR3* somatic mutations in bladder tumors and to determine their relationship with different clinicopathological characteristics for patients with urinary bladder cancer.

METHODS: This prospective study was conducted in Kashmir, India between 2008 and 2010. The paired tumor and adjacent normal tissue specimens of 65 consecutive patients with transitional cell carcinoma of the bladder were examined. The median patient age was 61 years (range, 38-80 years); the male:female ratio was 5:1. The DNA preparations were evaluated for the occurrence of *FGFR3* gene mutations by PCR-SCCP and DNA sequencing. Blood was also collected from all patients to rule out any germline mutation. Chi-square was used to compare the *FGFR3* gene mutation with the clinicopathological characteristics.

RESULTS: Somatic point mutations of the *FGFR3* gene aggregated to 21 of the 65 patients (32.30%). All mutations except 1 had been previously identified in various skeletal dysplasias. Codon S417Y mutation has not been previously identified in any disease and is reported for the first time. The pattern and distribution of *FGFR3* mutations were significantly associated with low-grade and low-stage tumors ($P < .05$). The frequency of mutation decreased significantly with an increase in the depth of tumor invasion ($P < .05$).

CONCLUSION: Our findings reveal that *FGFR3* mutations characterize a subgroup of superficial bladder tumors with low grade and low stage. The high incidence of *FGFR3* mutations can be an effective prognostic and predictive factor in distinguishing superficial from invasive bladder tumors.

KEYWORDS: Bladder carcinomas; Transitional cell carcinoma; Polymerase chain reaction.

CORRESPONDENCE: Mushtaq A. Siddiqi, Professor & Chairman, Department of Immunology and Molecular Medicine, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, 190011, India (vc.tmuk@gmail.com).

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Abbreviations and Acronyms

DNA = deoxyribonucleic acid
FGFR3 = fibroblast growth factor receptor 3
PCR = polymerase chain reaction
SSCP = single-strand conformation polymorphism
UCC = urothelial cell carcinoma.

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FGFR3 Germline Mutations Identified in Skeletal Dysplasia Significantly Cause Low-Grade and Low-Stage Bladder Cancer by Somatic Mutations

INTRODUCTION

Urinary bladder cancer ranks 9th in worldwide cancer incidence. It is the 7th most common malignancy in men and the 17th most common in women [1]. The American Cancer Society estimates that 70,980 adults were diagnosed with bladder cancer in 2009, leading to 14,330 adult deaths in the United States. Over 350,000 new cases occur across the world each year, with the highest incidence in industrialized countries and areas where infection with the parasite *Shistosoma haematobium* is endemic [2]. The worldwide age standardized incidence rate (ASR) is 10.1 per 100,000 for males and 2.5 per 100,000 for females [3].

Urothelial cell carcinoma (UCC) accounts for 5.6% of cancer in males and 1.8% of cancer in females in India, with an actual crude rate (ACR) incidence of about 1 in 174 men and 1 in 561 women [4]. A hospital-based study in Kashmir revealed that UCC has an annual incidence of 9.66 (2.46%), ranking 9th in all types of cancers [5]. We conducted a detailed study of the bladder cancer cases registered from 2005 to 2010 in the only tertiary care hospital in India. This study revealed that bladder cancer ranks as the 7th leading cancer and accounts for 5.9% of all prevalent cancers in the Kashmiri population (data unpublished).

The incidence of bladder cancer is nearly 4 times higher in men than in women [6]. Active smoking is the strongest environmental risk, contributing to more than 50% of cases [7]. Compared with the general population, light to moderate smokers are at 2 to 4 times greater risk and heavy smokers are at 5 times greater risk for bladder cancer [8]. Occupational exposure to aniline dyes and aromatic amines has been implicated as the second most common risk factor [9,10].

Molecular and pathological studies suggest that low-grade noninvasive and high-grade invasive UCC arise via distinct pathways [11,12]. A high proportion of patients with low-grade UCC develop recurrences, but usually with no progression to invasive disease [13,14]. At presentation, 70%-80% of UCC is superficial and yet to penetrate the epithelial basement membrane. Recently, activating mutations of fibroblast growth factor receptor 3 (*FGFR3*) were found in a significant proportion of UCCs [15-17]; this was considered an important finding. These mutations are significantly associated with low tumor grade and low tumor stage [18,19].

