A case series of pediatric survivors of anaplastic pleomorphic xanthoastrocytoma

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Abstract

Background. Anaplastic pleomorphic xanthoastrocytoma (APXA) is a rare subtype of CNS astrocytoma. They are generally treated as high-grade gliomas; however, uncertainty exists regarding the optimal therapy. Here, we report on 3 pediatric cases of APXA.

Methods. Our institutional database was queried for cases of APXA and 3 cases were identified. Surgical samples were processed for methylation profiling and chromosomal microarray analysis. Methylation data were uploaded to the online CNS tumor classifier to determine methylation-based diagnoses to determine copy number variations (CNVs).

Results. Two patients were male, 1 female, and all were aged 12 years at diagnosis. All underwent a gross total resection (GTR) and were diagnosed with an APXA. Immunohistochemical analysis demonstrated that 2 cases were BRAF V600E positive. Methylation-based tumor classification supported the APXA diagnosis in all cases. CNV analyses revealed homozygous \textit{CKDN2A} deletions in all and chromosome 9p loss in 2 cases. All patients received radiation therapy (54 Gy in 30 fractions) with concurrent temozolomide. Two patients received maintenance chemotherapy with temozolomide and lomustine for 6 cycles as per the Children's Oncology Group ACNS0423. The third patient recurred and went on to receive a second GTR and 6 cycles of lomustine, vincristine, and procarbazine. All are alive with no evidence of disease >4 years post-treatment completion (overall survival = 100%, event free survival = 67%).

Conclusions. The natural history and optimal treatment of this rare pediatric tumor are not well understood. This case series supports the use of adjuvant chemoradiotherapy in the treatment of APXA. The genetic landscape may be informative for optimizing treatment and prognosis.

Key Points

- APXA are exceedingly rare pediatric CNS tumors of which the biology is not well understood.
- Methylation-based tumor classification supported the APXA diagnosis in all cases.
- These 3 cases of long-term survivors of APXA support the use of adjuvant chemoradiotherapy in the treatment of APXA.
Importance of the Study

APXA is an exceedingly rare CNS tumor in children and there is a limited understanding of the natural history of these lesions along with no consensus regarding optimal treatment. Our study describes 3 cases of long-term survivors of APXA treated using a high-grade glioma treatment approach. Methylation-based tumor classification supported the APXA diagnosis in all cases. As our understanding of the molecular nature of APXA improves, a standardized approach to the treatment of these tumors may be developed.

Methods

Clinical Cohort

All patients treated for APXA between 2005 and 2019 at British Columbia Children’s Hospital (BCCH) in Vancouver, Canada were identified. Clinical data regarding patient demographics, clinical variables, diagnostic testing and pathological diagnosis, treatments provided, and current status were collected retrospectively. This study was approved by the University of British Columbia Clinical Research Ethics Boards.

Pathology Analysis

Each tissue sample was analyzed by a neuro-pathologist at our centre at the time of the original diagnosis. Anaplasia was defined based on current WHO criteria. Criteria included a mitotic index ≥5 mitoses/10 high power field. One case included evidence of tumor necrosis in pathology review.

Tissue Sample Processing

DNA was extracted from fixed paraffin-embedded tissue samples for all 3 patients. DNA was bisulfite converted

Table 1. Tumor Characteristics and Treatment

| Case | Sex | Age  | Tumor Location | Surgery | Chemotherapy | Radiation            |
|------|-----|------|----------------|---------|--------------|----------------------|
| 1    | M   | 12 years | Left temporal | GTR     | Concurrent temozolomide with radiation 90 mg/m² and temozolomide (200 mg/m²) × 2 cycles, then 6 cycles of lomustine, vincristine, and procarbazine | 54 Gy in 30# to surgical bed |
| 2    | M   | 12 years | Right parietal | GTR     | COG Protocol ACNS 0423* | 54 Gy in 30# to surgical bed |
| 3    | F   | 12 years | Left temporal | GTR     | COG Protocol ACNS 0423* | 54 Gy in 33# to surgical bed |

GTR, gross total resection.

*Children's Oncology Group (COG) Protocol ACNS 0423 protocol involves temozolomide with radiation therapy and maintenance with temozolomide and lomustine.
and profiled on the Illumina Infinium Human Methylation EPIC (850K) array (Illumina) to obtain whole-genome DNA methylation data. Methylation data were subsequently submitted to the publicly available German Cancer Research Center (DKFZ) brain tumor methylation-based classifier v11b4 (https://www.molecularneuropathology.org/mnp) and classification results along with MGMT promoter methylation status were downloaded. Copy number variation (CNV) plots were obtained using raw methylation data. Methylation data from these patients were plotted together with data from the cohort of patients used to develop the DKFZ CNS tumor classifier on a t-distributed Stochastic Neighbor Embedding (tSNE) plot to verify classification results. Snap frozen tumor samples from Case 1 and 2 were processed for genome-wide chromosomal microarray analysis (CMA).

