Fibroblast Growth Factor Receptor 3 Is a Rational Therapeutic Target in Bladder Cancer

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Abstract

Activating mutations of fibroblast growth factor receptor-3 (FGFR3) have been described in approximately 75% of low-grade papillary bladder tumors. In muscle-invasive disease, FGFR3 mutations are found in 20% of tumors, but overexpression of FGFR3 is observed in about half of cases. Therefore, FGFR3 is a particularly promising target for therapy in bladder cancer. Up to now, most drugs tested for inhibition of FGFR3 have been small molecule, multityrosine kinase inhibitors. More recently, a specific inhibitory monoclonal antibody targeting FGFR3 (R3Mab) has been described and tested preclinically. In this study, we have evaluated mutation and expression status of FGFR3 in 19 urothelial cancer cell lines and a cohort of 170 American patients with bladder cancer. We have shown inhibitory activity of R3Mab on tumor growth and corresponding cell signaling in three different orthotopic xenografts of bladder cancer. Our results provide the preclinical proof of principle necessary to translate FGFR3 inhibition with R3Mab into clinical trials in patients with bladder cancer.

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Introduction

Cancer of the bladder will be newly diagnosed in an estimated 73,510 individuals and will cause 14,880 cancer related deaths in 2012 in the United States (1). This makes it the fourth most common cancer in men and the ninth most common in women. About 70% of cases are non-muscle invasive and have a high propensity to recur, giving bladder cancer the highest recurrence rate of any cancer (2). This translates clinically into intensive surveillance and multiple interventions over many years, explaining the high cost of managing bladder cancer (3). The other 30% of cases are muscle invasive tumors, for which the 5-year survival is approximately 50%. Both disease entities are marked by a paucity of new discoveries leading to novel therapies with clinical utility.

Fibroblast growth factor receptor-3 (FGFR3) is a particularly promising target for therapy in bladder cancer. Activating FGFR3 mutations have been described in approximately 75% of low-grade papillary bladder tumors (4). Mutations are found in only 20% of muscle invasive tumors, but FGFR3 overexpression is observed in 50% of these tumors (5, 6). The most common mutation (S249C), located on exon 7, results in the substitution of serine at codon 249 with a cysteine residue (7, 8). This leads to constitutive ligand-independent dimerization of the receptor and activation of downstream proliferative pathways (9, 10).

Recently, a number of small molecule receptor tyrosine kinase inhibitors have been developed to target FGFR3 (11). These small molecule inhibitors, including BGJ398 (Novartis), TKI258/CHIR258 (Dovitinib, Novartis; refs. 12–14), AZD4547 (Astra Zeneca) PD173074 (Pfizer), and BMS-582664 (Bristol–Myers Squibb; ref. 15), generally target all members of the FGFR family, vascular endothelial growth factor receptor 2 (VEGFR2), and other tyrosine kinases (11, 12). This general FGFR and VEGF pathway inhibition is associated with significant diarrhea, nausea, fatigue (16, 17), dyspnea (18), and abdominal pain (16–18), as well as thrombocytopenia and thromboembolic events (16). Hyperphosphatemia-mediated soft tissue calcification has been a further hurdle impeding preclinical development of pan-FGFR inhibitors (11). The FGFR pathway is involved in normal phosphate and vitamin D homeostasis, particularly through FGF23 signaling in the bone and kidney (19). Disruption of this pathway with pan-FGFR inhibitors leads to hyperphosphatemia and deposit of calcium phosphorous in the soft tissues, including the vasculature, smooth muscle, and renal tubules. These adverse effects may be avoided with specific FGFR3 inhibition.

The clinical development of these inhibitors has focused on multiple myeloma, which harbors specific translocations [t(4;14)(p16;q32)] that activate FGFR3 (20), and on hepatocellular carcinoma (12), but little work has been...
done in bladder cancer. Most recently, an inhibitory monoclonal antibody targeting FGFR3 specifically (R3Mab) has been developed and undergone preliminary testing in preclinical subcutaneous models of bladder cancer, where it has shown activity on both wild-type and mutated FGFR3 (21). In this study, we have focused on specific FGFR3 inhibition with R3Mab in an orthotropic bladder cancer model. The orthotopic model provides an appropriate organ-specific microenvironment that should allow better translation into clinical trials.