Activating mutations of *FGFR3* are found in the germline in several autosomal dominant human skeletal dysplasia syndromes, including achondroplasia, hypochondroplasia, and thanatophoric dysplasia [20,21]. The same activating point mutations that accounted for the skeletal anomalies in these syndromes were found in multiple myeloma [22] and carcinomas of the bladder [15-18], prostate [23], and cervix [16].

FGFR3 belongs to a family of structurally related tyrosine kinase receptors. Fibroblast growth factor receptors regulate cell growth, differentiation, and angiogenesis [24,25]. Somatic mutations of the gene were reported in approximately 40% of the bladder tumors analyzed. *FGFR3* mutations were localized in exons 7, 10, and 15, which represent putative "hotspot" regions within the gene [16,17]. Mutations in *FGFR3* have been shown to be dominant and to result in constitutive activation of the receptor by increasing its stability [26,27]. Therefore, it seems likely that they contribute to the malignant phenotype.

Although the *FGFR3* gene has been linked to somatic musculoskeletal disorders, more research is needed into the various signal pathways of this gene, particularly in bladder carcinogenesis. The purpose of the present study was to analyze the frequency and distribution of *FGFR3* somatic mutations in bladder tumors and to determine their relationship with different clinicopathological characteristics for patients with urinary bladder cancer.

METHODS

Study Design

This prospective study was conducted at the Sher-I-Kashmir Institute of Medical Sciences (SKIMS) in Kashmir, India, between 2008 and 2010. The Medical Ethical Committee of SKIMS Deemed University approved the study. All patients signed the written informed consent.

Participants

A total of 65 consecutive patients in the urological surgery department who underwent transurethral resection of bladder tumors (TURBT) and radical prostatocystectomy were included in this study. Their median age at the time of diagnosis was 61 years (range, 38-80 years); the male:female ratio was 5:1. Fifty patients (76.9 %) were smokers and 15 (23.1%) were nonsmokers. The majority of patients (70%) lived in a rural environment. Almost all patients presented with hematuria.

Procedures

Tumor grade and stage. Information on tumor grade and stage was obtained for all patients. The diagnostic slides were reviewed by a panel of 2 expert pathologists to confirm the diagnosis and ensure uniformity of classification criteria. All of the samples resected by the urological surgeon were histologically proven transitional cell carcinoma (TCC) of the bladder, except for 1 case of adenocarcinoma. A recurrence was defined as the presence of histologically proven bladder cancer

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at a positive cystoscopy after a complete previous resection. Venous blood (3-5 mL) was collected from each patient in ethylenediaminetetraacetic acid (EDTA) as a control, to rule out any germline mutation.

Deoxyribonucleic acid (DNA) extraction. Samples of both the tumor and adjacent normal tissue were collected. Samples were snap-frozen immediately and stored at -80°C. DNA from both types of tissue was extracted using DNeasy Blood and Tissue Kit (Qiagen GmbH; Hilden, Germany), according to the manufacturer's enclosed protocol.

