Novel *GOPC(FIG)-ROS1* fusion in a pediatric high-grade glioma survivor

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Pediatric high-grade glioma is a rare tumor characterized by high mortality. The authors report the first case of a high-grade glioma associated with a *GOPC(FIG)-ROS1* fusion in a pediatric patient. The patient underwent gross-total resection at the age of 4 years, followed by adjuvant high-dose chemotherapy and autologous hematopoietic stem cell rescue. At 30 months after transplantation, she remains disease free.

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HIGH-GRADE gliomas account for only 8%–12% of pediatric central nervous system (CNS) tumors, but are characterized by high mortality and morbidity, with only 5%–15% of patients surviving longer than 5 years.^{1,21} These tumors include anaplastic astrocytoma, anaplastic oligodendroglioma, glioblastoma, anaplastic ependymoma, and anaplastic pleomorphic xanthoastrocytoma, among others.¹⁵ Current treatment of pediatric high-grade gliomas includes maximal safe resection followed by adjuvant chemotherapy and radiation therapy.

Diagnosis and treatment of gliomas in the modern era incorporates molecular classification with histology, as seen in the revision to the WHO guidelines for the diagnosis of CNS tumors.¹⁵ Characterization of tumor samples using chromosomal microarray platforms can be used to identify copy number alterations, including gains, losses, and amplification, and targeted or whole genome sequencing assays can be used to elucidate specific mutations and fusions that may be oncogenic. This allows for detection of

genetic aberrations that are diagnostic and subgroup classification that may be associated with outcome. The majority of high-grade gliomas demonstrate known alterations in glial oncogenes and tumor suppressor genes, resulting in a defect in at least 1 of 3 molecular core pathways involving *RBI*, *TP53*, and/or receptor tyrosine kinases, as well as alterations in chromatin remodeling and telomere maintenance.¹⁷

The spectrum and incidence of histological subtypes of pediatric high-grade gliomas and their underlying genetic aberrations differ from those of adult high-grade gliomas. For example, adult high-grade gliomas frequently demonstrate *IDH* mutations and amplification of oncogenes such as *EGFR*. In contrast, high-grade gliomas in children and young adults are frequently characterized by histone mutations *H3F3A* p.K27M and p.G34M.^{13,15,18–20} Midline diffuse gliomas often contain the *H3F3A* p.K27M mutation and have a worse outcome. Pediatric high-grade gliomas outside of the midline structures often contain the alterna-

ABBREVIATIONS CNS = central nervous system; FFPE = formalin-fixed, paraffin-embedded; RT-PCR = reverse transcriptase–polymerase chain reaction; RTK = receptor tyrosine kinase.

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tive *H3F3A* p.G34M mutation, which may also be seen in other embryonal neoplasms and is frequently associated with *TP53* alterations.²⁰

We present the case of a 4-year-old girl with an unusual high-grade glioma who remains disease free 2.5 years following gross-total resection with adjuvant high-dose chemotherapy and autologous hematopoietic cell rescue without radiation therapy. Chromosomal microarray analysis of the primary tumor demonstrated 3 to 4 copies of the majority of chromosomes and an interstitial deletion in 6q22.1 that resulted in a *GOPC(FIG)-ROS1* fusion. We report the first example of a pediatric high-grade glioma with the *GOPC-ROS1* rearrangement and discuss implications for future therapy.

Case Report

History and Examination

A previously healthy 4-year-old girl presented to her pediatrician with a 6-month history of progressively increasing left-sided weakness resulting in falls and ataxia. An outpatient MRI study under anesthesia was ordered and obtained 3 months later. MRI demonstrated a large hypercellular, enhancing mass in the right lateral ventricle with invasion of the thalamus, significant mass effect on adjacent structures, and associated obstructive hydrocephalus (Fig. 1A). The child was admitted to the hospital from MRI. On physical examination she had ptosis and a left facial droop. Her muscle strength was 4/5 in the left upper and lower extremities. She had decreased dexterity of her left hand. She predominantly used her right side; there was concern for left-sided neglect, as passive movement exceeded active movement. She dragged her left leg when walking. Based on her signs, symptoms, and imaging findings, urgent surgery was recommended.

Operative Course

The patient was taken to the operating room for a right parietal craniotomy for resection of the tumor. She was positioned in a three-quarter prone position. Intraoperative navigation was synchronized. Baseline preoperative motor and sensory evoked potentials were diminished on the left compared with the right. A right parietal craniotomy was performed. A transsulcal approach was used to gain access to the right lateral ventricle where the tumor was immediately visible, and a specimen was sent to pathology. Intraoperative pathological examination revealed a hypercellular tumor with mitotic figures, consistent with a high-grade astrocytoma. The tumor was centrally debulked with the ultrasonic aspirator, and the margins were defined by careful microdissection. The tumor was followed all the way to the anterior horn of the lateral ventricle. There was no clear margin against the basal ganglia, where the ependymal surface was invaded. Following a presumed gross-total resection, intraoperative ultrasound was performed and did not reveal any residual tumor. Immediate postoperative MRI demonstrated gross-total resection of the mass (Fig. 1C).

