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Characterization of a KLK2-FGFR2 fusion gene in two cases of metastatic prostate cancer

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Abstract

Background—The fibroblast growth factor receptor (FGFR) signaling pathway is activated in multiple tumor types through gene amplifications, single base substitutions, or gene fusions. Multiple small molecule kinase inhibitors targeting FGFR are currently being evaluated in clinical trials for patients with FGFR chromosomal translocations. Patients with novel gene fusions involving FGFR may represent candidates for kinase inhibitors.

Methods—A targeted RNA-sequencing assay identified a *KLK2-FGFR2* fusion gene in two patients with metastatic prostate cancer. NIH3T3 cells were transduced to express the KLK2-FGFR2 fusion. Migration assays, Western blots, and drug sensitivity assays were performed to functionally characterize the fusion.

Results—Expression of the KLK2-FGFR2 fusion protein in NIH3T3 cells induced a profound morphological change promoting enhanced migration and activation of downstream proteins in FGFR signaling pathways. The KLK2-FGFR2 fusion protein was determined to be highly sensitive to the selective FGFR inhibitors AZD-4547, BGJ398, JNJ-42756943, the irreversible inhibitor TAS-120, and the non-selective inhibitor Ponatinib. The KLK2-FGFR2 fusion did not exhibit sensitivity to the non-selective inhibitor Dovitinib.

Conclusions—Importantly, the KLK2-FGFR2 fusion represents a novel target for precision therapies and should be screened for in men with prostate cancer.

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Conflict of interest SR participated in Advisory Boards for Incyte Corporation (2017), AbbVie, Inc. (2017), and QED Therapeutics (2018). SR received honoraria from IDT Integrated DNA Technologies (2017), Illumina (2018). The remaining authors declare that they have no conflict of interest.

Introduction

The fibroblast growth factor receptor (FGFR) family belongs to a superfamily of receptor tyrosine kinases [1]. FGFRs play essential roles in a variety of cellular processes including cell proliferation, survival, growth arrest, differentiation, migration, and apoptosis [2]. Given their critical role in numerous physiological processes, it is not surprising that perturbed FGFR signaling is frequently observed in cancer. Deregulation of the FGFR signaling cascade has been reported to occur through gene amplification, alternative splicing, aberrant FGF signaling, activating mutations, and chromosomal translocations. These genomic alterations have been reported in many tumor types including, but not limited to, non-small cell lung carcinoma, endometrial cancer, urothelial bladder carcinoma, intrahepatic cholangiocarcinoma, and prostate cancer [3–5]. Studies in cancer cell lines with activating FGFR alterations, including point mutations, amplifications, and gene fusions predict sensitivity to treatment with FGFR inhibitors [6, 7]. Thus, targeting aberrant FGFR signaling may be a novel and effective therapeutic strategy for patients with FGFR-driven cancers [6–10]. Several tyrosine kinase inhibitors, both non-selective and selective for FGFR, are being assessed in clinical trials for patients with metastatic cancer. While first-generation drugs inhibit FGFR kinases and related family members, such as FLT3, VEGFR, and cKIT [7, 11], second-generation inhibitors are more active specifically against FGFRs [6, 12, 13].

With approximately 160,000 new cases per year in the United States, prostate cancer is the most common cancer diagnosis in men, and remains the second most common cause of cancer mortality in men [14, 15]. There continues to be a need to develop therapies for patients with castrate-resistant metastatic disease [16]. Gene fusions involving ETS gene family members are highly prevalent in prostate cancer [17]. For instance, the *TMPRSS2-ERG* gene fusion is present in approximately 50% of prostate cancer cases [17]. While ETS gene fusions have been an attractive therapeutic target, drug development has been limited [18]. Interestingly, several recent studies have identified chromosomal translocations involving FGFR in prostate cancer [5, 19–21] suggesting the identification of a new molecular subset of prostate cancer that may be effectively treated with clinically available FGFR inhibitors, however, the complete landscape of FGFR alterations in prostate cancer remains uncharacterized.

Previously, we reported the detection of a KLK2-FGFR2 fusion gene in a patient with metastatic prostate cancer using our SpARKFuse Assay [22]. In this study, we describe this case in addition to another case of metastatic prostate cancer harboring the identical KLK2-FGFR2 fusion. KLK2 is a serine protease, similar to prostate specific antigen (PSA), but differing in enzymatic activity and expression, perhaps most strongly associated with higher grade and stage prostate cancer [23]. We hypothesize that the KLK2-FGFR2 fusion results in driving FGFR2 expression and downstream signaling activity, which promotes prostate cancer growth and metastasis. We address the impact of the KLK2-FGFR2 fusion through studies in NIH3T3 cells while also addressing the potential impact of novel agents targeting FGFR. Our findings highlight the need for comprehensive molecular testing for FGFR alterations in patients with prostate cancer and the potential clinical benefits of FGFR targeted therapies.

Materials and methods

Patient samples

This study was approved by The Ohio State University Institutional Review Board (OSU-13053,). Informed consent was obtained from patients for high-throughput sequencing (tumor and blood). OSU-SpARK-Fuse, a targeted RNA based next generation sequencing assay to detect gene fusions, was performed on tumor biopsy specimens as previously described [22].