Polymerase chain reaction (PCR). Exons 7, 10, and 15 of the *FGFR3* gene containing hotspot codons were amplified using previously described specific primers [28]. They were: (1) exon 7F, 5″–AGTGGCGGTGGTGGTGA GGGAG-3″ and 7R 5′-TGTGCGTCACTGTACACCTTGCAG-3″; (2) exon 10F, 5′-CAACGCCCA TGTCTTTGCAG-3″ and 10R, 5-CGGGAAGCGGGAGATCTTG-3″; and (3) exon 15F, 5′-GACCGAGG ACAACGTGATG-3″ and 15R, 5″-GTGTGGGAAGGCGGTGTTG-3″. PCR amplification was carried out in a 50 µL volume container with 50 ng of genomic DNA, 1XPCR buffer containing 15 mM $MgCl_2$, 100 µM each of dATP, dGTP, dTTP, dCTP, and 1.5 U of *Taq DNA polymerase* (Biotools; Madrid, Spain), and 1 µM of forward and reverse primers (Genescript; Piscataway, NJ, USA). The thermal conditions were set as: initial denaturation at 95°C for 5 minutes, 35 cycles of 94°C for 40 seconds, a specific annealing temperature (exon 7, 65°C; exon 10, 62°C; exon 15, 60°C) for 40 seconds and 72°C for 40 seconds, and final extension at 72°C for 7 minutes. The PCR products were run on 2% agarose gel and analyzed under an ultraviolet illuminator. The single-strand conformation polymorphism (SSCP) analysis of the amplicons of exon 7, 10, and 15 was performed on 6% nondenaturing polyacrylamide gel (PAGE) utilizing nonradioactive silver staining [29]. The purified PCR amplicons of the tumor samples showing mobility shift on SSCP analysis and randomly chosen normal samples were used for direct DNA sequencing, using the automated DNA sequencer ABI Prism 310 Genetic Analyzer (Applied Biosystems, Life Technologies; Carlsbad, CA, USA).

Data Analysis

Chi-square was used to compare the *FGFR3* gene mutation with the clinicopathological characteristics, using SPSS-IBM (SPSS, Inc; Chicago, IL, USA). Comparisons with a probability value < .05 were considered statistically significant.

RESULTS

Patient Characteristics

Table 1 contains the clinicoepidemiological characteristics of the patients with bladder cancer. The majority of patients had either G2 (41.5%) or G3 (38.4) cancer. The cancer was superficial in 61.5% and muscle-invasive in 38.5% of the patients, and occurred primarily in the right (46.1%) and left (38.5%) posterolateral areas. Tumor size was ≤ 3 cm for 53.8% and > 3 cm for 46.2% of the patients, respectively. The vast majority of patients (92.3%) had no lymph node involvement. For 75.3 % of the patients, the cancer was not recurrent. The pT stage was evenly divided across participants for pTa, pT1, and pT2.

Somatic Point Mutations

In this study, single-strand conformation polymorphism and DNA sequencing were used to analyze the regions of *FGFR3* harboring the point mutations in a series of 65 patients with bladder carcinoma. Overall mutations in exon 7, 10, and 15 of the *FGFR3* gene identified in this study aggregated to 32.3% (21 out of 65). Table 2 contains the clinicoepidemiological characteristics of the 21 patients with missence mutations and the mutant phenotypes of the *FGFR3* gene. There were 6 transitions and 15 transversions. Of the 6 transitions, 3 were A → G transitions and 3 were C → T transitions; of the 15 transversions, 12 were C → G transversions, 2 were G → T transversions, and 1 was a T → A transversion. Table 3 contains the point mutations in the FGFR3 gene. We detected 6 different single-nucleotide substitutions in 21 of the 65 bladder carcinomas. These mutations affected codons 248, 249, 372, 375, 417, and 652, according to FGFR3b isoform numbering (Figure 1a; Figure 1b; Figure 1c). All 6 types of mutations identified in bladder carcinomas (except for 1 in codon 417) were identical to the germinal-activating mutations that are responsible for thanatophoric dysplasia, a lethal form of dwarfism (Table 3). Mutations were found more frequently in UCCs of lower grade and lower stage. Five of these 6 mutations were located in the extracellular domain (codons 248 and 249) or transmembrane domain (codons 372, 375, and 417). The 6th mutation, located in the kinase domain (codon 652), resulted in the replacement of a positively charged residue by a negatively charged residue. The codon S249C mutation was the most frequent; it was found in 12 of the 21 mutated samples (57.1%). The codon S417Y mutation is reported for the first time. It resulted in replacement of serine into tyrosine. Six mutations were detected in recurrent cases (4 in S249C); the remaining mutations were found in 15 nonrecurrent cases (Table 2). The matched constitutional DNA contained the wild-type sequence in every case, demonstrating the somatic nature of these mutations in bladder cancer.

