# *IDH1/2* mutation is a prognostic marker for survival and predicts response to chemotherapy for grade II gliomas concomitantly treated with radiation therapy

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**Abstract.** Reliable prognostic biomarkers of grade II gliomas remain unclear. This study aimed to examine the role of mutations of isocitrate dehydrogenase (*IDH1/2*), 1p/19q co-deletion, and clinicopathological factors in patients with grade II glioma who were primarily treated with radiotherapy or chemoradiotherapy after surgery. Seventy-two consecutive patients, including 49 cases of diffuse astrocytomas (DA), 4 oligodendrogliomas (OL) and 19 oligoastrocytomas (OA), who underwent treatment from 1991 to 2010 at a single institution were examined. The overall survival (OS) of the DA patients (8.3 years) was significantly shorter than that of the OL and OA patients (11.7 years). *IDH1/2* mutations were found in 46.9% of the DA patients and 82.6% of the OL and OA patients. The progression-free survival (PFS) and OS of the patients with *IDH1/2* mutations (8.4 and 16.3 years) were significantly longer than those of the patients without *IDH1/2* mutations (3.3 and 4.5 years). Among the patients with *IDH1/2* mutations, those who were initially treated with chemoradiotherapy including nimustine hydrochloride (ACNU), had significantly longer PFS than those treated with radiotherapy alone, whereas no significant difference in PFS was observed between the chemo-radiotherapy and radiotherapy groups in the patients without *IDH1/2* mutations. Oligodendroglial tumors, age <40 years, initial Karnofsky performance status (KPS) ≥80, and *IDH1/2* mutations were favorable prognostic factors regarding PFS and OS. *IDH1/2* mutation was a predictive factor of response to chemoradiotherapy in grade II gliomas. Patients with *IDH1/2* mutations may benefit more from chemoraiotherapy than those without *IDH1/2* mutations.

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## Introduction

World Health Organization (WHO) grade II gliomas (low-grade gliomas) are slow-growing tumors that include several subtypes, such as diffuse astrocytomas, mixed oligoastrocytomas, and oligodendrogliomas (1). The 10- and 20-year survival rates for patients with grade II glioma are reported to be 48 and 22% (2), reflecting the malignant potential of these tumors in long-term survival. Radiotherapy is often the treatment of choice for patients with incompletely resected grade II gliomas. However, the timing of radiotherapy for patients with these malignancies remains controversial and no difference in overall survival (OS) between groups receiving early and delayed radiation has been reported (3). Moreover, the efficacy of chemoradiotherapy for grade II gliomas is largely unknown. The addition of procarbazine, lomustine, and vincristine (PCV) therapy to radiotherapy for grade II gliomas conferred a significant increase in OS and progression-free survival (PFS) of >2 years in the Radiation Therapy Oncology Group (RTOG) 9802 study (4), suggesting that chemoradiotherapy might be effective for a subset of these patients.

Several studies have attempted to identify prognostic factors for grade II gliomas. To date, older age, astrocytic histology, the presence of neurologic deficits before surgery, larger tumor diameters, and tumors crossing the midline have been proposed as unfavorable prognostic factors (5-9). Several genetic markers, such as 1p/19q codeletion or mutations of the isocitrate dehydrogenase 1 and 2 genes (*IDH1/2*), have also been associated with patient survival. Oligodendrogliomas typically show 1p/19q codeletion (≤70%) (10,11), and its presence is reported to predict longer survival in oligodendroglial tumors (12). The 1p/19q codeletion is also a statistically significant predictor of prolonged survival in patients with astrocytomas (13). Furthermore, 1p/19q codeletion was associated with longer survival in all types of adult gliomas, independent of age and diagnosis (14,15). On the other hand, 1p/19q codeletion did not appear to be a sensitive prognostic biomarker in patients with either grade II astrocytic

or oligodendroglial tumors who did not receive radiotherapy or chemotherapy after surgery (16).

Mutations of the *IDH1/2* genes are common events in gliomas (17), especially among grade II gliomas, where *IDH1* mutations are observed in 70-80% of cases (11,17,18). Glioblastomas and anaplastic astrocytomas (WHO grade III) with IDH1/2 mutations have more favorable prognoses than those with a wild-type phenotype (17). Several studies have indicated that *IDH1/2* mutations are significantly associated with positive prognosis and chemosensitivity in low-grade gliomas (19,20), whereas others have reported that *IDH1/2* mutations were not associated with prognosis (21,22). Thus, the prognostic or predictive values of these genetic markers in grade II gliomas remain controversial.

In the present study, the clinicopathological factors, including age, Karnofsky performance status (KPS), histology, extent of resection, radiotherapy, chemoradiotherapy, largest tumor diameter, and MIB-1 staining index, as well as *IDH1/2* mutations and 1p/19q codeletion, were analyzed in grade II gliomas and correlated with the clinical course of the patients. Oligodendroglial tumors, age <40 years, initial KPS ≥80, and *IDH1/2* mutations were favorable prognostic factors for PFS and OS. The IDH1/2 mutation was a predictive factor of response to chemoradiotherapy in grade II gliomas.

## Materials and methods

*Patients and tissue collections*. The data were collected from 72 patients who were found with WHO grade II gliomas at the first surgery. These included 49 diffuse astrocytomas and 23 oligodendroglial tumors, including 4 oligodendrogliomas and 19 oligoastrocytomas (male-female, 40:32; median age, 39.0 years). These consecutive cases were diagnosed and treated between 1991 and 2010 at the National Cancer Center Hospital in Japan. The clinical records of the patients were reviewed, and the data on the extent of tumor resection were obtained from the surgical report. Total or subtotal resection was defined as the removal of 90% or more of the tumor based on the surgeon's clinical report. Fifty-eight patients (80.6%) underwent initial surgeries followed by radiotherapy (22.2%) or chemoradiotherapy with ACNU (58.3%). Three patients with total or subtotal removal and 11 with partial resection or biopsy (19.4%) were followed-up without radiotherapy until progressive disease. Of the remaining patients, those who underwent initial treatment between 1991 and 2006 were treated with chemoradiotherapy and those treated between 2007 and 2010 underwent radiotherapy alone based on our treatment protocols. The radiation doses were 60 Gy before 2006 and 54 Gy after 2007. The chemotherapy in the diffuse astrocytoma cases consisted of ACNU administered twice during radiotherapy and 3 additional doses every 2 months after radiotherapy. The patients with oligodendroglial tumors received ACNU + VCR (vincristine) twice during radiotherapy, and thereafter, PAV [ACNU + VCR + PCZ (procarbazine)] was administered in 3 cycles every 2 months after radiotherapy. Each patient was worked up by MRI every 3-4 months until 2 years from the initial treatment and then every 6 months after 2 years. Progression was determined when the MRI showed a new enhancing lesion with Gd-DTPA, a new high intensity lesion or an obvious increased lesion (at least 20% larger than previous MRI in diameter) on T2/FLAIR images. Clinical deterioration of a patient was also determined as progression.

