Case Report

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Anaplastic glioneuronal tumor with *KIAA1549/BRAF* fusion

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Summary **Glioneuronal tumors are usually low-grade and have favorable prognosis. The anaplastic glioneuronal tumor with *KIAA1549/BRAF* fusion has not yet been documented. This article reports a case of glioneuronal tumor with anaplasia and *KIAA1549/BRAF* fusion to illuminate the importance of *KIAA1549/BRAF* fusion in high-grade glioneuronal tumors. A ten-year-old boy presented with one year of headache and three months of blurry vision and proptosis. Ophthalmologic evaluation revealed bilateral papilledema. Magnetic resonance imaging showed a large mixed cystic and solid mass in the left frontal lobe of cerebrum. Histologic analysis demonstrated a neoplasm with pseudopapillary growth pattern, focal necrosis, microcalcification, and brisk mitotic activity with a high Ki67 labeling index of focally up to 20%. Immunohistochemical assessment identified a mixed glial and neuronal neoplastic cell population. Molecular studies revealed a *KIAA1549/BRAF* fusion. The histological and molecular changes are consistent with an anaplastic glioneuronal tumor with *KIAA1549/BRAF* fusion. In view of the fact that the effective, targeted therapies for the tumors with *KIAA1549/BRAF* fusion are available, detection of *KIAA1549/BRAF* fusion for high-grade glioneuronal tumors is clinically helpful.**

Keywords: Glioneuronal tumor, *KIAA1549/BRAF* fusion, anaplasia, central nervous system

1. Introduction

Glioneuronal tumors are a group of neoplasms composed of mixed glial and neuronal cells. According to the WHO classification, this group of tumors includes papillary glioneuronal tumor, rosette-forming glioneuronal tumor, diffuse leptomeningeal glioneuronal tumor, ganglioglioma, dysembryoplastic neuroepithelial tumor (DNT), and desmoplastic infantile ganglioglioma (*1*). These tumors are low-grade, except for anaplastic ganglioglioma (WHO grade III) and a minor subset of diffuse leptomeningeal glioneuronal tumors, and have favorable prognosis.

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Several underlying genomic alterations have been identified in glioneuronal tumors (*1*). Gangliogliomas possess *BRAF* V600E mutation and *BRAF* fusion to *FXR1*, *KIAA1549* or *MACF1*. Reduced expression of *LDB2* gene is also found in the neuronal component of this type of tumors. Anaplastic gangliogliomas harbor *CDKN2A* deletion or gain/amplification of *CDK4*. *PIK3CA* and *FGFR1* mutations as well as *KIAA1549/BRAF* fusion are identified in some of rosette-forming glioneuronal tumors (*2*). Approximate three-fourths of diffuse leptomeningeal glioneuronal tumors show *KIAA1549/BRAF* fusion, and either 1p or 1p19q chromosomal deletion is identified in some of these tumors as well. *BRAF* V600E mutation is documented in approximately 30% of DNTs. Minority of desmoplastic infantile gangliogliomas have *BRAF* V600E mutation. Papillary glioneuronal tumors exhibit *SLC44A1-PRKCA* fusion in a high portion of the cases, and *FGFR1* N546K mutation is also reported in one case of this tumor (*3*).

KIAA1549/BRAF fusion has not been reported in

high-grade glioneuronal tumors so far in the literature. Here, we discuss a case of glioneuronal tumor with anaplasia and *KIAA1549/BRAF* fusion.

2. Case Report

2.1. *Clinical history*

A ten-year-old boy presented to hospital with one year of headache and three months of blurry vision and proptosis. Ophthalmologic examination demonstrated bilateral papilledema. Magnetic resonance imaging (MRI) with and without contrast showed a mixed cystic and solid mass measuring 7.7 × 7.2 × 8.9 cm in the left frontal lobe with significant mass effect upon the left lateral ventricle, 1.7 cm of left to right midline shift, and right lateral hydrocephalus (Figure 1). A near total resection was performed. The resulted tissue was submitted for pathology evaluation. The patient is currently receiving continuous care in an outside hospital and in a stable condition.

