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CDKN2A/B Loss is Associated with Anaplastic Transformation in a Case of *NTRK2* Fusion-positive Pilocytic Astrocytoma

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Abstract

Pilocytic astrocytomas are typically grade I astrocytomas, only rarely progressing to anaplastic counterparts [1]. In the case of anaplastic pilocytic astrocytomas, some are associated with neurofibromatosis type 1 (NF1) [2], others are associated with radiation treatment [2], and the remainder appear de novo. These de novo tumours can be particularly challenging to distinguish from glioblastomas, which are grade IV and carry a worse prognosis. Here we report an unusual case of malignant transformation of a pilocytic astrocytoma in the absence of *NF1* alterations or radiation treatment.

Keywords

Astrocytoma; Glioma; Mutation

A two -year-old girl presented with several weeks of emesis in the morning and right eye deviation. Head magnetic resonance imaging (MRI) demonstrated a 6 cm heterogeneous right cerebellar mass (Figure 1) with tonsillar herniation and obstructive hydrocephalus. She underwent gross total tumour resection but developed MRI findings concerning for recurrence 10 months later. A 6 mm nodule, near the resection cavity, was subsequently resected 12 months from the initial surgery. After both resections, no adjunct therapy was given.

On histology, the initial resection specimen demonstrated some areas with classic features of pilocytic astrocytoma, including oval to piloid cells, biphasic architecture, and occasional Rosenthal fibres and eosinophilic granular bodies. Other areas demonstrated increased cellularity with anaplastic features, including a focal pseudo-papillary structure (Supplemental Figure 1). Within these anaplastic areas, the cells demonstrated a more epithelioid appearance (Figure 1), with moderate eosinophilic cytoplasm and increased mitotic activity (focally up to 8 mitoses/10 high power fields).

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Conflicts of Interest

The authors have no conflicts of interest to disclose. The study was carried out according to the ethical requirements of the Children's Hospital *of* Philadelphia and UCSF.

The differential diagnosis was broad: Immunohistochemical stains did not show INI-1 loss or BRG-1 loss, and were negative for inhibin, Melan-A, cytokeratin, and transthyretin, arguing against atypical teratoid/rhabdoid tumour, hemangioblastoma, melanoma, or choroid plexus neoplasm. A CD45 stain showed scattered lymphocytes but was negative in tumour cells, excluding lymphoma.

Immunohistochemical stains demonstrated staining for S-100 in both parts. There was strong GFAP staining in the classic-appearing areas, with decreased staining in the regions of anaplasia (Figure 1), supporting a glial neoplasm. Synaptophysin stain showed weak to moderate positivity throughout but did not identify any ganglion cell or neurocytic component. Neurofilament protein stain demonstrated a predominantly solid growth pattern with entrapped axons noted only at the periphery. The MIB-1 labelling index was estimated at up to 1% in classic areas, and up to 7% in the anaplastic regions.

To better assess the two distinct areas of the tumour, next generation sequencing (NGS) was performed on the tumour specimen, with the classic pilocytic area and the anaplastic part of the tumour tested separately at the Children's Hospital *of* Philadelphia utilizing a custom-designed targeted NGS panel that includes sequencing of the coding regions of 237 cancer genes for mutations and copy number variations and RNA-sequencing of 106 fusion gene partners for cancer associated fusion genes (https://www.testmenu.com/chop/ Tests/785964, last accessed 3/22/2018). Both regions demonstrated the presence of a novel *KANK1-NTRK2* fusion. In addition to the fusion, the regions with classic features of pilocytic astrocytoma showed a gain of several genes on chromosome 9p (defined as the presence of greater than two copies of the given region), including *JAK2*, *CD274*, *CDKN2A/B* and *PAX5*. Interestingly, the regions with an anaplastic appearance demonstrated complicated copy number changes on chromosome 9p including gains of *JAK2*, *CD274*, and *PAX5*, and losses of *CDKN2A/B* with homozygous deletion identified in a fraction of cells, as well as gain of additional genes on chromosome 9q, including *GNAQ*, *ABL1*, *NTRK2*, and *TSC1* (Supplemental Table 1). Correspondingly, a p16 stain showed strong staining in classic regions and loss of staining in the majority of tumour cells in the anaplastic regions (Figure 1). Of note, no mutations in *NF1*, *H3F3A*, or *HIS1H3B* were identified. Additional testing was performed separately for MGMT promoter methylation status. Both the classic pilocytic areas and the anaplastic-appearing regions of the initial tumour lack MGMT promoter methylation.