RNA isolation, RT-PCR, and Sanger sequencing

RNA was isolated from cell lines using the Quick-RNA Mini Prep Kit (Zymo) and cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad). cDNA was PCR amplified with KLK2-FGFR2 fusion specific primers (IDT). Primer sequences are as followed: Forward-*5′-CATGTGGGACCTGGTTCTCT-3′* and Reverse-5′-CCTGCTTA *AACTCCTTCCCG-3′*. Amplified PCR product was purified using PureLink Quick PCR Purification Kit (Invitrogen) and Sanger sequenced (The Ohio State University Comprehensive Cancer Center Genomics Shared Resource, Columbus, OH).

Cell culture

NIH3T3 and HEK293T cells were purchased from American Type Culture Collection (ATCC) and cultured in an incubator at 37 °C and 5% CO_2. NIH3T3 cells were maintained in DMEM (Gibco) media supplemented with 10% Bovine Calf Serum (Sigma Aldrich) and 1× Glutamax (Gibco by Life Technologies). HEK293T cells were maintained in DMEM (Gibco) media supplemented with 10% Tet System Approved Fetal Bovine Serum (Clontech) and 1× Glutamax (Gibco by Life Technologies). Cells were routinely subjected to mycoplasma testing using the e-Myco plus Mycoplasma PCR Detection Kit (Bulldog Bio). Cells also underwent routine short tandem repeat profiling to confirm identities.

cDNA plasmid generation, lentivirus production and transduction

The KLK2-FGFR2 fusion was generated and cloned into the pLVX-IRES-Puro vector (Clontech) by GenScript (Supplementary Fig. 1). Lentivirus production was conducted using the Lenti-X™ VSV-G Packaging Single Shots (Clontech) using HEK293T cells. NIH3T3 cells were transduced with KLK2-FGFR2 or Empty lentiviral vectors and transduced cells were selected in puromycin (1 ug/mL; Sigma) for 72 h prior to experiments.

Migration assay

Cells were serum-starved overnight. 5×10^4 cells were plated in serum free media in the upper chamber of an8.0 μm Transwell Insert (Corning Incorporated) with complete media in the receiver well and incubated at 37 °C in 5% CO_2 for 24 h. Following incubation, non-invading cells were removed from the upper surface of the insert and inserts were stained with 0.5% crystal violet stain in 20% methanol. Migrating cells were imaged by light microscopy and quantified by dissolving the crystal violet stain in 10% acetic acid and measuring absorbance was measured at 590 nm. Data are reported as mean ± SEM from three independent experiments. *P*-values were calculated using a Students *t*-test.

Western blotting

Western blot assays were performed using established protocols with the following antibodies: phospho-Akt (Ser473) 1:1000 (Cell Signaling 9271), Total Akt 1:1000 (Cell Signaling 9272), phospho-MEK1/2 1:5000 (Cell Signaling 9154), Total MEK1/2 1:5000 (Cell Signaling 9122), phospho-FGF Receptor (Tyr653/654) 1:500 (Cell Signaling 3471), FGF Receptor 2 (D4L2V) 1:500 (Cell Signaling 23328), p44/42 MAPK (Erk1/2) 1:5000 (Cell Signaling 9101), Total MAPK 1:5000 (Cell Signaling 9102), pFRS2 1:1000 (Cell Signaling 3864), anti-FRS2 (abcam ab10425), phospho-PI3 Kinase p85 (Tyr458)/p55 (Tyr199) 1:1000 (Cell Signaling 4228), PI3 Kinase p85 (19H8) 1:000 (Cell Signaling 4257), β-actin 1:10000 (Cell Signaling 4967) and GAPDH 1:10000 (Santa Cruz sc-25778).

Drug sensitivity assays

NIH3T3 cells were plated at 10,000 cells per well in 96-well plates in sextuplets. Cells were treated for 72 h with various selective and non-selective FGFR inhibitors ranging from0.01 to 5000 nM followed by quantification of viable cells using an MTS/PMS colorimetric assay. FGFR inhibitors tested include BGJ398, JNJ-42756493, AZD-4547, Ponatinib, Dovitinib, and TAS-120 (Cayman Chemical). The half maximal inhibitory concentration (IC_{50}) of each FGFR inhibitor was calculated with GraphPad Prism using a four-parameter dose-response curve from four independent experiments.

