IDH1 mutation of gliomas with long-term survival analysis

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Abstract. A recurrent mutation affecting codon 132 of the isocitrate dehydrogenase 1 (IDH1) gene has been found in ~5% of primary glioblastomas (GBMs), but in >70% of secondary GBMs or oligodendroglial and astrocytic tumors. We investigated IDH1 mutations in a series of 134 brain tumors to determine the prevalence and prognostic impact of IDH1 mutations. We also examined the correlations among histology, p53 and PTEN immunoeexpression, MGMT methylation status, 1p 19q co-deletion and EGFR gene amplification. The 134 brain tumors included 41 low-grade oligodendrogliomas (LOs), 47 anaplastic oligodendrogliomas (AOs) and 46 primary GBMs. Data showed that 53.7% (72/134) of cases showed mutations affecting codon 132 of IDH1, including 73.2% of LOs, 82.9% of AOs and three primary GBMs (6.5%). All IDH1 mutations were Arg132His. In a survival analysis, patients with IDH1 mutations had better survival compared to those with wild-type IDH1 (p<0.05) in LOs and AOs, but not in primary GBMs (p=0.587). In addition, in patients with both IDH1 mutation and MGMT methylation, p53 overexpression was a significant poor prognostic factor both in LOs and AOs. However, IDH1 mutation was not correlated with common genetic profiles that affect patient prognosis, including MGMT methylation, 1p 19q co-deletion, PTEN loss and EGFR amplification in LOs, AOs and GBMs. From our results, IDH1 mutation was an independent positive prognostic factor in LOs and AOs, especially in the absence of p53 overexpression.

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Abbreviations: EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; MGMT-MSP, O6-methylguanine-DNA methyltransferase methylation-specific polymerase chain reaction

Key words: isocitrate dehydrogenase, mutation, brain tumor, glioma

Introduction

In 2008, genome-wide mutational analysis with 22 glioblastomas (GBM) performed by Parson et al (1) found recurrent point mutations affecting the isocitrate dehydrogenase 1 (IDH1) and IDH2 genes. This novel point mutation was thus placed in the spotlight of brain cancer biology (2-29). The mutation in IDH1 consistently occurred in exon 4 at codon 132, where a CGT→CAT transition of a single amino acid from arginine to histidine (R132H) occurred (1,16), and less frequently in IDH2, at the corresponding amino acid R172 (28). IDH1 and IDH2 mutations have been found with high frequency in lower grade astrocytic and oligodendroglial neoplasms compared with in GBM (1,2,18,23,28). In addition, although a low incidence of IDH1 mutation was found in primary GBM, it is more frequently mutated in secondary GBM (2,28). The accumulating research regarding this IDH1 mutation has generated many insights (30-33); one is the prognostic usefulness of IDH1 mutation, as mutated tumors have a better prognosis. The other is that IDH1 mutation has already become an essential diagnostic marker for brain tumors (1,2,22,28). Also, in a multivariate analysis, IDH1 mutation was confirmed as an independent prognostic factor in patients with gliomas (18,23). Among the notable genetic profiles in gliomas, 1p 19q co-deleted genotype and MGMT methylation were tightly associated with IDH1 mutation, but IDH1 mutation was mutually exclusive with EGFR gene amplification and loss of chromosome 10 (23). Substantial research effort into IDH1 mutation has concentrated on its mechanistic role. Wild-type IDH1 catalyzed the oxidative carboxylation of isocitrate (ICT) to α-ketoglutarate (α-KG), yielding reduced nicotinamide adenine dinucleotide phosphate (NADPH) (23). However, mutated IDH1 in a tumor inhibited IDH1-mediated conversion of ICT to α-KG and induced hypoxia-inducible factor 1α (HIF-1α) (29). Moreover, mutated IDH1 acquired the ability to catalyze α-KG to R(-)-2-hydroxylglutarate [R(-)-2HG] (6,20). Identification of IDH1 mutation in gliomas not only improved physicians’ ability to predict disease progression but also prompted researchers to re-evaluate the disease entity. The study of IDH1 mutations will change the treatment options and drug regimens used in gliomas in the near future. Although the need for such research is increasing, no information is available regarding IDH1/IDH2 mutations in Korean brain tumor patients. Therefore, to determine the prevalence and prognostic impact of IDH1/IDH2 mutations in the Korean population,
we investigated a series of 134 glioma patients. Additionally, we compared IDH1 mutations with other genomic profiles commonly associated with gliomas.

