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Histological features in pediatric central nervous system tumors with *FGFR* alterations

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Abstract

Introduction: *Identification of genetic alterations in central nervous system (CNS) tumors provides diagnostic and prognostic information and allows identification of potential therapeutic targets. Next-generation sequencing (NGS) technologies currently used for molecular testing are costly and remain largely limited to major academic centers or reference labs. Identification of histologic or immunohistochemical correlates for particular molecular alterations can serve as surrogates and can help triage cases for subsequent NGS-based confirmation. Recently, adult IDH-wildtype adult glioblastomas (GBMs) with fibroblast growth factor receptor (FGFR) gene alterations were reported to show palisading monomorphic cells, delicate arcuate vasculature, and microcalcifications. We explored whether pediatric tumors with FGFR fusion also show these histologic features and whether these features could predict the presence of this gene alteration.*

Material and methods: *We reviewed pediatric CNS tumors with FGFR-fusions to retrospectively determine the presence/absence of the above-mentioned histological features in fusion-positive tumors.*

Results: *10 pediatric tumors with FGFR fusions were identified. Pediatric tumors demonstrated histologic and tumor type diversity, with diagnoses of pilocytic/pilomyxoid astrocytoma, pediatric-type oligodendroglioma, anaplastic astrocytoma, polymorphous low-grade neuroepithelial tumor of the young, rosette-forming glioneuronal tumor, and extraventricular neurocytoma.*

Conclusions: *Pediatric FGFR-fused CNS tumors demonstrate histologic features similar to their adult counterparts but also exhibit significant morphologic variability. As such, this histologic variability prevents the prediction of FGFR fusion and necessitates molecular testing for the identification of this alteration.*

Key words: *FGFR, TACC, polymorphous low-grade neuroepithelial tumor of the young (PLNTY), glioneuronal, astrocytoma, fusion testing, next-generation sequencing.*

Introduction

In 2016, the World Health Organization (WHO) Classification of Central Nervous System (CNS) Tumors adopted an integrative diagnostic approach incorporating molecular parameters into the classification of CNS tumor entities [14]. Molecular characteristics are likely to play an even more prominent role in the upcoming WHO classification with the possible introduction of several new categories of tumors defined by their molecular signature. In addition to aiding diagnosis, identification of genetic alterations also provides valuable prognostic infor-

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mation and, most importantly, can help in the identification of targeted therapy.

Next-generation sequencing (NGS) technologies are currently the most widely used for identification of molecular alterations in routine clinical practice but require high upfront infrastructure investment and have substantial running costs. The availability of NGS-testing is consequently still largely limited to large academic centers or reference labs. Identification of histologic or immunohistochemical (IHC) correlates can serve as surrogates for molecular alterations and can help triage cases for subsequent NGS-testing in select cases. This strategy has proved useful for *IDH*-mutant 1p19q-codeleted oligodendroglioma and *BRAF V600E*-positive epithelioid glioblastoma (GBM), for example [14]. In these tumors, careful histomorphologic examination and IHC studies can reliably select cases likely to harbor the molecular alteration. These can then be confirmed or disproved on targeted molecular testing [14]. Excellent molecular-histologic correlation also exists between *BRAF:KIAA1549* fusion and pilocytic astrocytoma and between *MYB:QKI* and angiocentric glioma. Many other tumors, however, show poor molecular-histologic correlation. Hence, for each tumor type, it is important to determine if and to what extent molecular-histological correlation exists. If such a correlation is high, it can assist the pathologist in triaging cases for confirmatory molecular testing [13].

Molecular-histologic correlation was recently demonstrated in a subset of adult GBMs when it was shown that adult *IDH*-wildtype adult GBMs with fibroblast growth factor receptor (*FGFR*) gene alterations show palisading monomorphic cells, delicate arcuate vasculature, and microcalcifications [3]. This was confirmed by several subsequent studies [1,2]. Whether *FGFR*-altered tumors show histomorphologic similarity outside of the adult *IDH*-wildtype group is less clear. In this study, we explore whether the histologic features reported for *FGFR*-altered *IDH*-wildtype adult GBMs are shared by pediatric tumors with *FGFR* fusion and if these characteristics can be used to predict *FGFR* fusions in individual cases.