The formalin-fixed paraffin-embedded tumor samples and frozen specimens, when available, were collected from the primary resection for all the patients who underwent surgery in the National Cancer Center and whenever possible for those operated at other hospitals. The samples were examined for *IDH1/2* mutations and 1p/19q codeletion only when sufficient material for DNA extraction was available at either the primary or secondary resection. The study was approved by the internal review board of the National Cancer Center. The detailed information for all the 72 patients is listed in Table I.

*Hematoxylin and eosin staining and immunohistochemical staining for MIB-1 and IDH1*. The surgical specimens were fixed in 10% formalin and embedded in paraffin. The hematoxylin and eosin-stained specimens were examined to determine the histological tumor type. The multiple serial sections were subjected to immunohistochemical analyses (IHC) to visualize local staining. Antigen retrieval was carried out by exposing the tissue sections to 15 min of microwave heating in 0.1-mol/l sodium citrate (pH 6.0). This was followed with immunostaining of the specimens with the streptavidin-biotin-peroxidase complex method (Vectastain, Vector Laboratories, Inc., Burlingame, CA, USA). The samples were incubated in human monoclonal antibodies against MIB-1 (Dako, Tokyo, Japan). Positive immunostaining results were detected with the diaminobenzidine reaction, and the slides were subsequently counterstained with hematoxylin, dehydrated, cleared, and mounted.

Cell counting was performed with the aid of a light microscope (Olympus Corp., Tokyo, Japan). Cell counting was done at a magnification of x400. At least 200 tumor cells were counted, and the results were expressed as the mean of the counts obtained from 3 different locations within each specimen. The MIB-1-stained cells were also counted, and the percentage of the MIB-1-stained cells was calculated within the observed field and expressed as the MIB-1 index.

Human monoclonal antibodies specific against IDH1-R132H and IDH1-R132S were used to identify these 2 types of IDH1 mutations (Medical & Biological Laboratories, Tokyo, Japan). Positive immunostaining results were detected with the diaminobenzidine reaction, and the slides were subsequently counterstained with hematoxylin, dehydrated, cleared, and mounted. The positive granular cytoplasmic staining of the tumor cells was evaluated for mutant *IDH1* (23).

*Extraction of nucleic acids*. The tumor samples were immediately frozen in liquid nitrogen and stored at -80˚C. A peripheral blood sample was drawn from each patient and stored at -80˚C. Total DNA was extracted from either frozen tissue samples or paraffin-embedded specimens and from the patients' blood with a DNeasy Blood & Tissue kit (Qiagen Sciences, Germantown, MD, USA) according to the manufacturer's instructions.

*Sequencing of IDH1/2*. A 129-base pair (bp) fragment of *IDH1* containing codon 132 or a 150-bp fragment of *IDH2* containing codon 172 was PCR amplified using the forward primer IDH1f (CGGTCTTCAGAGAAGCCATT) and reverse primer IDH1r (GCAAAATCACATTATTGCCAAC) for *IDH1* and the forward primer IDH2f (AGCCCATCATCTGCAAAAAC) and

reverse primer IDH2r (CTAGGCGAGGAGCTCCAGT) for *IDH2* (18). The thermocycling conditions consisted of 5 min at 95˚C, 35 cycles for 30 sec at 95˚C, 40 sec at 56˚C, and 50 sec at 72˚C, followed by 10 min at 72˚C. For confirmation, the forward primer IDH1fc (ACCAAATGGCACCATACGA) and reverse primer IDH1rc (TTCATACCTTGCTTAATGGGTGT) generating a 254-bp fragment and the forward primer IDH2fc (GCTGCAGTGGGACCACTATT) and reverse primer IDH2rc (TGTGGCCTTGTACTGCAGAG) generating a 293-bp fragment were used for amplification with the same thermocycling conditions (24). After the purification of the PCR products using the QIAquick PCR Purification kit (Qiagen), DNA sequencing for the *IDH1/2* gene was performed with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems), using the same primers as for PCR.

*1p and 19q status by fluorescence in situ hybridization*. For fluorescence *in situ* hybridization (FISH), the tumor sections were deparaffinized in Hemo-De (Falma, Tokyo, Japan), dehydrated with 100% ethanol, and digested using a Paraffin Pretreatment kit (Vysis-Abbott, Tokyo, Japan) according to the manufacturer's protocol. Each section was hybridized with LSI 1p36/1q25 and LSI 19q13/19p13 probes (Vysis-Abbott). The probes and target DNA were denatured individually at 72˚C for 5 min, followed by 2 overnight incubations at 37˚C. Posthybridization washes were carried out in standard saline solution twice, and the sections were air-dried. The nuclei were counterstained with 4,6-diamidino-2-phenylindole. The sections were analyzed using a fluorescence microscope (Biorevo BZ-9000, Keyence, Japan).

The 1p or 19q deletions were considered present when the population of the cells with single 1p36 or single 19q13 was <50% of the cells with double 1p36 or double 19p13, respectively. At least 100 non-overlapping nuclei were counted per hybridization.

*1p and 19q status by multiplex ligation-dependent probe amplification analysis*. We used the SALSA P088 kit (MRC Amsterdam, The Netherlands) containing 16 1p probes (6 probe at 1p36), 8 19q probes, and 21 control probes specific to other chromosomes, including 2 probes for 19p. Information regarding the probe sequences and ligation sites can be found at http://www.mlpa.com. Multiplex ligation-dependent probe amplification (MLPA) analysis was performed as described previously (25,26). The 1p36 or 19q deletions were considered present when 5 of 6 markers for 1p36 and 5 of 8 markers for 19q in each chromosome arm had normalized ratios <0.75.

*Statistical analysis*. All the statistical analyses, including the Kaplan-Meier survival analysis, were performed using the JMP ver. 8 software (Tokyo, Japan). The multivariate analysis with Cox regression, which was used to assess the independent prognostic factors for all the 72 cases, was performed only for the variables with $p<0.1$ and which included the data obtained in the univariate analysis for all the patients. A similar analysis was performed for 58 cases with radiotherapy or chemoradiotherapy.

