Dual activating \textit{FGFR1} mutations in pediatric pilomyxoid astrocytoma

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\textbf{Abstract}

\textbf{Background:} Pilomyxoid astrocytomas are an aggressive subtype of astrocytoma, not graded by WHO, frequently located in hypothalamic/chiasmatic region, affecting diencephalic structures, and characterized by shorter survival and high recurrence rates. Pilomyxoid astrocytoma management remains controversial, with pathologic tissue diagnosis and relief of mass effect being the main goals of surgery while avoiding treatment-related morbidity, including vision loss, panhypopituitarism, and hypothalamic dysfunction. Chemotherapy (typically vincristine and carboplatin) in all pediatric patients and radiation therapy in pediatric patients over 5 years of age are used for treatment.

\textbf{Methods:} We report clinical presentation, surgical management, and whole exome sequencing results in a pediatric patient with the subtotally resected pilomyxoid astrocytoma.

\textbf{Results:} We identified two somatic activating missense mutations affecting \textit{FGFR1}, including \textit{FGFR1} p.K656E and \textit{FGFR1} p.V561M. While the former is a known hotspot mutation that is both activating and transforming, the latter has been described as a gatekeeper mutation imparting resistance to FGFR inhibitors. Interestingly, both mutations were present with similar variant allele frequency within the tumor.

\textbf{Conclusion:} Similar variant allele frequencies of \textit{FGFR1} p.K656E and \textit{FGFR1} p.V561M mutations in our patient's tumor suggest that these mutations may have occurred at similar time points. Use of FGFR inhibitors in addition to STAT3 or PI3K/mTOR inhibition may prove a useful strategy in targeting our patient's pilomyxoid astrocytoma.

\textbf{Keywords} FGFR inhibitor resistance, \textit{FGFR1} mutation, \textit{FGFR1} p.K656E, \textit{FGFR1} p.V561M, pilomyxoid astrocytoma, whole exome sequencing
1 | INTRODUCTION

Pilomyxoid astrocytomas represent an aggressive subtype of astrocytoma originally described in 1999, are characterized by shorter survival and higher recurrence rates than pilocytic astrocytomas, and are not assigned a grade based on the most recent WHO consensus (Brat et al., 2007; Louis et al., 2016; Tihan et al., 1999). These tumors are commonly located in hypothalamic/chiasmatic regions, affecting diencephalic structures (Brat et al., 2007; Komotar, Burger, et al., 2004). Radiographically, pilomyxoid astrocytomas are commonly solid enhancing lesions that can be distinguished from pilocytic astrocytomas based on significantly higher rCBV, as well as dynamic susceptibility contrast perfusion and diffusion weighted imaging, and have a higher incidence of leptomeningeal involvement and spread (Alkonyi et al., 2015; Ho et al., 2020). These tumors have been described in supratentorial locations, and less than 20 have been reported to involve the spinal cord (Almubarak et al., 2019). Histologically, pilomyxoid astrocytomas are cellular monomorphic lesions with angiocentric architecture within myxoid background, containing cells with hyperchromatic pleomorphic elongated nuclei and fibrillary processes, and focally infiltrative into the surrounding brain. From the genomic perspective, these tumors often harbor mutations affecting MAPK pathway (BRAF p.V600E, OMIM#164757) and FGFR signaling (FGFR1 p.N546, FGFR1 p.K656, OMIM#136350), mutations in IDH1 (p.R132, OMIM#147700), IDH2 (p.R172, OMIM#147650), NF1 (OMIM#613113) and PTPN11 (p.E76K, OMIM#176876), BRAF-KIAA fusions due to tandem 7q34 band duplication, and copy number alterations in chromosomes 5, 7 and 11 (Fuller et al., 2010; Jones et al., 2013; Roth et al., 2016; Yde et al., 2016). Several of these alterations, namely BRAF-KIAA fusions and FGFR1 mutations, have been previously reported in thalamic/hypothalamic pilomyxoid astrocytomas to portend poor prognosis (Colin et al., 2013; Fernandez et al., 2003; Jeon et al., 2008; Komotar et al., 2004; Pehlivan et al., 2020). Management of pilomyxoid astrocytomas remains controversial, with diagnosis and relief of mass effect being the main goals of surgery to avoid treatment-related morbidity, including vision loss, panhypopituitarism, and hypothalamic dysfunction; radiation is generally reserved for tumor recurrences and children older than 5 years of age (Goodden et al., 2014; Kano et al., 2009; Komotar, Mocco, et al., 2004; Massimi et al., 2007; Tsugui et al., 2009). Primary surgery has been reported to not result in any significant clinical benefit if pilomyxoid features were present within the tumor, or in children younger than two years of age (Hidalgo et al., 2019). We report a case of a pediatric patient diagnosed with a large suprasellar/chiasmatic pilomyxoid astrocytoma, requiring subtotal resection with a goal of brainstem decompression, followed by chemotherapy. Whole exome sequencing (WES) results of patient’s germline DNA and tumor DNA were performed to identify somatic mutations and copy number variations in the tumor tissue.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

