Case Report

Novel BRAF Alteration in a Sporadic Pilocytic Astrocytoma

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Pilocytic astrocytoma (PA) is the most frequently encountered glial tumor (glioma or astrocytoma) in children. Recent studies have identified alterations in the BRAF serine/threonine kinase gene as the likely causative mutation in these childhood brain tumors. The majority of these genetic changes involve chromosome 7q34 tandem duplication, resulting in aberrant BRAF fusion transcripts. In this paper, we describe a novel KIAA1549:BRAF fusion transcript in a sporadic PA tumor associated with increased ERK activation and review the spectrum of BRAF genetic alterations in this common pediatric low-grade central nervous system neoplasm.

1. Introduction

Pilocytic astrocytomas are the most common nonmalignant brain tumor in the pediatric population. Children with the Neurofibromatosis type 1 (NF1) inherited cancer predisposition syndrome are prone to the development of these glial cell neoplasms, such that 15–20% of affected individuals will develop gliomas involving the optic pathway, hypothalamus, and brainstem [1]. Molecular analysis of these tumors has revealed biallelic inactivation of the NF1 tumor suppressor gene, resulting in loss of NF1 protein (neurofibromin) expression. However, sporadic PA tumors do not exhibit mutational inactivation of the NF1 gene, suggesting that other genetic mutations are responsible for the genesis of these histologically-identical low-grade brain tumors in the general population [2].

Over the past several years, the molecular basis for these nonsyndromic pediatric brain cancers has been elucidated with the identification of signature molecular changes involving the BRAF serine/threonine kinase gene. The most frequently encountered genetic alteration is a tandem duplication of the BRAF gene on chromosome 7q34, leading to fusion of the KIAA1549 gene to the carboxyl terminal region of the BRAF gene containing the kinase domain. This molecular change has been reported in 50–65% of sporadic pilocytic astrocytoma and is more frequent in cerebellar (~80%) tumors. The majority of these alterations involve fusions between KIAA1549 exon 16 and BRAF exon 9, KIAA1549 exon 15 and BRAF exon 9, and KIAA1549 exon 16 and BRAF exon 11 [3–9], while less common alterations include tandem duplications involving SRGAP3 and RAF1 or FAM131B and BRAF [10, 11]. In this paper, we describe a novel KIAA1549-BRAF fusion event in a sporadic pediatric pilocytic astrocytoma.

2. Case Presentation

The patient was a 14-year-old boy who presented with a 6-month history of headache that progressed to a two-day period of nausea, vomiting, and ataxia. Magnetic resonance imaging (MRI) at that time showed a cystic mass in the cerebellum compressing the fourth ventricle (Figure 1(a)). He was taken to the operating room where a gross total resection was performed. Neuropathological review revealed a classic pilocytic astrocytoma with alternating areas of compact and loose tissue architecture (Figure 1(b)). The compact areas were composed of piloid neoplastic cells containing numerous Rosenthal fibers and few eosinophilic granular bodies (Figure 1(c)), while the paucicellular areas
Figure 1: Molecular characterization of a novel KIAA1549:BRAF fusion alteration in a sporadic pediatric pilocytic astrocytoma. (a) Axial T1-weighted 1.5-Tesla gadolinium-enhanced MRI scan reveals a cystic lesion in the cerebellum with a peripheral enhancing nodule (arrow). Hematoxylin and eosin staining demonstrates a classic pilocytic astrocytoma with compact and loose areas (b), including Rosenthal fibers (arrow) and eosinophilic granular bodies (arrowhead) (c). The tumor is composed of cells with strong GFAP expression (d) and rare Ki-67 immunoreactivity (arrowhead; e). Direct amplification of RNA from this tumor demonstrates a 599 bp fragment, which creates a novel fusion KIAA1549:BRAF transcript in which exon 16 of the KIAA1549 gene is joined to BRAF sequences in the middle of exon 10. The bars below the predicted amino acid sequence correspond to BRAF exon 10 (red), BRAF exon 11 (green), and KIAA1549 exon 16 (blue) (f). Immunostaining with phospho-ERK-Thr202/Tyr204 antibodies demonstrates increased ERK activation in the PA tumor (bottom panel). Normal adult human frontal lobe (NB) from an autopsy specimen was included as reference tissue in the top panel (g). Western blot demonstrates 282-fold increase in ERK activation (phospho-ERK-Thr202/Tyr204, p-ERK; Cell Signaling Technologies, catalog no. 4370S) in the tumor (PA) relative to normal human brain (NB). Total ERK is included as internal control for protein loading (h).
were largely myxoid with scattered pleomorphic tumor cells, often containing multiple nuclei. Consistent with the glial nature of this tumor, there was diffuse and strong glial fibrillary acidic protein (GFAP) expression in the neoplastic cells (Figure 1(d)). The Ki67 labeling (proliferative) index was <1% (Figure 1(e)), and mitotic figures were not identified. Upon two-year followup, there was no evidence of recurrent tumor on MRI. To identify the molecular alteration in this pilocytic astrocytoma, RNA was extracted from a snap-frozen tumor specimen using the RNeasy mini-kit (QIAGEN), reverse transcribed, and amplified by PCR using BRAF and KIAA1549 primers as previously reported [8]. Both strands of the resulting novel 599 base pair (bp) product were directly sequenced on an ABI 3730xl DNA Analyzer. In contrast to previously reported KIAA1549:BRAF alterations, this tumor harbored a novel fusion transcript in which exon 16 of the KIAA1549 gene was fused to sequences within exon 10 of the BRAF gene (Figure 1(f)), generating a protein product in which the BRAF kinase domain is intact. This would result in a molecule in which the carboxyl terminal kinase domain is not bound by the amino terminal BRAF regulatory domain and is thus "constitutively" active, leading to downstream MEK and ERK activation. Consistent with this prediction, we found increased ERK activation using activation-specific (phospho-Thr202/Tyr204) antibodies in the tumor by both immunohistochemistry (Figure 1(g)) and Western immunoblotting (Figure 1(h)).