## Results

*Progression-free and OS*. The PFS and median OS times for all the 72 grade II glioma patients were 5.8 and 10.3 years, respectively (male-female, 40:32; median age, 39.0 years; Table I).

Table I. Characteristics of patients with grade II gliomas.

| Characteristic | No. of patients | Years | Percentage (%) |
|---|---|---|---|
| Sex | | | |
| Male | 40 | | 55.6 |
| Female | 32 | | 44.4 |
| Age (years) | | | |
| Median | | 39 | |
| Range | | 21-75 | |
| Histology | | | |
| Astrocytoma | 49 | | 68 |
| Oligodendroglioma | 4 | | 6 |
| Oligoastrocytoma | 19 | | 26 |
| Extent of removal | | | |
| Total removal | 12 | | 16.7 |
| Subtotal removal | 2 | | 2.8 |
| Partial removal | 27 | | 37.5 |
| Biopsy | 31 | | 43.1 |
| Largest diameter of initial tumor (cm) | | | |
| <6 | 40 | | 55.6 |
| ≥6 | 32 | | 44.4 |
| Initial KPS | | | |
| <80 | 4 | | 5.6 |
| ≥80 | 68 | | 94.4 |
| MIB-1 index (%) | | | |
| Median | 3 | | |
| *IDH* mutation | | | |
| Mutation | 42 | | 58.3 |
| Wild-type | 30 | | 41.7 |
| Loss of 1p/19q | | | |
| 1p/19q codeletion | 15 | | 25.0 |
| 1p deletion | 24 | | 40.0 |
| 19q deletion | 23 | | 38.3 |
| Initial radiotherapy | | | |
| + | 58 | | 80.6 |
| - | 14 | | 19.4 |
| Initial chemotherapy | | | |
| ACNU | 44 | | 61 |
| TMZ | 2 | | 3 |
| None | 26 | | 36 |
| PFS (years) | | | |
| Median | | 5.8 | |
| Overall survival (years) | | | |
| Median | | 10.3 | |

Table II. Univariate analyses of progression-free survival time and overall survival time of patients with grade II gliomas.

| Variable | No.of cases | PFS (95% CI) | p-value (log-rank) | OS (95% CI) | p-value (log-rank) |
|---|---|---|---|---|---|
| Histology | | | | | |
| Astrocytoma | 49 | 3.6 (2.1-7.7) | 0.08 | 8.3 (4.2-NR) | 0.04 |
| Oligodendroglioma/ oligoastrocytoma | 23 | 8.3 (4.3-14.4) | | 11.7 (8.1-18.2) | |
| Age | | | | | |
| <40 years | 38 | 7.0 (3.6-9.3) | 0.2 | NR (8.0-NR) | 0.02 |
| ≥40 years | 34 | 3.1 (1.8-8.9) | | 4.3 (3.9-16.3) | |
| *IDH* mutation | | | | | |
| Mutation | 42 | 8.4 (3.2-10.2) | 0.04 | 16.3 (9.6-18.2) | 0.004 |
| Wild-type | 30 | 3.3 (1.7-7.0) | | 4.5 (3.9-10.0) | |
| Extent of removal | | | | | |
| Total and subtotal removal | 14 | 10.4 (2.5-14.4) | 0.1 | 18.3 (4.1-18.3) | 0.08 |
| Partial removal and biopsy | 58 | 4.3 (2.3-8.3) | | 10.0 (5.2-16.3) | |
| Largest diameter of initial tumor (cm) | | | | | |
| <6 | 40 | 7.7 (2.3-10.4) | 0.2 | 10.0 (8.0-NR) | 0.7 |
| ≥6 | 32 | 4.3 (2.1-8.3) | | 10.3 (5.1-16.3) | |
| Initial KPS | | | | | |
| <80 | 4 | 0.6 (0.4-8.4) | 0.01 | 1.7 (0.5-10.3) | 0.0006 |
| ≥80 | 68 | 6.8 (3.1-8.9) | | 11.7 (8.0-18.2) | |
| MIB-1 index | | | | | |
| <4% | 33 | 8.1 (2.3-8.9) | 0.6 | 9.6 (5.1-NR) | 0.6 |
| ≥4% | 21 | 4.3 (1.8-NR) | | NR (3.9-NR) | |
| 1p/19q | | | | | |
| 1p/19q codeletion (+) | 15 | 6.8 (2.2-NR) | 0.4 | 11.7 (4.3-11.7) | 0.2 |
| 1p/19q codeletion (-) | 45 | 3.6 (2.3-8.4) | | 8.3 (4.4-NR) | |
| 1p | | | | | |
| 1p deletion | 24 | 5.8 (2.5-9.3) | 0.96 | 11.7 (4.2-11.7) | 0.9 |
| Intact | 36 | 4.2 (2.1-10.2) | | 9.6 (4.4-NR) | |
| 19q | | | | | |
| 19q deletion | 23 | 7.0 (4.2-9.3) | 0.5 | 11.7 (4.5-11.7) | 0.5 |
| Intact | 37 | 3.1 (1.9-10.2) | | 8.3 (3.9-NR) | |
| Initial radiotherapy | | | | | |
| + | 58 | 4.3 (2.9-8.9) | 0.98 | 8.3 (5.1-18.2) | 0.2 |
| - | 14 | 7.7 (2.5-9.1) | | 11.7 (4.2-16.3) | |
| Initial treatment | | | | | |
| Radiotherapy alone | 16 | 2.9 (0.7-4.3) | 0.01 | 4.2 (2.7-5.1) | 0.0002 |
| Chemoradiotherapy | 42 | 8.1 (3.2-10.2) | | 18.2 (8.1-18.2) | |

NR, PFS or median survival time is not reached; CI, confident interval.

These patients were initially treated with surgery followed by radiotherapy (22.2%) or chemoradiotherapy (58.3%). The median follow-up time for all the 72 patients was 6.4 years, and it was 7.6 years for the patients treated with chemoradiotherapy (n=42) and 4.0 years for those who underwent radiotherapy alone (n=16).