Among the 21 mutations found in this study, 3 were found in G1 tumors (50% of the G1 tumors) and 13 were found in G2 tumors (48% of the G2 tumors); mutations were only found in 4 (20%) and 1 (12.5%) of the G3 and G4 tumors, respectively (Table 1). There was a significant difference between mutations

Table 1. Clinicoepidemiological Characteristics of Patients With Bladder Cancer; Mutant Phenotypes of the *FGFR3* Gene; Probability of Significant Differences.
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Characteristic	Cases (N = 65)		Mutants M (n = 21)		Wild Type		*P*
	n	%N	n	%n	n	%n	
Sex							
Male	55	84.4	17	30.9	38	69.1	.572
Female	10	15.6	7	40.0	6	60.0	
Age, years							
≤ 50	20	30.0	5	25.0	15	75.0	.401
> 50	45	70.0	16	35.5	29	64.5	
Dwelling							
Rural	45	70.0	15	33.3	30	67.7	.604
Urban	20	30.0	8	40.0	12	60.0	
Smoking status							
Smoker	50	76.9	15	33.0	35	77.0	.468
Nonsmoker	15	23.1	6	40.0	9	60.0	
Differentiation grade							
G1	6	7.6	3	50.0	3	50.0	
G2	27	41.5	13	48.1	14	51.9	.044
G3	24	38.4	4	20.0	20	80.0	
G4	8	12.3	1	12.5	7	87.5	
Histological type							
Superficial	40	61.5	17	42.5	23	57.5	.026
Muscle-invasive	25	38.5	4	16.0	21	84.0	
Site							
Right posterolateral	30	46.1	11	36.6	19	63.5	
Left posterolateral	24	36.9	7	29.1	17	70.9	.576
Bladder neck	4	6.1	2	50.0	2	50.0	
Orifide	7	10.7	1	14.2	6	85.8	
Size							
≤ 3 cm	45	53.8	17	37.7	28	62.3	.157
> 3 cm	20	46.2	4	20.0	16	80.0	
Lymph node status							
No	60	92.3	20	33.3	40	67.7	.540
Yes	5	7.6	1	20.0	4	80.0	
Status							
Nonrecurrent	49	75.3	15	30.6	34	69.4	.609
Recurrent	16	24.7	6	37.5	10	62.5	
Stage							
pTa	21	32.3	12	57.1	9	40.9	.008
pT1	22	33.8	6	27.2	16	72.7	
pT2	22	33.8	3	13.6	19	86.4	

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Table 2. Clinicoepidemiological Characteristics of the Patients with Missence Mutations; Mutant Phenotypes of the *FGFR3* Gene (n = 21). doi: 10.3834/uij.1944-5784.2010.12.07t2