This study was approved by the Human Investigations Committee and the Human Research Protection Program at Yale University. Patient’s blood and tumor tissue were collected after obtaining written informed consent from parents. Tumor specimens were evaluated by the board-certified neuropathologist. Immunohistochemical staining was used to identify the lesion as previously described (Louis et al., 2016).

2.2 | Exome sequencing and data analysis

Genomic DNA from blood and tumor tissue was extracted from formalin-fixed paraffin embedded blocks via standard techniques, and sequenced at the Yale Center for Genome Analysis (YCGA) as previously described. xGEN Exome Research Panel v1.0 kit (Integrated DNA Technologies, Coralville IA) was used to capture coding sequences, which were then sequenced on Illumina NovaSeq6000 whole-exome sequencing platform with 2x100 bp reads. Downstream analysis for the alignment, duplicate marking, realignment, and base quality recalibration was performed in concordance with “GATK Best Practice” recommendations. Somatic single nucleotide variant (SNV), insertion/deletions (INDEL) and copy number variations (CNV) were identified as reported before (Kundishora et al., 2020). Mean coverage of 124.9X was achieved for blood and 121.2X for the tumor tissue. Several somatic mutations were identified, including FGFR1 p.K656E (NM_023110.3:c.1966A>G) and FGFR1 p.V561M (NM_001174064.2:c.1681G>A).

3 | RESULTS

3.1 | Clinical presentation

Patient is an 18-month-old otherwise healthy boy who presented with acute onset nausea, vomiting, and gait instability, resulting in a fall on the day of presentation. On arrival to the ED, vital signs were notable for hypertension BP 119/69 and tachypnea RR 28. On examination, patient was noted to have macrocephaly (head circumference of 51 cm, over 98%ile for age) and unsteady gait with veering to the right while walking. Pertinent imaging findings are shown in Figure 1. Patient underwent
bifrontal craniotomy for subtotal tumor resection and brainstem decompression. Final pathology returned as pilomyxoid astrocytoma, with tumor histology showing a moderately cellular glial tumor characterized by piloid monomorphous cells within a markedly myxoid background, mild nuclear atypia, endothelial hypertrophy (mild vascular proliferation), and absence of Rosenthal fibers and eosinophilic granular bodies (Figure 2). Immunohistochemical staining showed an elevated proliferative index of 5%–8%. Tumor was IDH1 (p.R132H) negative, strongly GFAP positive, EMA negative; the tumor retained nuclear ATRX (OMIM#300032) staining, and less than 2% of tumor cells showed p53 staining. BRAF p.V600E mutation and KIAA1549-BRAF fusion were not detected in the tumor (Figure 2). While not typically described as a part of pilomyxoid astrocytomas, the presence of minimal necrosis in our patient's tumor was likely related to recent hemorrhage within tumor prior to presentation. At the time of discharge, patient was interactive and watching TV, moderate verbal output, full strength on the right side, and able to squeeze fingers in left hand and moving left leg with antigravity strength.

Pediatric hematology oncology team recommended repeat MRI brain total spine w wo contrast at 2 weeks post-operatively; MRI brain showed expected residual, MRI spine showed no evidence of metastatic disease. Port was placed for initiation of chemotherapy (vincristine and carboplatin) tentatively planned to start 1 month after surgery, with possible need for proton beam radiation upon recurrence. LP was completed for staging, with no evidence of metastatic disease. Over time, patient exam continued to improve, including speaking in short sentences, feeding self with spoon and fork, using straw and sippy cup, standing independently, walking with left AFO with hip support, using left shoulder and elbow. He has tolerated chemotherapy 4 weeks on, 2 weeks off and is currently on maintenance cycle 2 week 4 of carboplatin and vincristine without evidence of progression for over 6 months post resection, while remaining on Keppra for seizure prophylaxis, Synthroid for hypothyroidism, hydrocortisone for adrenal insufficiency, and ddAVP for diabetes insipidus.