*Progression-free and OS times according to clinical factors*. The univariate analysis (Table II) showed that the patients with oligodendroglial tumors (n=23) had longer OS than those with diffuse astrocytoma (n=49; p=0.04). The PFS and OS were 3.6 and 8.3 years, respectively, in the patients with diffuse astrocytoma, and 8.3 and 11.7 years, respectively, in the patients with oligodendroglioma or oligoastrocytoma (Fig. 1A and B). The patients younger than 40 years (n=38) had longer OS than those who were 40 years or older (n=34; p=0.02). The PFS and median survival time of the patients in the younger age groups were 7.0 years and still not reached, respectively, whereas the PFS and OS of the patients in the older age groups were 3.1 and 4.3 years, respectively. The patients with an initial KPS score ≥80 (n=68) had significantly longer OS (p=0.0006) and PFS (p=0.01) than those with a KPS score <80 (n=4). The PFS and OS of the patients with a KPS score ≥80 were 6.8 and 11.7 years, respectively, and those of the patients with a KPS score <80 were 0.6 and 1.7 years, respectively. The patients in the total or subtotal resection (≥90% removal) groups (n=14; median age, 34.0 years) tended to have longer OS than those in the partial (<90%) removal or biopsy groups (n=58; median age, 41.0; p=0.08). The PFS and OS were 10.4 and 18.3 years, respectively, in the patients in the total or subtotal resection groups and 4.3 and 10.0 years, respectively, in the patients in the partial resection or biopsy groups. The patients who were initially treated with chemoradiotherapy after surgery showed significantly longer PFS (p=0.01) and OS (p=0.0002) than those treated with radiotherapy alone (Fig. 1C and D). The PFS and OS of the patients who were initially treated with radiotherapy after surgery (n=16) were 2.9 and 4.2 years, respectively, and the PFS and OS of the patients who were initially treated with chemoradiotherapy after surgery (n=42) were 8.1 and 18.2 years, respectively. According to MIB-1 staining index, there was no significant difference of survival between groups with cut-off point at 4, 8 and 15% in our study.

*Presence of 1p/19q codeletion, 1p deletion, and 19q deletion and survival*. The presence of 1p/19q deletions was determined for 25 or 26 primary resections and for 7 or 2 secondary resection samples by MLPA or FISH, respectively. The 1p/19q codeletion was observed in 15.9% (7/44) of the astrocytomas and 50% (8/16) of the oligodendroglial tumors. The OS of the patients with 1p/19q codeletion was 11.7 years, and the OS of those without 1p/19q codeletion was 8.3 years (p=0.2; Fig. 1E and F). In the patients with astrocytic tumors, the median survival time of those with 1p/19q codeletion was not reached and the OS of those without 1p/19q codeletion was 6.3 years (p=0.5). The OS of the patients with 1p/19q codeletion was 11.7 years, and the OS of those without 1p/19q codeletion was 10.3 years in the oligodendroglial tumors (p=0.5). The presence of 1p/19q codeletion, 1p deletion, or 19q deletion was not correlated with the PFS or OS time (Table II).

Table III. Mutation of *IDH1/2*.

| | Diffuse astrocytoma (%) | Oligodendroglioma (%) | Oligoastrocytoma (%) |
|---|---|---|---|
| *IDH1/2* mutation by sequence | | | |
| IDH1 R132H | 13 (26.5) | 2 (50.0) | 5 (26.3) |
| IDH1 R132S | 1 (2.0) | 0 (0.0) | 0 (0.0) |
| IDH2 R172K | 1 (2.0) | 0 (0.0) | 0 (0.0) |
| Wild-type | 15 (30.6) | 1 (25.0) | 2 (10.5) |
| IDH mutation by IHC | | | |
| IDH1 R132H | 8 (16.3) | 1 (25.0) | 11 (57.9) |
| IDH1 R132S | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Mutation (-) | 11 (22.4) | 0 (0.0) | 1 (5.3) |
| Total | 49 (100) | 4 (100) | 19 (100) |
| Mutation | 23 (46.9) | 3 (75.0) | 16 (84.2) |
| Wild-type | 26 (53.1) | 1 (25.0) | 3 (15.8) |

IHC, immunohistochemical staining.

*IDH1/2 mutations and survival in the whole series*. *IDH1/2* mutations were determined in 55 samples at the primary resection and 17 at the secondary resection by IHC alone for 32 cases (44.4%) and by direct sequencing in 40 cases (55.6%). IDH1/2 mutations were found in 46.9% (23/49) of the astrocytomas, 84.2% (16/19) of the oligoastrocytomas, and 75.0% (3/4) of the oligodendrogliomas (Table III).

The patients with *IDH1/2* mutations (n=42) had longer PFS (p=0.04) and OS (p=0.004) than those without IDH1/2 mutations (n=30; Table II). The PFS and OS of the patients with *IDH1/2* mutations were 8.4 and 16.3 years, respectively, and the PFS and OS of the patients without *IDH1/2* mutations were 3.3 and 4.5 years, respectively (Fig. 1G and H). The diffuse astrocytoma patients with *IDH1/2* mutations (n=23) tended to have longer survival times than those without *IDH1/2* mutations (n=26), although the difference was not significant (p=0.08). The median survival time of the diffuse astrocytoma patients with *IDH1/2* mutations was not reached and that of the diffuse astrocytoma patients without *IDH1/2* mutations was 4.4 years. The oligodendroglial tumor patients with *IDH1/2* mutations also tended to have longer, though not significant, survival times (p=0.1).

The survival of the patients with *IDH1/2* mutations and 1p/19q codeletion was longer than that of the patients with neither *IDH1/2* mutations nor 1p/19q codeletion (11.7 vs. 4.4 years, respectively), although the difference did not reach statistical significance (p=0.1). Furthermore, a combined *IDH1/2* and 1p/19q status did not correlate with the PFS and OS of the patients who were initially treated with chemoradiotherapy after surgery regardless of the histological tumor type.