2.2. *Histopathology*

Intraoperative cytological study showed mixed cell population including cells with regularly shaped nuclei, moderate eosinophilic cytoplasm, and cell processes (astrocytes); cells with small hyperchromatic nuclei and small amount of eosinophilic cytoplasm, and no obvious cell process (oligodendrocyte-like cells); and cells with round or oval nuclei and scant or inconspicuous cytoplasm (neurocytes). There were no bipolar cells in the cytological smear preparation. Histological examination of the tumor using hematoxylin and eosin staining revealed a hypercellular neoplasm with diffuse pseudopapillary growth pattern. The neoplastic cells around hyalinized vessels were flattened or cuboidal with moderate nuclear pleomorphism and cell processes and in single or pseudostratified layer overlying hyalinized vascular cores. In the interpapillary area, there were small to medium size neoplastic cells, and some of them had eccentrically located nuclei and eosinophilic cytoplasm. There were no definitive nucleolus-bearing dysplastic ganglioid cells in the specimen examined. Multifocal microcalcifications were present, and scattered hemosiderin-laden macrophages were noted. Focal necrosis was evident. There was focal brisk mitotic activity in the tumor. Neither Rosenthal fibers nor eosinophilic granular bodies were present. Immunohistochemical studies revealed that Ki67 labeling index was diffusely increased with several foci of up to 20%. Neoplastic cells mostly surrounding the vascular cores co-expressed glial fibrillary acid protein (GFAP) and S100 (data not shown), while neoplastic cells mostly residing in the interpapillary area were reactive with OLIG2 antibody. Multiple foci of neoplastic cells majorly located in the interpapillary area expressed synaptophysin, neuron specific enolase (NSE) (data not shown), and NeuN (data not shown). There were no cells expressing neurofilament protein (NFP) by immunohistochemical study. Chromochranin-A expression was absent. There was a tendency that the GFAP-positive cells were more concentrated perivascularly, while the cells expressing synaptophysin, NSE, NeuN, and OLIG2 mostly resided in the interpapillary area (Figure 2).

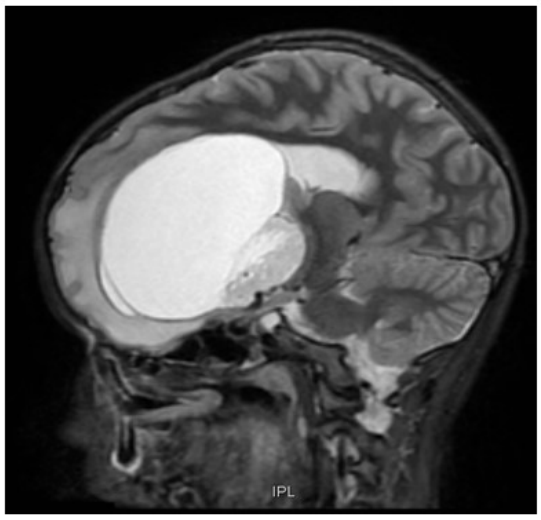

Figure 1. Magnetic Resonance Imaging, T2-weighted, showed a cystic and solid mass in the left frontal lobe.

2.3. *Molecular and cytogenetic study*

Molecular and cytogenetic studies were performed on this case. The next-generation DNA sequencing that evaluates 30 genes for point mutations, small insertions, and deletions as well as 24 genes for copy number changes carried out the molecular studies by the Molecular & Genomic Pathology Laboratory, University of Pittsburgh (*4*). These genes include *IDH1/2*, *ATRX*, *BRAF*, *KRAS*, *PTEN*, *TERT*, *p53*, *AKT1*, *CDK6*, *CIC*, *CDKN2A*, *CTNNB1*, *DDX3X*, *EGFR*, *FUBP1*, *H3F3A*, *HRAS*, *KLF4*, *KRAS*, *MET*, *MYC*, *MYCN*, *NF1/2*, *NRAS*, *PIK3CA*, *PTCH1*, *RB1*, *SETD2*, and *SMO*. This sequencing also screened for 16 subtypes of *BRAF* and *FGFR3* gene fusions as well as *EGFRvIII* structural alterations. Methylation status of the *MGMT* gene promoter was assessed with methylation specific polymerase chain reaction (MS-PCR) by the Molecular & Genomic Pathology Laboratory, University of Pittsburgh. Cytogenetic studies for chromosomal abnormalities, particularly 1p and/or 19q deletion, were performed using the nuclear in situ hybridization technique by the Cytogenetics Laboratory, Robert Wood Johnson Medical School. The above studies demonstrated a *KIAA1549/BRAF* fusion. There were no other molecular and cytogenetic alterations (Table 1).

