Expression of IDH1 Mutant Protein R132H and SDHB in Adult and Pediatric Gliomas

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Abstract

Isocitrate dehydrogenase 1 (IDH1) and Succinate dehydrogenase (SDH) mutant tumors may have increased levels of hypoxia inducible factor (HIF), which leads to a tumoral pseudohypoxic profile. Increased HIF levels may promote tumor progression by the activation of numerous cellular processes including resistance to apoptosis, vascular remodeling and angiogenesis. A high frequency of IDH1 mutations has been detected in adult but not in pediatric gliomas. However, loss of SDH expression has not been investigated in gliomas. To further explore the profile and possible contribution of IDH1 and SDHB to glioma tumorigenesis, we investigated the expression of IDH1-R132 mutant and SDHB in a series of 94 histologically confirmed pediatric and adult gliomas. There were 13 diffuse intrinsic pontine gliomas and 81 adult gliomas: 10 low-grade astrocytomas, 8 oligodendrogliomas, 4 ependymomas and 59 glioblastomas (GBMs). Pediatric brain stem gliomas were negative for mutant IDH1 (0/13) and showed preserved SDHB expression (13/13). IDH1 mutant tumors included 6/10 astrocytomas, 5/8 oligodendrogliomas, 0/4 ependymomas and 13/59 GBMs. No adult or pediatric glioma exhibited loss of SDHB expression. IDH1 mutations are frequently present in low- and high-grade gliomas, but not in pediatric brain stem gliomas. It appears that IDH1, but not SDHB, contributes to HIF stabilization in adult gliomas. However, IDH1 or SDHB alterations do not appear to play a role in pediatric gliomagenesis.

Keywords: Gliomas; DIPG; IDH1; SDHB; HIF; GBM

1. Introduction

Gliomas account for 28% of primary brain tumors and 80% of primary malignant brain tumors (Ostrom et al., 2013). Astrocytomas are classified by the World Health Organization (WHO) as Grades I through IV. Pilocytic astrocytoma (WHO grade I) is one of the most common gliomas in...
children. In contrast, glioblastoma multiforme (GBM, WHO grade IV) is the most common malignant primary CNS glioma in adults. For the past 30 years there has been minimal progress in the survival of patients with high-grade gliomas, in part due to cell-specific multi-drug resistance, radioresistance, lack of predictive preclinical models, and the blood-brain barrier. However, in the past decade there has been a significant increase in the understanding of the early molecular events in gliomas. One of the most significant recent discoveries in the genetic aspects of gliomas development has been the identification of IDH1/IDH2 mutations in a large number of cases (Yan et al., 2009). Interestingly, patients with IDH1/IDH2 mutant tumors have a better prognosis than patients with IDH WT tumors.

The IDH1 enzyme participates in the Kreb’s cycle and catalyzes the decarboxylation of isocitrate into α-ketoglutarate reducing NADP to NADPH in the process. The point mutations in IDH1 (R132) and IDH2 (R172) discovered in gliomas were shown to cause a loss in enzyme activity (Yan et al., 2009). Alternatively, it has been proposed that the mutated enzyme facilitates the conversion of α-ketoglutarate to R(2)-2-hydroxyglutarate (2HG), a metabolite that may contribute to cancer development (Dang et al., 2009). Also, Zhao et al. proposed that IDH1 mutations lead to a reduction in enzymatic activity that contributes to increased levels of the hypoxia inducible factors (HIF) , facilitating tumor growth in hypoxic conditions (Zhao et al., 2009). Increased HIF levels may promote tumor progression by the activation of numerous cellular processes including resistance against apoptosis, vascular remodeling and angiogenesis.

SDHB is another enzyme in the Kreb’s cycle that has been associated with tumor development, in particular in pheochromocytomas/paragangliomas and gastrointestinal stromal cell tumors (GIST) (Astuti et al., 2001; Gill et al., 2010). In addition to SDHB mutations in pheochromocytomas, a rare occurrence of IDH1 mutant pheochromocytoma has been reported (Gaal et al., 2010). This highlights the tumorigenic potential of IDH1 and SDHB and suggests the existence of a shared mechanism by which mutations in these two enzymes contribute to cancer development. Recently, it was proposed that SDHB and IDH mutations are associated with DNA hypermethylation in cancer (Killian et al., 2013). A significant number of adult gliomas have IDH1 mutations, in contrast, pediatric gliomas are wild type (WT) for IDH1 (Ballester et al., 2013; Yan et al., 2009). However, SDHB gene expression has not been investigated in adult or pediatric gliomas. We used immunohistochemistry to further explore SDHB and IDH1-R132 expression in a group of pediatric and adult gliomas.