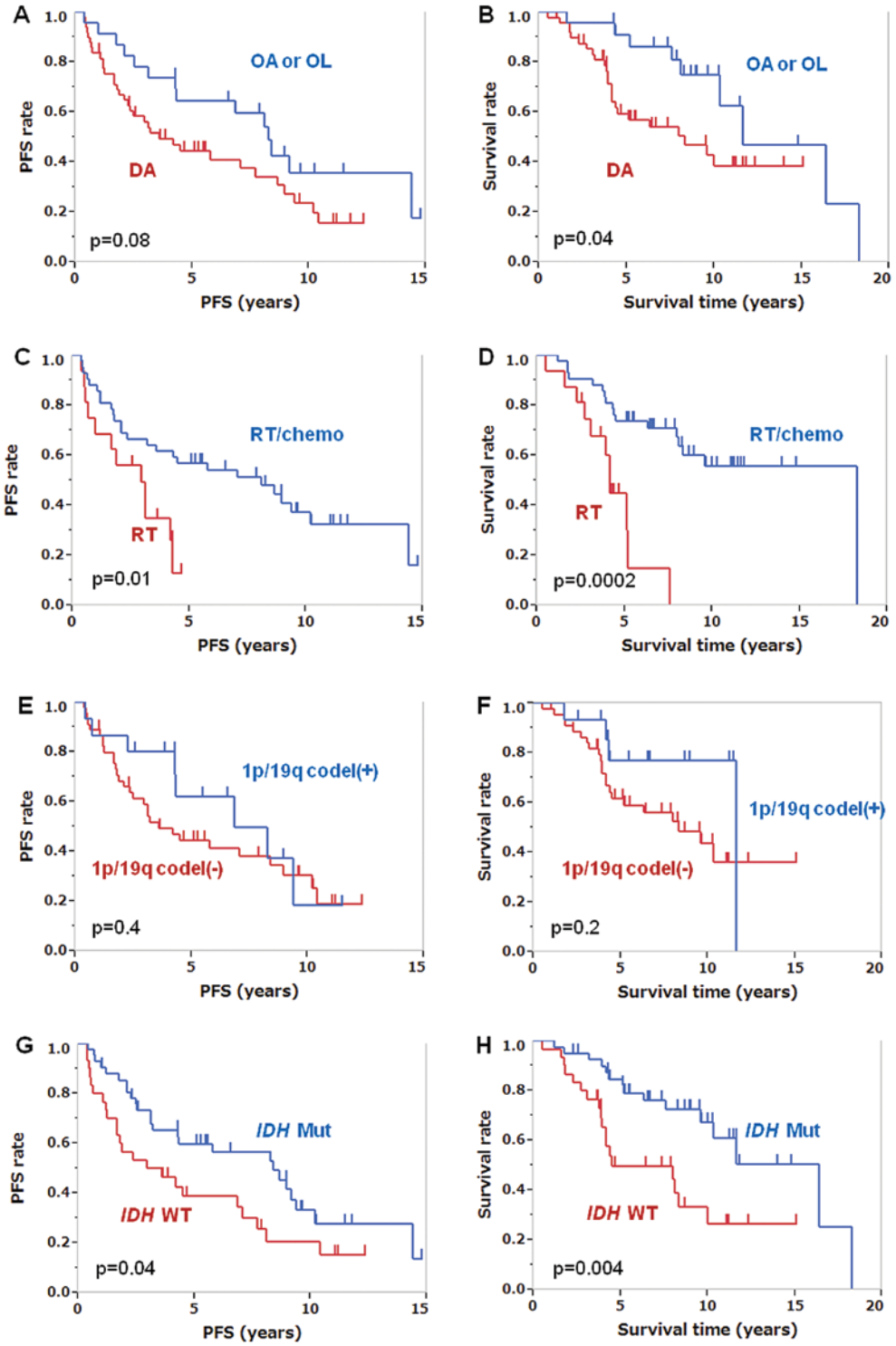


Figure 1. Kaplan-Meier survival curves of the patients with WHO grade II gliomas grouped according to genetic and clinical factors associated with overall survival (OS) and progression-free survival (PFS) by univariate analysis. The survival estimates were calculated according to the following variables: (A) PFS, diffuse astrocytoma (DA) versus oligodendroglial tumors (OA or OL); (B) OS, diffuse astrocytoma (DA) versus oligodendroglial tumors (OA or OL); (C) PFS, radiotherapy (RT) versus chemoradiotherapy (RT/chemo); (D) OS, radiotherapy (RT) versus chemoradiotherapy (RT/chemo); (E) PFS, 1p/19q codeletion (codel) (+) or (-); (F) OS, 1p/19q codeletion (codel) (+) or (-); (G) PFS, IDH1/2 mutation (mut) or wild-type (WT); and (H) OS, IDH1/2 mutation (mut) or wild-type (WT).

In the total or subtotal resection group, the patients with *IDH1/2* mutations had longer OS than those without *IDH1/2* mutations (p=0.04; Fig. 2A). The OS of the patients with *IDH1/2* mutations (n=6, 2 diffuse astrocytomas, 3 oligoastrocytomas, and 1 oligodendrogliomas) was 18.2 years; to date, 5 are still alive and 1 is dead. The OS of the patients without *IDH1/2* mutations (n=8, 7 astrocytomas and 1 oligoastrocytoma) was 8.0 years. In the partial resection or biopsy group, the patients with *IDH1/2* mutations had longer OS than those without *IDH1/2* mutations in the partial resection or biopsy group (p=0.01; Fig. 2B). The OS of the patients with *IDH1/2* mutations (n=36, 21 diffuse astrocytomas, 13 oligoastrocytomas, and 2 oligodendrogliomas)