**Results**

**Case Descriptions**

Three patients with grade III APXA were treated at British Columbia Children’s Hospital (BCCH) in Vancouver, Canada, from 2007 to 2019 (Table 1).

**Case 1**

The first case is a previously well, developmentally normal 12-year-old male who presented with a 1-week history of headaches and vomiting. A CT scan and MRI demonstrated an irregularly enhancing lesion in the left temporal lobe with surrounding edema with enhancement of the dura. He underwent a left craniotomy with gross total tumor resection and pathology was consistent with a diagnosis of an APXA. Immunohistochemistry demonstrated BRAF V600E and CD34 positivity.

CMA was consistent with a homozygous deletion of CDKN2A at 9p21.3, as well as the presence of additional CNAs that included a loss of one copy of chromosomes 9, 10 and the Y chromosome. This patient underwent cranial RT (54 Gy in 30#) to the surgical bed with concurrent temozolomide 90 mg/m²/day followed by temozolomide maintenance at 200 mg/m² for 2 cycles. On post-RT imaging at the end of the first 2 cycles, he was found to have progressive disease of a small postoperative residual lesion. Therefore, he underwent a second GTR followed by cyclophosphamide 2 g followed by 6 cycles of lomustine, vincristine, and procarbazine maintenance therapy. He is currently alive without recurrent disease 12 years post-treatment completion.

**Case 2**

The second case is a developmentally normal, 12-year-old boy with a history of migraine headaches who presented with focal seizures that had become generalized over 2 months. Neuroimaging demonstrated a right frontoparietal mass with calcifications. The patient underwent a gross total resection where the diagnosis of APXA was confirmed. Immunohistochemistry was negative for BRAF V600E.

CMA was consistent with a homozygous deletion of CDKN2A, as well the presence of additional CNAs that included a gain of 1q, an interstitial deletion of at 10p14p12.31, a gain of chromosome 12 with an interstitial deletion at 12q15q23.1 and a terminal deletion at 18q22.2q22.3.

He received RT (again 54 Gy in 30#) to the surgical bed, concurrent temozolomide with RT, and then maintenance chemotherapy with temozolomide and lomustine for 6 cycles (Children’s Oncology Group [COG] Protocol ACNS 0423). He had a continued complete response on post-treatment imaging and remains well and disease-free 11 years post-treatment completion.

**Case 3**

The third case is a previously well, developmentally normal, 12-year-old girl. She presented with intermittent headaches and phonophobia over the prior 12 months as well as a focal seizure leading up to diagnosis. MRI demonstrated a mass in the superolateral left temporal

| Immunohistochemistry | CMA | Methylation Profiling | Selected CNV Results |
|----------------------|-----|-----------------------|----------------------|
|                      |     |                       |                      |
| **Case 1**           |     |                       |                      |
| Positive             | Positive | Yes (anaplastic) PXA | Loss of chromosome 9, Homozygous del CDKN2A gene |
|                      |         | (anaplastic) PXA      |                      |
|                      |         | Unmethylated          |                      |
|                      |         |                       |                      |
| **Case 2**           |     |                       |                      |
| Negative             | Negative | Yes (anaplastic) PXA | Homozygous del CDKN2A gene |
|                      |         | (anaplastic) PXA      |                      |
|                      |         | Unmethylated          |                      |
|                      |         |                       |                      |
| **Case 3**           |     |                       |                      |
| Positive             | Positive | NA (anaplastic) PXA  | Loss 9p, Homozygous del CDKN2A gene |
|                      |         | (anaplastic) PXA      |                      |
|                      |         | Unmethylated          |                      |
|                      |         |                       |                      |

APXA, anaplastic pleomorphic xanthoastrocytoma; CMA, chromosomal microarray; CNV, copy number variation; DKFZ, brain tumor methylation classifier developed at the German Cancer Research Center; MGMT, O[6]-methylguanine-DNA methyltransferase; NA, not available; tSNE, t-distributed Stochastic Neighbor Embedding.
lobe, centered near the gray-white matter junction with a central enhancing soft tissue component surrounded by a cystic component.

She underwent a gross total resection and pathology was consistent with an APXA with BRAF V600E immunohistochemistry positivity shown within the tumor. She was treated with the same approach as Case 2 with RT (54 Gy in 33#) to the surgical bed plus concurrent temozolomide with RT followed by maintenance chemotherapy with temozolomide and lomustine for 6 cycles (COG Protocol ACNS 0423). She had a continued complete response on post-treatment imaging and remains well and disease-free 4 years post-treatment completion.

