GOPC-ROS1 Fusion Due to Microdeletion at 6q22 Is an Oncogenic Driver in a Subset of Pediatric Gliomas and Glioneuronal Tumors

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Abstract

ROS1 is a transmembrane receptor tyrosine kinase proto-oncogene that has been shown to have rearrangements with several genes in glioblastoma and other neoplasms, including intrachromosomal fusion with GOPC due to microdeletions at 6q22.1. ROS1 fusion events are important findings in these tumors, as they are potentially targetable alterations with newer tyrosine kinase inhibitors; however, whether these tumors represent a distinct entity remains unknown. In this report, we identify 3 cases of unusual pediatric glioma with GOPC-ROS1 fusion. We reviewed the clinical history, radiologic and histologic features, performed methylation analysis, whole genome copy number profiling, and next generation sequencing analysis for the detection of oncogenic mutation and fusion events to fully characterize the genetic and epigenetic alterations present in these tumors. Two of 3 tumors showed pilocytic features with focal expression of synaptophysin staining and variable high-grade histologic features; the third tumor aligned best with glioblastoma and showed no evidence of neuronal differentiation. Copy number profiling revealed chromosome 6q22 microdeletions corresponding to the GOPC-ROS1 fusion in all 3 cases and methylation profiling showed that the tumors did not cluster together as a single entity or within known methylation classes by t-Distributed Stochastic Neighbor Embedding.

Key Words: 6q22, Astrocytoma, Brain tumor, GOPC, Pediatric glioma, ROS1.

INTRODUCTION

ROS1, located at 6q22.1, encodes a transmembrane receptor tyrosine kinase that under normal circumstances binds to growth factors and undergoes dimerization and phosphorylation with transmission of growth signals downstream through intracellular second messenger systems (1). Oncogenic ROS1 fusion was originally discovered in a glioblastoma (GBM) cell line (U118MG) in the late 1980s (2–4). Subsequent work demonstrated this original alteration to be due to an intrachromosomal microdeletion of 240–250 kilobases (kb), resulting in fusion of GOPC and ROS1 in this cell line (5).

This del(6)(q22;q22) and the resulting rearrangement produces a constitutively active tyrosine kinase fusion product that has been demonstrated to be sufficient to produce neoplastic transformation and tumor development in both cell culture and murine models (4–7). Since its discovery, several studies have identified this particular alteration as a relatively uncommon tumor driver in a small subset of GBM and low-grade gliomas, including very rare pediatric tumors (6, 8, 9). GOPC-ROS1 fusion has also been identified in several other cancers, including lung adenocarcinoma (10–12) and cholangiocarcinoma (13), and ROS1 fusions with other partners have been discovered in many other tumor types (14). Another pathogenic fusion, ZCCHC8-ROS1, was found to be the underlying oncogenic driver in single a case of...
congenital GBM (15). Analysis of The Cancer Genome Atlas (TCGA) dataset also suggested that GOPC-ROS1 fusion may result in shorter overall survival in adult patients with GBM (6). While this gene fusion product may be difficult to readily detect due to the size of the microdeletion at 6q22 and the inability of break-apart FISH probes to identify this particular rearrangement, commercially available immunohistochemical stains for ROS1 are often positive in cases with ROS1 rearrangement with any number of fusion partners (6, 11, 12).

In this study, we identified 3 pediatric patients with clinically similar supratentorial brain tumors, diagnosed as pilocytic astrocytoma, glioneuronal tumor, and/or GBM after initial resection. Two of the 3 cases had strikingly similar histology, including mixed astrocytic and oligodendrogli-like features, with diffuse glial fibrillary acidic protein (GFAP) staining and focal islands of synaptophysin-reactive, NeuN-negative cells. Case 1 appeared to be low-grade on histologic exam of the initial resection specimen; however, it recurred with features suggestive of anaplasia. Case 2 had focal high-grade features on the initial resection specimen. Case 3 had high-grade astrocytic features with diffusely infiltrating components. Methylation profiling failed to classify these tumors to known tumor entities present in the current version of the methylation classifier with a high degree of certainty. Subsequent whole-genome copy number profiling demonstrated microdeletions at 6q22, and DNA and RNA sequencing using next generation sequencing (NGS) methods demonstrated GOPC-ROS1 fusion in all 3 cases.