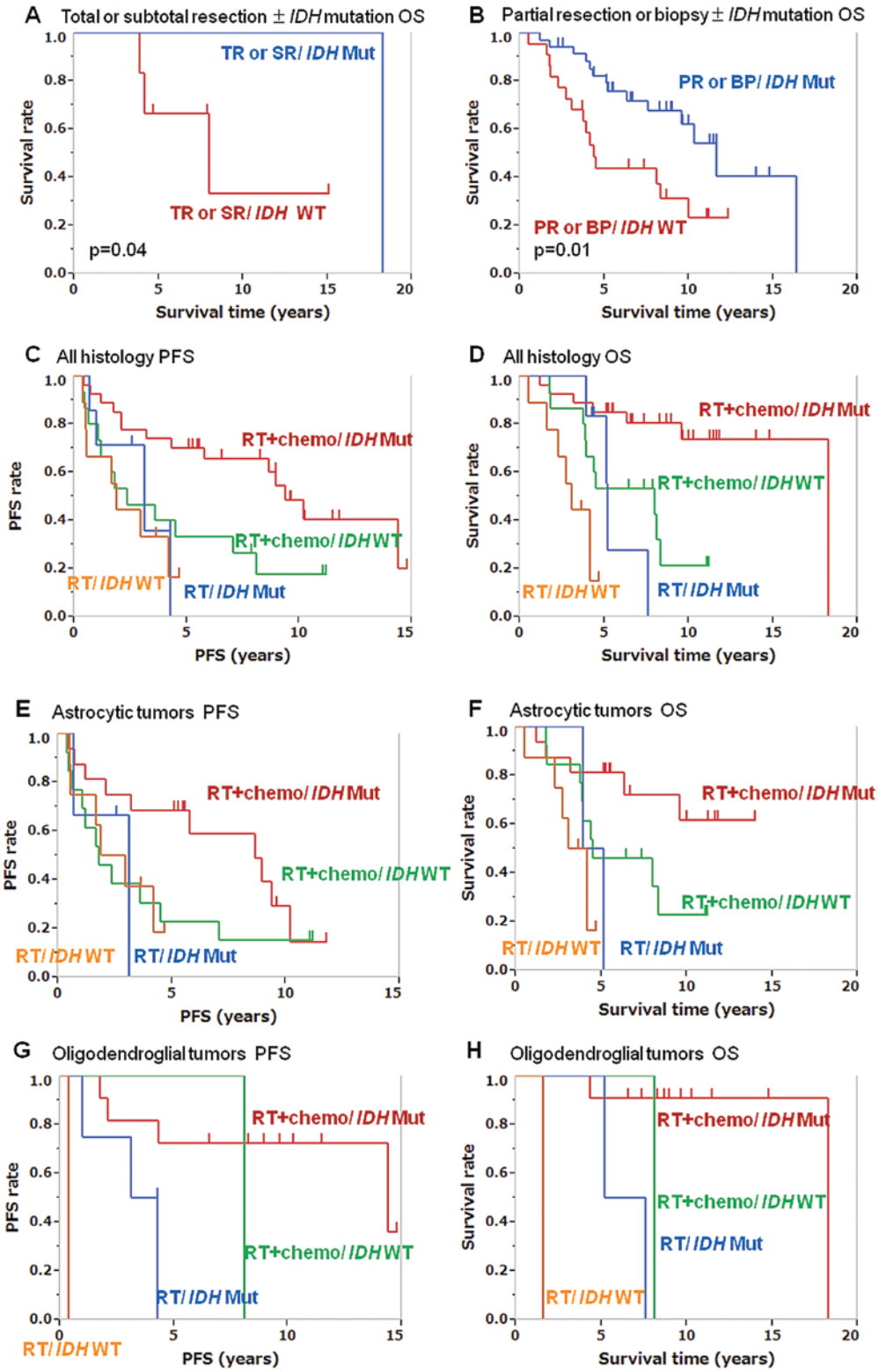


Figure 2. (A and B) Kaplan-Meier survival curves of the patients in the total or subtotal tumor resection (TR or SR) (A) and partial resection or biopsy (PR or BP) (B) groups according to the *IDH1/2* status. (C-H) Kaplan-Meier survival curves of the patients who were initially treated with radiotherapy (RT) and chemotherapy (chemo) or radiotherapy alone according to *IDH1/2* status associated with the overall survival (OS) and progression-free survival (PFS) by univariate analysis: (C) PFS of all the WHO grade II gliomas, (D) OS of all the WHO grade II gliomas, (E) PFS of the diffuse astrocytomas, (F) OS of the diffuse astrocytomas, (G) PFS of the oligodendroglial tumors, (H) OS of the oligodendroglial tumors.

was 11.7 years, and that of the patients without *IDH1/2* mutations in these groups (n=22, 19 diffuse astrocytomas, 2 oligoastrocytomas, and 1 oligodendroglioma) was 4.4 years.

*IDH1/2 mutations and survival in the patients who underwent chemoradiotherapy after surgery.* Among the grade II glioma patients who were initially treated with chemoradiotherapy

Table IV. PFS and OS in patients with radiotherapy or chemoradiotherapy according to IDH1/2 status.

| Variable | | No. of cases | PFS (95% CI) | OS (95% CI) |
|---|---|---|---|---|
| All grade II gliomas | | | | |
| RT+chemo | Mut (+) | 27 | 9.3 (4.3-NA)[a,b] | 18.2 (9.6-18.2)[c,d] |
| RT+chemo | Mut (-) | 15 | 2.3 (0.6-7.0)[a-,b] | 8.0 (3.8-8.3)[c,d] |
| RT only | Mut (+) | 7 | 3.1 (0.7-4.3)[b] | 5.1 (3.9-7.5)[b,d,e] |
| RT only | Mut (-) | 9 | 1.9 (0.4-4.2) | 3.1 (0.5-4.2)[b,e] |
| Diffuse astrocytoma | | | | |
| RT+chemo | Mut (+) | 16 | 8.6 (2.1-10.2)[f] | NR (6.3-NR)[g,h] |
| RT+chemo | Mut (-) | 13 | 1.8 (0.6-4.5)[f] | 4.5 (3.8-NR)[g,h] |
| RT only | Mut (+) | 3 | 3.1 (0.7-3.1)[f] | 4.5 (3.9-5.1)[h] |
| RT only | Mut (-) | 8 | 2.4 (0.5-NR) | 3.6 (0.5-NR) |
| Oligodendroglioma/oligoastrocytoma | | | | |
| RT+chemo | Mut (+) | 11 | 14.4 (2.1-NR)[e] | 18.2 (NR)[a] |
| RT+chemo | Mut (-) | 2 | 8.1 (NR)[e] | 8.1 (NR)[a] |
| RT only | Mut (+) | 4 | 3.7 (1.0-4.3)[e,i] | 6.3 (5.1-7.5)[a,i] |
| RT only | Mut (-) | 1 | 0.4 (NR)[i] | 1.6 (NR)[i] |

NR, PFS or median survival time is not reached; CI, confident interval; RT, radiotherapy; chemo, chemotherapy; mut, mutation. [a]p=0.02; [b]p=0.01; [c]p=0.004; [d]p=0.008; [e]p=0.03; [f]p=0.1; [g]p=0.06; [h]p=0.07; [i]p=0.05.

Table V. Multivariate analyses of PFS and OS of patients with all grade II gliomas.

| Variable | No. of cases | PFS hazard ratio (95% CI) | PFS p-value (Cox) | OS hazard ratio (95% CI) | OS p-value (Cox) |
|---|---|---|---|---|---|
| Histology | | | | | |
| Diffuse astrocytoma | 49 | 1 | 0.1 | 1 | 0.02 |
| Oligodendroglioma/ oligoastrocytoma | 23 | 0.576 (0.262-1.186) | | 0.290 (0.086-0.815) | |
| *IDH* mutation | | | | | |
| Wild-type | 31 | 1 | 0.08 | 1 | 0.01 |
| Mutation | 41 | 0.558 (0.289-1.068) | | 0.365 (0.155-0.819) | |
| Age (years) | | | | | |
| ≥40 | 34 | 1 | 0.5 | 1 | 0.02 |
| <40 | 38 | 0.802 (0.440-1.460) | | 0.400 (0.175-0.877) | |
| Extent of removal | | | | | |
| Partial removal and biopsy | 58 | 1 | 0.1 | 1 | 0.2 |
| Total and subtotal removal | 14 | 0.556 (0.222-1.217) | | 0.463 (0.107-1.403) | |
| Initial KPS | | | | | |
| <80 | 4 | 1 | 0.01 | 1 | 0.0002 |
| ≥80 | 68 | 0.179 (0.063-0.640) | | 0.045 (0.011-0.198) | |

CI, confident interval.

after surgery, those with *IDH1/2* mutations had significantly longer PFS and OS than those without *IDH1/2* mutations (PFS: p=0.02, OS: p=0.004; Fig. 2C and D; Table IV).