2. Materials and Methods

Samples from 94 gliomas were included in the study. H&E stained slides were reviewed for confirmation of diagnosis by a neuropathologist (MMQ). Samples included 13 pediatric diffuse pontine gliomas (autopsy material), and 81 adult gliomas: 10 low grade astrocytomas, 8 oligodendrogliomas, 4 ependymomas and 59 glioblastomas (GBMs). A total of 4 multitumor tissue blocks including the 94 cases were prepared as previously described (Miettinen, 2012). Immunohistochemistry for IDH1-R132 mutant (1:250 citric buffer,pH 6.0, Dianova) and SDHB (1:1000, EDTA, pH 8.0, Abcam) proteins was performed. Several pathologists (LYB, PPA, JL and MMQ) reviewed the results of the immunohistochemical stains.
3. Results

All 94 cases were classified based on their predominant cell type, presence or absence of necrosis, mitotic figures, nuclear atypia, and endothelial cell proliferation (Figure 1A-1D). Immunohistochemistry showed that all pediatric brain stem gliomas were negative for mutant IDH1 protein expression (0/13) (Figure 2A). Among the 81 adult cases, IDH1 mutant tumors included 60% (6/10) of astrocytomas, 62% (5/8) of oligodendrogliomas, 0% (0/4) of ependymomas and 22% (13/59) of GBMs (Figure 2B-2D). SDHB expression was preserved in 100% (13/13) of pediatric brain stem gliomas and 100% (81/81) of adult gliomas (Figure 3A-3D). The results of the immunohistochemical findings are summarized in Table 1.

Fig. 1. Morphology of gliomas. H&E stain shows pediatric brain stem glioma (A, 400x), ependymoma (B, 400x), high-grade oligodendroglioma (C, 400x), and glioblastoma (D, 200x).
Fig. 2. Expression of IDH1-R132 mutant in gliomas. Mutant IDH1 was not detected in pediatric brain stem gliomas (A, 400x) or ependymomas (B, 400x) but was present in oligodendrogliomas (C, 400x), and glioblastomas (D, 400x).

Table 1 Summary of IDH1 mutation and SDHB expression in adult and pediatric gliomas.

<table>
<thead>
<tr>
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<th>N</th>
<th>IDH1-R132H</th>
<th>SDHB</th>
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<tbody>
<tr>
<td>Pediatric gliomas</td>
<td>13</td>
<td>0/13</td>
<td>13/13</td>
</tr>
<tr>
<td>Adult gliomas</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Low grade astrocytomas</td>
<td>10</td>
<td>6/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Ependymomas</td>
<td>4</td>
<td>0/4</td>
<td>4/4</td>
</tr>
<tr>
<td>Oligodendrogliomas</td>
<td>8</td>
<td>5/8</td>
<td>8/8</td>
</tr>
<tr>
<td>Glioblastomas</td>
<td>59</td>
<td>13/59</td>
<td>59/59</td>
</tr>
</tbody>
</table>
Fig. 3. Expression of SDHB in gliomas. No loss of SDHB expression was identified in pediatric brain stem gliomas (A, 400x), ependymomas (B, 400x), oligodendrogliomas (C, 400x), or glioblastomas (D, 400x).

4. Discussion

Somatic mutations in the catalytic domain of IDH1/2 appear to be among the earliest events in gliomagenesis and may contribute to malignant transformation in a subset of cases (Ichimura, 2012). A single conserved codon (R132), located on the IDH1 active site, was found to be mutated in more than 70% of low-grade gliomas (Yan et al., 2009). The R132 mutation was found to dominantly inhibit wild-type IDH1 and potentially alter the succinate to αKG ratio leading to increased HIF availability, similar to that seen in SDH-mutant tumors (Zhao et al., 2009). Another hypothesis links IDH1 mutations to epigenetic deregulation and a hypermethylated status in gliomas (Turcan et al., 2012). A similar hypermethylation status has been linked to SDHB mutations in several tumor linages (Killian et al., 2013).

In the present study, we evaluated the expression of the IDH1-R132 and SDHB in 94 gliomas, and found all pediatric brain stem gliomas negative for mutant IDH1-R132 (0/13) and SDHB expression was preserved in all cases (13/13). In contrast, adult IDH1 mutant tumors included 6 astrocytomas, 5 oligodendrogliomas, and 13 GBMs. Therefore, in contrast to pediatric gliomas, IDH1-R132 mutation is frequently present in adult gliomas.
SDH, a component of the tricarboxylic acid cycle and the complex II of the electron transport chain, controls HIF availability by regulating the activity of enzymes responsible for limiting HIF half-life, prolyl hydroxylases (PHDs) (Selak et al., 2005). PHDs are oxygen-dependent enzymes that use hydroxylation to target HIF for degradation via the ubiquitin proteasome pathway. A recent study shows that a HIF-related gene expression signature is common to SDH and von Hippel-Lindau (VHL) mutated tumors, including paragangliomas and pheochromocytomas. Both, canonical HIF-1α and HIF-2α target genes were overexpressed in the SDH/VHL cluster, suggesting that a global HIF deregulation accounts for this common profile (López-Jiménez et al., 2010). To our knowledge, this is the first report evaluating SDHB expression in adult and pediatric gliomas. In the present study, we found that all 94 glioma cases showed preserved SDHB expression. The results of the study suggest that IDH1, but not SDHB, plays a role in adult gliomagenesis.

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