Fibroblast growth factor receptor (FGFR) gene: pathogenesis and treatment implications in urothelial carcinoma of the bladder

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ABSTRACT
Dysregulation of fibroblast growth factor receptors (FGFRs) has been implicated in several human malignancies, including urothelial carcinoma. In urothelial carcinoma, the oncogenic role of mutated FGFR is mediated by the RAS-mitogen-activated protein kinase pathway, resembling the effects observed with activated HRAS. Activating somatic mutations of FGFR3 are clustered in three hotspots in exons 7, 10 and 15, and are almost always missense mutations leading to amino acid substitution in the external, transmembrane or intracellular regions of the receptor. A fusion of FGFR3 to transforming acid coiled-coil containing protein 3, FGFR3 amplification and alternative splicing leading to aberrant FGFR3 activation are less common molecular alterations. In April 2020, the Food and Drug Administration (FDA) approved the first targeted FGF therapy, erdafitinib, in patients with locally advanced or metastatic bladder cancer who have progressed on platinum-based chemotherapy. Herein, we reviewed the normal structure and function of FGFR. We also explored its role in the development of urothelial carcinoma and major developments in the FGFR-targeted therapy.

INTRODUCTION
In the USA, carcinoma of the urinary bladder is the fourth leading cancer diagnosis in men, with an estimated incidence of 81400 new cases in 2020. Multiple genetic and environmental factors contribute to its development, including hereditary cancer syndromes, exposure to a carcinogenic chemical compound, smoking and infections. Histologically, carcinoma of the urinary bladder is heterogeneous, with urothelial carcinoma being the most common. It is known that urothelial carcinoma has a high propensity for divergent differentiation, which may, in part, be reflective of the differences in the underlying molecular pathways.

The development and progression of urothelial carcinoma follow at least two major pathways, non-invasive and invasive diseases. The former is usually low grade, papillary and with a high propensity for multiple recurrences; the latter is usually high grade, flat and has the major mortality impact of the disease. Generally, low-grade urothelial carcinoma predominantly follows the fibroblast growth factor receptor 3 (FGFR3)/RAF/RAS signalling pathway, while the carcinoma in situ and high-grade invasive disease follows the p53/retinoblastoma pathway, which both are reported to be mutually exclusive by some studies.

Although the concept of two different pathways involved in the tumourigenesis exists, the possibility of genetic progression from a low-grade FGFR3-mutated to high-grade TP53-mutated tumours have been investigated. In a study by Lott et al., 45% of inverted papillomas had FGFR3 mutations, whereas none had TP53 mutations, supporting the concept that both low-grade and high-grade urothelial neoplasms arise in a background of distinct molecular pathways.

In this review, we summarise the current understanding of the FGFR pathway alteration, its relationship to the pathogenesis of urothelial carcinoma and the treatment implications.

FGFR structure and function
The FGFRs are a family of tyrosine kinases that constitute four different receptors: FGFR1–FGFR4. These receptors are encoded by different genes; however, they all share a high sequencing identity. They are located at the cell membrane and are formed of extracellular, transmembranous and intracellular domains. The diversity between the FGFRs is mostly attributed to the alternative splicing of the mRNA sequence that produces the extramembranous domain. This domain is formed of one peptide signalling region, two to three immunoglobulin-like domains (IgL-D) and a hallmark of a serine-rich sequence of the acidic box between IgL-D1 and IGL-D2. IgL-D1 and acid box are thought to have a role in receptor autoinhibition, while IgL-D2 and IgL-D3 are important for finding to fibroblast growth factor (FGF) ligands. Additionally, IgL-D3 also has three isoforms (a, b and c), that are formed of alternative splicing of exons 7, 7/8 and 8/9, respectively. IgL-D3b and IgL-D3c splice variants are observed in FGFR1–FGFR3, while only IgL-D3b variants are observed in FGFR4. This alternative splicing also contributes to the receptor specificity, whereby FGFR1b–FGFR3b are predominantly epithelial, while FGFR1c–3c is mesenchymal.

