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IDH-1 polymorphisms in pilocytic astrocytomas

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ABSTRACT

As of 2016, isocitrate dehydrogenase (IDH)-1 and IDH-2 mutations are part of the definition of an oligodendroglioma and may be seen in a significant subset of grade II-IV fibrillary astrocytomas. Reports of IDH-1 and IDH-2 alterations in pilocytic astrocytomas have been rare. This study reports two cases of pilocytic astrocytomas which harbored IDH-1 polymorphisms (G105G) (c.315C > T) discovered on polymerase chain reaction (PCR) testing and sequencing. The first was encountered in a 21-year-old male with a right orbital frontal pole mass. The second occurred in a 19-year-old female with a right frontal tumor. Neither tumor stained with antibody to IDH-1 (R132H). No BRAF V600E immunostaining, minimal p53 staining (< 5%) and no loss of ATRX staining was noted in both cases. The significance of the IDH-1 findings at this juncture is uncertain. Misdiagnosis of the tumor as a fibrillary astrocytoma or oligodendroglioma due to the presence of an IDH alteration should be avoided.

1. Introduction

Pilocytic astrocytomas are low grade tumors which can arise at any age but are most frequently encountered in pediatric patients. Distinction of pilocytic astrocytoma from diffuse astrocytoma and oligodendroglioma is important. The 2016 WHO Classification of Tumours of the Central Nervous System redefined oligodendrogliomas as tumors, which in addition to having morphology consistent with what had been previously described as oligodendroglioma, also need to demonstrate evidence of an isocitrate dehydrogenase (IDH)-1 or IDH-2 mutation along with evidence of codeletion on chromosomes 1p and 19q [1]. A subset of diffuse astrocytomas are also noted to show evidence of IDH mutations, although generally, they do not demonstrate evidence of full arm deletions on chromosomes 1p/19q [2-4].

Pilocytic astrocytomas may show some morphologic features which resemble fibrillary astrocytomas or oligodendrogliomas but are generally considered unique tumors and do not display the typical molecular findings of oligodendroglioma or diffuse astrocytoma. They show their own distinctive molecular findings, most notably evidence of BRAF abnormalities – either a mutation in the BRAF V600E or evidence of KIAA1549-BRAF fusion [5-7]. The purpose of this study is to review a series of pilocytic astrocytomas which underwent both immunostaining with antibody to IDH-1 (R132H) and polymerase chain reaction (PCR) testing looking for evidence of IDH-1 or IDH-2 mutation.

2. Materials and methods

Institutional Review Board Approval was obtained prior to commencement of the study. The surgical pathology files were searched for astrocytic tumors that had IDH-1(R132H) staining and IDH-1/-2 PCR testing performed from June 2016–June 2019. Seven pilocytic astrocytomas, WHO grade I tumors were identified out of 366 astrocytic neoplasms that had both of these tests performed. Of these seven pilocytic astrocytomas, two were found to have an IDH-1 alteration by PCR and are the focus of this study. Clinical information on these two patients was obtained by review of the medical record.

For each of those two cases, a panel of antibody stains were evaluated including BRAF V600E (1:500 dilution; EMD Millipore. Burlington, MA), IDH-1 (R132H) (1:40 dilution. Histo BioTec. Miami Beach, FL), p53 (1:20 dilution. Agilent DAKO, Santa Clara, CA), ATRX (1:200 dilution. Sigma Aldrich. St. Louis, MO), and Ki-67 (prediluted. Ventana. Indianapolis, IN).

IDH-1/-2 PCR testing was performed in all seven pilocytic astrocytomas. DNA was extracted from tissue sections cut from the formalin-fixed, paraffin-embedded block and library construction was done utilizing the custom Cancer Hotspot Panel v.1 (Life Technologies, Grand Island, NY). DNA sequencing of gene mutation hotspot regions was performed on the MiSeq instrument (Illumina, San Diego, CA). NextGENe software (Softgenetics, State College, PA) was used to analyze FASTQ files to identify hotspot mutations if present.