MATERIALS AND METHODS

Clinical Histories

Case 1

A 4-year-old previously healthy female patient presenting with seizure and rapid decline of mental status was found to have a 3.1 × 2.8 × 2.5 cm tumor in the left thalamus with focal contrast enhancement extending into the left basal ganglia, left temporal lobe, and left ventricle, resulting in hydrocephalus (Fig. 1A, B). She underwent ventriculoperitoneal shunt placement and resection of the tumor which showed pilocytic-like morphology with low proliferative activity. The patient was treated with carboplatin and vincristine. Over the next 3 years, there was slow, progressive increase in tumor size (Fig. 1C, D) requiring tumor debulking and repeat administration of carboplatin and vincristine with additional vinblastine. The results of the second surgery showed similar histology but the tumor then had higher-grade features, including increased proliferative activity and focal necrosis. Two years later, after additional tumor growth she received left-sided laser ablation of the thalamic tumor and repeat biopsy for additional molecular characterization, followed by 4 cycles of Temodar and Avastin, complicated by myelosuppression, with radiologic evidence of further tumor progression (Table).

Case 2

A 5-year-old previously healthy male patient presented with 3–4 weeks of somnolence, emesis, and headaches was
<table>
<thead>
<tr>
<th>Case #1</th>
<th>Surgery 1</th>
<th>4</th>
<th>Female</th>
<th>Focally enhancing 3.1 × 2.8 × 2.5 cm left thalamic/basal ganglia/medial temporal lobe/ventricular mass with hydrocephalus</th>
<th>Mildly hypercellular astrocytic morphology with oligodendrogial-like areas and microcystic areas, rare EGBs identified, no ganglion cells; 0 mits/10 HPF</th>
<th>GFAP+, OLIG2+, focal synaptophysin+, focal weak EMA+, IDH1 R132H-, BRAF V600E-, p53-, ATRX-retained, H3K27M-, Ki-67 0%–2%</th>
<th>N/A</th>
<th>N/A</th>
<th>Low-grade glioma, most consistent with pilocytic astrocytoma</th>
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<tr>
<td>Surgery 2</td>
<td>7</td>
<td>Residual/recurrent nodular enhancing 2.4 × 1.8 × 1.7 cm bilateral thalamic mass with extension into the left lateral and third ventricle</td>
<td>Hypercellular astrocytic morphology with microvascular proliferation and necrosis, rare EGBs identified, 4 mits/10 HPF</td>
<td>GFAP+, OLIG2+, focal synaptophysin+, focal weak EMA+, IDH1 R132H-, BRAF V600E-, p53-, ATRX-retained, H3K27M-, Ki-67 15%</td>
<td>KIAA1549-BRAF fusion negative</td>
<td>N/A</td>
<td>Glioma with increased proliferation, most consistent with pilocytic astrocytoma</td>
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<td>Surgery 3</td>
<td>9</td>
<td>Residual/recurrent nodular enhancing 3.8 × 2.3 × 2.2 cm bilateral thalamic and periatrial mass</td>
<td>Hypercellular astrocytic morphology with microcystic areas, microvascular proliferation, and focal necrosis, no Rosenthal fibers or EGBs identified, 3 mits/10 HPF</td>
<td>GFAP+, OLIG2+, focal synaptophysin+, focal weak EMA+, IDH1 R132H-, BRAF V600E-, p53-, p16-retained, ATRX-retained, H3K27M-, Ki-67 20%</td>
<td>GOPC-ROS1 fusion; CHEK2 (I157T) mutation</td>
<td>Loss of chromosomes 1 and 5; deletion of C19MC; microdeletion at 6q22</td>
<td>Glioneuronal Tumor with anaplastic features and GOPC-ROS1 fusion</td>
<td></td>
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<tr>
<td>Case #2</td>
<td>Surgery 1</td>
<td>5</td>
<td>Male</td>
<td>Heterogeneously enhancing 4.7 × 4.3 × 4.0 cm mass predominantly involving the right anterior lateral ventricle</td>
<td>Hypercellular astrocytic morphology with oligodendrogial-like areas and myxoid areas, infiltrative features, 3 mits/10 HPF</td>
<td>GFAP+, focal synaptophysin+, faint EMA+, neurofilament-in tumor cells but present in background axons, Ki-67 20%–30%</td>
<td>GOPC-ROS1 fusion</td>
<td>Loss of chromosomes 1 and 5; deletion of C19MC; microdeletion at 6q22</td>
<td>Glioneuronal Tumor with anaplastic features and GOPC-ROS1 fusion</td>
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(continued)
found to have a 4.7 × 4.3 × 4.0 cm heterogeneously enhancing intraventricular mass involving the right anterior lateral ventricle with an inferior portion projecting to the foramen of Monro, resulting in obstructive hydrocephalus (Fig. 1E, F). He was obtunded with a dilated right pupil and required emergency ventricular drain placement. The patient underwent gross total resection of the right frontal intraventricular mass. Pathology was reported to be a glioneuronal tumor with focal high-grade features. His postoperative course was complicated by a pseudomeningocele, which was subsequently treated by overseeing his incision and placement of a lumbar drain. Since his resection, he has had no evidence of recurrence on MRI imaging and has not required further treatment thus far.