An important finding is that the patients who were initially treated with chemoradiotherapy after surgery and had *IDH1/2* mutations showed significantly longer PFS and OS than those

Table VI. Multivariate analyses of PFS and OS of patients with all grade II gliomas with radiotherapy ± chemotherapy.

| Variable | No. of cases | PFS hazard ratio (95% CI) | PFS p-value (Cox) | OS hazard ratio (95% CI) | OS p-value (Cox) |
|---|---|---|---|---|---|
| Histology | | | | | |
| Diffuse astrocytoma | 40 | 1 | 0.2 | 1 | 0.2 |
| Oligodendroglioma/ Oligoastrocytoma | 18 | 0.549 (0.209-1.290) | | 0.490 (0.133-1.445) | |
| *IDH* mutation | | | | | |
| Wild-type | 24 | 1 | 0.05 | 1 | 0.01 |
| Mutation | 34 | 0.467 (0.215-0.999) | | 0.316 (0.117-0.793) | |
| Age (years) | | | | | |
| ≥40 | 28 | 1 | 0.5 | 1 | 0.5 |
| <40 | 30 | 0.758 (0.362-1.559) | | 0.745 (0.300-1.808) | |
| Extent of removal | | | | | |
| Partial removal and biopsy | 47 | 1 | 0.03 | 1 | 0.08 |
| Total and subtotal removal | 11 | 0.364 (0.118-0.918) | | 0.356 (0.080-1.120) | |
| Initial treatment | | | | | |
| Radiotherapy alone | 16 | 1 | 0.04 | 1 | 0.002 |
| Chemoradiotherapy | 42 | 0.408 (0.182-0.948) | | 0.198 (0.073-0.529) | |

CI, confident interval.

treated with radiotherapy alone with *IDH1/2* mutations. The PFS and OS of the patients with *IDH1/2* mutations who were initially treated with chemoradiotherapy after surgery (n=27) were 9.3 and 18.2 years, respectively, and the PFS and OS of those treated with radiotherapy alone with *IDH1/2* mutations (n=7) were 3.1 and 5.1 years, respectively (PFS, p=0.01; OS, p=0.008). In the oligodendroglial tumors, the PFS and OS of the patients with *IDH1/2* mutations who were initially treated with chemoradiotherapy (n=11) were 14.4 and 18.2 years, respectively, and the PFS and OS of those treated with radiotherapy alone with *IDH1/2* mutations (n=4) were 3.7 and 6.3 years, respectively (PFS: p=0.03, OS: p=0.02; Fig. 2G and H). Similar tendencies, although not reaching statistical significance, were observed in the astrocytic tumors (PFS: p=0.1, OS: p=0.07; Fig. 2E and F).

The *IDH1/2* status had no impact on the PFS of all the grade II glioma or diffuse astrocytoma patients who underwent radiotherapy alone. No significant difference in PFS was observed between the radiotherapy and chemoradiotherapy groups in the grade II glioma patients without *IDH1/2* mutations. Chemoradiotherapy did not prolong the PFS of the patients without *IDH1/2* mutations in the astrocytic and oligodendroglial tumors.

*Multivariate analysis*. Oligodendroglial tumors (hazard ratio (HR)=0.29, p=0.02), age <40 years (HR=0.40, p=0.02), initial KPS ≥80 (HR=0.045, p=0.0002), and *IDH1/2* mutations (HR=0.37, p=0.01) were favorable prognostic factors for OS time, as determined by the multivariate analysis, of the 72 patients included in the study (Table V). The *IDH1/2* mutation status was not a prognostic factor for PFS when all the patients were considered, including those who did not undergo initial radiotherapy or chemotherapy (p=0.08). In contrast, total or subtotal tumor resection (HR=0.36, p=0.03), chemoradiotherapy (HR=0.41, p=0.04), and *IDH1/2* mutations (HR=0.47, p=0.05) were favorable prognostic factors for PFS, as determined by the multivariate analysis, of the patients who were initially treated with radiotherapy or chemoradiotherapy (Table VI). Histological appearance was not a prognostic marker for PFS in this series (p=0.2) compared with *IDH1/2* mutations (p=0.05).

## Discussion

WHO grade III and IV astrocytomas with *IDH1/2* mutations have more favorable prognoses than those with wild-type *IDH1/2* (17). *IDH1/2* mutations, 1p/19q codeletion, and *MGMT* promoter methylation are pivotal prognostic factors in anaplastic oligodendroglial tumors treated with radiotherapy or chemoradiotherapy (EORTC 26951) (27). However, the impact of *IDH1/2* mutations and/or 1p/19q codeletion as biomarkers in grade II gliomas remains controversial. The present study was therefore aimed at identifying prognostic and/or predictive factors in grade II gliomas.

*The presence of IDH1/2 mutations is a favorable prognostic marker for OS*. The results of the univariate analysis revealed that the presence of *IDH1/2* mutations was a prognostic factor of longer OS (p=0.004) and PFS (p=0.04) in the entire patient cohort and among the patients who underwent with or without radiation therapy after initial surgery with or without chemotherapy. The multivariate analysis revealed that the presence of

*IDH1/2* mutations was associated with prolonged PFS (p=0.05) and OS (p=0.01) in the patients who initially underwent radiotherapy with or without chemotherapy. Our results suggest that *IDH1/2* mutations may be involved in the response to genotoxic therapy, such as radiotherapy or chemotherapy, and may act as a prognostic factor for chemotherapy or radiotherapy in grade II gliomas. There are currently increasing numbers of reports showing that *IDH1/2* mutations are prognostic markers for several malignancies, including grade II gliomas. Houillier *et al* (19) reported that the presence of *IDH1/2* mutations is a significant prognostic marker for OS and chemosensitivity in low-grade glioma patients who were initially treated with temozolomide (TMZ) before any other treatment except surgery. Hartmann *et al* (16) reported that the *IDH1* mutation was a prognostic factor for PFS and OS in grade II glioma patients who underwent radiotherapy or chemotherapy after surgery. In our study, the presence of *IDH1/2* mutations was demonstrated by multivariate analysis to be a favorable prognostic factor (p=0.01) for OS but not a prognostic marker for PFS (p=0.08) in whole cohort, which included 14 patients who did not receive initial radiotherapy. Our finding that *IDH1/2* status did not affect PFS was in line with the findings reported by Hartmann *et al* (16) or Houillier *et al* (16,19), who showed that *IDH1* mutations did not affect the PFS in grade II glioma patients who did not receive radiotherapy or chemotherapy alone after surgery. Kim *et al* (21) and Mukasa *et al* (22) reported that the presence of *IDH1/2* mutations was not a prognostic factor for the survival of patients with low-grade glioma in univariate or multivariate analyses. The treatment of those patients was not fully described in their reports.