Results

Clinical description of two patients with metastatic prostate cancer harboring a KLK2-FGFR2 fusion

Patient One—In early 2015, a 60-year-old male presented with hematuria, lumbar back and perineal pain, dysuria, and urinary frequency and urgency. Prior PSA screening over 5 years showed values < 1.00 ng/mL with a rise to 2.47 ng/mL at time of biopsy. His pathology showed a poorly differentiated prostate adenocarcinoma, with intraductal features, Gleason scores 10 (5 + 5) and 9 (5 + 4) in 5 of 6 cores. Involved cores showed 70–100% cancer. The immunohistochemical stains were positive for cytokeratin AE1/AE3, CAM5.2, MOC-31, CK20 (focal), NKX3.1, AMACR-p504s (focal), PSA (focal), PSAP (focal), androgen receptor, CDX-2, villin (weak, rare cells), synapto-physin (focal), and chromogranin (rare cells) and negative for CK7, TTF-1, Napsin A, GATA3, and CD56. Staining for Ki-67 demonstrated positive nuclear staining in 90–95% of the tumor cells and a mucicarmine stain was negative. He was soon hospitalized for acute retention and severe prostatitis. Initial staging scans showed indeterminate bilateral pulmonary nodules, a questionable density in the right inferior pubic ramus, and pelvic lymphadenopathy. A lymph node biopsy confirmed the presence of metastatic disease. He was started on combined androgen deprivation with bicalutamide and leuprolide with the addition of docetaxel. After one cycle of treatment he developed neutropenic fever and prostatitis and required hospital admission for treatment of sepsis. He demonstrated rapid disease progression over a few months with emergence of new metastases involving the liver, spine, ribs, calvarium and retroperitoneal lymph nodes (Fig. 1a, b). Tumor markers showed elevations of PSA to a maximum of4.38 ng/mL, elevated CEA to 841 ng/mL (nl 0–5.0 ng/mL), neuron specific enolase to 83 ng/mL (nl <10.8 ng/mL), and Chromogranin A to

95/ng/mL (nl <93 ng/mL). He underwent biopsy of a liver metastasis for molecular testing with a targeted RNA based next generation sequencing assay [22]. The results of RNA sequencing demonstrated the presence of a *KLK2-FGFR2* gene fusion (Fig. 1e). The fusion involved exon 1 of *KLK2* and exons 4 to 17 of *FGFR2*. The expression of *KLK2* is regulated by androgen receptor and has been shown to be correlated with increased cellular proliferation and decreased apoptosis in castrate-resistant prostate cancer (CRPC) specimens [24, 25]. Unfortunately, the patient was hospitalized multiple times for cancer-related pain and fevers, which precluded him from receiving additional therapy. He was discharged from the hospital with home hospice care and passed away thereafter.

Patient two—In February 2011, a 61-year-old male presented with gross hematuria and a PSA at 3.2 ng/mL and abnormal digital rectal exam. He underwent a prostate biopsy revealing Gleason 9 adenocarcinoma with staging showing no evidence of metastasis. He underwent prostatectomy with Gleason 4 + 5 = 9 disease, with lymphovascular and perineural invasion, negative margins, and absent lymph node involvement (pT3aN0, Mx). His post-operative PSA was0.32 ng/mL and was started on androgen deprivation therapy with GNRH antagonist and external beam radiotherapy (7020 cGy) to the prostate fossa. However, within a few months his PSA rose, with restaging scans showing liver metastasis, and no impact of abiraterone. Taxotere was initiated with a PSA of 999 ng/ml with a rapid response to undetectable PSA (<0.01). Subsequent PSA rise to 72 was treated with enzalutamide with a 10-month response and nadir PSA of 14. After progression, he was referred to The Ohio State University where he underwent a new tumor biopsy, which was subjected to molecular profiling with a targeted RNA based next generation sequencing assay (SpARKFuse) [22]. Like our previous patient, the results of RNA-sequencing demonstrated a *KLK2-FGFR2* gene fusion with an identical breakpoint resulting in identical fusion proteins (Fig. 1e). This fusion was reported in our OSU-SpARKFuse validation paper but did not include functional characterization or assessment of prevalence. Based on the presence of this fusion, this patient was determined to be eligible for a clinical trial enrolling patients with advanced solid cancers harboring genomic alterations involving *FGFR* family members 1–4, *KIT* and *RET* (). Patients on this study receive molecular matched therapy with the oral multi-kinase inhibitor ponatinib. Our patient achieved control of his cancer and demonstrated stable disease on ponatinib for approximately 4 months. While on ponatinib therapy, he exhibited an initial decrease in PSA levels from 2378 to 1842 ng/mL after cycle one with subsequent rise, in parallel to dose reduction due to toxicity. Unfortunately, after 4 months his hepatic metastases progressed. PSA levels increased to 3435.07 ng/mL, and therapy was discontinued (Fig. 1c, d). Additional salvage therapy unfortunately could not halt rapid disease progression, and he passed away shortly after cessation of ponatinib.

FGFR2 is highly expressed in patients harboring the *KLK2-FGFR2* gene fusion

Having identified the presence of a KLK2-FGFR2 fusion gene in two patients with advanced metastatic prostate cancer, we next investigated whether this fusion resulted in the amplification of FGFR2 expression. In these two patients, >95% of sequencing reads at the FGFR2 break-point support the KLK2-FGFR2-fusion while <5% of reads support wildtype FGFR2 (Fig. 2a, b). Consistent with this apparent amplified expression of the fusion allele, both KLK2-FGFR2 positive patients had dramatically increased FGFR2 expression when

compared with a cohort of 15 other prostate cancer patients seen at OSU (Fig. 2c). Expanding this analysis to include 499 The Cancer Genome Atlas (TCGA) prostate cancer patients, gene expression data confirmed that FGFR2 expression was also significantly increased in both primary and metastatic sites of a single KLK2-FGFR2 positive patient (Fig. 2d, Supplementary Data 1). These findings suggest that the KLK2-FGFR2 fusion effectively amplifies FGFR2 expression in these patients and that these patients would benefit from FGFR targeted therapy.