Materials and methods

Case selection. Tumor tissue was from human brain tumor specimens diagnosed in the Department of Neuropathology at the Seoul National University Hospital from 1999 to 2011. This study included 41 oligodendrogliomas (LO), 47 anaplastic oligodendrogliomas (AO), and 46 primary GBM. This study was approved by the Institutional Review Board of Seoul National University Hospital (H-1201-037-394). DNA extraction and PCR amplification for IDH1 sequencing. Tumor areas were manually microdissected from 6-µm unstained histological sections. DNA was isolated from tumor tissue using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Template DNA (1 µl) was added to 100 µl of PCR reaction solution [10 µl 10X MG™ Taq-HF buffer, 10 µl 2 mM Mg²⁺-dNTP mixture, 5 µl 10 pmol primer (2X), 1 µl MG-Taq-HF polymerase, distilled water]. IDH1 forward primer (5'-ACC AAA TGG CAC CAT ACG A-3') and reverse primer (5'-GCA AAA TCA CAT TAT TGC CAA C-3') generated a 130-bp PCR product; IDH2 forward primer (5'-GCT GCA GTG GGA CCA CTA TT-3') and reverse primer (5'-TGT GGC CTT GTA CGT CAG AG-3') generated a 293-bp PCR product; IDH1 forward primer (5'-ACC AAA TGG CAC CAT ACG A-3') generated a 130-bp PCR product; IDH2 forward primer (5'-GCT GCA GTG GGA CCA CTA TT-3') and reverse primer (5'-TGT GGC CTT GTA CGT CAG AG-3') generated a 293-bp PCR product (Table I).

PCR amplification was performed using AmpliTaq Gold PCR Master Mix (Applied Biosystems, Inc., Foster City, CA). The reaction mixture was subjected to an initial denaturation at 95°C for 10 min, followed by 35 cycles of amplification consisting of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 60 sec.

Direct sequencing. Purified PCR products were sequenced using two IDH1 primers, as described in Table I. Sequencing was performed using a BigDye terminator cycle sequencing kit v.3.1. (Applied Biosystems). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems).

Immunohistochemistry. The primary antibodies used in the immunohistochemical study of formalin-fixed, paraffin-embedded sections are summarized in Table II, based on our previous reports (35,36).

Fluorescence in situ hybridization (FISH). Analysis of chromosome 1p, 19q deletion, and EGFR gene status was conducted by FISH using Vysis probes, as per our previous report (35,36).

O6-methylguanine-DNA methyltransferase (MGMT) methylation-specific polymerase chain reaction (MSP) analysis. Analysis of methylation of the MGMT promoter was performed using the methylation-specific polymerase chain reaction (MSP) technique, as described previously (37).

Statistical analyses. Fisher's exact test was used to examine associations between IDH1 mutations and genetic alterations. The t-test was used to assess the relationship of IDH mutations with the presence or absence of genetic alterations with age. Overall survival of patients with LO, AO was estimated by the Kaplan-Meier method and compared using a log-rank test. All statistical analyses were performed with SPSS version 18 (SPSS Inc., Chicago, IL, USA).

Results

IDH1 mutation frequencies in various brain tumors. The patients ranged in age from 3 to 71 years (mean 41.3 years). The male-to-female ratio was 1.5:1. We analyzed DNA from 134 formalin-fixed, paraffin-embedded tissue samples from archival surgical specimens. We found 72 (53.7%) mutations in codon 132 of IDH1 (Fig. 1). All were G395A (Arg132His). IDH1 mutation frequencies differed according to histologic subtype, affecting 30 (73.2%) of 41 LO, 39 (82.9%) of 47 AO and three (6.5%) of 46 primary GBM cases at IDH1 codon 132. These results are summarized in Table III.

Oligodendroglioma (LO). The 41 LO patients ranged in age from 23 to 69 years (mean 41.1 years). They were operated on from 1999 to 2009. The follow-up duration was 9.9 to 130.5 months. Tumor recurrence occurred in 14 cases, and one case was lost to follow-up. Of the 14 patients with recurrence, 12 had an IDH1 mutation. Of the 26 patients without recurrence, 18 had an IDH1 mutation. Of the 26 patients whose samples were subjected to p53 immunostaining, 8 revealed simultaneous p53 expression and IDH1 mutation. Sixteen of 20 patients whose samples were subjected to PTEN immunostaining expressed PTEN and the IDH1 mutation. The 1p 19q co-deletion was found in 32 of 38 patients by FISH. Of the 32 patients with the 1p 19q co-deletion, 22 had the IDH1 mutation. None of our LO revealed EGFR gene amplification or high polysomy. In LO, we analyzed the relationship between the IDH1 mutation and factors, such as age, sex, recurrence and 1p 19q co-deletion. There was no statistically
significant correlation between *IDH1* mutation status and other factors except p53. In patients with both *IDH1* mutation and *MGMT* methylation, p53 immunoexpression was a significant negative prognostic factor (*p*=0.049) (Fig. 3).