### Table 1: Summary of reported BRAF fusion transcripts.

<table>
<thead>
<tr>
<th>Fusion partner</th>
<th>BRAF</th>
<th>Number of cases</th>
<th>% cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIAA1549 exon 16</td>
<td>exon 9</td>
<td>136</td>
<td>62.4</td>
</tr>
<tr>
<td>KIAA1549 exon 15</td>
<td>exon 9</td>
<td>47</td>
<td>22.6</td>
</tr>
<tr>
<td>KIAA1549 exon 11</td>
<td>exon 11</td>
<td>29</td>
<td>12.3</td>
</tr>
<tr>
<td>KIAA1549 exon 18</td>
<td>exon 10</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>KIAA1549 exon 19</td>
<td>exon 9</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>KIAA1549 exon 16</td>
<td>exon 10*</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>FAM131B</td>
<td>exon 9</td>
<td>3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Current paper.

This proposed tumorigenicity is attributed to constitutive activation of the BRAF kinase domain as a result of the removal of the amino terminal inhibitory domain, leading to increased signaling to its immediate downstream effectors, MEK and ERK. Similar to other BRAF mutations, this novel KIAA1549:BRAF molecular alteration is also associated with increased ERK activity. However, the exact mechanism by which deregulated MEK/ERK activation resulting from KIAA1549:BRAF leads to pilocytic astrocytoma development is unclear. In this regard, several groups have shown that the expression of constitutively active (oncogenic) BRAF (BRAFV600E; V600E mutation within the BRAF activation domain) in human astrocytes and glial progenitor cells leads to cellular senescence in vitro [12], and neither oncogenic BRAFV600 nor RAF1 expression in mice results in glioma formation in vivo [13, 14]. However, forced expression of the kinase domain of BRAFV600, but not of wild-type BRAF (as exists in KIAA1549:BRAF fusion protein products), is transforming in primary human astrocytes in vitro and can induce tumors in mice in vivo [13].

NF1-associated pilocytic astrocytomas also exhibit increased ERK activation as a result of mutation loss of the NF1 tumor suppressor protein, neurofibromin. In primary mouse astrocytes, loss of neurofibromin Ras GTPase activating protein (GAP) activity leads to high levels of Ras effector (ERK, AKT) activation. However, Nf1 genetically engineered mouse optic glioma growth is attenuated by inhibiting AKT pathway signaling [15]. In these studies, inhibition of AKT-mediated mammalian target of rapamycin (mTOR) activity using the macrocide rapamycin resulted in reduced optic glioma volume and proliferation. In light of these observations, the molecular mechanism shared by BRAF activation and neurofibromin loss will require further experimental investigation.

In this regard, future studies will likewise be required to determine precisely how BRAF activation leads to glioma formation either alone or in concert with other genetic or stromal (microenvironment) changes. Despite these seemingly contradictory experimental observations, the identification of BRAF as a seminal genetic alteration in pilocytic astrocytoma sets the stage for therapeutic trials aimed at restoring deregulated BRAF/RAF signaling in this common pediatric brain tumor.

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**References**


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