3.2 | Genomic analysis

Whole exome sequencing identified a total of 15 somatic mutations, including nine missense mutations. Interestingly,
we identified two activating mutations affecting FGFR1, including **FGFR1** p.K656E (NM_023110.3:c.1966A>G) and **FGFR1** p.V561M (NM_001174064.2:c.1681G>A) (Table S1). Variant allele frequencies for **FGFR1** p.K656E and **FGFR1** p.V561M mutations were 38.9% and 31.4%, respectively. Somatic CNV analysis together with the loss-of-heterozygosity (LOH) analysis did not reveal any amplification/deletion or LOH events, including focal amplifications and deletions such as the ones seen frequently in **CDKN2A** (OMIM#600160) deletions. Germline analysis was unrevealing.

4 | DISCUSSION

The FGFR family includes four transmembrane tyrosine kinase (TK) receptors FGFR1-4 as well as FGFR1/FGFR5 that does not have a TK domain, all highly evolutionarily conserved, activated by FGF ligands 1–18 that bind extracellular immunoglobulin-like domains, and involved in cell survival, migration, differentiation, and fate, as well as angiogenesis, embryonic development and tumorigenesis (Turner & Grose, 2010). FGFR is structurally homologous to PDGFR, VEGFR, and other TKRs (Hubbard & Till, 2000). Upon ligand binding and receptor dimerization, FGFR kinase domains undergo transphosphorylation, resulting in the downstream activation of MAPK, PI3K/AKT, STAT, and PLCγ pathways. FGFR1, specifically, is highly expressed and thought to drive proliferation of multipotent stem cells in the subventricular zone of the developing human brain (Fu et al., 2003). Concordantly, midline pilocytic astrocytomas involving thalamus or brainstem and harboring **FGFR1** mutations are thought to originate from the subventricular zone (Jones et al., 2013). Spontaneous hemorrhage has been reported in up to 24% of pilomyxoid astrocytoma cases (Ishi et al., 2020; Karthigeyan et al., 2019; Linscott et al., 2008). Recent studies show an association of **FGFR1** mutations with spontaneous hemorrhage in low grade gliomas in pediatric patients (Ishi et al., 2020); it is curious that our patient had intralesional hemorrhage on presentation. Although the
mechanism remains unclear, it does not appear to be related to Ki67 proliferation index or microvascular proliferation, but is potentially mediated by FGFR1 effects other than the MAPK pathway (Kartheigeyan et al., 2019; Linscott et al., 2008). The mutation we identified, FGFR1 p.K656E affecting tyrosine kinase domain of FGFR1 is a gain-of-function hotspot mutation that is both activating (i.e., resulting in constitutively active receptor signaling) and transforming (Hart et al., 2000), and has been previously reported in neuroepithelial tumors, rosette forming glioneural tumors and multiple other cancers within COSMIC database (Forbes et al., 2017; Gessi et al., 2014; Helsten et al., 2016; Jones et al., 2013; Rivera et al., 2016). Another mutation we identified, FGFR1 p.V561M, has not been described in primary brain tumors. However, this mutation has been previously described in stem cell leukemia/lymphoma syndrome (SCLL) patients as one of the mechanisms of FGFR inhibitor resistance, and has been shown to impart FGFR1 inhibitor resistance via activation of STAT3 signaling in patients with non-small-cell lung cancer(Cowell et al., 2017; Ryan et al., 2019).