FGFR signaling regulates a variety of cellular pathways including cell proliferation, differentiation, and survival. *FGFRs* form a family of four highly conserved transmembrane receptor tyrosine kinases (*FGFR1-4*), and alterations in 3 *FGFR* genes *(FGFR1/FGFR2/FGFR3*) have been reported in a variety of pediatric and adult tumors [4,7,16,17]. Recently, a subset of adult *IDH*-wildtype GBMs was found to harbor gene fusion events involving *FGFR1/FGFR3* with the transforming acidic coiled-coil (*TACC*) domains of *TACC1* or *TACC3* genes [3,5,19]. Recognition of this alteration carries particular clinical significance as inhibitors of *FGFR*s have recently been developed and these could represent a promising therapeutic option for patients with *FGFR* alterations [5]. Studies have shown that inhibitors of kinase activity can block tumor growth in a preclinical model of gliomas with the *TACC3:FGFR3* fusion and clinical response has been documented in a few patients [5]. The *TACC* gene family includes 3 individual genes, including *TACC1, TACC2*, and *TACC3*. *TACC* domains promote dimerization and constitutive activation of *FGFR* leading to hyperphosphorylation and constitutive activation of the kinase domain promoting oncogenesis [15,19,23].

Characteristic histologic features reported for *FGFR3:TACC3* gliomas in adults include a monomorphic population of small tumor cells with ovoid nuclei, perivascular pseudorosettes, microcalcifications, nuclear palisading, and/or an endocrinoid ("chicken-wire") capillary network [3]. All cases show absence of cytoplasmic *IDH1 R132H* immunostaining, low p53 nuclear immunolabelling and retained nuclear ATRX expression. The most common cytogenetic alterations include: gain of chromosome 7p/loss of chromosome 10q, absence of *EGFR* amplification except in rare cases [3,5,9], and frequent *CDKN2A* homozygous deletion [5,9]. Thus morphology and routine cytogenetic tests can provide clues to the presence of *FGFR3* fusions in adult gliomas, the majority of which are GBMs, *IDH*-wildtype, WHO grade IV [3]. This correlation between histology and the presence of *FGFR3:TACC3* alteration is not perfect and molecular testing still needs to be performed to confirm the fusion. While the vast majority of these tumors feature an *FGFR3*:*TACC3* fusion, rare cases showing *FGFR3* fusion with a non-*TACC3* gene partner (such as *CAMK2A*) have also been reported [9].

Various other pediatric and adult tumors have been reported to have *FGFR* fusions [4,7,17]. While a majority of pilocytic astrocytoma (PA) show *BRAF* fusions or mutations, a small proportion feature *FGFR* fusions [12,16,18,21]. *FGFR1:TACC1* fusion is a frequent event in extraventricular neurocytoma and has been found in up to 60% of molecularly-defined cases [22] with a smaller proportion of cases

showing *FGFR3:TACC3* alteration [22], as recently reviewed [1]. Similarly, the recently described tumor termed polymorphous low-grade neuroepithelial tumor of the young (PLNTY) also features *FGFR2* and *FGFR3* fusions in a large percentage of cases (still other cases are positive for BRAF *V600E* mutation) [11]. It is noteworthy that many of the histological features described by Bielle *et al*. [3] in the adult high-grade gliomas with *FGFR* fusion, such as uniformly small rounded nuclei with conspicuous perinuclear halo, perivascular pseudorosetting, nuclear palisading and calcifications, are also seen in PLNTY, although these are exclusively low-grade tumors [11]. Finally, it is important to realize that *FGFR* fusions are not exclusive to the central nervous system tumors since *FGFR3-TACC3* fusions have been found in non-CNS malignancies including urothelial, breast, endometrial lung and ovarian cancers [10].

We noted that no study has undertaken a direct analysis of the histologic features of *FGFR*-fused CNS tumors in the pediatric population. The purpose of this study is to, for the first time, directly compare histological features of pediatric tumors with *FGFR* fusions and ask whether identification of these histological features might predict which tumors would show the highest yield in terms of identification of fusions and thus allow for triaging of cases for molecular testing of the fusion status, since such testing can be costly, time consuming, and may require send out to a reference laboratory.

Material and methods

We identified CNS tumors with *FGFR* gene alteration *via* a retrospective database review of all pediatric patients in the Department of Pathology databases at Children's Hospital Colorado, 2010-present. Fusion testing became available at our institution in 2016; however, some older cases underwent testing at the clinical team's request. For the vast majority of cases (9 out of 10 cases), the initial surgery and subsequent management had been performed at our institution. One case had been biopsied at outside institutions and reviewed at our department for diagnostic consultation. Standard H&E stained sections were examined and the WHO 2016 classification of central nervous system tumors criteria were applied for all diagnoses, with the exception of PLNTY, which followed the diagnostic criteria outlined by Huse *et al*. [11].