Pathological Findings

Histopathological examination revealed a densely cell-

FIG. 1. A: Axial and coronal T2-weighted images (upper) demonstrating low signal intensity, also suggestive of hypercellularity; and axial diffusion-weighted image and apparent diffusion coefficient (ADC) map (lower) demonstrating a hypercellular mass centered in the right lateral ventricle with invasion of the right thalamus and mass effect on the basal ganglia. **B:** Axial T1-weighted images with (upper) and without (lower) contrast enhancement demonstrating homogeneous enhancement of the mass. **C:** Axial and coronal T2-weighted images obtained immediately after surgery demonstrating gross-total resection of the mass. **D:** Follow-up axial and coronal T2-weighted images obtained 2 years after surgery demonstrating no evidence of tumor recurrence.

ular tumor with a glial appearance. Tumor cells ranged from round to spindled, and a subpopulation of larger rounded nuclei were noted in some regions of the tumor. Zones of necrosis, frequent mitotic figures, and foci of microvascular proliferation were also present (Fig. 2A). Immunohistochemical analysis demonstrated a GFAP-positive portion of the tumor as well as a GFAP-negative portion, which had more predominant EMA staining. The majority of tumor cells showed nuclear OLIG2 immunopositivity, supporting a diagnosis of glioma.

Chromosomal microarray analysis was performed according to the manufacturer's protocols (Affymetrix OncoScan FFPE Assay).¹⁰

The Affymetrix OncoScan FFPE Assay utilizes Molecular Inversion Probe (MIP) technology to obtain accurate genome-wide copy number and loss-of-heterozygosity profiles using DNA from formalin-fixed, paraffin-embedded (FFPE) samples. The assay contains 220,000 probes across targeted cancer regions and the rest of the genome, allowing detection of copy number abnormalities at 50- to

FIG. 2. **A:** Photomicrograph of H & E–stained tumor section demonstrating findings typical of a high-grade glioma, including pseudopalisading necrosis, focal microvascular proliferation, hypercellularity, high mitotic activity, and nuclear atypia. Original magnification $\times 100$. **B:** Chromosome microarray analysis demonstrating copy number gains and losses as evidenced by changes in the log ratio ($\log_2$) in the *top panel* and differences in the allele tracks in the *bottom panel*. **C:** Microarray profiles of chromosome 6 illustrating the 6q22.1 deletion with breakpoints in *ROS1* and *GOPC*. **D:** Results of RT-PCR and Sanger sequencing, demonstrating a *GOPC(FIG)-ROS1* fusion. Figure is available in color online only.

100-kb resolution in approximately 900 cancer genes and at 300-kb resolution in other chromosomal regions. Patient hybridization results are compared with data derived from over 300 FFPE samples from unaffected tissues. Genomic linear positions are given relative to NCBI build GRCh37/hg 19 (February 2009).

GOPC(FIG)-ROS1 fusion was confirmed by reverse transcriptase–polymerase chain reaction (RT-PCR). RNA was extracted from frozen sections using the RNeasy Mini RNA Extraction Kit (Qiagen, Germany). First strand cDNA was synthesized from 200 ng of total RNA with the SuperScript III first-strand synthesis system (Invitrogen) with oligo(dT)₂₀, followed by amplification with gene-specific primers FIG-2F and ROS-GSP3.1 and sequencing of the PCR product.¹¹

As shown in Fig. 2B, there were copy number alterations of the majority of the chromosomes with 2 copies of chromosomes 1, 14, 19, and 21; 3 copies of chromosomes 2, 4, 5, 6, 7, 10, 11, 12, 13, 15, 18, 20, 22, and X; and 4 copies of chromosomes 3, 8, 16, and 17. This places the tumor in the near-triploid range for DNA content. The key finding was an interstitial deletion in 6q22.1 with breakpoints localized within the *ROS1* and *GOPC* genes (GRCh37chr6:117,642,494–117,889,493) (Fig. 2C). The deletion was postulated to result in a *GOPC-ROS1* fusion, which was confirmed by RT-PCR and Sanger sequencing (Fig. 2D).

Postoperative Course

Following surgery the patient had persistent left hemiparesis and was transferred to inpatient rehabilitation while undergoing chemotherapy as per Head Start III Regimen C. Four cycles of induction chemotherapy (carboplatin, vincristine, and temozolomide) were followed by a single cycle of myeloablative consolidation chemotherapy (car-

boplatin and thiopeta) and autologous stem cell rescue.⁸ At the conclusion of chemotherapy, repeat MRI showed no evidence of disease, and the patient's parents declined adjuvant conformal radiation therapy. At 30 months following surgery, the patient remains disease free without recurrence (Fig. 1D). On physical examination she is an alert, interactive child. She walks with an ankle-foot orthosis for residual left foot drop. She has some left upper-extremity neglect and has spasticity of the left hand. She attends regular classes in school and is active in swimming and ballet.

