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Pilocytic astrocytoma *vs.* ganglioglioma: Progression *vs.* misdiagnosis, and implications in BRAF testing

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ABSTRACT

Ganglioglioma (GG) is a mixed glio-neuronal tumour, comprised of a neoplastic glial component and dysplastic ganglion cells. GG is a tumour of unclear histogenesis; previous studies examining *BRAF* mutations and chromosome imprinting has provided evidence that both the neuronal and glial components likely arise from a common precursor. Both p.V600E mutation and *KIAA1549-BRAF* fusion have been described in pilocytic astrocytoma (PA) and GG, but they differ with regards to the rates of different *BRAF* alterations, and careful histological examination is an important component of patho-molecular correlations. More recently, cases of PA with gangliocytic differentiation (PA-GD) have been described, and these cases are thus far restricted to those with the *KIAA1549-BRAF* fusion.

Here, we describe three cases of GGs in patients with history of previously diagnosed PAs. The cases differ with respect to the chronologic intervals between the PA and the GG diagnoses. In two of the cases, where the PA-GG diagnostic intervals are less than ten years, pathological review revealed the older specimens to have been misdiagnosed as PAs. In the third case, where the interval spanned multiple decades, the GG was found to be positive for both BRAF p.V600E immunohistochemistry (IHC) and for the KIAA1549-BRAF fusion. Molecular study for the *BRAF* p.V600E mutation was negative, proving the IHC result to be a false-positive. Our case demonstrates that cases of GG can harbour the *KIAA1549-BRAF* fusion, even with positive BRAF p.V600E IHC results, and the case highlights a diagnostic challenge that may be encountered.

1. Introduction

Ganglioglioma (GG) is a mixed glio-neuronal tumor, comprised of dysplastic ganglion cells and neoplastic glial cells [1]. It is a well-differentiated, slow-growing tumour, accounting for about 0.4% of all CNS tumours [2,3]. The glial component is variable, and it may include cell types that resemble fibrillary astrocytoma, oligodendroglioma or pilocytic astrocytoma (PA) [1]. Immunohistochemically, the glial component is generally positive for GFAP expression, with faint or absent MAP2 immunoreactivity [4]. The neuronal component can also be highlighted by immunostains (ex. HU), and their dysmorphic features may include clustering and binucleation [1].

The histo-genesis of ganglioglioma remains unclear, but a number of lines of evidence support the hypothesis of a common progenitor for both the neuronal and glial components. *BRAF* p.V600E mutation has been reported in GGs (in 20–60%) [5]. While *BRAF* p.V600E protein appears to be mainly localized to ganglion cells, it has been reported to be expressed in the glial cells [6], suggesting that the mutation may be a founder mutation for both components. In an older study that examined GGs from female patients, it was observed that the same parental X chromosomes were inactivated in five out of seven cases in majority of the cells making up the tumours, also arguing for their development from a common progenitor cell, at least in those five cases [7]. *KIAA1549-BRAF* translocations are seen in pilocytic astrocytomas (ex. [8]), and have also been described in paediatric GGs [8,9]. In those cases without *BRAF* abnormalities, small studies employing a variety of methods, including spectral karyotyping (SKY), genome-wide high resolution single nucleotide polymorphism (SNP)-array and genome-wide copy number analyses, have reported various chromosomal abnormalities, but no clear driver genetic abnormalities [10,11].

Herein, we present three cases of GGs with varying timelines of reclassification/progression from the initial diagnosis of PAs. We compare and contrast the clinical and pathological features of the

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three cases, in consideration of different scenarios that the cases may present and examine the different *BRAF* abnormalities that were detected in these patients. These cases highlight the spectrum of *BRAF* abnormalities that can be seen in GGs and the importance of careful histological examination of these specimens.