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3. Results

Of the seven pilocytic astrocytomas identified during this time period that had both IDH-1 immunostaining and IDH-1/-2 PCR testing performed, only two tumors demonstrated evidence of and IDH-1 alteration. The other five patients included four females and 1 male who ranged in age from 13 to 68 years. These tumors were located in the temporal lobe ($N = 2$), cerebellum ($N = 1$), pons (N = 1), and suprasellar region (N = 1). BRAF V600E immunostaining was evaluated in four of these cases and was observed to be negative. More specific information regarding the two patients of interest is reported as follows.

3.1. Case 1

The patient was a 21-year-old male, previously in good health, who presented with a generalized tonic clonic seizure. Computed tomographic (CT) and magnetic resonance imaging (MRI) studies showed a partially calcified and enhancing tumor situated in the paramedian right orbital frontal pole. Based on the imaging studies, a diagnosis of a low grade glioma was favored. The patient underwent surgical gross total resection of the tumor. The tumor microscopically was marked by mild to focally moderate hypercellularity, comprised of spindled, piloid cells arranged against a fibrillary background (Fig. 1). Rosenthal fibers were focally noted within the tumor (Fig. 1). Areas with a microcystic appearance and looser arrangement of tumor cells were noted (Fig. 2). Focally, calcifications, as suggested by the imaging studies, was observed (Fig. 3). Mitotic activity and necrosis were not seen.

The tumor did not stain with antibody to IDH-1 (R132H). An IDH-1 G105G (c.315C > T) polymorphism was identified in the tumor on PCR testing. No variant was detected on IDH-2. The tumor did not stain with antibody to BRAF V600E. Less than 5% of tumor cells stained positivity with antibody to p53. Loss of ATRX staining, suggestive of a mutation, was not observed. A Ki-67 labeling index of approximately 3% was noted focally (Fig. 4).

A postoperative MRI 4 months later showed no evidence of recurrent tumor. No additional followup is available at the time of this writing.

3.2. Case 2

The patient was a 19-year-old female who presented 8 months prior to surgery with new onset generalized tonic clonic seizures. An initial electroencephalogram (EEG) study showed no abnormalities. A MRI study identified a 2.4 cm mass centered between the right superior and middle frontal area with adjacent edema. The tumor showed heterogenous hyperintensity on the T2-weighted images. The patient

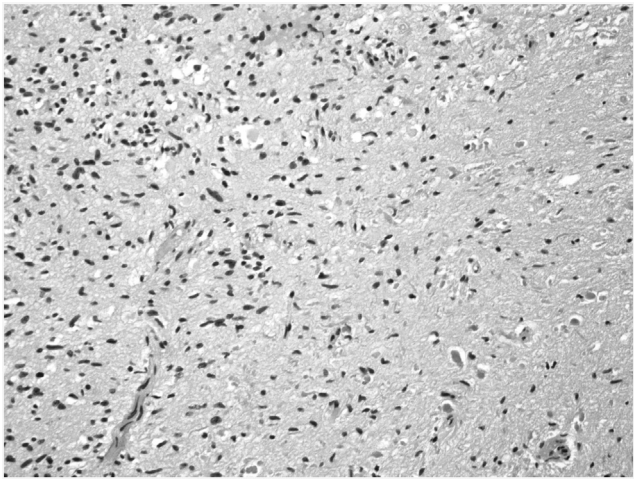

Fig. 1. Case 1. The tumor was marked by a proliferation of spindled cells arranged against a fibrillary background with scattered Rosenthal fibers (hematoxylin and eosin, original magnification 200×).

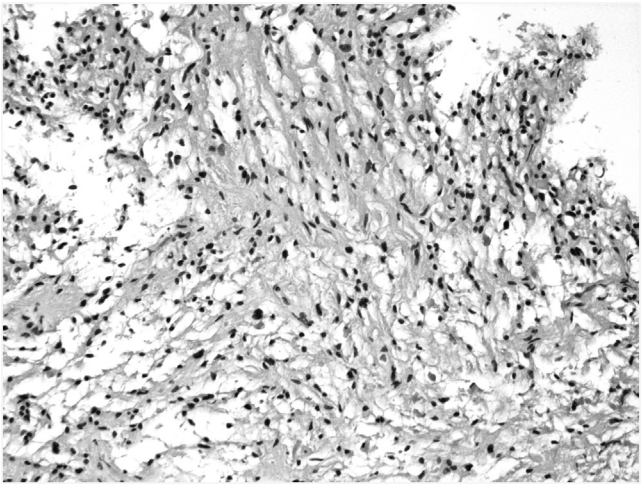

Fig. 2. Case 1. In areas of the tumor, cells were more loosely arranged (hematoxylin and eosin, original magnification 200×).

