The Fibroblast Growth Factor Receptor 3 (FGFR3) Mutation Is a Strong Indicator of Superficial Bladder Cancer with Low Recurrence Rate

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Abstract

We analyzed the possible prognostic value of the recently discovered fibroblast growth factor receptor 3 (FGFR3) mutations in bladder cancer. A FGFR3 mutation was found in 34 of 53 pT\textsubscript{a}-pT\textsubscript{1} bladder cancers, whereas none of the 19 higher-staged tumors had a mutation (P < 0.0001). In 57 patients with superficial cancer followed prospectively by cystoscopy for 12 months, 14 of 23 patients in the wild-type FGFR3 group developed recurrent bladder cancer compared with only 7 of 34 patients in the mutant group (P = 0.004). The recurrence rate per year was 0.24 for the FGFR3 mutant tumors and 1.12 for tumors with a wild-type FGFR3 gene. In addition, FGFR3 mutation status was the strongest predictor of recurrence when compared with stage and grade (P = 0.008). This is the first mutation in bladder cancer that selectively identifies patients with favorable disease characteristics. Our results suggest that the frequency of cystoscopic examinations can be reduced considerably in patients with FGFR3-positive tumors.

Introduction

Urinary bladder cancer is the fifth most common neoplasm in western society with ~54,400 new cases in the United States per year (1). In the majority of patients, bladder cancer is superficial (i.e., pT\textsubscript{a}-pT\textsubscript{1}) at first presentation. After TUR\textsuperscript{3} of superficial bladder cancer, patients are monitored by cystoscopy at regular intervals because the recurrence rate of superficial bladder cancer is up to 70% (2–4). Progression to invasive disease occurs in around 15–20% of patients (2, 3). Clinical and histopathological factors for prediction of tumor recurrence and progression of bladder cancer have been studied extensively (2–4). Tumor grade, stage, and recurrence rate are especially important. In addition, it has appeared that mutations in the tumor suppressor genes TP53 and RB are of additional value to assess aggressive tumor behavior (5). The FGFR3 is a glycoprotein composed of three extracellular immunoglobulin-like domains, a transmembrane domain, and a split tyrosine-kinase domain. Several reports have shown that constitutive activation of the FGFR3 gene by specific point mutations leads to congenital anomalies such as achondroplasia and thanatophoric dysplasia (6, 7). A frequent t(4;14)/(p16.3;q32.3) chromosomal translocation with the breakpoint near FGFR3 in multiple myelomas suggested an oncogenic role for the FGFR3 gene (8). However, an activating mutation in the FGFR3 gene occurred rarely in multiple myeloma (9). The same missense mutations (R248C, S249C, G372C, and K652E) that were observed in thanatophoric dysplasia were recently found in 9 of 26 bladder carcinomas and 3 of 12 cervix carcinomas (10). It has been shown that mutated FGFR3 can transform NIH 3T3 cells when targeted to the cell membrane (11). Because the FGFR3 gene is expressed in bladder cancer and normal uterine epithelium (10), it is likely that the mutant gene has an oncogenic role in bladder cancer pathogenesis. The present study assesses the possible prognostic value of the FGFR3 mutation in bladder cancer.

Materials and Methods

Patients. Seventy-two consecutive patients who underwent TUR at the University Hospital Rotterdam in 1998–1999 were entered into this study. The patients were not selected on any clinical or other parameter. All of the patients signed written informed consent. The Medical Ethical Committee of the Erasmus University and the University Hospital Rotterdam approved the study (MEC 168.922/1998/55). Median age at time of diagnosis was 65.4 years (range, 30–89); male:female ratio was 4.2:1. We examined each patient’s history and obtained 12-months clinical follow-up on a prospective basis, including a cystoscopic examination every 3 months, from all of the patients that were diagnosed with superficial bladder cancer at the time of FGFR3 mutation analysis. Grading of cancers was performed according to the WHO classification and staging according to the 1997 TNM classification guidelines (UICC 1997). A recurrence was defined as the presence of histologically proven bladder cancer at a positive cystoscopy after a complete previous TUR. The recurrence rate per year was defined as the number of recurrences divided by the total number of months of follow-up. The result was then multiplied by 12. Hence, the recurrence rate takes into account the clinical course of patients during a longer interval and not only the time to first recurrence.