Case 3

A 2-year-old previously healthy male patient presented with new-onset seizures and was found to have a 5.7 × 5.3 × 4.9 cm multinodular and diffuse, heterogeneously enhancing signal abnormality present throughout portions of the right temporal lobe, right frontal lobe, and involving the right subinsular white matter as well as the right basal ganglia and posterior limb internal capsule, with its epicenter at anterior inferior right temporal lobe, and without evidence of metastatic disease in the spine (Fig. 1G, H). He underwent partial resection and the pathology demonstrated features consistent with high-grade neuroepithelial tumor, aligning best with glioblastoma. He was started on chemotherapy as per Baby POG 1 (2 cycles of cyclophosphamide and vincristine followed by a third cycle of cisplatin and etoposide) for 18 months with radiographic improvement in his tumor, followed by one cycle of autologous hematopoietic stem cell transplant with carboplatin, thiotepa, and etoposide. He still has evidence of stable residual disease on imaging without any evidence of progression or subsequent seizure activity and remains completely intact neurologically at 5 years of age.

Histology and Immunohistochemistry

Hematoxylin and eosin (H&E)-stained slides were prepared from 4-μm-thick sections of formalin-fixed, paraffin-embedded (FFPE) tissue using standard protocols. Immunohistochemistry was performed on 4-μm-thick paraffin sections following heat-induced epitope retrieval using CC1 (Ventana, Tucson, AZ), then staining with GFAP (Leica Biosystems, Richmond, VA and Thermo Fisher Scientific, Waltham, MA), Olig2 (Cell Marque, Rocklin, CA), IDH1 R132H (Dianova, Hamburg, Germany), ATRX (Sigma-Aldrich, St. Louis, MO), p53 (Ventana and Leica Biosystems), synaptophysin (Leica Biosystems), neurofilament (Covance, Princeton, NJ and Leica Biosystems), epithelial membrane antigen (EMA) (Cell Marque), Histone H3 K27M (Sigma-Aldrich), INI1 (Cell Marque), Neu-N (Sigma-Aldrich), and Ki-67 (Dako, Carpenteria, CA and Cell Marque) on either a Ventana Benchmark XT or Ventana Benchmark Ultra automated stainer, using Ventana UltraView Universal DAB Detection kits.
Methylation Studies
DNA extraction was carried out using the automated Maxwell system (Promega, Madison, WI). DNA methylation was analyzed by the Illumina EPIC Human Methylation array, which assesses 850,000 CpG sites, according to the manufacturer's instructions at the NYU Molecular Pathology laboratory as described previously (16). Molecular subclassification and t-Distributed Stochastic Neighbor Embedding (t-SNE) visualization was performed utilizing the cloud-based DNA methylation classifier as described previously (17). In addition, the array data were used to calculate a low-resolution copy number profile, as previously described (18–23). "Gain" or "amplification" in the copy number profiles was determined by \( \log_2 \geq 0.3 \).