The *KLK2-FGFR2* gene fusion leads to changes in cell morphology, promotes migration, and activates downstream *FGFR* signaling pathways

To evaluate the biological significance of the *KLK2-FGFR2* fusion, we generated two stable NIH3T3 cell lines expressing either control vector or the fusion protein. We chose to use the NIH3T3 cells, as they are an easily transfectable mouse fibroblast cell line and readily used to explore FGF function as they do not express endogenous FGF ligands or FGF receptor (data not shown). Since the mechanism of action of this fusion is through extreme over expression, the presence of endogenous FGFR2 signaling could be a confounding variable. The presence of the KLK2-FGFR2 fusion was confirmed using RT-PCR and Sanger sequencing with primers spanning the fusion breakpoint (Fig. 3a). Interestingly, we noticed that the *KLK2-FGFR2* fusion induced significant morphological changes in NIH3T3 cells (Fig. 3b). NIH3T3 cells with the fusion appeared more spindle-like than control NIH3T3 cells transduced with empty vector. We subsequently investigated whether the *KLK2-FGFR2* fusion might promote a migratory phenotype. NIH3T3 *KLK2-FGFR2* cells demonstrated substantial chemotactic migration relative to NIH3T3 Empty cells (Fig. 3c). We hypothesized that the presence of the *KLK2-FGFR2* fusion drives prostate cancer oncogenesis through activation of downstream FGFR signaling pathways. To test this hypothesis, we assessed the phosphorylation of FGFR2 and its downstream signaling intermediates in NIH3T3 cells transduced with the fusion. There are four main signal transduction pathways downstream of FGFR including MAPK/MEK, PI3K/AKT, PLCγ, and STAT [26]. Western blot analyses demonstrated ectopic increase in MAPK/MEK, PI3K/AKT and expectedly FGFR2 signaling as evident by high levels of pMAPK/pMEK, pPI3K/pAKT and pFGFR/pFRS2 in fusion expressing cells (Fig. 3d). Thus, the *KLK2-FGFR2* fusion protein is a driver of hall-marks of oncogenesis.

The KLK2-FGFR2 gene fusion is selectively sensitive to FGFR inhibitors

Having established that *KLK2-FGFR2* fusion expression promotes oncogenesis through increased migration and activation of downstream FGFR signaling pathways, we hypothesized that this fusion may represent a novel therapeutic target. Because of the critical role FGFR plays in numerous tumor types, several small molecule inhibitors have been developed and have shown clinical efficacy in FGFR-driven tumors [6–10]. To evaluate the in vitro sensitivity of the KLK2-FGFR2 fusion to FGFR inhibitors, we treated NIH3T3 Empty and NIH3T3 KLK2-FGFR2 cells with increasing doses of FGFR inhibitors of interest or DMSO (vehicle) ranging from 1.0 nM to 20 μM and cell viability was assessed after 72 h. Treatment of NIH3T3 KLK2-FGFR2 cells with FGFR inhibitors resulted in substantial and reproducible inhibition of cell viability. Our results demonstrated that KLK2-FGFR2 cells were sensitive to the selective inhibitors AZD-4547 and BGJ398 with IC_{50}

values of 64.86 nM and 42.07 nM, respectively (Fig. 4a, b). For both of these inhibitors, maximum inhibition was achieved at 50% cell viability (Fig. 4a). KLK2-FGFR2 cells were exquisitely sensitive to the selective inhibitor JNJ-42756493 with an IC_{50} value of 34.83 nM (Fig. 4a, b). The selective, irreversible inhibitor TAS-120 demonstrated the lowest IC_{50} value at2.96 nM, however only 50% inhibition was achieved (Fig. 4a, b). Empty vector control cells (Empty) were not sensitive to AZD-4547, BGJ298, JNJ-42756493, or TAS-120 (Fig. 4a). Ponatintib also demonstrated complete inhibition of cell viability with an IC_{50} value of 239.88 nM (Fig. 4a, b). However, at the highest dose (5000 nM) of Ponatinib, the Empty cells were sensitive, which was not surprising as Ponatinib targets numerous kinases in addition to FGFR (Fig. 4a). Lastly, Dovitinib was largely ineffective against the fusion with an IC_{50} value at 534.56 nM, and at higher doses the viability of both the fusion and Empty cells were decreased (Fig. 4a, b). Taken together, these data demonstrate that the KLK2-FGFR2 fusion protein is exquisitely sensitive to a variety of FGFR inhibitors.