Table I. List of genes tested in ArcherDx FusionPlex Solid Tumor Panel

AKT3	*EWSR1*	*NOTCH1*	*PRKCA*
ALK	*FGFR1*	*NOTCH2*	*PRKCB*
ARHGAP26	*FGFR2*	*NRG1*	*RAF1*
AXL	*FGFR3*	*NTRK1*	*RELA*
BRAF	*FGR*	*NTRK2*	*RET*
BRD3	*INSR*	*NTRK3*	*ROS1*
BRD4	*MAML2*	*NUMBL*	*RSPO2*
EGFR	*MAST1*	*NUTM1*	*RSPO3*
ERG	*MAST2*	*PDGFRA*	*TERT*
ESR1	*MET*	*PDGFRB*	*TFE3*
ETV1	*MSMB*	*PIK3CA*	*TFEB*
ETV4	*MUSK*	*PKN1*	*THADA*
ETV5	*MYB*	*PPARG*	*TMPRSS2*
ETV6			

The Archer assay includes gene-specific primers to various exons (and some introns) in the above 53 genes and simultaneously detects and identifies fusions and other mutations associated with the listed genes.

Fusion testing was performed by the Colorado Molecular Correlates (CMOCO) Laboratory in the Department of Pathology at the University of Colorado Denver (UCD). Extracted nucleic acid samples were assessed using the *ArcherDx FusionPlex* (ArcherDx, Boulder, CO) Solid Tumor library preparation kit followed by sequencing on the Illumina platform [8,13]. This assay uses proprietary Anchored Multiplex PCR (AMP)-based library preparation to detect oncogenic gene isoforms and gene fusions regardless of the identity of the fusion partner. All cases were also tested on *ArcherDx VariantPlex* (ArcherDx, Boulder, CO) solid tumor panel which tests for mutations in 69 genes, including *IDH1* and *IDH2*. A complete list of genes covered on the fusion and mutational assay has been published previously [8] and provided as Tables I and II.

Clinical data for all cases were collected by review of the patient's electronic medical records on EPIC (Epic Systems Corporation, Verona, WI) and included the age, sex, clinical presentation, pre and post-surgical imaging findings, tumor location, extent of surgical resection, treatment modalities used, and survival.

Results

Ten cases of pediatric/young adult (less than 21 years of age) CNS tumors with FGFR alterations were identified. Of these cases, 6 involved *FGFR1* fusions or tyrosine kinase domain (TKD) duplica-

Table II. List of genes tested in ArcherDx VariantPlex Solid Tumor Panel

ABL1	*AKT1*	*ALK*	*APC*
AR	*ATM*	*AURKA*	*BRAF*
CCND1	*CCNE1*	*CDH1*	*CDK4*
CDKN2A	*CDKN2B*	*CSF1R*	*CTNNB1*
DDR2	*EGFR*	*ERBB2*	*ERBB3*
ERBB4	*ESR1*	*EZH2*	*FBXW7*
FGFR1	*FGFR2*	*FGFR3*	*FLT3*
FOXL2	*GNA11*	*GNAQ*	*GNAS*
H3F3A	*HNF1A*	*HRAS*	*IDH1*
IDH2	*JAK2*	*JAK3*	*KDR*
KIT	*KRAS*	*MAP2K1*	*MDM2*
MET	*MLH1*	*MPL*	*MYC*
MYCN	*NOTCH1*	*NPM1*	*NRAS*
PDGFRA	*PIK3CA*	*PIK3R1*	*POLE*
PTEN	*PTPN11*	*RB1*	*RET*
RHOA	*ROS1*	*SMAD4*	*SMARCB1*
SMO SRC	*STK11*	*TERT*	*TP53*
VHL			

tions, 1 pediatric patient had *FGFR2* fusion and 3 cases involved *FGFR3* gene alterations. Fusion partners of *FGFR* genes included *TACC1*, *TACC3*, *KIAA1598*, *THAP10* and *INA* genes. Clinicopathologic findings and fusion events are summarized in Table III.

CNS tumors with *FGFR* fusions were negative for co-occurring fusion or mutation events tested on our panels.