Sample Collection and DNA Extraction. The bladder tumors were subjected to careful microdissection after confirmation of the histopathological diagnosis. Venous blood (7 ml) was obtained from every patient to be used as control. DNA from paraffin-embedded, formalin-fixed neoplastic tissue was extracted using DNeasy Tissue kit (Qiagen GmbH, Hilden, Germany) according to enclosed protocol. DNA from venous blood was isolated using one-step DNAzol BD Reagent (Life Technologies, Inc., Grand Island, NY). We checked the accuracy of the microdissection procedure by LOH analysis in 18 of the 19 patients with ≥pT, tumors comparing the allele intensities of normal and tumor DNA of the same samples that were used for the FGFR3 mutation analysis. A 50% reduction of allele intensity was considered LOH. We used 23 microsatellite markers on 9 different chromosomes as described previously (12). Every LOH was confirmed in a second PCR.

FGFR3 Mutation Analysis. FGFR3 mutation analysis was performed by PCR-SSCA on tumor- and control blood DNA of all of the patients. Three regions of interest containing the four previously identified mutations were amplified by PCR. The primer sequences were the same as used by Capellen et al. (10). The following primer pairs were used: for exon 7, 5'-AGTGGCG-GTGGTGGTGGAAGGAG-3' and 5'-TGTGCCTGACTGACCTTCTGCA-3'; for exon 10, 5'-CAACGCCCAGTCTTTGCTAG-3' and 5'-CGGGAAG-CGGGATGTTTGG-3'; and for exon 15, 5'-GACGGACAGACAGCT-GATG-3' and 5'-TGTTGGGAAGGCGTGTTG-3'. All of the tumors with an aberrant band at SSCA were sequenced with T7 Sequenase v2.0 (Amersham life Science, Inc., Cleveland, OH) on both strands to check the identity of the

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3 The abbreviations used are: TUR, trans-urethreal resection; RB, retinoblastoma; FGFR3, fibroblast growth factor receptor 3; LOH, loss of heterozygosity; SSCA, single-strand conformation analysis.
mutations. Analysis of all of the samples was carried out in a blinded fashion, without knowledge of clinical or histopathological status.

**Statistical Analysis.** Statistical package for social sciences 8.0 (SPSS Inc., Chicago, IL) and StatXact version 2 (Cytel Software Corporation, Cambridge, MA) computer software were used for data collection and analysis. Fisher’s exact test was used to analyze the FGFR3 mutation in relation to stage, grade, highest stage, and prediction of tumor recurrence. Logistic regression analysis with the backward elimination method was used for comparison of variables to predict recurrence. All of the Ps are two sided. Statistical significance was assumed if $P < 0.05$.

**Results**

We performed a mutation analysis of the FGFR3 gene in a group of 72 bladder carcinomas from 72 consecutive patients. Thirty-seven patients were diagnosed with bladder cancer for the first time. All of the 72 bladder cancers were transitional cell carcinomas except for one squamous cell carcinoma that was pT2, grade 3.

SSCA detected aberrant bands (Fig. 1) in DNA samples from 34 tumors but not in DNA from corresponding venous blood, indicating the somatic nature of FGFR3 mutations in bladder cancer. Thirty mutations concerned the already described codon 249 mutation (S249C). Codon 248 (R248C) and 372 (G372C) mutations were found once and twice, respectively (Fig. 1, A, C, and D). An additional mutation, not observed in bladder cancer previously, was also detected (Fig. 1E). DNA sequence analysis revealed the point mutation leading to A393E, previously known in a hereditary skeletal syndrome (13). The two mutations, R248C and S249C, can be found in one amplification region. This region represented 91% of the observed FGFR3 mutations in our group of bladder cancers. The identity of all of the mutations was confirmed by DNA sequence analysis. Five samples from blood DNA were also sequenced as negative controls. No mutations were found in these samples. Fig. 1 shows that the five detected mutations can easily be identified by SSCA.