Like other tyrosine kinases, once bound to the activating FGF ligand through the extracellular domain, the receptor dimerises, enabling transphosphorylation and becomes activated. This, in turn, activates downstream transduction intracellular signalling pathways, including phospholipase C (PLC)γ, phosphatidylinositol 3-kinase (PI3K) –AKT (also known as protein kinase B (PKB)), and RAS-mitogen-activated protein kinase (MAPK) pathways.
activated is determined by multiple factors, including the nature of activating FGFR ligand and the type of receptor involved; however, no single cause relationship exists between ligand, receptor or pathway activated.\textsuperscript{24} For instance, the activation of the (extracellular signal-regulated kinase (ERK)1/2 and p38) MAPK pathways mediates FGF-induced growth arrest of chondrocytes, while promoting endothelial cells in angiogenesis.\textsuperscript{25–28}

In recent years, a growing interest has developed toward classifying tumours based on their molecular signature, including urothelial carcinomas. Using gene expression profiling, studies reported luminal, basal and other molecular subtypes of urothelial carcinoma.\textsuperscript{27–29} In a meta-analysis by Dadhania et al.,\textsuperscript{30} the superficial papillary urothelial tumours were exclusively luminal, while the invasive ones were almost equally divided into luminal and basal subtypes, concluding that the invasive tumours showing luminal expression signatures most likely represent a progression of superficial papillary urothelial tumours. This tumour subtype is enriched in epithelial markers, including high levels of FGFR3 and activating FGFR3 mutations.\textsuperscript{31}

\textbf{Role of FGFR in urothelial carcinoma}

Dysregulation of FGFRs has been implicated in different human malignancies, including urothelial carcinoma. In the urinary bladder, genetic alterations in FGFR1–FGFR3 have been implicated.\textsuperscript{32} FGFR1 alteration is reported in 7% of urothelial carcinomas, predominantly the FGFR1\textalpha variant, and switching from FGFR1\textalpha to FGFR1\textbeta correlates with increasing stage and grade of the tumour.\textsuperscript{33–35}

In urothelial carcinoma, the oncogenic role of the mutated FGFR3 is mediated by the RAS-MAPK pathway, resembling the effects observed with activated HRAS. Activating somatic mutations of FGFR3 have been detected in 50%–70% of papillary urothelial carcinomas. These mutations are clustered in three hotspots in exons 7, 10 and 15, and are almost always missense mutations leading to amino acid substitution in the external, transmembrane or intracellular regions of the receptor. The most common mutation (up to 70% of tumours harbouring FGFR3 mutations) occurs in exon 7, codon 249, replacing serine with cysteine, followed by codon 248 (up to 17% of tumours), replacing arginine for cysteine, while mutations in other exons are less common.\textsuperscript{36–39} These mutations can lead to ligand-independent dimerisation, autophosphorylation and activation of the receptor, or may alternatively decrease the lysosomal degradation pathways.\textsuperscript{40} FGFR3 mutations are common in low-grade tumours but have also been reported in high-grade tumours (figure 1).\textsuperscript{37–39} No significant difference between FGFR mutational hotspots was identified between low-grade and high-grade tumours, although a higher percentage of high-grade tumours harboured S249C point mutation in a study by Al-Ahmadi et al.\textsuperscript{37} In another meta-analysis study, the frequency of the FGFR3 mutations decreased with the increasing stage (65% in pT1a to 12% in pT2–pT4, and 70% in grade 1 to 19% in grade 3 tumours).\textsuperscript{38} These findings also coincide with subsequent studies where FGFR3 mutations were found in a subset of high-grade papillary urothelial carcinoma.\textsuperscript{37–39}

A fusion of FGFR3 to transforming acid coiled-coil containing protein 3 (TACC3) leads to constitutive tyrosine kinase activation, disruption of mitotic activity and aneuploidy.\textsuperscript{41–43} FGFR3 amplification and alternative splicing leading to aberrant FGFR3 activation are less common molecular alterations implicated in the proliferative process of urothelial carcinoma.

The frequency of FGFR3 mutations between upper and lower tract urothelial carcinomas was under investigation by many reports. The upper tract showed a higher rate of FGFR3 gene mutations when compared with the bladder, although this has not reached a statistically significant level in some reports.\textsuperscript{38–41} In a recently published study which included 479 upper tract urothelial carcinomas and 1984 bladder urothelial carcinomas, FGFR3 mutations were statistically more common in the upper tract versus in the bladder (21% vs 14%, p=0.002). Other FGFR3 alterations, including amplification and rearrangements, showed no significant difference between both groups.\textsuperscript{44} Additionally, Lynch syndrome patients had a significantly higher risk of upper tract urothelial carcinoma development.\textsuperscript{45} A significant proportion of these patients had FGFR3 R248C mutation, contrasting with the most common FGFR3 S249C mutation that was found to be related to APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like)-mediated mutagenesis.