The pediatric CNS tumors harboring *FGFR* fusions in our cohort possessed more diverse histologic and molecular features than those reported for adult high-grade gliomas with tumors. In addition to the 3 *FGFR3* fusion cases (with 3 different fusion partners, namely: *TACC3*, *INA* or *THAP10*), 6 cases of *FGFR1* structural alterations (3 with *FGFR1:TACC1* fusion, and 3 with TKD alterations) and 1 case of *FGFR2:KIAA1598* were found. These findings are summarized in Table III. Histologic and radiologic features were similarly varied with cases carrying histologic diagnoses of extra ventricular neurocytoma ($n = 1$), pilomyxoid astrocytoma ($n = 2$), pilocytic astrocytoma ($n = 1$), PLNTY ($n = 2$), diffuse astrocytoma ($n = 1$), pediatric-type oligodendroglioma ($n = 1$), anaplastic astrocytoma with Li Fraumeni syndrome (LFS) ($n = 1$) and rosette-forming glioneuronal tumor ($n = 1$) (Table III). In contrast to the adult cases with *FGFR* fusions, all of which are reported to be GBM, *IDH*-wild-type, WHO grade IV [3], 9/10 pediatric cases showed low-grade glioma/glioneuronal histology, with one showing anaplastic astrocytoma histology (Table III).

Despite the diversity in histologic diagnosis, there were unifying features. Pediatric cases in our cohort showed frequent nuclear palisading (Fig. 1A-C), small monomorphic round cells (Fig. 1D-F), frequent calcifications (Fig. 2A-C), thin vasculature (Fig. 2D-F), and a low-grade histology without mitotic figures or elevated MIB-1 (Ki-67) staining (except in case 9, an anaplastic astrocytoma in LFS).

Retrospective review of the histologic features showed four distinct histologic groups as described below.

The first group ($n = 3$) showed pilocytic or pilomyxoid histology with tumor cells manifesting round to oval nuclei and long fibrillary/piloid processes, prominent perivascular arrangement was seen in two cases with absence of Rosenthal fibers or EGBs – hence resembling pilomyxoid astrocytoma (Fig. 1A,B) – and one case was negative for perivascular tumor cell arrangement and positive for Rosenthal fibers (Fig. 1D) – hence consistent with pilocytic astrocytoma. While 2 of these cases showed no recurrence, case 3 with an *FGFR1 exon 18:10* fusion has died of disease. In this case, the resection was incomplete owing to the presence of the tumor in an eloquent region (suprasellar/hypothalamic) and the surgery being complicated by parenchymal hemorrhage and brain damage. Whether the poor outcome was due to the *FGFR1 exon 18:10* fusion or the sensitive location/incomplete surgical resection remains unclear.

The second group ($n = 2$) consisted of glioneuronal tumors with extensive Cluster of Differentiation 34 (CD34) staining. Both of these cases had small round oligodendroglia-like cells with thin arcuate blood vessels (Figs. 1E, 2A), microcalcifications (Fig. 2A, arrow), and patchy strong CD34 staining, features that are suggestive of PLNTY (Fig. 1E, inset). One of these cases showed *FGFR3:TACC3* and the other *FGFR2:KIAA1598* fusion, similar to what has been previously reported for PLNTY [11]. None of these cases has shown a recurrence in 9-13 months of post-resection follow up.

The third group ($n = 3$) showed an infiltrative histology (Figs. 1F, 2C-E). These cases had round or oval oligodendrocyte-like or astrocytic cells with variable perinuclear halos (Fig. 1F, 2C, D) and thin arcuate vasculature (Fig. 2D) with one case additionally showing calcifications (Fig. 2C, arrow). Unlike group 2, no CD34 staining was seen in these cases, ruling out PLNTY.

Table III. Clinical and pathologic features of patients with central nervous system (CNS) tumors harboring FGFR structural alterations