Discussion

In this paper, we report two patients with metastatic prostate cancer harboring a previously uncharacterized FGFR fusion gene, KLK2-FGFR2. Although our sample size is small, this fusion is recurrent and is associated with high-risk features, such as being poorly differentiated, short responses to standard therapy, and rapid mortality. In vitro characterization of the KLK2-FGFR2 gene fusion revealed that the fusion likely drives prostate cancer oncogenesis through hallmarks of enhanced migration as well as activation of the MAPK/MEK and PI3K/AKT signaling pathways. Of clinical interest, we demonstrated that the KLK2-FGFR2 fusion is sensitive to a variety of FGFR inhibitors, including AZD-4557, BGJ398, JNJ-42756493, TAS120 and ponatinib. Our in vitro studies demonstrate that the fusion is not equally sensitive to all FGFR inhibitors (selective, non-selective and irreversible) and warrants further exploration of the sensitivity of KLK2-FGFR2 to FGFR inhibition in orthotopic and patient derived xenograft models of prostate cancers. Collectively, our findings demonstrate that the *KLK2-FGFR2* fusion gene may represent a promising target for FGFR inhibition in fusion positive patients and provide rationale that patients with prostate cancer should be screened for FGFR fusions as they may be eligible for FGFR targeted therapeutic strategies.

Deregulation and activation of FGFR signaling has been identified in multiple cancers, including bladder, lung, biliary, breast, and others [4]. *FGFR1* amplification has been reported in 20% of lung squamous cell carcinoma cases [27]. *FGFR3* activating point mutations are reported in approximately 70% of low-grade urothelial carcinomas [28]. In addition to oncogenic fusions involving *FGFR3* in bladder cancers, other FGFR fusions have been reported in breast, biliary, prostate, and thyroid cancers [5]. The *TACC3* gene is the most common partner gene in FGFR fusions and is present in numerous tumor types [29]. Furthermore, additional rare and novel FGFR fusion partner genes including *ACSL5*, *PHGDH*, *APIP*, and *BAIAP2L1* have been described [30–33]. Thus, the complete landscape of FGFR gene fusions continues to expand as new fusion partners are being discovered. From a clinical perspective, the discovery and characterization of novel FGFR fusions may lead to treatment opportunities that would otherwise not be available for these patients.

A recent study by Hu et al., analyzed nearly 10,000 cancer samples from The Cancer Genome Atlas for the presence of fusions and identified two prostate adenocarcinoma cancer samples and one uveal melanoma cancer sample harboring the KLK2-FGFR2 fusion [21]. The authors also reported that the KLK2-FGFR2 fusion had high centrality scores supporting its role as a driver fusion [21]. Within prostate cancer, the KLK2-FGFR2 fusion is present in 0.4% of the TCGA cohort ($N = 499$) and 11.7% of our cohort ($N = 17$). These studies along with the findings in this study, support the role for further molecular profiling of prostate cancer patients for the presence of FGFR fusions.

Our two patients and the prostate tumor sample from the TCGA had the highest expression of FGFR2 within a cohort of patients. This is further supported by the findings of Wu et al. who identified an *SLC45A3-FGFR2* fusion in a prostate cancer patient. SLC45A3 is another prostate-specific androgen regulated gene and this fusion, which consists of the non-coding exon 1 of SLC45A3 fused to the entire coding region of FGFR2, is thought to drive the overexpression of the full length, wildtype FGFR gene. Expression of FGFR was substantially increased compared to a cohort of 84 prostate cancer tissues.

The clinical benefit of FGFR inhibitors for patients with FGFR fusions includes disease control rates as high as 60–80% in cholangiocarcinoma and urothelial cancer, but observations in fusion-positive prostate cancer patients have been limited thus far [34, 35]. Our first prostate cancer patient was unfortunately too ill to receive FGFR-directed therapy as he passed shortly after receiving his sequencing results. Thus, whether he would have responded to FGFR inhibition is unknown. The second prostate cancer patient was able to receive the FGFR inhibitor, ponatinib, on clinical trial. While on ponatinib, he achieved stable disease for 4 months based upon imaging, but unfortunately had disease progression and died shortly after therapy discontinuation. Our two prostate cancer patients harboring the *KLK2-FGFR2* fusion both had metastatic liver disease. While the rate of liver metastases in patients with prostate cancer is uncommon, there is a trend of increasing liver disease in patients receiving next generation anti-androgen therapies [36]. Furthermore, patients with CRPC who have liver metastases have a worse overall median survival when compared to patients with lymph node only, bone or lung metastases [37]. Efforts to fully molecularly profile patients with CRPC will need to be complemented with FGFR inhibitor trials to offer novel therapies for this subset. Additional basket trials for INCB054828, BGJ398, and other FGFR inhibitors will hopefully capture more prostate cancer patients harboring FGFR fusions.