Presence of multiple activating mutations within the same gene in a given tumor is uncommon, but has previously been reported in adult patients with EGFR driven gliomas (Bent et al., 2015; Eskilsson et al., 2018; Felsberg et al., 2017). There has been a recent report of an FGFR1 p.K656E mutation in a patient with a dysmyeloplastic neuroepithelial tumor (DNET) that was in cis with a germline FGFR1 p.R661P mutation, confirmed by in silico modeling (Rivera et al., 2016). Curiously, analysis of additional DNETs showed a high incidence of 55.5% (10/18) of multiple FGFR1 mutations in patients with DNETs driven by sporadic FGFR1 mutations, with 9 of 10 multiple mutants in cis (Rivera et al., 2016). Although our patient’s lesion does not harbor the same FGFR mutations and germline analysis was unrevealing, there may be a similar relationship or mechanism with the above mutations in DNETs. The mechanism of acquisition of multiple FGFR1 mutations remains unclear. Interestingly, variant allele frequencies for FGFR1 p.K656E and FGFR1 p.V561M in our patient's tumor were similar, indicating that these mutations may have occurred at similar timepoints. Our patient has not had any prior radiation or chemotherapy as evolutionary pressures to develop a second driver mutation in his lesion. Such pressure, however, could represent the preponderance of various mutations to activate different downstream pathways depending on the mutation type, for example, by varying affinity for secondary adapter proteins to activate PI3K versus MAPK pathways, or the ability to preferentially directly activate STAT versus PLCγ pathways depending on the type of mutation, resulting in dose-related effects. Rivera et al propose a mechanism whereby presence of multiple mutations results in balanced signaling, requiring multiple enabling mutations and resulting in benign tumor behavior (Rivera et al., 2016). A similar mechanism of enhanced oncogenicity resulting from combinations of functionally weak, infrequent mutations has been supported by recent pan-cancer analysis (Saito et al., 2020). This does not appear to be the case in our patient’s tumor, where one of the FGFR mutations is potently activating and transforming, and the other is sufficient to confer FGFR inhibitor resistance. Double FGFR mutations were reported in multiple developmental disorders including Crouzon, Pfeiffer and Kallman syndromes and achondroplasia, and their phenotypic severity appears proportional to FGF signaling in a dose-dependent manner (Goriely et al., 2005; Passos-Bueno et al., 1999; Turner & Grose, 2010). Of note, while in developmental disorders FGFR mutations are distributed along the entire FGFR gene, FGFR mutations associated with brain lesions tend to involve known hotspots, resulting in gain-of-function FGFR mutations (Goriely et al., 2005; Passos-Bueno et al., 1999; Turner & Grose, 2010).

Due to its widespread involvement in a variety of cancers including uterine, breast, endometrial, squamous lung, and ovarian affecting a significant number of patients, the ability to target FGFR is clearly beneficial (Dutt et al., 2011; Gozgit et al., 2012; Helsten et al., 2016; Weiss et al., 2010). A variety of TK inhibitors are available to inhibit FGFR and have been tested in preclinical and clinical trials including dovitinib (TKI258), lenvatinib (E7080), lucitanib (E3810), nintedanib (BIBF 1120), ponatinib (AP24534), regorafenib (BAY 73-4506), and others, including selective FGFR inhibitors NVP-BGJ398, AZD4547, JNJ-42756493; several are currently FDA approved for cancer treatment (ClinicalTrials.gov. U.S., 2020; Helsten et al., 2016). FGFR TKIs normally compete with ATP for the active binding site, their specificity dependent on interactions with the neighboring hydrophobic region guarded by the gatekeeper residue. Using TKIs is plagued with developing resistance, often as a result of acquiring gatekeeper residue mutations to a larger residue blocking access of the inhibitor to the hydrophobic pocket, thus effectively precluding TKI binding, while still allowing for ATP to bind (Azam et al., 2008; Sohl et al., 2015). Gatekeeper mutations in EGFR, PDGFR, Src, and cABL affect kinase activity and can cause cell transformation (Dudka et al., 2010). One of the two mutations identified in our patient, FGFR1 p.V561M, has been shown to impart resistance to FGFR1 inhibitor AZD4547 via activation of STAT3 with cellular transformation toward a mesenchymal phenotype, thus resulting in epithelial-mesenchymal transition (EMT) associated with increased cell proliferation, migration, invasion and anchorage-independent growth (Ryan et al., 2019). Several STAT3 inhibitors including AZD9150, napabucasin, TTI-101 are now being tested in clinical trials, and could be beneficial to treat our patient as a combination therapy with FGFR1 inhibitors (ClinicalTrials.gov. U.S., 2020; Yang, Lin, et al., 2019). Ryan et al. (2019) propose that gatekeeper mutations may exist as germline mutations increasing individual
susceptibility to cancer in general, or be present at baseline in a subset of tumor cells, possibly cancer stem cells. The tumor VAF for FGFR1 p.K656E and FGFR1 p.V561M mutations in our patient are similar, suggesting that this is not the case. Cowell et al propose that in stem cell leukemia/lymphoma syndrome (SCLL) patients FGFR inhibitor resistance can result from either FGFR1 p.V561M mutation or PTEN inactivation, with ectopic PTEN expression restoring sensitivity to FGFR inhibition, and therefore treatment with PI3K inhibitors showing additive effects on growth and survival with FGFR inhibition in vitro and in vivo (Cowell et al., 2017). Thus, inhibition of PI3K pathway is another feasible target as a combination therapy with FGFR1 inhibition in our patient; dual PI3K/mTOR inhibitors are available (ClinicalTrials.gov. U.S., 2020; Yang, Nie, et al., 2019).

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CONFLICT OF INTEREST

The authors report no conflict of interest in relation to the materials or methods used in this study, or the findings specified in this paper.

DATA AVAILABILITY STATEMENT

All data available upon request.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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