Next-Generation Sequencing
Targeted genome sequencing was performed on DNA isolated from FFPE tissue using NGS panels to evaluate 324 genes and gene rearrangements, microsatellite instability, and overall tumor mutation burden in case 1 (Foundation Medicine, Cambridge, MA). RNA was extracted and sequenced using a customized clinically validated and NY State approved RNAseq panel targeting 86 cancer related genes (NYU Fusion SEQer) using Anchored Multiplex PCR (ArcherDX, Boulder, CO) in case 2. For case 3, whole genome sequencing was performed on DNA isolated from FFPE tissue and normal peripheral blood. Libraries were prepared using NEBNext Ultra II DNA reagents (New England Biolands, Ipswich, MA). Paired-end 151-bp reads were generated on the Illumina HiSeq 4000 and reads were aligned to the human genome reference sequence (build GRCh37). Copy number variation was measured using VarScan (http://varsan.sourceforge.net/; last accessed August 1, 2019) and structural variation was analyzed using Lumpy (https://github.com/arq5x/lumpy-sv; last accessed August 1, 2019) and structural variation was analyzed using Lumpy (https://github.com/arq5x/lumpy-sv; last accessed August 1, 2019). In parallel, tumor RNA derived from FFPE underwent whole transcriptome sequencing. Paired-end 151-bp reads were generated on the Illumina HiSeq 4000 and reads were aligned to the human genome reference sequence (build GRCh38). Gene fusions were evaluated by an ensemble approach of 7 fusion callers.

RESULTS
Tumor Histology
Case 1

The first resection specimen showed features most consistent with pilocytic astrocytoma: There were atypical astrocytic areas, oligodendrogial-like areas, myxoid areas with microcystic changes, calcifications, and rare eosinophilic granular bodies, without any definitive ganglion cells. The tumor was diffusely positive for GFAP with focal islands of synaptophysin-positive, NeuN-negative cells and focal cytoplasmic EMA positivity. The tumor cells were negative for IDH1 R132H, BRAF V600E, and V53 and had retained ATRX expression. Ki-67 proliferation index was 0%–2%. Subsequent resection specimens from tumor recurrence demonstrated a similar overall morphology except there was microvascular proliferation, focal necrosis, and increased mitotic figures (up to 4/10 HPF) in the second specimen (Fig. 2A, B). The tumor was positive for GFAP (Fig. 2C), had islands of synaptophysin-positive cells (Fig. 2D), diffuse Olig2 reactivity (Fig. 2E), and was negative for IDH1 R132H, BRAF V600E, and p53, and had retained nuclear ATRX expression. Staining for neurofilament revealed no axons coursing through the tumor in any area, suggesting a mostly noninfiltrative process. Ki-67 proliferation index was ~15%–20% (Fig. 2F). All specimens were negative for H3K27M and none showed aberrant CD34 staining to suggest diffuse midline glioma or polymorphous low-grade neuroepithelial tumor of the young (PLNTY).

Case 2

The resection specimen demonstrated similar histology to case 1 with cords of astrocytic cells, myxoid change, and oligodendroglial-like areas (Fig. 2G, H). The tumor was diffusely positive for GFAP (Fig. 2I) with focal synaptophysin (Fig. 2J) and weak cytoplasmic EMA reactivity. There were areas with neurofilament-positive axons traversing the tumor, suggesting a focally infiltrative process (Fig. 2K). Ki-67 proliferation index was relatively low in the majority of the tumor, but focally elevated up to 20%–30% in a few foci (Fig. 2L). The majority of the tumor demonstrated low-grade fibrillar and neurocytic morphology with variability in the myxoid stroma and focal calcification. On H&E sections, some areas of the tumor were noted to have pleomorphism, hypercellularity, mitotic activity, and microvascular proliferation.