In summary, this work provides evidence that the KLK2-FGFR2 fusion represents a targetable event in patients with advanced metastatic prostate cancer. While limited to two cases of metastatic prostate cancer and a single cell line, this is the first study to our knowledge to describe that the fusion is dramatically overexpressed and is sensitive to FGFR inhibition. Overall, our findings highlight the need for comprehensive molecular testing for FGFR alterations in patients with prostate cancer as these patients may be eligible for targeted FGFR inhibition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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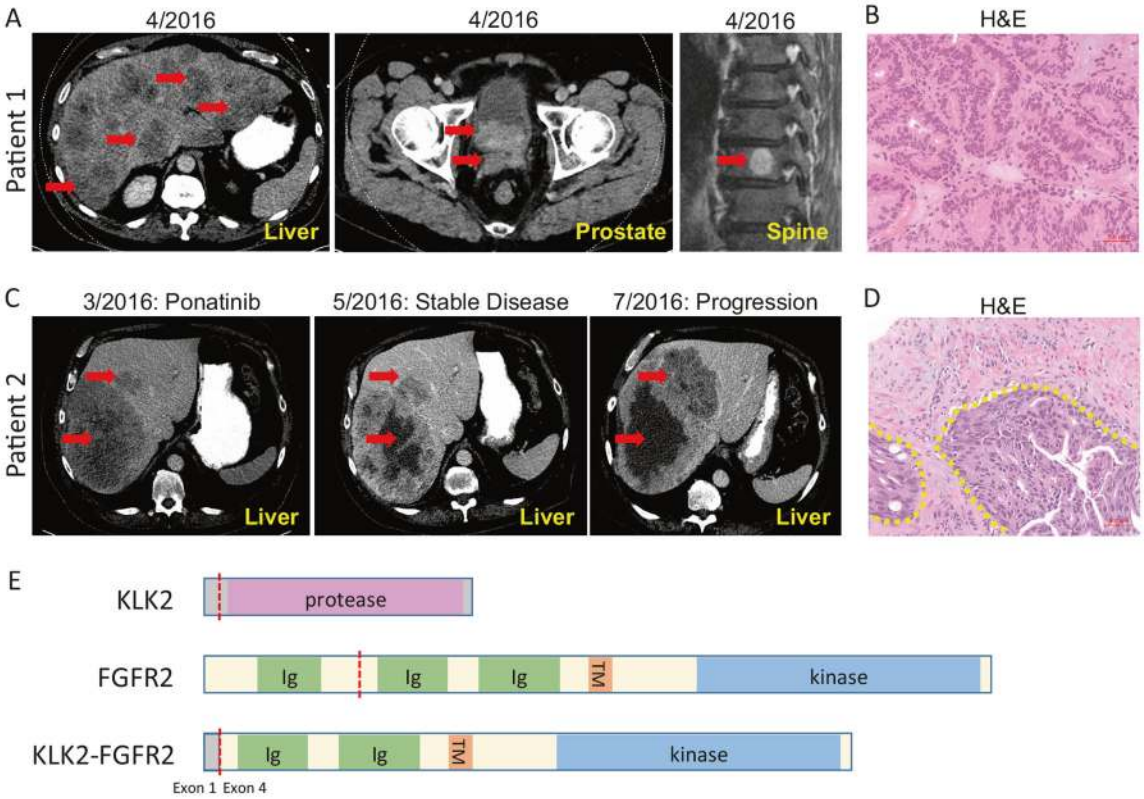


Fig. 1.

Clinical presentation of two patients with metastatic prostate cancer harboring a KLK2-FGFR2 fusion. **a** CT scans of patient one demonstrate widespread disease in the liver, prostate and spine as denoted by red arrows. **b** Hematoxylin & eosin (H&E) stained section of the pre-treatment core needle tumor biopsy. Tumor cells indicated by dashed yellow lines. **c** Patient with metastatic prostate cancer who failed prior therapy had a liver biopsy and sequencing revealed an FGFR2 gene fusion. He was enrolled on a Phase 2 trial for ponatinib (left panel) and had stable disease after two months on therapy (middle panel). After 4 months on therapy, he developed progression (right panel). **d** Hematoxylin & eosin (H&E) stained section of the pre-treatment core needle tumor biopsy. e Schematic of KLK2-FGFR2 fusion involving exon 1 of KLK2 and exons 4 to 17 of FGFR2. Red dashed lines indicate breakpoint