Overall, all 3 patients remain alive with no evidence of disease at more than 4 years of post-treatment completion (overall survival = 100%, event free survival = 67%).

Additional Molecular Analyses

Table 2 summarizes the results of immunohistochemical, methylation, and CMA for these cases. The DNA methylation-based tumor classification for all 3 tumors was that of an (anaplastic) PXA. These tumors were located within or in closest proximity to the (anaplastic) PXA tumors used to develop the DKFZ classifier on tSNE plotting, further supporting these classifications. All tumors had unmethylated MGMT promoters. The CNV analyses revealed homozygous CDKN2A deletions in all cases and 2 cases with 9p loss. Snap frozen case and control tumor samples were processed for genome-wide CMA and identified chromosome 9p deletions in Cases 1 and 2 (Figure 1).

Discussion

APXA is an exceedingly rare CNS tumor in children and there is a limited understanding of the natural history of these lesions along with no consensus regarding optimal treatment. Here, we report on 3 pediatric cases of APXA, who are all long-term survivors.

Reassuringly, all 3 cases in this series were accurately diagnosed histologically through neuropathology review alone, and later confirmed through molecular testing with methylation analysis for the purpose of this series. Although rare, APXA is a defined grade III entity in the 2016 update to the fourth edition of the WHO description of tumors of the central nervous system.1 Previously, APXA was challenging to diagnose accurately through histopathological features alone. In fact, the existing literature includes both pediatric and adult cases where APXA was either challenging to diagnose or misdiagnosed as either low-grade tumors or WHO grade 4 gliomas.3,12,18,24-26

Identifying molecular features unique to APXA may aid in the accurate diagnosis of these rare tumors. In pediatric low-grade gliomas (PLGGs), there is a broad genetic landscape, which carries some similarities to APXA. BRAF V600E mutation and homozygous deletion of CDKN2A are well described in PLGG and PXA. Recently, in a molecular analysis of 1000 PLGGs, BRAF V600E was the second most common genetic aberration and CDKN2A was the most common deletion within these tumors.27 In that review, 1.4% of gliomas were PXAs. Both of these molecular aberrations were seen in the tumors within our series. In contrast to the existing literature suggesting a negative prognostic significance of CDKN2A homozygous deletion in IDH-mutant lower-grade glioma and glioblastoma,28 our patients with CDNK2A homozygous deletion are long-term survivors without relapse.

Although molecular features are similar, typically APXA carries a very poor prognosis when compared to PLGG. The prognostic role of a GTR is not well understood in this rare tumor and therefore the optimal adjuvant therapy for patients who undergo a GTR versus subtotal resection is also not known. Among pediatric oncologists, debate exists as to whether APXA in children should be treated like a PLGG, especially in the setting of gross total resection, versus a therapeutic approach similar to high-grade gliomas. Prior to this series, 2 other author groups each described a 5-year-old child with APXA, who were treated with multimodal therapy.17,20 Unlike our cases, both of these patients had disseminated disease and therefore did
not undergo GTRs. In contrast to other cases of APXA, the 3 cases in this report are survivors of more than 4 years after completion of therapy with GTRs, high-dose RT, and chemotherapy. This suggests that these tumors respond to high-grade glioma therapy with multimodality therapy to producing a durable response and enabling long-term survival.

As our understanding of the natural history of APXA and their molecular features improve, a standardized approach to the treatment of these tumors must be developed. In a recent review of 500 pediatric low-grade gliomas, patients with BRAF V600E mutation had a poorer response to chemotherapy and both extent of resection as well as CDKN2A deletion independently predicted prognosis.29 With BRAF V600E mutation testing now becoming standard of care, there may be an increased role for consideration of BRAF and mitogen-activated protein kinase (MEK) inhibitors in the treatment of tumors with BRAF mutations. Currently, an open Children’s Oncology Group trial and Novartis international trial is evaluating the treatment of v600e mutant malignant glioma with radiation, dabrafenib, and trametinib following resection. Targeted therapy may allow improved cure rates, in addition to treatment modification to minimize long-term side effects of alkylators and potentially radiation therapy.

**Conclusions**

This rare pediatric tumor is not well understood. The genetic and epigenetic landscapes may be informative in determining optimal treatment and accurate prognosis. Furthermore, these tumors appear to be aggressive and requiring multimodality therapy to obtain a durable response and produce long-term survival.

**Keywords**

anaplasia | astrocytoma | children | pediatric

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