*The presence of IDH1/2 mutations is a predictive marker for PFS in the grade II glioma patients treated with chemoradiotherapy*. The patients who were initially treated with chemoradiotherapy after surgery showed significantly longer OS (p=0.0002) and PFS (p=0.01) than those treated with radiotherapy alone in our study. Chemoradiotherapy significantly prolonged PFS and OS compared with radiotherapy alone in all the grade II gliomas with *IDH1/2* mutations (p=0.01 and 0.0008, respectively), diffuse astrocytoma (p=0.1 and 0.07, respectively), and oligodendroglial tumors (p=0.03 and 0.02, respectively) in the univariate analysis. Chemoradiotherapy was shown by multivariate analysis (p=0.04) to significantly prolong the PFS of grade II glioma patients carrying *IDH1/2* mutations who underwent radiotherapy with or without concomitant chemotherapy (p=0.04). In contrast, there were no differences in PFS between the radiotherapy and chemoradiotherapy groups among the grade II glioma patients without *IDH1/2* mutations in the univariate analysis. PFS did not differ by *IDH1/2* status in the grade II glioma patients who underwent radiotherapy alone. However, the present study was limited by the small number of samples and the differences in the follow-up periods between the radiation and chemoradiotherapy groups (4 and 7.6 years, respectively). A prospective study including a larger patient cohort is required to obtain conclusive evidence that the presence of *IDH1/2* mutations is a predictive marker for chemoradiotherapy in grade II gliomas. Nonetheless, our results suggest that *IDH1/2* mutation is a predictive marker for chemoradiotherapy in grade II glioma patients and indicate that these patients may benefit from concurrent chemotherapy and radiotherapy compared with patients who do not carry *IDH1/2* mutations.

Mutations in *IDH1/2* result in the acquisition of new enzymatic activity that enables the NADPH-dependent reduction of α-ketoglutarate to 2-hydroxyglutarate, and the mutation confers oncogenic properties (28). *IDH1* mutations are early events in the development of astrocytomas and oligodendrogliomas (11). Another possible function of *IDH1/2* mutations is the dominant-negative inhibition of the oxidative decarboxylation of isocitrate as a result of the formation of a wild-type/mutant heterodimer (29). Cellular IDH1 levels are associated with the protection from apoptosis and cell death after exposure to reactive oxygen species or ultraviolet B-induced phototoxicity and *IDH1/2* functions in cellular defense reactions (30). Glioma cells with *IDH1/2* mutations may be vulnerable to irradiation and chemotherapeutic agents, which might explain why *IDH1/2* mutations could be a predictive and prognostic marker for grade II gliomas in patients receiving chemoradiotherapy. Our findings warrant a prospective large-scale clinical study addressing the efficacy of chemoradiotherapy in grade II glioma patients in association with *IDH1/2* status.

*Grade II glioma patients with wild-type IDH1/2 have poor prognoses even after total resection*. The extent of resection of tumors has been reported to be significantly associated with survival and recurrence of disease in low-grade glioma patients (9,31). In our study, the patients in the total or subtotal resection (≥90% removal) group tended to have longer survival times than the patients in the partial (<90% removal) or biopsy group (p=0.08). The patients without *IDH1/2* mutations had shorter OS than those with *IDH1/2* mutations in the total and subtotal resection groups (p=0.04) and in the partial and biopsy groups (p=0.01). Although the number of patients examined was small, we believe that this is a very important finding and that it indicates that patients without *IDH1/2* mutations may require more intensive treatment, such as chemoradiotherapy, even after total resection of the tumor.

*1p/19q codeletion is not a prognostic factor*. In our study, the OS and PFS in the diffuse astrocytomas with 1p/19q codeletion tended to be longer than those in the patients without 1p/19q codeletion, but the difference did not reach statistical significance. Furthermore, no significant differences were observed between the grade II glioma patients with regard to 1p/19q status. Prior studies reported that the presence of the 1p/19q codeletion was significantly associated with longer OS in low-grade gliomas (12,13,15,21,32). On the other hand, Houillier *et al* and Mukasa *et al* (19,22) reported that loss of 1p/19q was not a sensitive prognostic biomarker. Ichimura *et al* and Vogazianou *et al* reported that total 1p/19q loss is rare and that when present, it is associated with longer survival than other 1p/19q changes in adult gliomas independent of pathological diagnosis (14,15). Deletion of 1p or 19q was determined mainly by FISH analysis in our study, and this technique cannot discriminate between total and partial 1p/19q deletion, which might explain the discrepancy in the results.

*Clinicopathological factors in grade II gliomas*. The multivariate analysis showed that age ≥40 years (p=0.02), astrocytic tumors (p=0.02), initial KPS <80 (p=0.0002), and wild-type *IDH1/2* (p=0.01) were unfavorable prognostic factors in our series. These results are generally in line with previous reports

showing that older age, astrocytic histology, presence of neurologic deficits before surgery, largest tumor diameter, and tumors crossing the midline were important unfavorable prognostic factors for survival in adult patients with low-grade gliomas (5-9).

In conclusion, the multivariate analysis showed that age <40 years, oligodendroglial tumors, initial KPS ≥80, and *IDH1/2* mutations were favorable prognostic factors for survival of the grade II glioma patients. The presence of *IDH1/2* mutations was a prognostic factor for grade II glioma patients with radiotherapy. Furthermore, it is a predictive factor of response to chemoradiotherapy in grade II gliomas. Patients carrying *IDH1/2* mutations may benefit more from concurrent chemotherapy and radiotherapy compared with those without *IDH1/2* mutations.

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