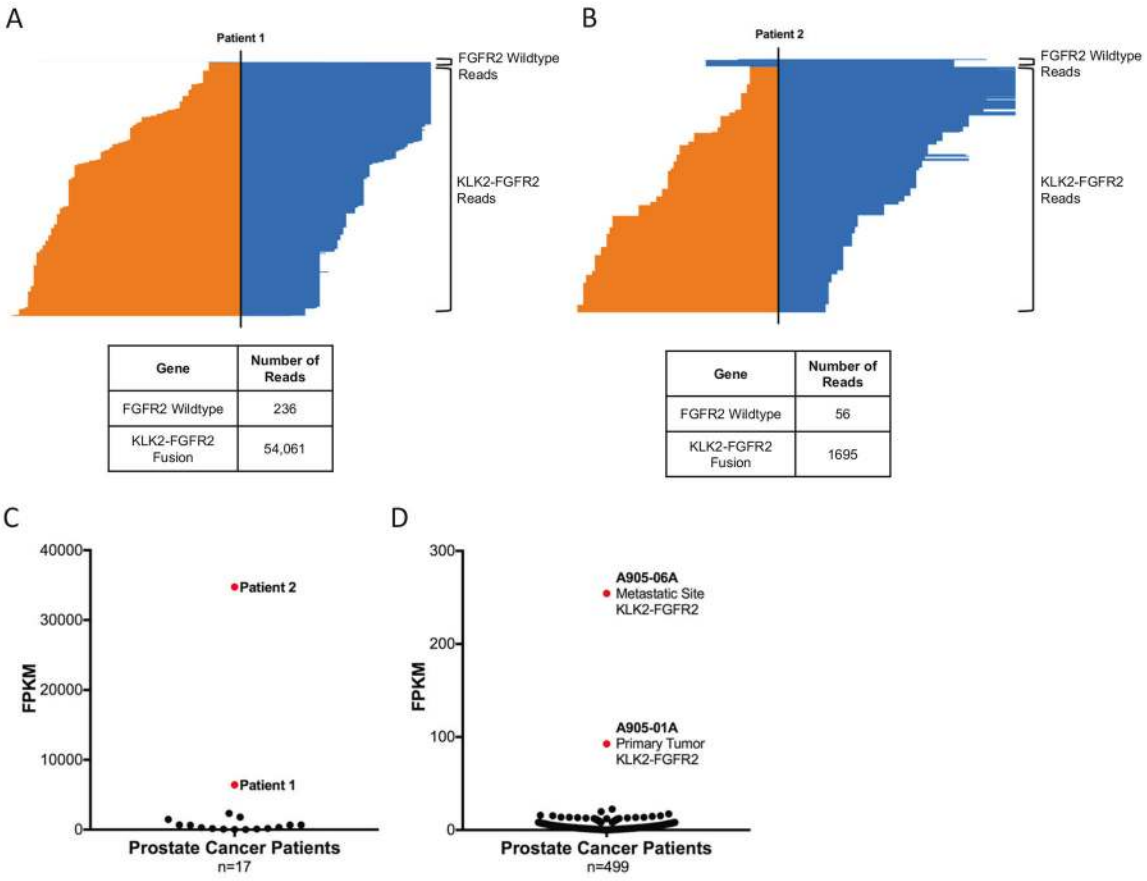


Fig. 2.

Expression of FGFR2 in patients harboring the *KLK2-FGFR2* gene fusion. **a**, **b** Patient 1 (a) and Patient 2 (b) FASTQ file raw reads containing the 15 bases of FGFR2 immediately following the fusion breakpoint were extracted, aligned, and colored based on the identity of the sequence (FGFR2 sequences are blue and KLK2 sequences are orange). A pileup of all reads is shown with the black vertical line representing the fusion breakpoint. The total number of reads sup-porting WT FGFR or the KLK2-FGFR2 fusion is listed in the table below. **c** FGFR2 expression was measured using a targeted RNA sequencing assay in a cohort of 17 prostate cancer patients from The Ohio State University. **d** FGFR2 expression (FPKM) for all TCGA prostate cancer patients (N = 499) assayed using exome capture (exact capture regions vary). Note that FPKM values are ~100 fold higher for SpARKFuse due to the capture region being approximately 1% of the size of the exome capture region. Data were downloaded from the Genomic Data Commons (gdc.cancer.gov). Expression levels are measured as FPKM (Fragments Per Kilobase per Million mapped reads)