**Anaplastic oligodendroglioma (AO).** The 47 AO patients ranged in age from 26 to 69 years (mean 44.9 years). Of the 12 patients with recurrence, 10 had the *IDH1* mutation. Of the 35 patients without recurrence, 29 had the *IDH1* mutation. Of the 42 patients whose samples were subjected to p53 immunostaining, 6 revealed simultaneous p53 expression and *IDH1* mutation. Twenty of 37 patients whose samples were subjected to PTEN immunostaining expressed PTEN and the *IDH1* mutation. The 1p 19q co-deletion was found in 40 of 47 patients by FISH. Of the 40 patients with the 1p 19q co-deletion, 33 had the *IDH1* mutation. Seven cases without the 1p 19q co-deletion had the *IDH1* mutation. EGFR FISH was performed in 47 cases. Forty-five of these revealed no amplification of the *EGFR* gene, and 37 of these had the *IDH1* mutation. Only 2 cases of AO showed *EGFR* FISH-positive (2 high polysomy). These two *EGFR* FISH-positive cases had the *IDH1* mutation. Thus, we could not see the mutual exclusion between *IDH1* mutation and EGFR positivity. Forty-one of 43 cases subjected to *MGMT* MSP revealed methylation of the *MGMT* promoter, and 35 of these had the *IDH1* mutation. In AO, there was no statistically significant correlation between the *IDH1* mutation and other factors, such as sex, recurrence, 1p 19q co-deletion, *EGFR* FISH. In the patients with both *IDH1* mutation and *MGMT* methylation, p53 immunoexpression was a significant negative prognostic factor as in LO (*p*=0.002) (Fig. 3).

Table III. *IDH1* mutation frequencies in 134 brain tumors.

<table>
<thead>
<tr>
<th>IDH1</th>
<th>Mutant</th>
<th>Wild</th>
<th>Total number</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LO</td>
<td>30</td>
<td>11</td>
<td>41</td>
<td>73.2</td>
</tr>
<tr>
<td>AO</td>
<td>39</td>
<td>8</td>
<td>47</td>
<td>82.9</td>
</tr>
<tr>
<td>Primary GBM</td>
<td>3</td>
<td>43</td>
<td>46</td>
<td>6.5</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>62</td>
<td>134</td>
<td>53.7</td>
</tr>
</tbody>
</table>

LO, low grade oligodendrogliomas; AO, anaplastic oligodendrogliomas; GBM, glioblastoma.
Glioblastoma (GBM). The 46 GBM patients ranged in age from 3 to 71 years (mean 39.6 years). Among the 41 patients without recurrence, two had the IDH1 mutation, and 39 did not. Of the 5 patients with recurrence, one had the IDH1 mutation. The 1p 19q co-deletion was not found in any of the 46 patients by FISH. Three of 46 cases without the 1p 19q co-deletion had the IDH1 mutation. EGFR FISH was positive in 7 (15.2%) out of 46 performed. One of 7 cases with EGFR gene amplification and 2 of 39 patients without EGFR gene amplification had the IDH1 mutation. Therefore, EGFR gene amplification and IDH1 mutation were not mutually exclusive (p=0.398), but it needs to be studied in more cases. Fourteen (30.4%) cases of 46 GBM performed MGMT MSP revealed methylation of the MGMT promoter. Two of 14 cases with MGMT methylation and one of 32 cases without methylation of the MGMT promoter had the IDH1 mutation. We analyzed the relationship between IDH1 mutation status and other factors, such as sex, recurrence, 1p 19q co-deletion, EGFR FISH and MGMT MSP and found no statistically significant correlation.