Therefore, we aimed to show the preclinical proof of principle that targeting FGFR3 with R3Mab is rational in bladder cancer. We have measured FGFR3 mutation and expression in an American cohort of patients with bladder cancer and have shown R3Mab activity in an orthotropic xenograft model of bladder cancer. We believe that this justifies the translation of FGFR3 inhibition with R3Mab into clinical trials in patients with bladder cancer.

Materials and Methods

Cell lines

Nineteen human bladder cancer cell lines were kindly provided by the Pathology Core of the Bladder Cancer SPORE at MD Anderson Cancer Center, including UM-UC1, UM-UC3, UM-UC4, UM-UC5, UM-UC6, UM-UC7, UM-UC10, UM-UC11, UM-UC12, UM-UC13, UM-UC14, UM-UC15, UM-UC16, UM-UC17, 253J-P, 253J-BV, RT4v6, and RT112. T24 was purchased from the American Type Culture Collection. All cell lines were cultured in Minimum Essential Medium (MEM), supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acids, 1% l-glutamine, and 1% sodium pyruvate (all purchased from Invitrogen). The cell lines were routinely grown and passaged at 37°C in a humidified atmosphere of 5% CO2.

Cell line identities were confirmed by DNA fingerprinting using the AmpFISTR Identifier Amplification Kit (Applied Biosystems) or the AmpFISTR Profiler PCR Amplification Kit (Applied Biosystems) protocols. The cell lines were monitored regularly for mycoplasma infection.

For in vivo studies, UM-UC1, UM-UC14, and RT112 cell lines underwent transduction with a lentiviral construct carrying the luciferase firefly gene for in vivo imaging. The luciferase plasmid contained a blastocidin-resistance gene enabling positive selection with 10 μg/mL blasticidin. The luciferase plasmid was introduced into the cell lines by transduction. These cell lines were chosen due to their high levels of FGFR3 expression and their growth characteristics in the orthotopic xenograft model.

Antibodies

FGFR3 polyclonal (sc-123), FRS2a (sc-8318), cyclin D1 (sc-718), cyclin E (sc-481), and vimentin (sc-5565) antibodies were purchased from Santa Cruz Biotechnologies. Antibodies detecting p-FGFR (#3471), p-FRS2 (#3864), p42/44MAPK (mitogen-activated protein kinase; #4965), phospho-p42/44-MAPK (#4370), AKT (#9272), phosphor-AKT (ser 473; # 2927S), ZEB-1 (Zinc finger E-box-binding homeobox 1; #3396), and cleaved caspase-3 (#9661) were purchased from Cell Signaling. Antiphospho-tyrosine 4G10 was obtained from EMD Millipore and E-cadherin antibody (#610181) was obtained from BD Biosciences. A monoclonal Ki-67 antibody for evaluation of proliferation of xenograft tumors was purchased from Thermo Scientific. For expression analysis of FGFR3 on the human tissue microarray (TMA), a polyclonal FGFR3 antibody (F0425) was obtained from Sigma-Aldrich.

Immunohistochemistry

Use of all human tissue was approved by the Institutional Review Board at MD Anderson Cancer Center and written consent was obtained from all patients. Paraffin-embedded sections of bladder were cut to 4 mm and placed on polylysine-coated microscope slides and baked overnight at 50°C in a dry oven. Sections were then deparaffinized in xylene for 2 x 5 minutes and rehydrated in alcohol for 2 x 5 minutes. Endogenous peroxidase activity was quenched by applying 2% hydrogen peroxide in 30% methanol for 10 minutes. Antigen retrieval was carried out by incubation in proteinase K (40 μg/mL PBS) for 15 minutes at 37°C in a humidified chamber. The sections were stained with primary antibody (Sigma Aldrich) overnight at 4°C. The bound primary antibodies were visualized by avidin–biotin complex assay (DAKO Corp.) with 3,3′-diaminobenzidine as a chromogen (DAKO) and hematoxylin as a counterstain. FGFR3 staining was indicated by characteristic brown staining. Sections from 4 normal human ureters were used as a negative control. Staining intensity was assessed on a scale from 0 (no staining) to 3 (strong staining) by a pathologist (B. Czerniak).