1.1. Case one

The patient initially received an operation at age 9 for excision of pilocytic astrocytoma (PA) involving the hypothalamus, which had recurred at ages 13 and 17. When the patient required another surgical resection at age 18, the resection specimen was noted for numerous eosinophilic granular bodies, in line with the previous diagnosis of PA (Fig. 1A). However, the specimen was also notable for the presence of dysmorphic neurons, often bi-nucleated and containing large nuclear pseudo-inclusions (Fig. 1B), and these dysplastic neurons were highlighted by immunostaining for HU (Fig. 1C). Immunostaining for IDH1 (R132H mutated) was completely negative (data not shown). BRAF p.V600E was strongly expressed in the tumour cells, being diffusely expressed in both the neuronal and glial cells (Fig. 1D). *KIAA1549-BRAF* translocation was not seen on molecular testing by the NanoString RNA hybridization assay.

The aforementioned findings triggered review of the pathology of the previous specimens (from ages 13 and 17). Although minor, the dysmorphic neuronal component could be noted in both specimens, and it was concluded that these cases were previously misdiagnosed, resulting in the amendment of the diagnoses to ganglioglioma.

At age 24, the residual tumour currently remains stable in size radiologically.

1.2. Case two

The patient initially presented as a 19-year-old student with profound visual loss. The workup for the symptoms discovered a large mass in the suprasellar and hypothalamic area. The initial management with surgical resection was necessarily incomplete due to the tumour anatomy. The tumour comprised of piloid astrocytes, largely composed of compact biphasic cells interspersed with numerous Rosenthal fibres and eosinophilic granular bodies. Minor component of areas with oligodendroglioma-like cells was noted. Mitotic figures were inconspicuous. The tumour was classified as a PA, as per the WHO classification at the time (2007 version). The surgery was followed by adjuvant radiation and chemotherapy (temozolomide in two separate courses).

The follow-up period for the patient involved repeat imaging, along with multiple CSF diversion (via ventriculo-peritoneal shunt) for symptoms attributable to hydrocephalus. Multiple ventriculostomies were also required. Unfortunately, about nine years after the initial surgery, the patient presented with diminished level of consciousness, confusion and short-term memory loss, and the MRI showed the presence of a suprasellar mass, concerning for growth of the residual tumour. Much of the mass was radiologically cystic, triggering multiple surgical attempts to decompress the cyst. Even after these attempts, it was evident that the mass was growing quickly; in a span of a month and half, the mass had increased from 3.8 × 2.8 cm to 4.7 × 3.5 cm, despite the cystic component having shrunken post-Omnaya reservoir. Based on these findings, another surgical debulking of the tumour was performed at age 28, to be followed by another course of adjuvant radiation.

When the latest resection was examined, the glial component was composed, once again, of piloid astrocytes, along with eosinophilic granular bodies, consistent with the previous diagnoses of PA (Fig. 2A), with the glial component expressing GFAP and MAP2 (data not shown). However, the larger specimen size afforded us the ability to appreciate the dysplastic neurons that were previously not seen. The dysmorphic neurons were often binucleated and were highlighted by immunohistochemistry for HU and chromogranin A (Fig. 2B, 2C). The presence of dysplastic neurons in addition to the glial component warranted amendment of