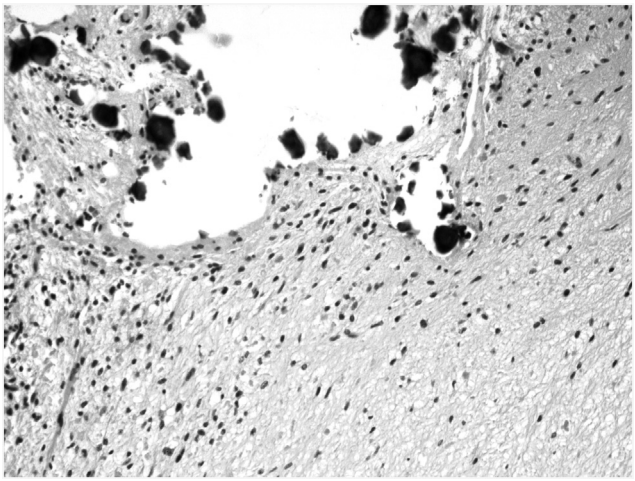

Fig. 3. Case 1. Focally, microcalcifications were noted in this patient's tumor (hematoxylin and eosin, original magnification 200×).

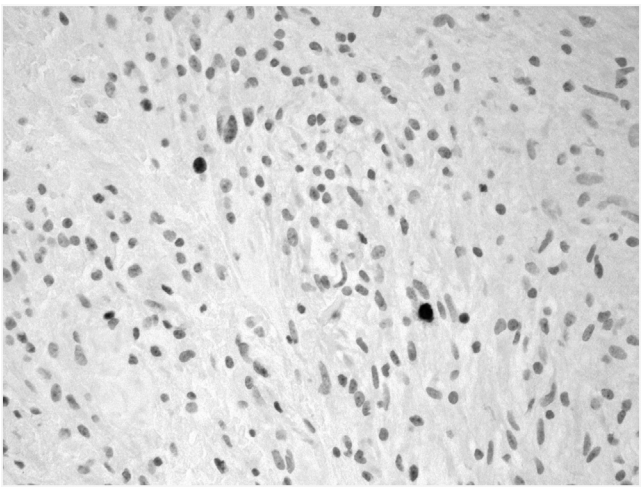

Fig. 4. Case 1. A Ki-67 immunostain showing occasional positive staining tumor cell nuclei; a labeling index of about 3% was focally noted in this tumor (original magnification 400×).

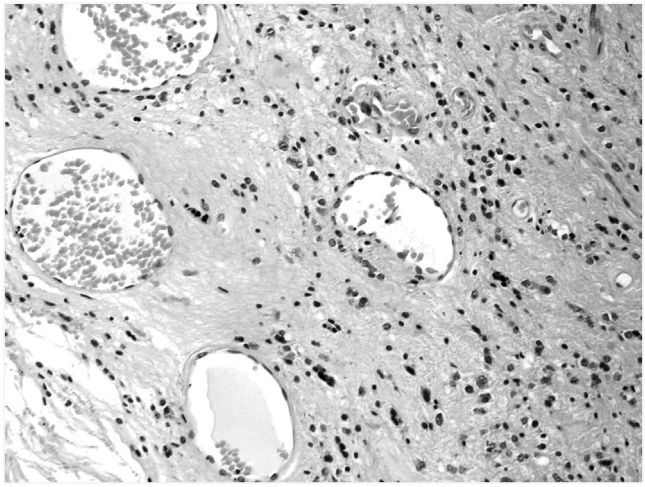

Fig. 5. Case 2. The tumor has a biphasic appearance with fibrillary areas with Rosenthal fibers juxtaposed to microcystic areas (lower left) (hematoxylin and eosin, original magnification 200×).

underwent gross total resection of the mass.