FGFR3 mutation analysis

DNA was isolated from the urothelial carcinoma cells and tumor samples using a genomic DNA extraction kit (Qiagen) according to the manufacturers’ instructions. Exons 7 and 10 were amplified by PCR using AmpliTaq Gold DNA polymerase (Applied Biosystems). The following primers were used: 5′-CGGCCATGCGCGTGGTG-3′ (sense) and 5′-AGCAACCGGGCTGCTTG-3′ (antisense) for exon 7 (22), and 5′-CTCTGCTGCTG-3′ (sense) and 5′-AGCCAGCTGGAACCTGGTA-3′ (antisense) for exon 10 (Sigma-Aldrich). Five pmol/L of each primer was added to the 20-μL reaction volume, and 1-μL dimethyl sulfoxide (DMSO) was added for exon 7. The following cycling variables were used: 95°C for 10 minutes, then 35 cycles of 95°C for 30 seconds, 65°C (exon 7) or 58°C (exon 10) for 30 seconds, and 72°C for 30 seconds, followed by a final incubation at 72°C for 10 minutes (22). Unincorporated primers and deoxynucleotides were removed using shrimp alkaline phosphatase and exonuclease I (U.S.
The ExactaCruz B system (Santa Cruz).

Immunoprecipitation was carried out using West Femto Maximum Sensitivity Substrate (Thermo Scientific). Thirty micrograms of protein were incubated with horseradish peroxidase-conjugated primary antibody at 4°C overnight. Blots were developed using an enhanced chemiluminescence (ECL) substrate system for the detection of horseradish peroxidase, SuperSignal enhanced chemiluminescence (ECL) substrate system for chemiluminescence detection (Thermo Scientific).

Bioluminescence was used to quantify tumor burden and was measured on the Xenogen IVIS Spectrum imaging system after intraperitoneal injection of 200 μg/kg luciferin (Caliper). Images were taken at 10 and 14 minutes after luciferin injection and the average counts were used for statistical analysis. Bioluminescence imaging was carried out on the 5th or 6th day, and mice were divided into equal treatment groups based on tumor burden (13–16 animals per group). Treatment was started the following day and imaging was repeated every 5 days. Tumor uptake rates in our orthotopic xenograft model were 100% for UM-UC14 and RT112, and 96% for UM-UC1 cells.

In vivo treatment

The FGFR3-targeting antibody R3Mab (21) was provided by Genentech. It was reconstituted in sterile water for injection day and imaging was repeated every 5 days. Tumor uptake rates in our orthotopic xenograft model were 100% for UM-UC14 and RT112, and 96% for UM-UC1 cells.

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Table 1. Patient and tumor characteristics for TMA analysis

<table>
<thead>
<tr>
<th></th>
<th>Male, n (%)</th>
<th>Female, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, range)</td>
<td>65 (37–85)</td>
<td>67 (40–81)</td>
</tr>
<tr>
<td>n</td>
<td>112 (77)</td>
<td>33 (23)</td>
</tr>
<tr>
<td>Tumor grade 1</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tumor grade 2</td>
<td>35 (31)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Tumor grade 3</td>
<td>76 (68)</td>
<td>29 (88)</td>
</tr>
<tr>
<td>Papillary</td>
<td>47 (42)</td>
<td>13 (39)</td>
</tr>
<tr>
<td>Nonpapillary</td>
<td>65 (58)</td>
<td>20 (61)</td>
</tr>
<tr>
<td>NMIBC</td>
<td>40 (36)</td>
<td>7 (21)</td>
</tr>
<tr>
<td>MIBC</td>
<td>72 (64)</td>
<td>26 (79)</td>
</tr>
</tbody>
</table>

Orthotopic bladder cancer xenograft model

All animal work was approved by the Institutional Review Board of the University of British Columbia (approval #A08-0733). Six-week-old male nude mice (Harlan Laboratories) were anesthetized with isoflurane (2 Vol.%), and analgesia was provided by subcutaneous injection with buprenophore and meloxicam (Boehringer Ingelheim). After disinfection of the abdominal wall with chlorhexidine, a low transverse laparotomy was made and the urinary bladder was extra-corporalized. Fifty μL of cell suspension containing 2.5 × 10^5 or 5.0 × 10^5 cells was inoculated using a 30 G needle directly into the bladder wall (24). The incision was closed with suture.