Patient ID	Age, years	Sex	Setting	Smoking Status	Grade	Stage	Lymph Node Status	Histo-pathological Type	Site	Type	Status	Exon	Cordon Number	Base Change	Amino Acid Change
AT2	70	M	Rural	smoker	3	pT2	Yes	muscle-invasive	RPL	TCC	Non	7	249	TCC→ TGC	Ser→ Cys
AT7	75	M	Rural	smoker	4	pT2	No	muscle-invasive	LRL	TCC	Rec	10	417	TCC→ ACC	Ser→ Tyr
AT8	43	M	Rural	non-smoker	2	pT1	No	superficial	RPL	TCC	Rec	7	249	TCC→ TGC	Ser→ Cys
AT12	58	M	Urban	smoker	2	pTa	No	superficial	LRL	TCC	Rec	7	249	TCC→ TGC	Ser→ Cys
AT15	38	M	Rural	smoker	2	pT2	Yes	muscle-invasive	RPL	TCC	Non	7	249	TCC→ TGC	Ser→ Cys
AT17	70	M	Urban	smoker	2	pT1	No	superficial	LRL	TCC	Non	7	249	TCC→ TGC	Ser→ Cys
AT22	51	M	Rural	smoker	3	pTa	No	superficial	LRL	TCC	Rec	7	249	TCC→ TGC	Ser→ Cys
AT25	68	F	Rural	smoker	2	pTa	No	superficial	RPL	TCC	Non	7	248	CGC→ TGC	Arg→ Cys
AT29	60	M	Rural	smoker	2	pTa	No	superficial	LRL	TCC	Rec	7	249	TCC→ TGC	Ser→ Cys
AT30	68	F	Urban	non-smoker	1	pTa	No	superficial	BN	TCC	Non	7	249	TCC→ TGC	Ser→ Cys
AT36	71	M	Urban	smoker	2	pTa	No	superficial	RPL	TCC	Non	15	652	AAG→ GAG	Lys→ Glu
AT39	44	M	Urban	non-smoker	2	pTa	No	superficial	RPL	TCC	Non	7	248	CGC→ TGC	Arg→ Cys
AT41	60	M	Urban	smoker	2	pTa	No	superficial	RPL	TCC	Non	7	249	TCC→ TGC	Ser→ Cys
AT46	55	M	Urban	smoker	3	pT1	No	superficial	LPL	TCC	Rec	10	372	GGC→ TGC	Gly→ Cys
AT51	41	M	Rural	non-smoker	2	pT1	No	superficial	BN	TCC	Non	10	375	TAT→ TGT	Tyr→ Cys
AT55	50	F	Urban	non-smoker	2	pT1	No	superficial	RPL	TCC	Non	7	249	TCC→ TGC	Ser→ Cys
AT57	66	M	Rural	smoker	2	pTa	No	superficial	RPL	TCC	Non	7	248	CGC→ TGC	Arg→ Cys
AT58	58	M	Urban	smoker	3	pT1	No	muscle-invasive	BN	TCC	Non	10	375	GGC→ TGC	Gly→ Cys
AT61	71	M	Rural	smoker	2	pTa	No	superficial	UO	TCC	Non	7	249	TCC→ TGC	Ser→ Cys
AT63	59	F	Rural	non-smoker	1	pTa	No	superficial	RPL	TCC	Non	7	249	TCC→ TGC	Ser→ Cys
AT65	77	M	Rural	smoker	1	pTa	No	superficial	RPL	TCC	Rec	10	375	TAT→ TGT	Tyr→ Cys

Abbreviations: BN, bladder neck; F, female; LPL, left posterolateral; M, male; Non, nonrecurrent; O, orifide; Rec, recurrent; RPL, right posterolateral; TCC, transitional cell carcinoma; UO, ureteric orifice.

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Figure 1a. Partial Nucleotide Sequences in Exon 7.
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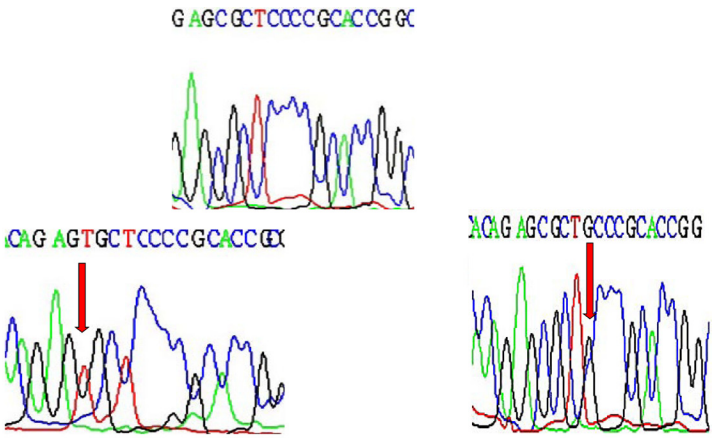


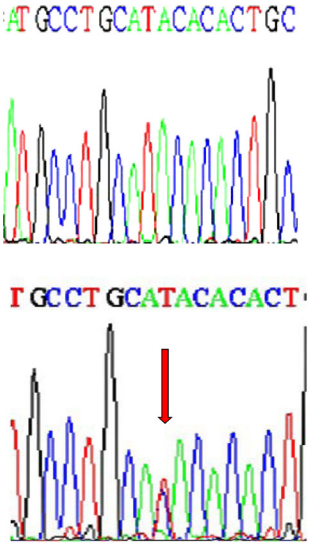
Partial nucleotide sequences in Exon 7 (forward) of the normal (above) and mutants in exon 7 of the *FGFR3* gene codon 248 (CGC → TGC) and codon 249 (TCC → TGC) (see Table 2). Red arrow points toward base change in mutants with respect to normal sequence.