Histologically, the tumor had a biphasic appearance with more fibrillar areas juxtaposed to areas which appeared more microcystic (Fig. 5). Occasional Rosenthal fibers were observed in the more fibrillar areas; eosinophilic granular bodies were not noted (Fig. 5). In areas of the tumor, thickened and fibrosed blood vessel walls were observed (Fig. 6). Focally, vascular proliferative changes (Fig. 7) and microcalcifications were seen. Mitotic activity and necrosis were not identified.

Similar to Case 1, the tumor did not stain with antibody to IDH-1 (R132H) (Fig. 8). An IDH-1 G105G (c.315C > T) polymorphism was identified in the tumor on PCR testing, identical to the one seen in Case 1. No variant was detected on IDH-2. The tumor did not stain with antibody to BRAF V600E. Less than 1% of tumor cells stained positively with antibody to p53. Loss of ATRX staining, suggestive of a mutation, was not observed. A Ki-67 labeling index of approximately 5% was noted focally.

At her most recent follow-up 10 months after surgery, there was no evidence of tumor recurrence on MRI and she had no evidence of seizures since surgery. She complained of occasional headaches in the region of the prior surgery.

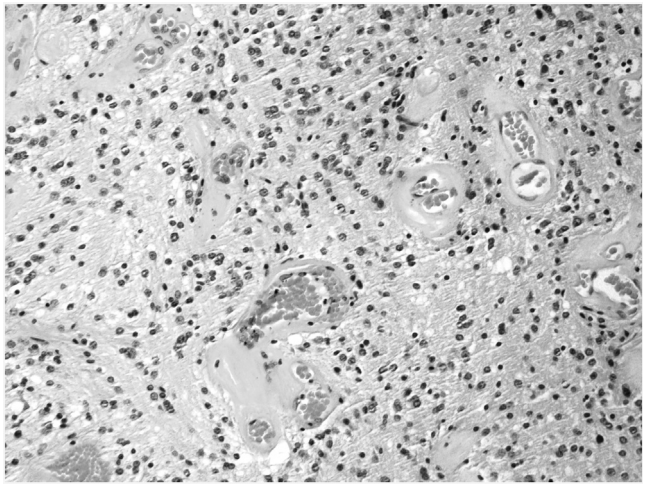

Fig. 6. Case 2. Vascular sclerosis was focally noted in the tumor (hematoxylin and eosin, original magnification 200×).

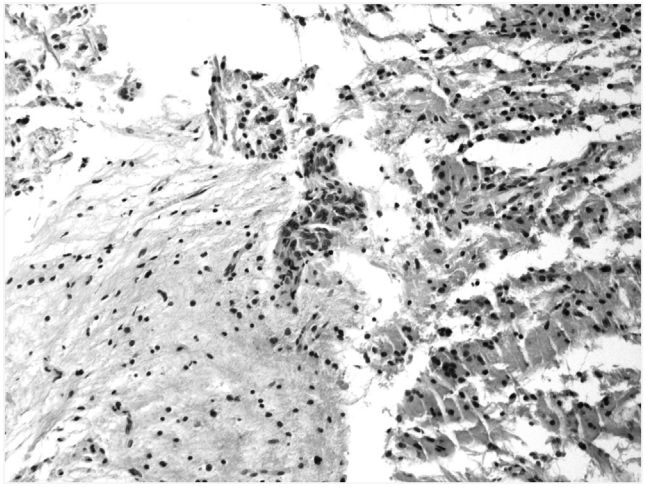

Fig. 7. Case 2. Vascular proliferation was also focally observed in this tumor (hematoxylin and eosin, original magnification 200×).