Discussion

With the integration of molecular parameters into the WHO classification of CNS tumors, molecular classification is transitioning from a research investigation to an essential clinical diagnostic assay. In the modern era of molecular profiling, the distinct genomic and epigenomic alterations in different subgroups of high-grade gliomas are guiding diagnosis and clinical decision making. In our patient, chromosomal microarray analysis revealed a near-triploid DNA content, with relative loss of chromosomes 1, 14, 19, and 21 and relative gain of chromosomes 3, 8, 16, and 17. These numerical alterations were consistent with a high-grade glioma. Identification of the deletion in 6q22.1 resulted in a *GOPC-ROS1* fusion.

Some cancers mediate proliferation and survival through the constitutive activation of receptor tyrosine kinases (RTKs). RTKs are transmembrane proteins that regulate growth and survival through autophosphorylation and consequent downstream signal transduction.⁵ In rare cases, glioblastomas have been shown to constitutively activate RTKs through the formation of oncofusion proteins. A deletion of 240 kb in chromosome 6q21–q22

has been shown to lead to the fusion of the housekeeping gene *FIG* (“Fused in glioblastoma”), now known as *GOPC*, with the RTK *ROS1*, which constitutively activates the tyrosine kinase.³ *ROS1* rearrangements have also been identified in non–small cell lung cancer and cholangiocarcinoma, which often contain the *GOPC(FIG)-ROS1* fusion gene.^{11,16} In the non–small cell lung cancer literature, *ROS1* rearrangements occur more frequently in younger patients, in the absence of known oncogenic drivers.⁷ The overall rate of *ROS1* rearrangements ranges from < 1% in ovarian cancers to nearly 8% in cholangiocarcinomas.⁷

Despite the delineation of the *GOPC(FIG)-ROS1* rearrangement in 2 cell lines belonging to a single glioblastoma patient, clinical cases are extremely rare. Analysis of The Cancer Genome Atlas Research Network (TCGA) data set reported by Brennan et al.² did not demonstrate cases with the *GOPC/FIG-ROS1* fusion. Lim et al.¹⁴ did not find a single *ROS1* rearrangement in 109 adult glioblastoma specimens, which had a high rate of *MGMT* gene methylation and *IDH1* mutations. It is possible that the fluorescence in situ hybridization (FISH) assay design in this study would preclude identification of the fusion, since it arises as a result of a deletion and not translocation. Das et al.⁶ reported a *GOPC(FIG)-ROS1* rearrangement in 15 adult glioblastoma specimens, but the denominator in the study is not clearly defined. The incidence of the *GOPC-ROS1* fusion in pediatric glioblastoma is unknown, but it should be noted that a *ZCCHC8-ROS1* fusion has been reported as an oncogenic driver in a case of congenital glioblastoma.⁴

Standard treatment for pediatric high-grade glioma has not been established but typically includes involved field irradiation followed by low-intensity maintenance chemotherapy, with essentially palliative intent.¹² Survival in a minority of children with high-grade glioma treated with dose-intensive chemotherapy without radiation has been reported.^{8,9} The patient described in this report had histopathological findings demonstrating a high-grade glioma, compatible with a glioblastoma, and underwent intensive chemotherapy and autologous stem cell transplant without conventional radiation therapy, which her parents opted to forgo.

This patient has experienced prolonged disease-free survival without radiation therapy. Although *ROS1* rearrangements are not known to be associated with prolonged survival alone, the identification of the *GOPC-ROS1* fusion within her tumor may provide insight into the pathophysiology of her disease. There is now a recognized subclass of non–small cell lung cancer with the *GOPC-ROS1* fusion.

Crizotinib is an FDA-approved *ROS1* inhibitor that could potentially target the *GOPC-ROS1* fusion. Experimental use of combination therapy consisting of crizotinib and temozolomide to desensitize and target *GOPC-ROS1* fusions in cell cultures from adult glioblastoma has had a profound antitumor effect in vitro and ex vivo.⁶ A similar benefit has been seen for a subclass of non–small cell lung cancer patients with the *ROS1* rearrangement who experienced prolonged survival on crizotinib. Crizotinib is being used as salvage therapy for adult and pediatric cancers.²² Further development and delineation of treatment guide-

lines for *ROS1* inhibitors may represent a promising modality for future study.^{6,7} With modern technology, identification of unique molecular subgroups within pediatric high-grade astrocytomas and analysis of their response to conventional therapy will be of paramount importance for developing focused treatment protocols.

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Disclosures

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

Author Contributions

Conception and design: Kiehna, Arnush, Robison, Biegel. Acquisition of data: all authors. Analysis and interpretation of data: all authors. Drafting the article: Kiehna, Arnush, Tamrazi, Cotter, Hawes, Robison, Biegel. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Kiehna. Administrative/technical/material support: Kiehna, Biegel. Study supervision: Kiehna, Biegel.

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