Case #	Age (yrs)	Sex	Molecular findings	Imaging findings	Histologic diagnosis	Clinical follow up	Follow up interval
Case 1	1	F	*FGFR1 exon 19:10 repeat*	Diffusely infiltrating tumor involving bilateral basal ganglia, optic pathways, septum pellucidum, medial left temporal lobe, and brainstem	Diffuse astrocytoma	Died of disease, rapid progression of invasive tumor	15 mth
Case 2	1	M	*FGFR1:TACC1*	Solid and cystic cervical spinal cord tumor	Pilomyxoid astrocytoma	Negative for tumor recurrence	3 yrs
Case 3	2	F	*FGFR1 exon 18:10*	Large suprasellar/ hypothalamic mass extending into the right frontal lobe, third ventricle, interpeduncular and prepontine cistern	Pilomyxoid astrocytoma	Died of disease; incomplete resection of tumor followed by intratumoral and parenchymal hemorrhage and subsequent brain damage	9 mth
Case 4	4	M	*FGFR1 exon 19:10 repeat*	Left thalamic tumor extending into internal capsule, caudate, globus pallidus, midbrain and the medial left temporal lobe	Pediatric-type oligodendroglioma	Died of disease, progressive interval growth of tumor	24 mth
Case 5	6	F	*FGFR2:KIAA1598*	Right insular cortex cystic and solid mass with haziness to the subjacent white matter	PLNTY	No residual tumor	13 mth
Case 6	9	M	*FGFR1: TACC1*	Acute bleeding at presentation with hyperdense mass of posterior temporal lobe	Extraventricular neurocytoma	Negative for tumor recurrence	25 mth
Case 7	11	F	*FGFR3:TACC3*	Medial right temporal lobe heterogeneous mass with numerous small cystic components	PLNTY	Negative for tumor recurrence	9 mth
Case 8	16	F	*FGFR1: TACC1*	Midbrain, dorsal tegmentum and tectum non-enhancing mass	Pilocytic astrocytoma	Negative for tumor recurrence	26 mth
Case 9	19	M	*FGFR3:INA*	Right frontal lobe non-enhancing lesion with interval growth first identified on screening MRI	Anaplastic astrocytoma in LFS	Negative for tumor recurrence	7 yrs
Case 10	21	F	*FGFR3:THAP10*	Intraventricular mass, right frontal horn of lateral ventricle	Rosette-forming glioneuronal tumor	Negative for tumor recurrence	8 yrs

mth – months, yrs – years, PLNTY – polymorphous low-grade neuroepithelial tumor of the young, LFS – Li Fraumeni syndrome

Two of these cases showed *FGFR1 exon 19:10 repeat* fusion and one showed *FGFR3:INA* fusion; all were *IDH1/2* wildtype (tested by IHC as well as mutational analysis) and negative for 1p/19q codeletion (tested by FISH). One of these cases (case 9) occurred in the context of known history of Li-Fraumeni syndrome and had frankly anaplastic histology. The third case in this group was diagnosed as a pediatric-type oligodendroglioma and was confirmed to be *IDH*-wild-type without 1p/19q codeletion.