At the time of recurrence, the tumour demonstrated histologic features similar to the anaplastic areas of the initial resection (Supplemental Figure 2). The *KANK1-NTRK2* fusion was still present, as well as the gains on chromosome 9 (Supplemental Table 1), and a heterozygous *CDKN2A/2B* deletion, suggesting expansion of an anaplastic clone. To our knowledge, other than *NTRK2* fusion, none of the chromosome 9 gains have been previously reported in pilocytic astrocytoma, including those examined with whole genome sequencing analysis [3]. It remains unclear which, if any, may have played a role in the rapid recurrence of this tumour.

The most common genetic alteration in pilocytic astrocytoma is *BRAF-KIAA* fusion; however, it was not identified in this case. Instead, the tumour had a unique alteration, in the

form of a *KANK1-NTRK2* fusion; *NTRK2* fusion can occur in a subset of pilocytic astrocytomas without *BRAF-KIAA* fusions [4, 5]. This fusion likely provides an alternative mechanism for MAPK pathway activation, which has been observed in virtually all pilocytic astrocytomas [3, 6].

Loss of p16 expression, as would occur in the setting of *CDKN2A/2B* deletion, has been correlated with more aggressive behaviour in pilocytic astrocytomas [7], and one study identified homozygous *CDKN2A* deletions in 20% of histologically anaplastic pilocytic astrocytomas [8]. Broader studies across all paediatric low-grade gliomas have identified *CDKN2A* inactivation/deletion in over half of secondary high-grade gliomas arising from paediatric low-grade gliomas [9-10], as well as 17% of paediatric low grade gliomas without BRAF V600E mutations [11], although the implications for prognosis are not always as clear in patients with tumours lacking BRAF V600E mutation [12]. A recent report focusing solely on anaplastic astrocytomas with piloid features identified *CDKN2A/B* deletion in 80% of their cohort [13]. In our case, the presence of this deletion solely in anaplastic areas, in combination with atypical cytology and increased mitotic index, suggests anaplastic transformation within a pilocytic astrocytoma. In one case report of a pilocytic astrocytoma with spontaneous anaplastic transformation, BRAF V600E mutation was combined with a heterozygous *CDKN2A* deletion [14], also suggesting *CDKN2A* deletion as a potential driver for anaplastic transformation. Additionally, through the use of the comprehensive NGS-based testing in both components, this case provides evidence supporting *CDKN2A* inactivation as a mechanism for anaplastic transformation in pilocytic astrocytomas, including those with rarer initiating mutations or fusions.

Interestingly, a model system for progression of PXA identified a combination of *CDKN2A* deletion combination with a broader gain of chromosome 9 in mouse xenografts studying PXA progression, and more studies are needed to identify how each may play role in progression of low grade gliomas [9].

ATRX mutations have been correlated with more aggressive behaviour in NF1-associated gliomas, including pilocytic astrocytomas [15]. NGS revealed wildtype *ATRX* in the initial tumour (both low and high-grade regions), as well as the recurrence. Additionally, immunohistochemical staining performed on the initial resection confirmed the presence of ATRX expression. Another study identified *PTEN* mutations in approximately one third of cases [8]. In that study, the three cases identified with *p16* mutation additionally harboured *PTEN* mutations [8]; however, no *PTEN* mutations were identified in the current case.

In many previously reported pilocytic astrocytomas with malignant transformation, there was a prior history of pilocytic astrocytoma treated with radiotherapy. Interestingly, a recent report looking at anaplastic astrocytoma with piloid features identified a history of radiation in only 5% of such cases within their cohort [13], suggesting that radiation is not a requirement for anaplastic transformation. Our case represents anaplastic transformation at initial presentation, with no prior history of radiotherapy. Through analysis of both classic-appearing and anaplastic-appearing regions of the tumour, we are able to provide unique evidence for features present at tumour initiation versus those implicated in tumour progression. This case demonstrates a novel *KANK1-NTRK2* fusion, which likely provides

a mechanism for activation of the MAPK pathway. Finally, this case demonstrates *CDKN2A/B* deletion in the anaplastic-appearing areas and recurrence, providing unique evidence to support the hypothesis that loss of *CDKN2A* can serve as a mechanism of anaplastic transformation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Drs. Mariarita Santi, Brian Harding, Arie Perry, and Giselle López performed histologic and immunohistochemical analysis of the tumour. Dr. Marilyn Li performed the genomic analysis of the tumour. Dr. Giselle López wrote the manuscript. All authors reviewed the manuscript and provided edits.