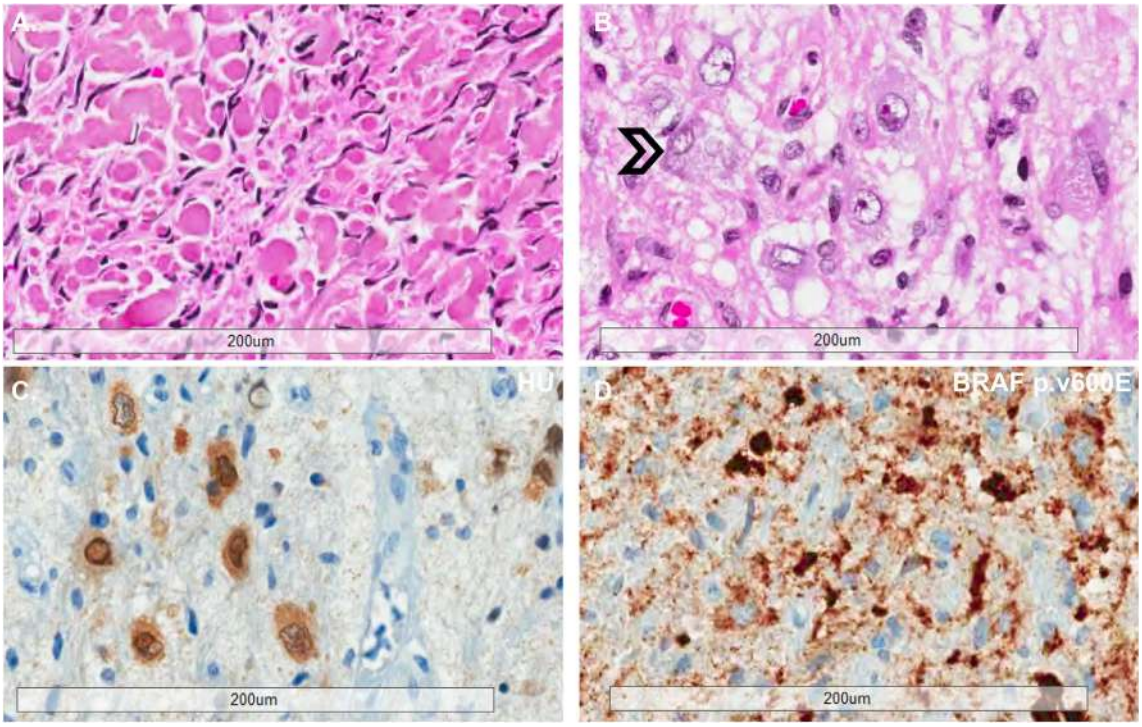

Fig. 1. Case one. A) Representative section from the resection specimen, highlighting the pilocytic astrocytoma-like component of the tumour. Hematoxylin phloxine saffron (HPS) stain. B, C) Representative HPS section (B) showing the dysplastic neurons, including binucleated ganglion cells (arrow head), with immunostaining for HU shown in C). D) Immunostaining for BRAF (V600E). Bar = 200 μm.