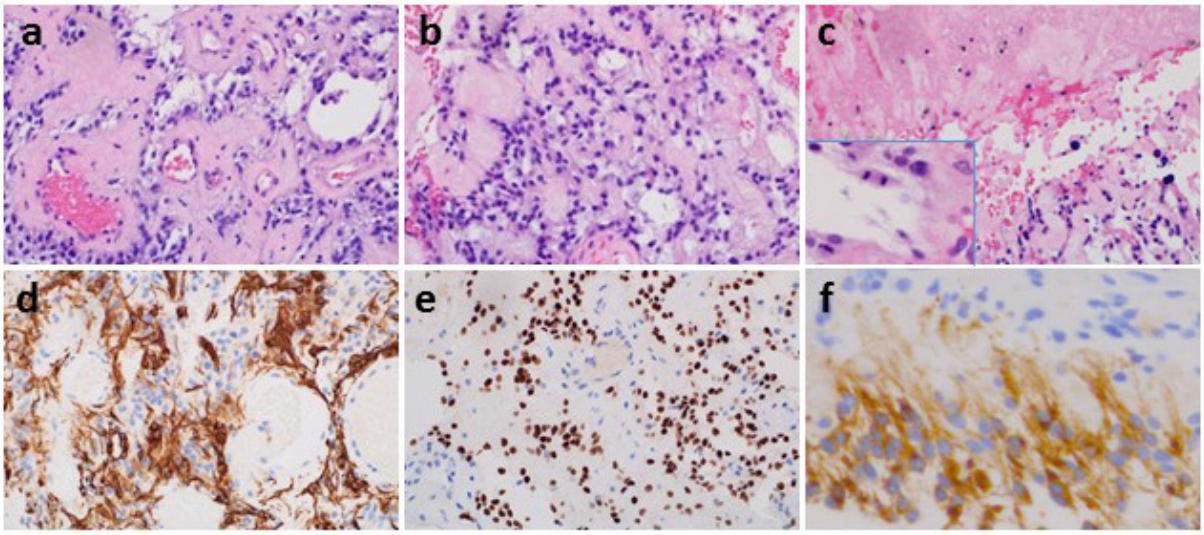

Figure 2. The lesion showed a cellular tumor with mixed cell population, pseudopapillary growth pattern (**a** and **b**), focal necrosis (**c**), and brisk mitotic activity (insert [3 mitotic figures] in **c**). Immunohistochemical studies demonstrated a mixed glial and neuronal cell population (**d-f**). **a-c**) H+E, 400×; **d**) GFAP immunostaining, 400×; **e**) OLIG2 immunostaining, 400×; **f**) Synaptophysin immunostaining, 600×.

Table 1. Molecular and cytogenetic study

Test	Result
Next-generation DNA Sequencing (GlioSeq)	*KIAA1549-BRAF* fusion
Methylation-specific PCR	Negative for MGMT promoter hypermethylation
Nuclear in situ hybridization	Negative for 1p and/or 19q chromosomal deletion

3. Discussion

The histopathological and molecular findings of this tumor are consistent with an anaplastic glioneuronal tumor with *KIAA1549/BRAF* fusion. Previous studies have revealed that the primary central nervous system (CNS) tumors can harbor *KIAA1549/BRAF* fusion (*5*). The *KIAA1549/BRAF* fusion has been considered as a characteristic genomic alteration of pilocytic astrocytoma in recent years. However, our case does not have the essential histological features such as bipolar glial cells, Rosenthal fibers, eosinophilic granular bodies, and unique biphasic histologic pattern to satisfy the classification of pilocytic astrocytoma. Instead, this tumor contains significant glial and neuronal components and exhibits a diffuse pseudopapillary growth pattern, which is more like those seen in a papillary glioneuronal tumor. Interestingly, the *KIAA1549/BRAF* fusion is also commonly seen in glioneuronal tumors. However, up to date, this genomic change has only been identified in several low-grade glioneuronal tumors such as ganglioglioma, rosette-forming glioneuronal tumor, and diffuse leptomeningeal glioneuronal tumor (*1,2,6*). Our case demonstrates that the *KIAA1549/BRAF* fusion exists in a high-grade glioneuronal tumor.

The v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) gene is located at chromosome 7 (7q34) and encodes BRAF protein. The latter is a serine/threonine protein kinase in the downstream of RAS-RAF-MEK-ERK signaling pathway, also known as MAPK/ERK pathway, and a signal transducer between extracellular growth stimulus and cellular response. Three RAF proteins (ARAF, BRAF, and CRAF/c-RAF-1) are the first activators of RAS downstream. RAS-GTP association and binding of RAS-binding domain to the N-terminal regulatory region of RAF kinase result in recruitment of the RAF proteins to cell membrane and structure alteration of the RAF protein, leading to RAF activation. RAF subsequently activates MEK and ERK via phosphorylation, which in turn activates transcription factors Elk-1, c-Fos, and c-Myc; promoting cellular differentiation and proliferation (*7*). BRAF is the most potent activator of MEK/ERK. Regulation of the MAPK/ERK pathway is important for the balance between extracellular signaling and gene transcription. Dysregulation of this pathway leads to tumorigenesis (*8*). The *KIAA1549/BRAF* fusion results in a tandem duplication involving chromosome 7q34. This fusion retains the C-terminal BRAF protein kinase and the substituted N-terminal which does not have the BRAF auto-regulatory domain, leading to constitutive activation of the BRAF kinase domain and hyperactivity of the MAPK/ERK pathway (*9*).

The clinical prognostic significance of *KIAA1549/BRAF* fusion for primary brain tumors seems to be dependent on histological type, location, and age of diagnosis. There is a debate over the clinical relevance of *KIAA1549/BRAF* fusion status. Some studies have reported better clinical outcome for a pediatric low-grade astrocytoma with *KIAA1549/BRAF* fusion (*10-12*), while others failed to find similar results (*9,13,14*). Despite the inconclusive prognostic significance of *KIAA1549/BRAF* fusion, detection of its presence is still useful in view of that the effective, targeted therapies for *KIAA1549/BRAF* fusion are clinically available (*15*).

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