Case 3

The resection specimen aligned best with GBM, and demonstrated multiple hypercellular discrete-appearing nodular components, as well as surrounding areas of extensive infiltration, with pleomorphic round, ovoid, and bipolar elongated cells (Fig. 2M). The tumor cells formed secondary structures, with areas of prominent perineuronal, perivascular, leptomeningeal, and subpial infiltration. No obvious oligodendroglial-like or myxoid/microcystic areas were identified. There was focal microvascular proliferation but no definite necrosis was seen (Fig. 2N). The tumor cells were partially positive for GFAP (Fig. 2O) within a background of synaptophysin-reactive neuropil and entrapped neurons (Fig. 2P). The tumor cells were also partially positive for Olig2 (Fig. 2Q) and negative for EMA. IDH1 R132H and H3K27M were both negative, p53 showed patchy weak positivity in scattered tumor cells, and ATRX and INI-1 were both retained, suggesting wildtype status of these 5 genes. Neurofilament was focally positive with background axons and NeuN was positive in scattered entrapped neurons, further supporting an infiltrative process, and no evidence of neuronal differentiation by tumor cells was detected. Scattered mitotic figures were present (1–4/10 HPF), and Ki-67 proliferation index was elevated, multifocally as high as 30%–40% (Fig. 2R).
FIGURE 2. Histologic findings in the second resection specimen of Case 1 showing H&E sections with varying histologic features (A, B), diffuse GFAP staining (C), focal islands of synaptophysin staining (D), diffuse Olig2 reactivity (E), and brisk Ki-67 proliferation rate (F). Histologic findings in Case 2 showing H&E sections with varying histologic features (G–H), diffuse GFAP staining (I), islands of synaptophysin staining (J), neurofilament-positive axons (K), and Ki-67 proliferation rate (L). Histologic findings in Case 3 showing a hypercellular glial/astrocytic proliferation with focal endothelial proliferation (M, N), diffuse-patchy GFAP staining (O), background synaptophysin staining (P), partial Olig2 nuclear staining (Q), and elevated Ki-67 proliferation index (R). (G) is taken at a total magnification of 100×, (L) is taken at a total magnification of 400×, all other images are taken at a total magnification of 200×. All scale bars: 200 μm.
Methylation Analysis and t-SNE
Specimens from tumor recurrence in case 1 and initial resection in cases 2 and 3 each underwent whole genome DNA methylation profiling, classification, and t-SNE cluster analysis (www.molecularneuropathology.org); however, none of the cases matched well with any known entity included in the classifier (V11b4, optimal score >0.9). Case 1 classified best with anaplastic pilocytic astrocytoma, albeit poorly (0.49 calibrated score) and had unmethylated MGMT promoter. Case 2 classified poorly with pilocytic astrocytoma (0.4 calibrated score) and also had unmethylated MGMT promoter. Case 3 did not match any known methylation class and had unmethylated MGMT promoter. t-SNE plots demonstrated that these 3 cases did not cluster together as a distinct new entity but instead clustered with various other methylation classes including different methylation subclasses of low-grade glioma and pleomorphic xanthoastrocytoma (PXA; Fig. 3).

Copy Number Profiling
Each case had large scale gains and losses as well as more focal alterations. Case 1 demonstrated heterozygous loss of chromosomes 3, 8, 11, 17, 18, and 22q, as well as gain of chromosome 7, 9, 14q, and 20, deletion of the genomic region around C19MC, and microdeletion at 6q22 (the region encompassing both GOPC and ROS1) (Fig. 4A). Case 2 demonstrated homozygous loss of chromosome 1 and heterozygous loss of chromosome 5 and with deletion of C19MC, along with microdeletion at 6q22 (Fig. 4B). Case 3 demonstrated microdeletion at 6q22 (Fig. 4C).

Whole Genome and Whole Exome NGS Panel
Targeted NGS panel at Foundation Medicine identified the CHEK2 1157T variant as well as GOPC-ROS1 fusion (GOPC [NM_020399] exon 8; ROS1 [NM_002944] exon 35) in case 1, confirming the results of the copy number profiling analysis. The tumor tissue was found to maintain microsatellite stability and a low mutational burden of ~1 mutation/megabase. Targeted NGS panel at ArcherDX demonstrated GOPC-ROS1 fusion (NM_020399) exon 4; ROS1 (NM_002944) exon 36) in case 2 (Fig. 5). In case 3, GOPC-ROS1 fusion (GOPC [NM_020399] exon 4; ROS1 [NM_002944] exon 36) was identified by RNA sequencing, confirming the WGS copy number and structural variation analysis. No other clearly significant genetic variation was detected.