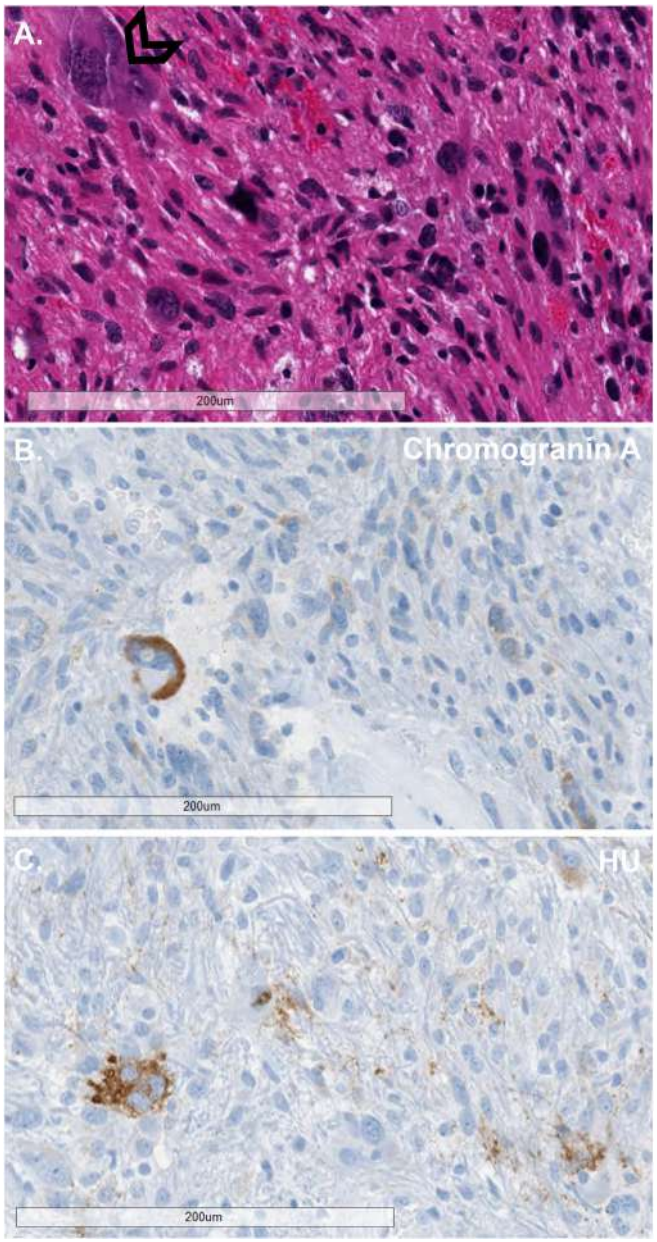

Fig. 2. Case two. A) Representative section from the resection specimen, showing the pilocytic astrocytoma-like component of the tumour, with the dysplastic neurons (arrow head). HPS stain. B, C) HU (B) and chromogranin (C) immunostaining, highlighting the dysplastic neurons seen in A). Bar = 200 μm.

the previous diagnosis as a ganglioglioma, with pilocytic astrocytoma component. BRAF (p.V600E, by immunostaining) was negative in both components (data not shown). The NanoString assay for the *KIAA1549-BRAF* translocation was inconclusive due to RNA degradation, even after multiple attempts.

The patient was discharged about two weeks after the last surgery, having had marked improvement neurologically.

1.3. Case three

This female patient had a remote history significant for a posterior fossa brain tumour resected as a child, which left her blind at age 7. PA was the initial diagnosis rendered, and the initial tumour was not available for review. The patient's past medical history was also notable for scleroderma and interstitial lung disease.

She later presented at age 59 with a 2-year history of progressive posterior fossa symptoms, namely headache, balance difficulties, dizziness and "fogginess" in her head. Radiology showed a multi-cystic posterior fossa lesion with an enhancing nodular component along the posterior vermis of the cerebellum. The patient underwent surgical resection, with the presumed pre-operative diagnosis of PA recurrence.

The resection specimen received consisted of fragments of tissue, most of which comprised of piloid astrocytes, with occasional eosinophillic granular bodies, with dysmorphic, often bi- and multinucleated cells were noted in clusters (Fig. 3A). The dysmorphic cells expressed HU (Fig. 3B) and synaptophysin, revealing their neuronal lineage. Immunostaining for BRAF (p.V600E) showed diffuse, granular staining in the cytoplasm throughout the lesion (Fig. 3C). Thus, considering the presence of the dysplastic neuronal cells and immunostaining for BRAF p.V600E, the diagnosis of ganglioglioma was rendered. Interestingly, when the case was retrospectively examined for the *KIAA1549-BRAF* translocation, the case was positive for the translocation. The IHC positivity was thus followed up by mutant allele specific for *BRAF* p.V600E, and the mutation was undetectable, proving false positive IHC results.