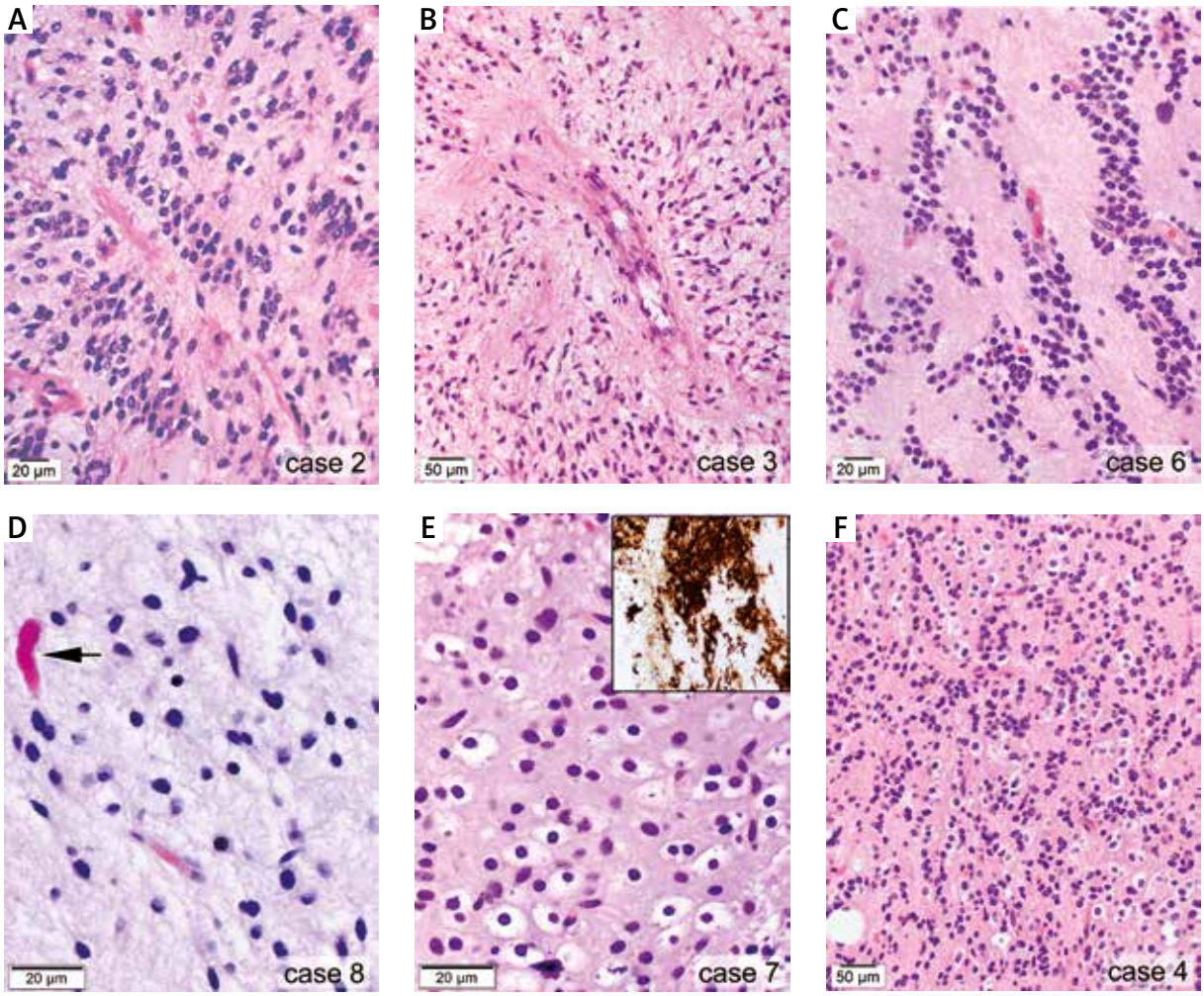

Fig. 1. FGFR altered pediatric central nervous system (CNS) tumors show monomorphic round cells and nuclear clustering: despite histologic variability, majority of cases showed monomorphic round cells and nuclear clustering. **A-C**) 6 cases with histologic diagnoses of pilomyxoid astrocytoma (**A, B**), extraventricular neurocytoma (**C**), pilocytic astrocytoma (**D**), PLNTY (**E**) and pediatric-type oligodendroglioma (**F**) all showing tumor cells with small round monomorphic nuclei. Nuclear palisading/clustering and perivascular arrangement in tumors with histologic diagnoses varying from pilomyxoid astrocytoma (**A-B**) and extraventricular neurocytoma (**C**). **D**) Arrow highlights a Rosenthal fiber. **E**) Inset shows patchy strong CD34 staining supporting the diagnosis of PLNTY.

Finally, the last group consisted of unique cases (n = 2). One case showed neurocytic rosettes with small round (NeuN+) cells arranged around neurocytic rosettes (Figs. 1C, 2F) and surrounded by microcalcification (Fig. 2B). This case showed *FGFR1:TACC1* fusion and was diagnosed as extra ventricular neurocytoma. The last case (case 10) was consistent with rosette-forming glioneuronal tumor.

It is not clear whether *FGFR* fusion status effects prognosis in tumors independent of histologic features [18]. Anecdotally, however, we do note that in our small cohort of 10 pediatric examples, 3 patients with *FGFR1:exon 18 or exon 19* fusions, involving the thalamus, hypothalamus and basal ganglia, died of disease (cases 1, 3 and 4), while the rest are free of disease (Table III). Whether this is due to the surgically sensitive/eloquent nature of midline tumors or the presence of the *FGFR1:exon 18 or exon 19* fusion is unclear.

In summary, most but not all *FGFR* fused tumors in the pediatric population show at least some characteristic histologic features including small mono-