All of the 34 mutations were found in the 53 pTa bladder cancers (64%), whereas none of the 4 pT1 and 15 higher-staged tumors had a mutation in the gene (Table 1A). Furthermore, all of the tumors with a mutation in the FGFR3 gene were grade 1 or 2. The mutation did not occur in 19 grade 3 tumors ($P < 0.0001$). The stages of these 19 G3 tumors were pT4 in 3, pT3 in 1, and $\geq$ pT2 in 15 cases, respectively. When we compared the patient’s history (median history of 5.4 years; range, 0.8–26.9 years) with histopathological and FGFR3 mutation status, we observed that patients with only a pT4 history, had a mutation in 33 (73%) of 45 cases. In patients whose history revealed at least 1 pTa tumor, the FGFR3 mutation occurred in only 1 (8%) of 12 tumors ($P < 0.0001$; Table 1B). These results suggest that the FGFR3 mutations are linked with a disease course in which lower-staged (i.e., pTa) tumors prevail.

To further investigate the link between FGFR3 mutations and stage, we performed a prospective follow-up analysis. Twelve months follow-up after mutation analysis revealed that bladder cancer recurrence was far more frequent in the group of patients whose initial tumors were wild type with respect to the FGFR3 gene ($P = 0.004$; Table 2). It should be noted that only patients ($n = 57$) diagnosed with superficial bladder cancer (i.e., pTa, pT1) at time of mutation analysis were included in this follow-up analysis because patients with invasive tumors underwent cystectomy or radiotherapy. Moreover, in logistic regression analysis with the backward elimination method, using recurrence as a dependent variable and tumor stage, grade, highest stage, and FGFR3 mutation status as independent variables, the FGFR3 mutation status remained as the only significant ($P = 0.008$) predictor of recurrence.

We also determined the number of recurrent tumors per patient. In 14 of the 23 patients that were wild type for the FGFR3 gene, 24 TURs revealed recurrent bladder cancer during the 12 months of follow-up. In 17 of the removed tumors FGFR3 mutation analysis was performed. No mutations were found. Two patients, both with a wild-type FGFR3 gene, had already progressed to invasive (i.e., $\geq$ pT2) disease after 3 months. They were removed from subsequent cystoscopical follow-up. On the other hand, in 7 of the 34 patients

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**Table 1**  
Tumor stage and FGFR3 mutation status  

<table>
<thead>
<tr>
<th>Stage</th>
<th>Wild type</th>
<th>Mutation</th>
<th>$P^a$</th>
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<tr>
<td>pTa</td>
<td>19</td>
<td>34</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>pT1</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$\geq$ pT2</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 2**  
Relation between disease recurrence within 12 months in superficial (i.e., pTa, pT1) bladder cancer patients and the FGFR3 mutation status ($n = 57$)  

<table>
<thead>
<tr>
<th>FGFR3 mutation analysis</th>
<th>Wild type</th>
<th>Mutation</th>
<th>$P^a$</th>
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<tbody>
<tr>
<td>12 months follow-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence-free</td>
<td>9</td>
<td>27</td>
<td>0.004</td>
</tr>
<tr>
<td>Recurrence</td>
<td>14</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>

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$^a$ Ps were determined by two-sided Fisher’s exact test calculated for $3 \times 2$ tables.

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Fig. 1. PCR-SSCA of the FGFR3 gene. The figure shows examples of the FGFR3 gene mutations in our population. Paired lanes are normal blood DNA (N) and tumor DNA (T) from the same patient. Solid arrowheads, mutations leading to R248C (patient A), S249C (patient B), G372C (patient C), G372C (patient D), and A393E (patient E). In patient B, the same activating point mutation (S249C) was found in the original (T1) and the recurrent tumor (T2). In patient C, an additional silent mutation (G to T transition) at nucleotide position 1113, adjacent to codon 372, was observed. The codon and nucleotide numbering corresponds to the cDNA open reading frame of the FGFR3b isoform. This isoform is expressed in epithelium and contains two amino acids more than the FGFR3c isoform expressed in bone.

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Bladder cancer is a common type of cancer that has a high incidence but, more importantly, requires major clinical attention after initial treatment. Nowadays, patients are monitored intensively by cystoscopy for possible recurrence. Standard practice after TUR of bladder cancer is to screen patients with a cystoscopic examination every 3–4 months for 2 years and every 6–12 months thereafter. On the basis of incidence numbers and recurrence rate (1–4), it can be calculated that >500,000 cystoscopies per year are performed in the United States alone.