Bioluminescence was used to quantify tumor burden and was measured on the Xenogen IVIS Spectrum imaging system after intraperitoneal injection of 200 μg/kg luciferin (Caliper). Images were taken at 10 and 14 minutes after luciferin injection and the average counts were used for statistical analysis. Bioluminescence imaging was carried out on the 5th or 6th day, and mice were divided into equal treatment groups based on tumor burden (13–16 animals per group). Treatment was started the following day and imaging was repeated every 5 days. Tumor uptake rates in our orthotopic xenograft model were 100% for UM-UC14 and RT112, and 96% for UM-UC1 cells.

In vivo treatment

The FGFR3-targeting antibody R3Mab (21) was provided by Genentech. It was reconstituted in sterile water for
injection at a concentration of 10 mg/mL and stored at 4°C. Control treatments included phosphate-buffer saline (PBS) and an isotype control antibody (human IgG1). All agents were prepared freshly in PBS immediately before each treatment session and injected intraperitoneally.

**Evaluation of proliferation and apoptosis in orthotopic xenograft tumors**

Representative tumors derived from orthotopic xenografts were stained for Ki-67 and caspase-3, as markers of proliferation and apoptosis. Staining of paraffin-embedded tumor sections (4 mm) was conducted on a Ventana Discovery XT autostainer platform (Ventana Medical Systems) with an enzyme-labeled biotin streptavidin system and a solvent-resistant 3,3′-diaminobenzidine Map kit by using primary antibodies at a concentration of 1:500 for Ki-67 and 1:50 for caspase-3 at 37°C and an incubation time of 1 hour. After application of the secondary antibody, a hematoxylin counterstain was carried out. The average count of positively stained cells in 10 high-power fields was evaluated on each section.

**Statistics**

Statistical analysis was carried out using the Chi-square testing for analysis of immunohistochemistry (IHC), in addition t test and ANOVA were conducted for in vitro and in vivo experiments. Significance was defined at values of \( P \) less than 0.05. Data were expressed as mean
Statistical analysis was conducted using GraphPad Prism 4.03 (GraphPad Software, Inc.).

### Results

#### Immunohistochemistry

A human bladder cancer TMA containing cores of 153 tumor samples from patients with urothelial carcinoma of the bladder (Table 1) was stained for total FGFR3 expression. The median age of these patients was 65 (range 37–85) years and 77% were male. Tissue was obtained both from cystectomy and transurethral resection specimens. The intensity of staining was graded from 0 (no staining) to 3 (strong staining). Tumors were classified as invasive (T1-T4 in 68%) or noninvasive (Ta in 32%), and as low (28%) or high (72%) grade. Expression of FGFR3 was found in approximately 70% of both low- and high-grade tumors, as well as equally between invasive and noninvasive tumors. High levels of FGFR3 were observed in 36% of low-grade tumors compared with 22% of high-grade lesions (\( P = 0.047; \) Fig. 1A). No significant difference was found for invasive versus noninvasive tumors. There was no evidence of staining in normal urothelium.

#### Mutation analysis

A total of 170 fresh-frozen samples from an independent cohort of patients with urothelial carcinoma of the bladder were analyzed for mutations in the FGFR3 gene by direct sequencing. The median age of the patients was 66 (range 41–87) years and 77% were male. The stage and grade of tumors are reflected in Table 2. FGFR3 mutations in exon 7 or exon 10 were found in 26% of all samples and 56% of low-grade tumors. Low grade, noninvasive tumors showed more than a 4-fold increased rate of FGFR3 mutation compared with high-grade, invasive tumors (Table 2). Similar analysis revealed a S249C mutation (exon 7) in 4 of 19 cell lines, and a R248C (exon 7) and Y375C (exon 10) mutation in each one of these cell lines. The remaining cell lines contained a wild-type FGFR3 gene (Table 2).