Survival analysis. We analyzed the prognostic impact of IDH1 mutation in LO, AO, and GBM. The follow-up duration was 9.93-130.5 months, 3.4-166.6 months and 9 days to 79.8 months in LO, AO and GBM. The median survival was 68.4, 54.2 and 19.7 months in LO, AO and GBM, respectively. The overall survival rate was 82.9, 78.7 and 0% in LO, AO and GBM, respectively. In GBM, 1-, 2- and 3-year survival rates were 60.9, 28.4 and 13.0%, respectively. In LO, overall survival was higher in IDH1-mutated LO, compared with non-mutated LO (p=0.03; Fig. 2A). Also, in AO, overall survival was higher in IDH1 mutated AO compared with non-mutated AO (p=0.013; Fig. 2B). In contrast to LO and AO, overall survival was higher in IDH1 non-mutated GBM, compared with mutated GBM (p=0.587; Fig. 2C), but this result was not statistically significant, because IDH1 mutated GBM was only 3 cases. Also, progression-free

Table IV. The relationship of IDH1 mutations to genetic alterations according to age.

<table>
<thead>
<tr>
<th>Entity</th>
<th>IDH1</th>
<th>Number</th>
<th>Mean age (SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LO</td>
<td>Mutant</td>
<td>30</td>
<td>41.67 (10.1)</td>
<td>0.578</td>
</tr>
<tr>
<td></td>
<td>Wild</td>
<td>11</td>
<td>39.55 (12.34)</td>
<td></td>
</tr>
<tr>
<td>AO</td>
<td>Mutant</td>
<td>39</td>
<td>45.77 (10.22)</td>
<td>0.856</td>
</tr>
<tr>
<td></td>
<td>Wild</td>
<td>8</td>
<td>45.5 (11)</td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>Mutant</td>
<td>3</td>
<td>48.67 (10.26)</td>
<td>0.456</td>
</tr>
<tr>
<td></td>
<td>Wild</td>
<td>43</td>
<td>39.37 (21.04)</td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation; LO, low grade oligodendrogliomas; AO, anaplastic oligodendrogliomas; GBM, glioblastoma.
survival was not statistically different between IDH1 mutated and non-mutated tumors in the LO (p=0.708; Fig. 2D), AO (p=0.938; Fig. 2E), and GBM (p=0.173; Fig. 2F) groups. We analyzed survival in patients with both IDH1-mutated and MGMT methylated LO and AO according to p53 and PTEN expression. The overall survival and progression-free survival of patients with p53-negative tumors were significantly longer than in those with p53-positive tumors (p=0.049 and 0.002; Fig. 3A and B). However, PTEN expression did not affect the patients' survival.

Discussion

Recently, IDH1 mutation has been recognized as a strong prognostic factor in brain tumors. In low-grade astrocytic and oligodendrogial tumors (WHO grades II and III), the impact on prognosis was magnified several times. However, no Korean studies evaluating IDH1 mutation frequencies in brain tumors had previously been conducted, so we attempted to discover the IDH1 mutation frequency and prognostic impact in a Korean patient population. The methods for detection of IDH1 mutation have changed. Recently, a monoclonal antibody that detects the R132H IDH1 mutation was developed. This antibody has been applied to everyday pathologic practice (5). Immunohistochemical research with this monoclonal antibody is easy, and inexpensive, but has some limitations. The best way to identify the IDH1 mutation is by direct sequencing; thus, we performed direct sequencing instead of immunohistochemistry for IDH1. According to our data, WHO grades II and III astrocytic and oligodendrogial tumors exhibit high mutation frequencies. Although the case numbers were insufficient to reflect tumor frequencies, our IDH1 mutation results were similar to other published results. In accordance with other studies, we found that IDH1 mutation was a strong prognostic factor in oligodendroglioma and anaplastic oligodendroglioma. Detection of the IDH1 mutation was extremely helpful in brain tumors. Using the same approach with the 1p 19q co-deletion, IDH1 mutation was an excellent prognostic marker. The usefulness of the IDH1 mutation in brain tumors has been reported previously. In addition to its predictive utility, IDH1 also has possibilities as a diagnostic marker. IDH1 is a powerful differential diagnostic marker for round and clear-cell brain tumors mimicking oligodendroglioma, including dysembryoplastic neuroepithelial tumor (DNT), extraventricular clear-cell brain tumors mimicking oligodendroglioma, including dysembryoplastic neuroepithelial tumor (DNT), extraventricular clear-cell brain tumors mimicking oligodendroglioma, including dysembryoplastic neuroepithelial tumor (DNT), and other genetic profiles commonly associated with gliomas. These results remain to be proven in a larger study.

Acknowledgements

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