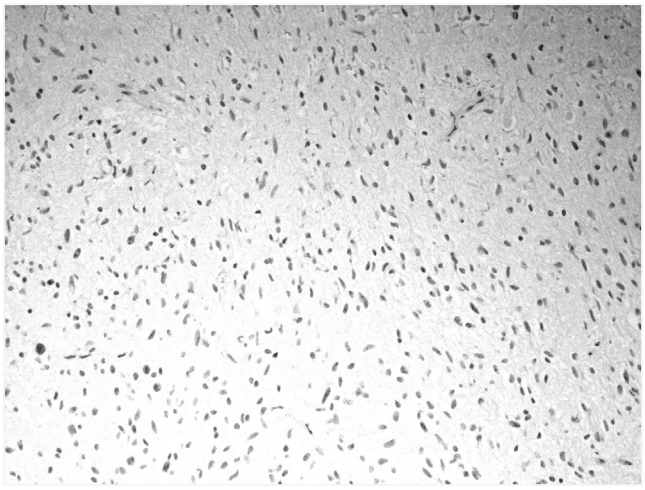

Fig. 8. Case 2: Similar to case 1, the tumor did not stain with antibody to IDH1 (R132H). (original magnification 200×).

4. Discussion

IDH-1 and IDH-2 mutations are a well recognized diagnostic feature of oligodendrogliomas and may also be encountered in a subset of fibrillary astrocytomas [1-4]. Hartmann and colleagues in a series of 1010 gliomas reported in 2009, prior to the requirement of an IDH mutation for the diagnosis of oligodendroglioma, noted IDH-1 mutations in 716 cases and IDH-2 mutations in 31 cases [4]. In another study from 2009 by Yan et al., IDH-1 mutations were noted in > 70% of WHO grade II and III astrocytomas, oligodendrogliomas and glioblastomas that developed from lower grade tumors [2]. A Similarly high rates of IDH mutations were observed in studies done by others [3,8,9].

A few of these large studies examined a spectrum of brain tumors, including some pilocytic astrocytomas. In their study, Chen and colleagues studied 40 pilocytic astrocytomas and found no evidence of an IDH-1 (R132H) clone H09 mutation by immunohistochemistry in any of the tumors evaluated [8]. They also found no evidence of an IDH-1 or IDH-2 mutations in any of the 26 tumors which had sequencing analysis performed [8]. In the Balss et al. study, 41 pilocytic astrocytomas were evaluated for an IDH-1 mutation on codon 132 by PCR amplification and direct sequencing; a mutation was observed in only 1 tumor [3]. Details regarding that case were not provided in the paper [3]. In a series of 70 pilocytic astrocytomas reviewed by Korshunov et al., none

of the tumors were noted to have either an IDH-1 or IDH-2 mutation by direct sequencing [10]. 70% of the cases in their series demonstrated evidence of a BRAF-KIAA1549 fusion [10].

In an evaluation of 65 pilocytic astrocytomas by PCR and sequencing, Cruz et al. noted that 9 tumors showed alterations in IDH-1 and 2 tumors showed an alteration in IDH-2 [11]. Most commonly, an IDH-1 polymorphism (G105G), similar to the findings in the two patients in the current study, were identified [11]. Similar polymorphisms have been noted in some patients with acute myeloid leukemia who have a worse prognosis [12]. The significance of this finding in pilocytic astrocytomas is uncertain at this juncture. The other observed mutations noted by Cruz and coworkers included two IDH-1 mutations: R132H (c.395G > A) in one case and R109K (c.326G > A) in one case [11]. One mutation on IDH-2 was also noted, R172S (c.516G > C). [11].

Two additional cases of IDH-1 (R32H) mutated pilocytic astrocytomas were reported in 2016 [13,14]. Medress and coworkers described an 86-year-old woman with a cerebellar pilocytic astrocytoma which demonstrated positive immunostaining with antibody to IDH-1 (R132H) [13]. The tumor showed no evidence of a BRAF V600E mutation or BRAF fusion [13]. The second case report described a 72-year-old woman with a cerebellar pilocytic astrocytoma which demonstrated a mutation by sequencing [14]. Similarly, BRAF V600E and BRAF fusion abnormalities were also not identified in this case [14].

Due to the relative infrequency of IDH-1 and IDH-2 alterations in pilocytic astrocytomas thus far reported, the significance of these findings is uncertain. The implications of these findings, however, are important for two main reasons. First, it is important to be aware that they do occasionally occur in astrocytoma types other than fibrillary astrocytomas grade II-IV and their presence should not necessarily equate with a diagnosis of a fibrillary astrocytoma. Second, there are potential implications in terms of targeted therapeutic approaches in the form of inhibitors that may in the future prove useful in the management of mutated tumors [15].

Declaration of competing interest

None.

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