#### Expression of FGFR3 in bladder cancer cell lines in relation to epithelial/mesenchymal markers

A panel of 12 human bladder cancer cell lines was screened for RNA and protein expression of FGFR3 by qPCR and Western blot. Based on prior work linking growth factor receptor activity to epithelial-to-mesenchymal (EMT) differentiation and invasive potential

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**Table 2. FGFR3 mutation analysis of tumor samples and urothelial carcinoma cell lines**

<table>
<thead>
<tr>
<th>Tumor samples</th>
<th>Low grade</th>
<th>High grade</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninvasive</td>
<td>28/50 = 56%</td>
<td>2/18 = 11%</td>
<td>30/68 = 46%</td>
</tr>
<tr>
<td>Invasive</td>
<td>4/7 = 57%</td>
<td>10/95 = 11%</td>
<td>14/102 = 22%</td>
</tr>
<tr>
<td>Total</td>
<td>32/57 = 56%</td>
<td>12/113 = 11%</td>
<td>44/170 = 26%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exon 7 mutation: 31 (70%)</th>
<th>Exon 10 mutation: 13 (30%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon</td>
<td>Nucleotide position/base change</td>
</tr>
<tr>
<td>7</td>
<td>746 C &gt; G</td>
</tr>
<tr>
<td>10</td>
<td>1114 G &gt; T</td>
</tr>
<tr>
<td>10</td>
<td>1117 A &gt; T</td>
</tr>
<tr>
<td>10</td>
<td>1124 A &gt; G</td>
</tr>
<tr>
<td>10</td>
<td>1156 T &gt; C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Exon 7</th>
<th>Exon 10</th>
<th>Cell lines</th>
<th>Exon 7</th>
<th>Exon 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>253J-P</td>
<td>wt</td>
<td>wt</td>
<td>UM-UC7</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>253J-BV</td>
<td>wt</td>
<td>wt</td>
<td>UM-UC10</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>RT4 v6</td>
<td>wt</td>
<td>wt</td>
<td>UM-UC11</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>RT112</td>
<td>wt</td>
<td>wt</td>
<td>UM-UC12</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>T24</td>
<td>wt</td>
<td>wt</td>
<td>UM-UC13</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>UM-UC1</td>
<td>wt</td>
<td>wt</td>
<td>UM-UC14</td>
<td>S249C</td>
<td>wt</td>
</tr>
<tr>
<td>UM-UC3</td>
<td>wt</td>
<td>wt</td>
<td>UM-UC15</td>
<td>S249C</td>
<td>Y375C</td>
</tr>
<tr>
<td>UM-UC4</td>
<td>wt</td>
<td>wt</td>
<td>UM-UC16</td>
<td>S249C</td>
<td>wt</td>
</tr>
<tr>
<td>UM-UC5</td>
<td>wt</td>
<td>wt</td>
<td>UM-UC17</td>
<td>S249C</td>
<td>wt</td>
</tr>
<tr>
<td>UM-UC6</td>
<td>R248C</td>
<td>wt</td>
<td></td>
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</table>

± SE, if not differently noted. Statistical analysis was conducted using GraphPad Prism 4.03 (GraphPad Software, Inc.).
of bladder cancer cell lines, the panel was divided in noninvasive/epithelial cell lines and invasive/mesenchymal cell lines (25). Expression of FGFR3 was associated with detection of E-cadherin, a marker of epithelial differentiation. Mesenchymal cell lines, defined by expression of ZEB-1 and vimentin and loss of E-cadherin, showed lower levels of FGFR3 (Fig. 1B and C).
**In vitro targeting of FGFR3**