Final Pathologic Diagnosis
Case 1: Glioneuronal tumor with anaplastic features and GOPC-ROS1 fusion; Case 2: Glioneuronal tumor with anaplastic features and GOPC-ROS1 fusion. Case 3: Glioblastoma with GOPC-ROS1 fusion (Table). Methylation studies were performed did not demonstrate definitive clustering with any clinically validated tumor entity.

All patients are currently alive as of this report. Patient 1 has been started on an ROS1 inhibitor (Lorlatinib) without evidence of further tumor progression. The Foundation Medicine Panel was unable to determine if the CHEK2 1157T mutation was somatic or germline, and the patient’s family was given genetic counseling with regard to the CHEK2 mutation, which has been associated with a variety of other cancers, including GBM, breast cancer, colorectal carcinoma, and uterine carcinoma, among others (24–29).

DISCUSSION
Herein, we describe 3 cases of pediatric glioma with unusual histology, all harboring GOPC-ROS1 fusions with corresponding microdeletions at 6q22. Tumors with this genetic rearrangement typically fall into the category of adult astrocytoma, although rare pediatric cases with similar findings have been previously identified (6, 9). All 3 tumors were supratentorial masses, and cases 1 and 2 showed prominent extension into the lateral ventricles. The histologic findings overlapped with pilocytic astrocytoma on H&E in cases 1 and 2, showing astrocytic regions with admixed areas of oligodendroglial-like areas, myxoid change, microcystic areas, and calcifications. Immunohistochemically, they were both diffusely positive for GFAP with unusual islands of synaptophysin-positive, NeuN-negative cells, suggesting a biphasic tumor type. Both had relatively low overall proliferation indices and mitotic counts on the initial resection specimens; however, the subsequent recurrence in case 1 had higher-grade features including microvascular proliferation, focal necrosis, increased mitotic rates, and increased Ki-67 index. There was evidence of at least focal tumor infiltration and focal increased mitotic activity in case 2. Case 3 had somewhat different histologic appearances, with a rather unusual (for diffuse astrocytoma) discretely multinodular architecture in some areas, while also demonstrating significant extensively infiltrating components away from the more cellular nodules, and a different immunohistochemical profile (compared with cases 1 and 2), without any morphologic or immunohistochemical evidence of neuronal differentiation detected, but with an increased mitotic index and focal microvascular proliferation.

On the basis of their variable methylation profiles that do not match closely to any known entity, t-SNE plotting was performed to determine whether these 3 cases form a distinct methylation entity as some neuroepithelial tumors with distinct fusions do (30, 31); however, these 3 cases did not cluster as a distinct entity (Fig. 3). It is important to note, however, that many of the entities included in the initial cluster profiles have wider spread clusters that could account for the difference in these cases and a larger number of cases may be needed to establish the signature for classification.

The molecular mechanism underlying ROS1 fusion proteins driving oncogenesis has been well studied over the past 30 years. GOPC, originally named FIG (Fused in Glioblastoma), is a constitutively expressed housekeeping gene coding for a protein associated with the Golgi apparatus with a possible role in vesicular transport (32). ROS1 is a transmembrane receptor tyrosine kinase proto-oncogene that...
functions to mediate extracellular growth factor ligand signals via phosphorylation of second messenger systems (5). These 2 genes are in close proximity to one another on chromosome 6q22.1, such that microdeletions of 240–250 kb in this region can result in chromosomal truncation and fusion of the 2 genes, leading to the regulatory domain of GOPC being fused to the kinase domain of ROS1 (5).

Although the process underlying 6q22 microdeletions resulting in GOPC-ROS1 fusion is not fully understood, the resulting protein has been shown to be sufficient to initiate neoplastic transformation in astrocyte cell culture and cause malignant neoplasms in murine models (5–7, 33). Fusions of ROS1 with GOPC and a myriad of other fusion partners have been demonstrated in a number of different cancers, including meningioma, ependymoma, low-grade glioma, GBM, and several peripheral cancers; however, these are thought to be more rare in pediatric gliomas (5, 6, 8, 11, 12, 34, 35).