in lower-grade and higher-grade tumors ($P < .05$), indicating a connection between the *FGFR3* mutation pattern and low-stage bladder tumors. Mutations were detected in 12 of 65 patients (57.1%) with pTa tumors and 6 of 65 (27.2%) patients with pT1 tumors; a significantly lower number of mutations were present in pT2 or higher stages of bladder tumors ($P = .008$) (Table 1). The distribution of *FGFR3* mutation as a function of the histological type of tumors (ie, superficial versus invasive) is shown in Table 1. A total of 17 out of 21 mutations (80.9%) were found in superficial tumors; 4 of 65 mutations (19%) were found in muscle-invasive cancer. This difference in mutation frequency between superficial and muscle-invasive tumors was statistically significant ($P < .05$). SSCP also identified abnormally migrating bands in exon 15. Later sequencing revealed that this band shift was caused by a single nucleotide base change that did not alter the amino acid. It was a synonymous polymorphic change that resulted in threonine in both cases.

DISCUSSION

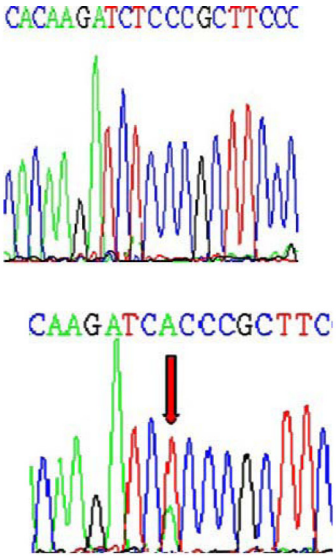
Among the different types of cancers screened, bladder cancer appears to have the highest frequency of *FGFR3* mutations. Capellen et al [16] identified mutations in 9 of 26 (35%) bladder

Figure 1b. Partial Nucleotide Sequences (Reverse) in Exon 10.
doi: 10.3834/uij.1944-5784.2010.12.07f1b



Partial nucleotide sequences (reverse) of the normal (above) and mutants (below) in exon 10 of the *FGFR3* gene codon 375 (TAT → TGT) (see Table 2). Red arrow points toward base change in mutants with respect to normal sequence.ard base change in mutants with respect to normal sequence.

Figure 1c. Partial Nucleotide Sequences (Forward) in Exon 10.
doi: 10.3834/uij.1944-5784.2010.12.07f1c



Partial nucleotide sequences (forward) of the normal (above) and mutants (below) in exon 10 of the *FGFR3* gene codon 417 (TCC → ACC) (see Table 2). Red arrow points toward base change in mutants with respect to normal sequence.

Table 3. Point Mutations in the *FGFR3* Gene in Patients With Bladder Carcinoma (N = 65).
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codon	nt position	Exon	Mutation	Tumors (n)	Frequency (%)
248	742	7	CGC → TGC	3	14.0
249	746	7	TCC → TGC	12	57.1
372	1114	10	GGC → TGC	1	4.7
375	1124	10	TAT → TGT	3	14.2
417	1250	10	TCC → ACC	1	4.7
652	1954	15	AAG → GAG	1	4.7

tumors that were identical to those found in thanatophoric dysplasia (R248C, S249C, G372C, and K652E). *FGFR3* mutations vary from 31% to 60% in bladder tumors [15-18]. The most frequent mutation in bladder tumors found so far is codon S249C. Germinal point mutations resulting in *FGFR3* activation are responsible for dwarfism syndromes. Surprisingly, similar *FGFR3* mutations have also been implicated in tumorigenesis. The mechanisms by which mutations occur in the germline or somatically in the bladder may be different, possibly reflecting a different balance between replicative errors and induced DNA damage.