Our results show that FGFR3 mutations occur in nearly 50% of bladder cancers. The mutations were exclusively observed in superficial tumors and absent from invasive carcinomas. Moreover, bladder cancer recurrence rates were dramatically lower for tumors with a mutant FGFR3 gene. We, therefore, conclude that the FGFR3 mutations identify a large cohort of bladder cancer patients with favorable disease characteristics. As a consequence, molecular FGFR3 mutation analysis represents a novel, technically simple, and potentially powerful tool for the adjustment of clinical management in bladder cancer. Nowadays, all patients undergo frequent cystoscopy to monitor their disease. Our results suggest that the frequency of this uncomfortable, invasive, and expensive diagnostic procedure can be reduced considerably in patients with FGFR3-positive tumors.

We observed a difference in mutation frequencies between primary and recurrent superficial tumors in our study. It has been noted by others that patients who already have recurrent disease continue to develop recurrences more often than patients with a primary tumor (2, 4). We suggest that this difference in mutation percentage is caused by the fact that patients who are cured after a single TUR. ~30%, do not, by definition, take part in the group of patients with recurrent disease. Combining these observations, we feel that it is likely that the group of patients with primary bladder cancer containing a FGFR3 mutation encompasses patients who will not develop a recurrence at all. Thus, the FGFR3 mutation itself, being an indicator of superficial bladder cancer with a low recurrence rate, is at least part of the explanation of the differences between these groups. Capellen et al. (10) were the first to report on somatic FGFR3 mutations in bladder cancer. They found 9 mutations in 26 tumors. Three of the nine mutations occurred in invasive (i.e., ≥pT2) tumors. The stage distribution of the wild-type tumors was not given in their study. In contrast, we found no mutations in invasive tumors. In addition, the correlation between the presence of a mutation and low stage was highly significant in our study. To ensure that no mutations were missed by contamination of benign cells in the invasive tumors, we also performed LOH analysis on these DNA samples. Multiple allelic losses were observed in 17 of 18 evaluable tumors (results not shown). These losses supported the hypothesis that the DNA samples used for mutation and LOH analysis indeed contained DNA that was derived mainly from tumor cells.

Thus, the apparent discrepancy between our study and that of Capellen et al. (10) remains difficult to explain. It may be attributable to the relatively small number of tumors examined by Capellen et al. (10).

The finding that tumors with FGFR3 mutations are less likely to lead to recurrences can be explained by the hypothesis that such tumors shed cells with a lower frequency than those tumors that do not carry a FGFR3 mutation. In the seven patients with recurrence in the mutant FGFR3 group, six of eight tumors occurred at the same region in the bladder, and six of eight recurrent lesions were single growths. In contrast, in most of the 14 patients who had recurrence in the nonmutant group, multiple tumors were found, and these tumors occupied several different sites in the bladder. These preliminary data indeed suggest that superficial bladder tumors without a FGFR3 mutation shed cells more easily and/or that these cells are better equipped to reimplant into the bladder epithelium. In addition, it may be possible that the FGFR3-mutated tumors proliferate not as fast as the nonmutated tumors. Additional experiments are required to test these hypotheses.

Molecular markers for bladder cancer may provide information to be used in clinical decision making. The incidence of TP53 gene mutations and an altered expression of the RB gene product appeared to be much higher in invasive, high-grade bladder cancers than in superficial low-grade ones (14, 15). These reports suggest that these molecular markers are relatively late events in disease pathogenesis and identify tumors with aggressive biological behavior. The clinical value of these two tumor suppressor genes is of importance to patients presenting with invasive bladder cancer (15, 16) and for prediction of progression in patients with high-grade superficial bladder cancer. Barton Grossman et al. (5) advocated stratification of pT1 bladder cancer patients based on TP53 and RB status. Their results suggest that patients with normal protein expression for both genes can be managed conservatively, whereas patients with alterations in one, and particularly in both genes, require more aggressive treatment to prevent progression to invasive disease (5). In contrast, chromosome 9q deletions, determined by LOH analysis, are found with similar frequency in superficial and invasive bladder cancer (17, 18) and, therefore, cannot be used for prognostic purposes. The FGFR3 gene is the first gene identified in bladder cancer to be mutated selectively in
those cancers that are characterized by favorable clinical parameters. Future studies should determine whether a combination of \( TP53 \), \( RB \), and \( FGFR3 \) gene analyses could lead to a more accurate prediction of the disease course with regard to recurrence and progression. Furthermore, treatment strategies may also be determined based on these molecular markers.

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**References**

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