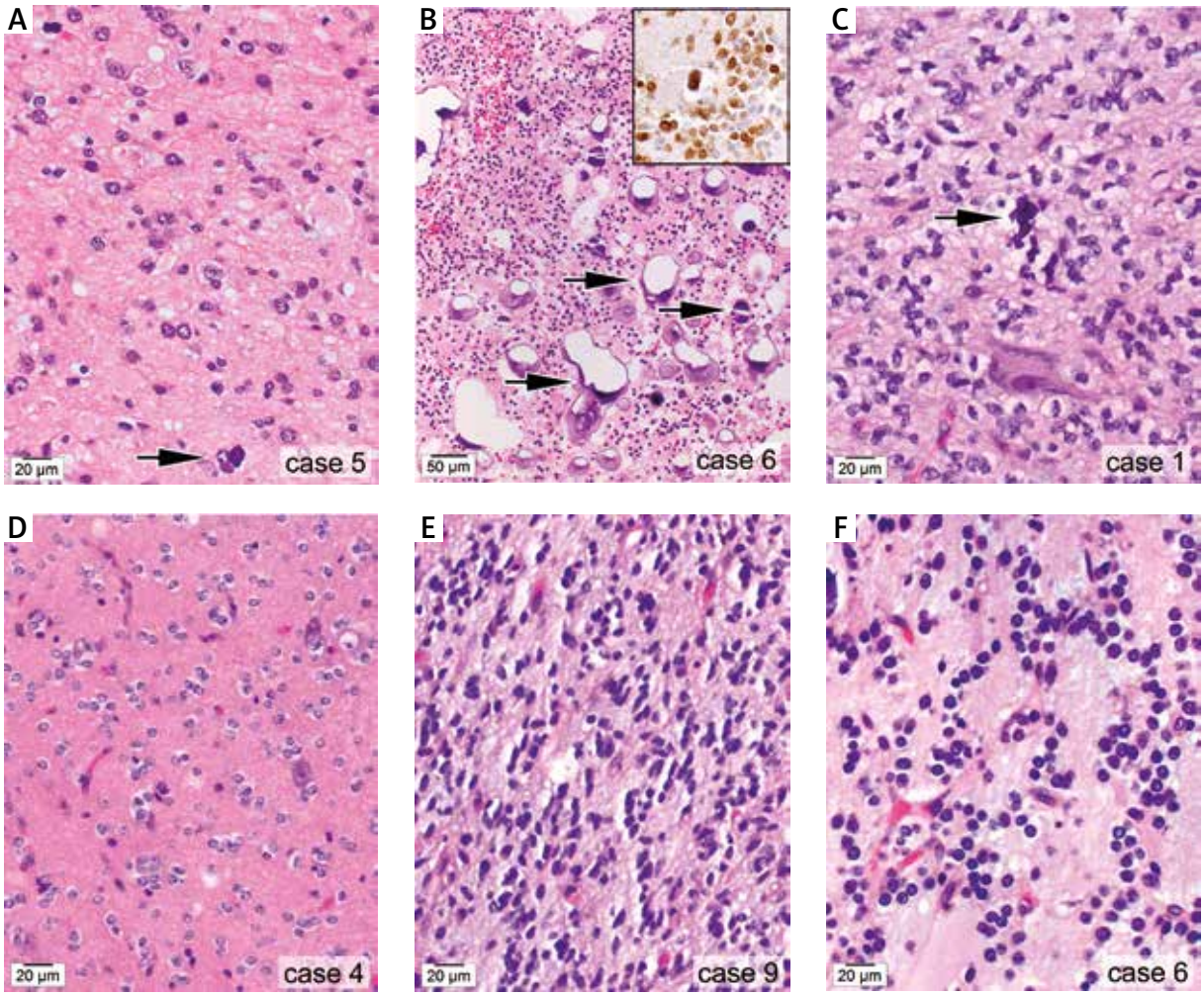

Fig. 2. FGFR altered pediatric central nervous system (CNS) tumors show frequent microcalcifications and delicate arcuate vasculature: **A-C**) Microcalcifications (arrows) were frequently seen in cases diagnosed as PLNTY (**A**), extraventricular neurocytoma (**B**), diffuse astrocytoma (**C**). Inset in panel B shows NeuN staining confirming the neurocytic differentiation in the extraventricular neurocytoma. **D-F**) thin delicate arcuate capillary network in cases with histologic diagnosis of pediatric-type oligodendroglioma (**D**), anaplastic astrocytoma in LFS (**E**) and extraventricular neurocytoma (**F**). **D**) Note the large entrapped neurons with normal cytological appearance and even placement of Nissl substance, excluding consideration of ganglioglioma.

morphic cells, fine arcuate vasculature, and microcalcification, histologic features that are reported for adult high-grade gliomas with *FGFR3:TACC3* fusion.

Discussion

In this study, we report the histological features of pediatric CNS tumors with *FGFR* fusion, adding to the growing literature that these are mostly low-grade glial and glioneuronal tumors. We determine that although the histological features reported by Bielle *et al*. [3] in adult *IDH*-wildtype GBMs are present to some degree in pediatric tumors, they are far from uniform and not archetypal enough to allow histological prediction of the fusion for adult gliomas and especially not for pediatric CNS tumors.