In the present study, the frequency of mutations in a series of 65 confirmed bladder tumors aggregated to 32.3%. The most common mutations found in our study were S249C and Y375C, representing highly activated forms of the receptor (Table 3). These mutations, called *dimerisation mutations*, generate an additional cysteine residue that allows ligand-independent dimerisation of the receptor. Similarly, 2 other less common mutations in our group (S248C and G372C) generate a novel cysteine residue that is predicted to cause dimerisation, phosphorylation, and downstream signaling. K652E mutation is predicted to cause conformational change in the kinase domain, leading to constitutive activation of the kinase activity of the receptor [30]. The codon S417Y single mutation found in this study has never been reported in any form of skeletal dysplasia or in any other cancer, including bladder cancer. The novel mutation was found in exon 10 in the in *FGFR3* gene in the transmembrane region of the fgfr3, where other mutations like codon 372, 375, 380, and 393 have already been reported in many studies. In contrast with the lower mutational frequencies in thanatophoric dysplasia, the high frequency of 249, 248, and 375 codon mutations that was observed in the present and other studies reflects differences in etiology. Most of the *FGFR3* mutations in bladder cancer could be caused by carcinogens, whereas the germinal mutations in thanatophoric dysplasia are spontaneous mutations that preferentially create C-to T transitions in CG dinucleotides.

Although initial analysis suggested an association with recurrence rate in superficial and invasive tumors [28], this association has not been confirmed in other studies [31,32]. In the present study, no appreciable difference was found in the distribution of mutations in patients with recurrent and nonrecurrent cancers (37.5% versus 30.6%, respectively). This finding was in contrast to some studies that showed *FGFR3* mutation associated with either low tumor recurrence [19] or higher recurrence rate [33].

It is now clear that mutation of *FGFR3* in bladder cancer is strongly associated with low tumor grade and low tumor stage; several studies describe very similar profiles [34,35]. In the present study, a significantly higher number of mutations were present in the lower stages of bladder tumors ($P = .008$) (Table 1). Among 21 mutations found, 16 mutations were detected in low-grade tumors, whereas 5 were observed in high-grade tumors. These mutation outcomes were in accordance with previous studies [15,18]. Therefore, *FGFR3* is the first gene found to be preferentially mutated in pTa tumors. Our findings confirm that *FGFR3* has a predictive and prognostic role in tumors of low stage and low grade and that there is a significant decrease in the prevalence of mutations as depth of invasion and differentiation of the bladder tumor increases. Because *FGFR3* mutation expression is linked to superficial papillary tumors, it is possible that *FGFR3* signaling maintains a differentiated phenotype that is incompatible with the development of more aggressive tumors. This idea was also proposed following the recent finding that *TP53* and *FGFR3* mutations are almost mutually exclusive in bladder tumors [36]. It seems possible that *FGFR3* mutations are an early event in the development of superficial papillary bladder cancer. In most cases, this mutation may provide a protection from the progression to muscle invasion. In a recent study on prostate cancer by Hernández et al [23], *FGFR3* gene alteration was observed (9 of 112 patients; 8%). This finding was in contrast to the results of 2 previous studies, where no mutation was found [37,38]. Hernández et al also showed involvement of the *FGFR3* gene in low-grade and low-stage tumors of prostate cancer, which is consistent with our findings. Association of *FGFR3* mutations with an early stage of tumor development or a less-aggressive form of disease in bladder cancer contrasts with multiple myeloma, where *FGFR3* mutation appears to be associated with tumor progression [39]. Currently, the signaling pathway(s) downstream of *FGFR3* in normal urothelial cells are not known, nor are those stimulated in bladder cancer cells

with mutation. However, it is predicted that the Ras/MAPK and PI3K pathways are signal transduction pathways for the mutant *FGFR3* tumors [40-43]; their role needs to be further evaluated. Similarly, nothing is yet known about the major effects of *FGFR3* mutation on the urothelial cell phenotype. This information will be pivotal to clearly understanding the consequences of receptor mutation during tumor development.

CONCLUSION

Our study shows that the *FGFR3* mutational spectrum characterizes the low-grade and low-stage pathway of bladder tumorigenesis. The prevalence of *FGFR3* and *P53* have an inverse relation [35], whereas the *FGFR3* and *RAS* families of gene mutation are mutually exclusive in bladder cancer [33]. Thus, a high incidence of *FGFR3* mutations can be an effective prognostic factor and a useful tool in standard management of a subgroup of bladder tumors of low grade and low stage. Our findings emphasize the need to conduct further studies to investigate *FGFR3* as a prognostic marker in a larger series.

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Conflict of Interest: none declared.

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