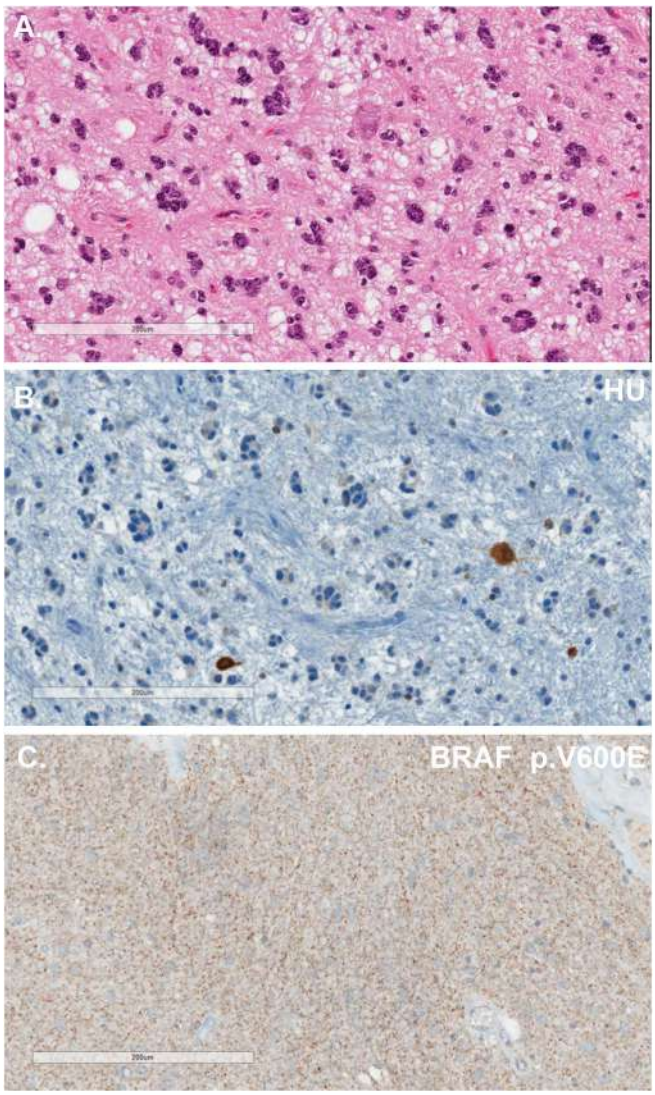

Fig. 3. Case three. A) Representative section from the resection specimen, showing the pilocytic astrocytoma-like component of the tumour, with the dysplastic neurons. HPS stain. B) HU immunostaining, highlighting the dysplastic neurons seen in A). C) Immunostaining for BRAF (V600E). Bar = 200 μm.

The patient remained radiologically stable two years post-surgery, with the MRI being notable for non-specific changes.

2. Discussion

PA and GG are both low-grade, circumscribed gliomas, with, generally speaking, favourable outcomes [3,12], and our series of three cases demonstrate the challenges of distinguishing the two entities. Part of the challenge is related to identifying the gangliocytic component, as demonstrated by the first two cases in this series. With the help of immunostains, some of which may not be routinely available, we were able to change the diagnoses to GG for the two cases.

On the other hand, our last case was a peculiar diagnostic challenge, as the case was positive for BRAF V600E (by IHC), while harbouring the *KIAA1549-BRAF* translocation. In the spectrum of PA and GGs, *BRAF* p.V600E mutation, *BRAF* duplication and translocation (*KIAA1549-BRAF*) have all been described [9,13]. In our case, the IHC immunoreactivity was proven to be false positive with mutant allele-specific amplification. While either the *KIAA1549-BRAF* fusion or the *BRAF* p.V600E mutation may coexist with IDH mutations [14], the two *BRAF* alterations are likely to be mutually exclusive. We noted that the BRAF p.V600E immunoreactivity in case three was weaker and more granular (compared to case one), but our number of cases is insufficient for any IHC-molecular correlation. The number of reported cases of GGs harbouring the *KIAA1549-BRAF* fusion is small [8,14], and false positive IHC results may be a contributing factor.

Ultimately, the diagnosis remains GG for our case three, and we are still left to postulate on the possible relationship between the patient's childhood history of PA and the current GG. Interestingly, Gupta *et al.* divided pediatric GGs into two groups—classic GG *vs.* PAs with focal gangliocytic differentiation (PA-GD) [9]. In their classification, the *BRAF* p.V600E mutation was limited to the classic GGs, while *BRAF* duplication and *KIAA1549-BRAF* fusions were limited to cases of PA-GD. As well, one recent report described a possible progression of PA to GG; the report described a 4-year old patient, initially diagnosed with a cerebellar PA, which harboured the *BRAF* p.V600E mutation [15]. When the tumour recurred, the second resection specimen showed histology compatible with GG, with BRAF p.V600E immunoreactivity in both the neuronal and glial components. Through whole exome sequencing, Fiset *et al.* were able to show that the two lesions shared a number of genetic lesions, including *BRAF* p.V600E, and the GG harboured additional mutations (such as a truncating mutation in *MAP2*). In our patient, considering the long chronological gap between the first diagnosis of PA and the "recurrence" of the tumour as GG, the story may represent either a misdiagnosis of GG (as in the first two cases), a PA-to-GG "progression", a *de novo* case of GG (unrelated to the previous PA diagnosis), or a case of a PA-GD in an adult patient. This case highlights the diagnostic challenge in the PA *vs.* GG distinction, and highlights the importance of interpreting ancillary study results, be it IHC or molecular, in the context of histomorphology and patient history.

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