Pediatric CNS tumors with *FGFR* fusions, in contrast to adult high-grade gliomas with this fusion [3], are a more histologically and molecularly heterogeneous cohort, as we and others [4,13] have shown. Thus, the fusion status is more difficult to predict, although certain histological features shared with their *FGFR*-fused adult counterparts do exist that

can aid in prompting molecular testing. In contrast to adult examples with this fusion [3], pediatric examples have almost exclusively been low-grade, yet, as we have demonstrated, still share overlapping morphological features of monomorphic nuclei, microcalcifications, nuclear palisading, and arcuate vasculature. These features are shared by several types of low-grade tumors and thus result in more diverse diagnoses, such as diffuse astrocytoma, anaplastic astrocytoma, pilocytic and pilomyxoid astrocytoma, PLNTY, neurocytoma, and rosette-forming glioneuronal tumor.

Thus, in pediatric tumors, while we show that *FGFR* fusions can be suspected based on these histological features in a variety of different WHO 2016 diagnoses, nevertheless, fusion testing is recommended in all pediatric tumors, given the possibility of identifying therapeutically targetable alterations in this age group in order to eschew use of more toxic chemo- and radiotherapies [13]. Of note, while the tumor types in our cohort were mostly low-grade and thus more aggressive therapies are often not necessary initially after first resection, unfavorable anatomical location may lead to the inability to achieve significant surgical resection and continued tumor growth may contribute to progressive symptomatology at a later time period. Often, some therapy becomes necessary during the course of the disease. Thus, similar to our work with gangliogliomas of the brainstem with BRAF *V600E* mutation, making them amenable to targeted therapy [6], the situation may arise that targeted therapy is also necessary in *FGFR* fusion-bearing low-grade pediatric tumors in unfavorable anatomical locations. Indeed, although the number of cases in our study is small, we did observe anecdotally that the only deaths in our cohort of 10 pediatric patients were in those tumors that were located in unfavorable/eloquent anatomical locations (Table III).

Within our cohort, the fusions we encountered parallel those in the literature, as reviewed by Bale [1]. Specifically, the cases of PLNTY showed *FGFR3-TACC3* or *FGFR2-KIAA1598* fusion. Both of these fusions (in addition to cases showing *FGFR2-CTNNA3* fusion or BRAF *V600E* mutation) have been reported previously in PLNTY [11]. The extraventricular neurocytoma similarly showed *FGFR1:TACC1* fusion which is the most common alteration reported in this tumor [22]. Finally, the single case of rosette-forming glioneuronal tumor in our cohort showed *FGFR3:THAP10* fusion. It is noteworthy that the largest case series of this rare tumor reported *FGFR1* hotspot mutations in all cases with a majority exhibiting co-occurring *PIK3CA* and/or *NF1* gene mutations [20]. Given the similarities between *FGFR1* and *FGFR3* genes and a shared downstream signaling pathway, our findings are consistent with those of Sievers and colleagues showing activation of mitogen-activated protein kinase (MAPK) pathway in this tumor [20]. We further note that *FGFR* fusions in our study occurred in the absence of any other identifiable fusion or mutation events (for a complete list of genes tested, see Tables I and II).

A recent paper, published during the preparation of this manuscript, shows that the presence of *FGFR* alterations, mostly *FGFR1-TACC1* fusion, in pediatric posterior fossa pilocytic astrocytomas correlate with oligodendroglial morphology, namely small monomorphic cells with round and partly hyperchromatic nuclei, perivascular halos, and calcifications [21]. This and other studies suggest the presence of shared histologic features in *FGFR*-fused tumors.

A major limitation, that might be a consideration, is that this is a single institution study with a relatively small sample size. For each type of *FGFR* alteration, a single or a small set of cases is included. Conversely, however, multi-institution studies looking at histomorphologic features can suffer from inter-observer bias. Hence, one of the strengths of this study is that all cases were diagnosed at a single institution and are more likely to be homogeneous in interpretation of histologic features.

We conclude that while pediatric tumors with *FGFR* fusions do show monomorphic *oligodendroglia*-like nuclei, arcuate vasculature, and microcalcifications, similar to those described by Bielle *et al*. in adult tumors, the features are too variable in extent to histologically predict the presence of fusion in pediatric tumors since the *FGFR*-fusion positive group comprises so many different diagnostic entities, including but not limited to pilocytic astrocytoma, pilomyxoid astrocytoma, PLNTY, extraventricular neurocytoma, rosette forming glioneuronal tumor, and pediatric-type oligodendroglioma.

Our final conclusion is that broad mutational and fusion testing remains necessary for pediatric patients with any glioneuronal CNS tumor, despite the cost and time burdens. We therefore recommend that fusion testing be performed for all pediatric glioneuronal tumors, regardless of histological

features; unfortunately, histological triaging of cases will miss examples with this potentially targetable fusion.

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Disclosure

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