We have not encountered additional cases with this particular fusion in our database of ~4000 profiled tumors, suggesting that these tumors are relatively rare in pediatrics, consistent with a previous study of 282 pediatric gliomas that identified only a single case with GOPC-ROS1 fusion and another with CEP85L-ROS1 fusion (8). Because of prior difficulty in identifying this rearrangement by FISH and other methods (6, 36, 37), it is possible that this rearrangement is more common in both adult and pediatric glioma cohorts than has been recognized to date, including in large TCGA studies and others (36, 38, 39).

These pediatric tumors, all with unusual morphology, immunohistochemical profiles, and uncertain methylation profiles, are very difficult to classify and have difficult to predict clinical behavior. The similar H&E morphology in cases 1 and 2, similar biphasic immunohistochemical staining patterns, and identical molecular aberrations on both copy number profiling analysis and next-generation sequencing panels suggest that they may represent similar or perhaps related entities, however that was not confirmed by t-SNE analysis. Given the small number of cases in this report, it is not currently certain if these tumors represent a previously unrecognized tumor entity or possibly a distinct subtype of anaplastic pilocytic astrocytoma or glioneuronal tumors. It is also possible that GOPC-ROS1 fusion acts as a driver in

FIGURE 3. t-Distributed Stochastic Neighbor Embedding (t-SNE) plot showing clustering of the 3 cases included in this report (arrows) in relation to other tumor entities, including diffuse leptomeningeal glioneuronal tumor (DLGNT), pilocytic astrocytoma/ganglioglioma (LGG PA/GG ST), midline pilocytic astrocytoma (LGG PA MID), rosette-forming glioneuronal tumor (LGG RGNT), ganglioglioma (LGG GG), and pleomorphic xanthoastrocytoma (PXA). Notably, despite a common fusion driver, these 3 tumors did not form a distinct cluster.
histologically diverse brain tumors. Our results suggest, at least, that this different genetic profile warrants further investigation, given the potential for better therapeutic modalities. Therefore, RNAseq studies are warranted in tumors that exhibit described histology and do not match with known entities.

With increasing emphasis on precision medicine, many recent studies have evaluated the potential for targeting oncogenic drivers in diverse tumor types. In vitro and ex vivo models of GBM have shown increased response to the more conventional GBM therapy temozolomide when used in combination with crizotinib, a targeted ROS1-inhibitor (37). Clinical trials for crizotinib and another ROS1-inhibitor, entrectinib, in lung cancer patients with ROS1 fusion have also shown promising results (40, 41). More recent studies with lorlatinib, a newer tyrosine kinase inhibitor with

**FIGURE 4.** Copy number plots showing large scale chromosomal gains and losses and focal chromosome 6q22 deletion (arrows) in Case 1 (A), Case 2 (B), and Case 3 (C).
better-blood brain barrier permeability, improved outcomes in patients with lung cancers harboring ROS1 rearrangements who had brain metastases (42–44) and in mouse models of GOPC-ROS1 fusion-positive GBM (6). It is also notable that a patient with a very similar tumor was treated, apparently successfully, with high-dose chemotherapy and autologous hematopoietic stem cell transplant (9). These advancements in molecular diagnosis and therapeutic options underscore the importance of recognizing unusual oncogenic drivers in pediatric brain tumors such as those with GOPC-ROS1.

FIGURE 5. Summary of GOPC-ROS1 gene fusion in Case 2. (A) A schematic representation of the 6q22 region with the GOPC and ROS1 loci. The red arrows indicate the boundaries of the 240 kb deletion leading to GOPC and ROS1 fusion (both genes are transcribed in the same direction). (B) Anchored multiplex PCR (AMP) enabled next generation sequencing (NGS) reveals both fusion partners GOPC (exon 4) and ROS1 (exon 36) and the fusion breakpoint. (C) Representation of supporting reads and coverage spanning the fusion breakpoint.

REFERENCES