\textbf{FGFR3-targeted therapy}

Most urothelial carcinomas are non-muscle invasive, while up to 25% are muscle invasive. The standard first-line therapy for patients with muscle-invasive urothelial carcinoma is
tumours. Only urothelial carcinoma and cholangiocarcinoma were assessed in patients with different advanced or refractory solid tumours. Loriot et al.49 In a multicentre phase I study, erdafitinib response was observed in 27% in cholangiocarcinoma in patients with metastatic disease. In a phase II trial, erdafitinib was approved for the treatment of adult patients diagnosed with metastatic urothelial carcinoma (NCT04197986) for the adjuvant treatment in patients with urothelial carcinoma.44 52 Assessing the efficacy in cases of urothelial carcinoma is currently in phase III trial (ClinicalTrials.gov identifier: NCT04228042) for the treatment of advanced or metastatic urothelial carcinoma.

FGFR3 genetic alterations is relatively common in urothelial carcinoma.47 48 The response rate was 44% in patients with metastatic disease. In a phase II trial, erdafitinib was approved for the treatment of adult patients diagnosed with advanced or refractory solid tumours.49 50 Interestingly, erdafitinib sensitivity is related only to FGFR overexpression. In tumour cell lines that harboured RAS or RAF mutations, erdafitinib lacked its sensitivity indicating that downstream alterations of RAS or RAF pathways can overcome the effects of FGFR inhibition.49 In a multicentre phase I study, erdafitinib response was assessed in patients with different advanced or refractory solid tumours.49 Only urothelial carcinoma and cholangiocarcinoma responded to erdafitinib. The objective response rate was 46% in urothelial carcinoma and 27% in cholangiocarcinoma in patients with FGFR genomic alterations. The response rate was <10% in all other tumour subtypes. Loriot et al.51 (ClinicalTrials.gov number: NCT02365597) reported the use of erdafitinib was associated with tumour response in 40% of patients who had locally advanced and unresectable or metastatic urothelial carcinoma with FGFR alterations, including 59% of patients who had undergone prior immunotherapy. In the same study, a slightly higher response rate was observed in patients with upper tract when compared with lower tract disease (43% vs 39%, respectively), although the difference was not statistically significant. On 12 April 2020, the FDA has approved Qigens Therascreen FGFR RT-PCR Kit as a companion diagnostic for erdafitinib. A summary of the FGFR point mutations and fusion targets is presented in table 2.

Infrafatinib is another FGFR-targeted drug. Like erdafitinib, the most observed responses to infrafatinib (BGJ398) were in patients with cholangiocarcinoma and urothelial carcinoma. A phase II trial (ClinicalTrials.gov identifier: NCT04233567) assessing the efficacy of infrafatinib in treating advanced or metastatic solid tumours in patients with FGFR genetic alterations is undergoing.52 Assessing the efficacy in cases of urothelial carcinoma is currently in phase III trial (ClinicalTrials.gov identifier: NCT04197986) for the treatment of advanced or metastatic urothelial carcinoma with susceptible FGFR3 genetic alterations. Recently, Necchi and his colleagues found a modest enrichment of FGFR3 alterations in the upper urothelial tract relative to that of the urinary bladder.44 53

### Table 1 Fibroblast growth factor receptor inhibitors undergoing clinical trials for treatment of urothelial carcinoma

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### Table 2 Therascreen FGFR RT-PCR Kit assay targets

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<td>c.1118A&gt;G</td>
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**Gene fusions**

**Fusion ID** | **Genes involved** | **Genomic breakpoints** | **Exons**
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<tr>
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 FGFR, fibroblast growth factor receptor; TACC3, transforming acid coiled-coil containing protein 3.
carcinoma and activating FGFR3 mutations and/or fusions in the upper tract (n=8) and the urinary bladder (n=59). The authors reported a disease control rate of 100% (n=8/8) and 59.3% (n=35/59) in both groups, respectively. This difference in the response rate was likely attributed to the notable differences in genomic alterations between these upper and lower tract groups of diseases.

Rogaratinib is an FGFR selective inhibitor. Its efficacy also correlates strongly with FGFR mRNA expression levels. Preliminary data from an ongoing phase II/III clinical trial (ClinicalTrials.gov identifier: NCT03410693) comparing rogaratinib (BAY1163877) and chemotherapy in patients with FGFR-positive locally advanced or metastatic urothelial carcinoma reported that in patients with FGFR1–FGFR3 mRNA-positive urothelial carcinomas, rogaratinib had an efficacy comparable to standard chemotherapy; however, subgroup analysis suggested rogaratinib to be more active in patients with an FGFR3 DNA alteration (objective response rate of 52% and 27% with rogaratinib and chemotherapy, respectively).

CONCLUSION

FGFR plays an essential role in the normal cellular transduction pathways through the bindings of the FGF. A deeper knowledge of its role in papillary urothelial carcinoma has led to the identification and development of several FGFR therapeutic targets, including a recently FDA-approved drug, erdafitinib.

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