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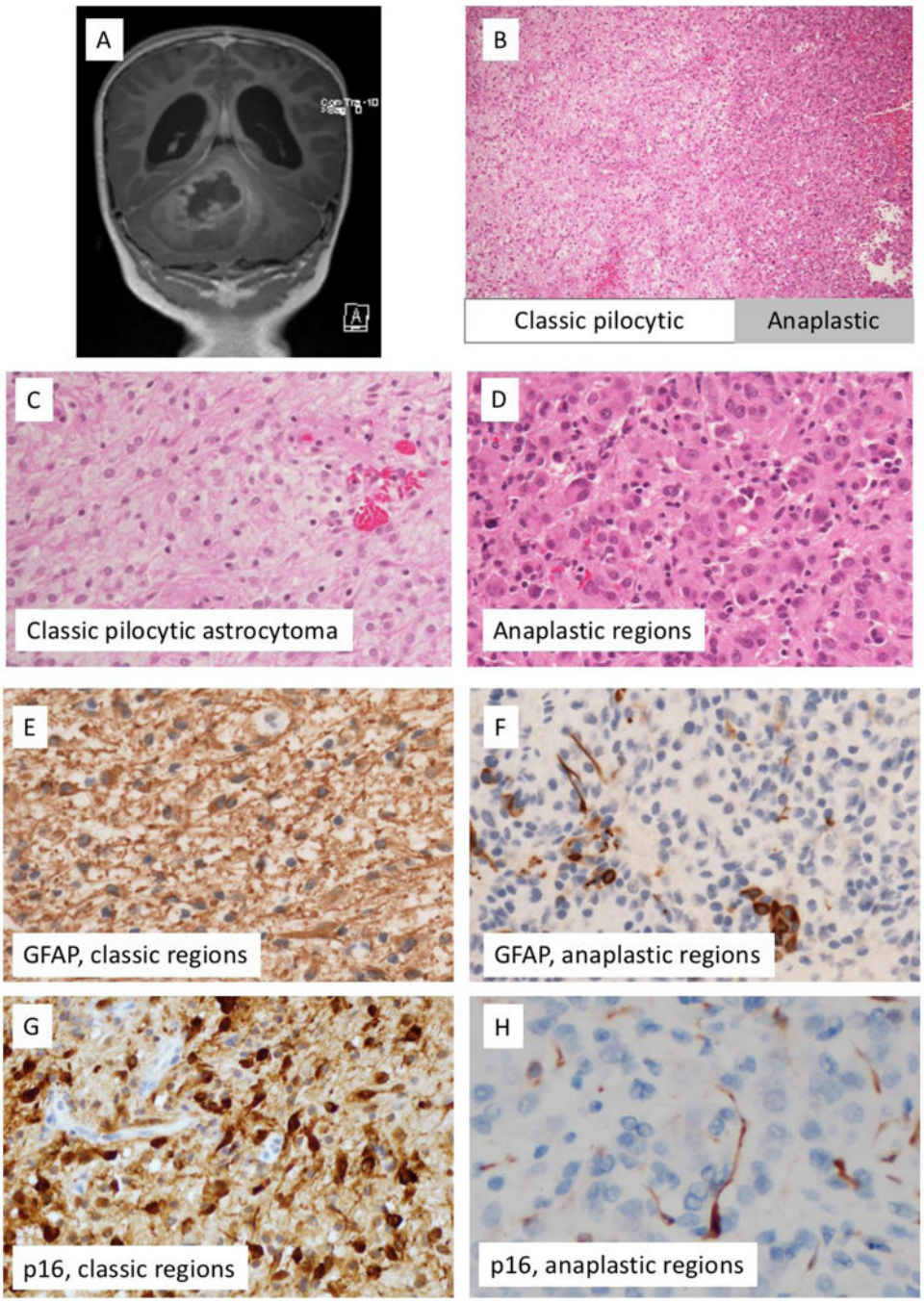

Figure 1.

Findings at time of initial resection. A) Pre-resectionT1-postgadolinium magnetic resonance imaging, B) H&E-stained section, interface of classic-appearing and anaplastic-appearing regions. C) Higher resolution H&E-stained section, region with classic features of pilocytic astrocytoma. D) Higher resolution H&E-stained section, anaplastic region. E) GFAP immunohistochemical staining shows strong diffuse positivity in classic pilocytic regions (E), but only scattered positive cells in anaplastic regions (F). On p16 immunohistochemical

stains, the regions with classic features of pilocytic astrocytoma demonstrate diffuse positivity (G), while anaplastic regions demonstrate loss of p16 expression (H).