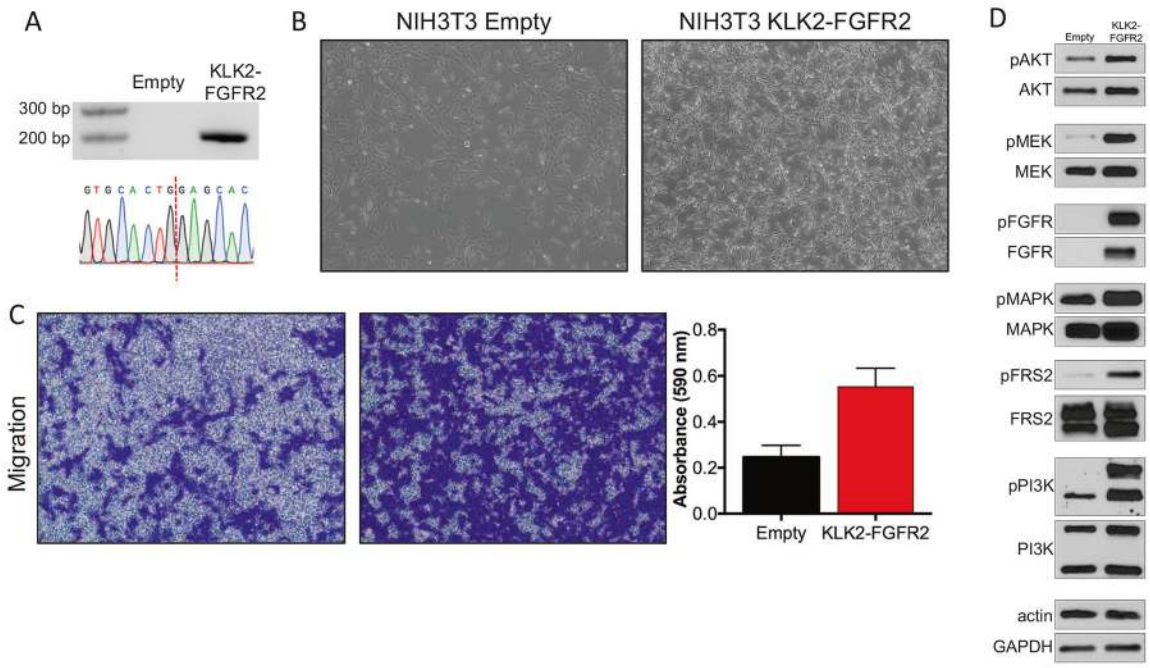
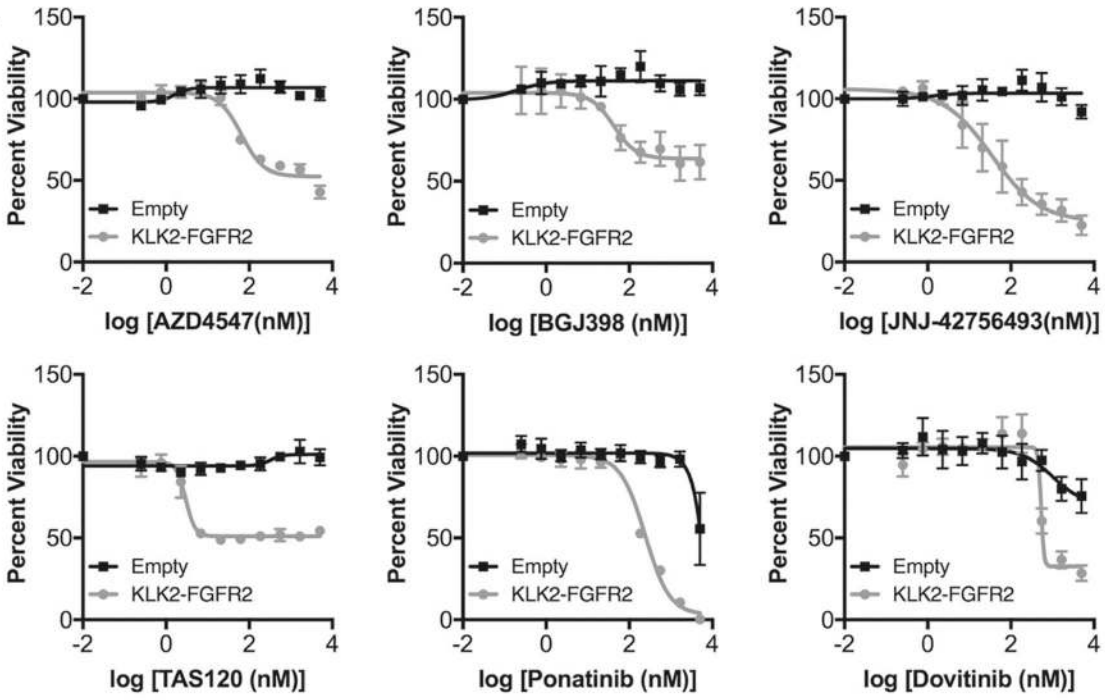


Fig. 3.

KLK2-FGFR2 changes cell morphology, promotes migration and activates downstream FGFR signaling pathways. **a** RT-PCR confirmed the presence of the KLK2-FGFR2 fusion in the NIH3T3 KLK2-FGFR2 cells. The fusion was not detected in the control vector (Empty) transduced cells. Chromatogram traces from Sanger sequencing of the NIH3T3 KLK2-FGFR2 cells confirmed the presence of the fusion. The dashed red line indicates the break point. **b** NIH3T3 Empty and KLK2-FGFR2 cells were visualized for morphological changes. **c** Transwell chemotactic migration assays were performed on NIH3T3 Empty and KLK2-FGFR2 cells. Migrated cells were fixed and stained with crystal violet for visualization. Bar graphs depict the absorbance of migrated cells stained with crystal violet (mean ± SEM; *$p < 0.05$). Data from three independent experiments are shown. d Total cell lysates from NIH3T3 Empty and NIH3T3 KLK2-FGFR2 cells were prepared and subjected to Western analysis with antibodies against: pAKT, AKT, pMEK, MEK, pFGFR, FGFR, pMAPK, MAPK, pFRS2, FRS2, pPI3K, PI3K, β-actin, and GAPDH

A



B

FGFR Inhibitor	IC_{50} (nM)	95% CI (nM)
AZD4547	64.86	44.06 to 114.82
BGJ398	42.07	12.97 to 895.36
JNJ-42756493	34.83	12.56 to 217.77
TAS120	2.96	-infinity to 4.23
Ponatinib	239.88	187.50 to 318.42
Dovitinib	~534.56	Very Wide

Fig. 4.

KLK2-FGFR2 fusion is sensitive to FGFR inhibitors.a IC_{50} curves of NIH3T3 Empty and KLK2-FGFR2 cells treated with FGFR inhibitors. $N = 4$ experiments. Selective inhibitors include AZD4547, BGJ398 and JNJ42756493. TAS120 is a selective irreversible FGFR inhibitor. Ponatinib and Dovitinib are multi-kinase inhibitors. **b** IC_{50} values and 95% confidence intervals (CI) are reported for each inhibitor from the curves seen in (a)