FGFR3 pathway activity was verified in UM-UC1 urothelial cancer cells, which express a high level of wild-type FGFR3. Stimulation of these cells with the ligand FGF-1 resulted in phosphorylation of FGFR3 and activation of the downstream signaling cascade in a time-dependent manner. Phosphorylation of the receptor was followed by phosphorylation of FRS-2, and increasing activation of both p-Erk 1/2 and p-Akt (Fig. 2A). In the same cell lines, inhibition of FGFR3 with R3Mab abrogated receptor phosphorylation, as well as phosphorylation of FRS-2 and Erk1/2 (Fig. 2B). A similar experiment was conducted in UM-UC14, which harbors an S249C mutation in exon 7 of FGFR3, but here an immunoprecipitation was conducted with anti-FGFR3 and subsequent blotting with antiphospho-tyrosine as an alternative method to show FGFR3 phosphorylation (Fig. 2C). Treatment with R3Mab in regular growth medium resulted in a concentration-dependent growth inhibition in 3 of 4 different tumor cell lines in a crystal violet assay (Fig. 2D). UM-UC1 displayed the most pronounced antiproliferative response, whereas the highly mesenchymal and invasive cells UM-UC3, UM-UC13, T24, and 253J-BV, all of which do not express appreciable levels of FGFR3, showed no response. RT4V6, RT112 (both high expression of wild-type FGFR3), UM-UC16 (low expression of S249C-FGFR3), and UM-UC14 (modest expression of S249C-FGFR3) showed only modest response.

**In vivo treatment of orthotopic bladder cancer xenografts**

UM-UC14, RT112, and UM-UC1 cells were orthotopically injected into the bladder wall of athymic nude mice. Successful tumor inoculation was verified by bioluminescence imaging on the 5th or 6th day, and the mice were divided into equal groups based on tumor burden. They were then treated with active agent (R3Mab) or control every 72 hours and bioluminescence was repeated every 5 days.

Two different dose levels were tested in UM-UC14 xenografts, including 15 mg/kg and 30 mg/kg, and compared with a saline-treated group (IgG1 control not available for this experiment). Tumor growth, 30 days after inoculation, showed a dose-dependent inhibition of tumor growth with a reduction in tumor growth of 22% and 33%, respectively, (Fig. 3A). Evaluation of representative tumor samples harvested from this experiment showed inhibition of FGFR3 phosphorylation by immunoprecipitation of FGFR3 and subsequent immunoblotting with antiphospho-tyrosine (Fig. 3B).

In mice-bearing orthotopic RT112 xenografts, R3Mab treatment at a dose of 30 mg/kg was compared with both a saline control group and a nontargeting human IgG1-control. The IgG1 had no effect on tumor growth, whereas mice treated with R3Mab showed a reduction in tumor growth by 59.2% compared with IgG and 57.2% compared with saline controls (Fig. 3C).

**In vivo,** UM-UC1 tumors orthotopically injected into the bladder of nude mice showed a reduction in tumor growth of 83.9% when treated with R3Mab compared with a saline control every 72 hours and bioluminescence was repeated every 5 days.
with mice injected with IgG and 84.1% compared with saline controls (Fig. 4A and B). Evaluation of whole bladder samples by Western blotting at the end of the experiment showed a significant inhibition of FGFR3 phosphorylation and downstream signaling, including p-FRS2, p-ERK1/2, cyclin D1, and cyclin E (Fig. 4C). Overall, this indicates that the observed tumor growth reduction is due to highly effective FGFR3 pathway inhibition by R3Mab in vivo (Fig. 4D). The inhibition of tumor growth is related to antiproliferative effects of R3Mab expressed in a reduced Ki-67 proliferative index (Fig. 4E), whereas no difference has been observed for expression of cleaved caspase-3 in xenograft tumors (data not shown) as a marker for apoptosis.
Discussion

We have shown a high rate of FGFR3 mutation and overexpression in an American cohort of patients. We have also shown that FGFR3 signaling is active in preclinical models of bladder cancer, and that specific inhibition of FGFR3 with a monoclonal antibody induces growth arrest in orthotopic bladder cancer xenografts. Our data builds on prior experience with the same inhibitor in subcutaneous models (21), yet advances this therapeutic strategy to meet a significantly higher bar of efficacy in the orthotopic setting. We believe that this provides the proof of principle required to investigate this agent or a similar novel FGFR3 inhibitor in specific clinical trials for bladder cancer.

We see a lower rate of FGFR3 mutations in low-grade tumors (4, 26, 27) and a higher rate of FGFR3 overexpression in high-grade tumors (5) compared with prior reports. This is most likely related to patient selection, which in turn is a reflection of the highly specialized clinical setting in which these tumor samples were collected. There are also likely differences in IHC methodology and assessment of staining. A slight increase in mutation rate would be expected if exon 15 had also been assessed.

A limitation of current preclinical models of bladder cancer is the inability to model low-grade, noninvasive disease. Although we possess bladder cancer cell lines with FGFR3 mutations, these mutations are found only in highly invasive cell lines that are not representative of nonmuscle invasive disease. RT4 is the only cell line that is low grade, but even it is invasive and it expresses nonmuscle invasive disease. RT4 is the only cell line that are not representative of disease. Although we possess bladder cancer cell lines with FGFR3 mutations, these mutations are found only in highly invasive cell lines that are not representative of nonmuscle invasive disease. RT4 is the only cell line data with FGFR3 overexpression in both noninvasive and invasive bladder cancer. We have shown that targeting FGFR3 in RT4v6 with excellent growth inhibition (data not shown). We and others are developing primary xenografts using fresh patient samples in an attempt to overcome our inability to model nonmuscle invasive disease.

The optimal disease state to test FGFR3 inhibition is open to debate. While it would seem logical to target the activating mutations in nonmuscle invasive disease, it will be challenging to give systemic therapy to this patient population, especially as FGFR3 mutations indicate a favorable prognosis (8, 28, 29). A trial with gefitinib [an oral epidermal growth factor receptor (EGFR) inhibitor] failed to accrue patients in Canada at least in part because physicians and patients did not accept systemic therapy for NMIBC (NCT00352079). The currently available multityrosine kinase inhibitors that target FGFR3 are all marred with concerns regarding systemic toxicity that would severely limit their use in this setting (11). R3Mab may prove to be better tolerated, since it should avoid the hypophosphatemia and soft-tissue calcification associated with pan-FGFR inhibitors. It remains to be proven whether these agents and especially R3Mab are efficacious when administered intravesically.

Our results suggest that R3Mab should be tested in patients with muscle invasive bladder cancer (MIBC) whose tumors show FGFR3 overexpression. There is a pressing need for new treatments in this lethal variant of bladder cancer, and our findings would support testing FGFR3 inhibition in conjunction with systemic cytotoxic chemotherapy. Whether FGFR3 mutations are relevant in the context of MIBC remains to be shown. Our cell line investigations reveal little drug activity in invasive cells with FGFR3 mutations, with the exception of UM-UC14. We observed a reduced growth inhibitory effect in UM-UC14 compared with prior reports (21, 30). This may be a difference in the model systems (orthotopic versus heterotopic) or potentially related to phenotypic drift in different strains of UM-UC14. Regardless of cell line data, any clinical trial would have to include assessment of FGFR3 mutation status and expression in every tumor. Patient selection based on these parameters may be an important factor in subsequent patient selection. We are also investigating mechanisms of resistance to R3Mab in preclinical models with the intention of potentially using this information for patient enrichment in clinical trials. Since FGFR3 seems to drive growth through AKT and ERK1/2 (31), it is likely that there is redundancy with other growth factor receptor pathways (32) much as we have described with EGFR and PDGFR-ß (33).

We have confirmed the results of prior reports on FGFR3 mutation and expression patterns in urothelial carcinoma of the bladder: FGFR3 is frequently mutated in noninvasive bladder cancer and frequently overexpressed in both noninvasive and invasive bladder cancer. We have shown that targeting FGFR3 with a specific inhibitory monoclonal antibody (R3Mab) effectively abrogates FGFR3 signaling pathway activation with a resultant decrease in tumor growth. This inhibitor is highly efficacious in a selection of bladder cancer cell lines and orthotopic xenografts. These are promising results that warrant translation into clinical trials in patients with bladder cancer.

Disclosure of Potential Conflicts of Interest

D.J. McConkey has a commercial research grant from AstraZeneca; has ownership interest (including patents) in Apsowell, Inc; and is a consultant/advisory board member for Apsowell, Inc. J. Qing has ownership interest (including patents) in Genentech. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: K.M. Gust, D.J. McConkey, A. Ashkenazi, C.P. Dinney, P.C. Black


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.M. Gust, S. Averty, P.K. Hegarty, P.C. Black

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