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ORIGINAL ARTICLE

Immunohistochemistry on IDH 1/2, ATRX, p53 and Ki-67 substitute molecular genetic testing and predict patient prognosis in grade III adult diffuse gliomas

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Abstract The molecular subgrouping of diffuse gliomas was recently found to stratify patients into prognostically distinct groups better than histological classification. Among several molecular parameters, the key molecules for the subtype diagnosis of diffuse gliomas are IDH mutation, 1p/19q co-deletion, and ATRX mutation; 1p/19q co-deletion is undetectable by immunohistochemistry, but is mutually exclusive with ATRX and p53 mutation in IDH mutant gliomas. Therefore, we applied ATRX and p53 immunohistochemistry instead of 1p/19q co-deletion analysis. The prognostic value of immunohistochemical diagnosis for Grade III gliomas was subsequently investigated. Then, the same immunohistochmical diagnostic approach was expanded for the evaluation of Grade II and IV diffuse glioma prognosis. The results indicate immunohistochemical analysis including IDH1/2, ATRX, p53, and Ki-67 index is valuable for the classification of diffuse gliomas, which is useful for the evaluation of prognosis, especially Grade III gliomas and lower-grade gliomas (i.e., Grade II and III).

Keywords ATRX · Glioma · IDH · Immunohistochemistry · Ki-67 index · p53

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Introduction

Anaplastic astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas (i.e., Grade III gliomas) can be divided into 3 prognostically different groups with respect to *IDH* and *ATRX* mutations and 1p/19q status [1, 2]; this molecular subgrouping stratifies patients into prognostically distinct groups better than histological classification. Consequently, the inclusion of molecular parameters in the WHO definition of brain tumors is being planned and has been forwarded as the "ISN-Haarlem" consensus [3]. Moreover, Caccellari et al. [4] classified diffuse glioma (Grade II–IV) into 7 different subtypes on the basis of clinical and various molecular/genetic parameters using TCGA data.

These molecular subtypes are clinically important, because treatment strategies can be planned with respect to molecular subtype in conjunction with WHO grade. The two major ways to identify these subtypes in a tumor sample are direct interrogation of the mutated DNA itself and immunohistochemical analysis to assess the effects of the mutated genes on proteins. Immunohistochemistry is a longstanding, affordable, robust, and widely available technology. Therefore, immunohistochemical approaches with defined protocols and materials have become an essential means of assessing molecular genetic changes and thus, a critical component of current practice [5]. Among several molecular parameters, the key molecules for the subtype diagnosis of diffuse gliomas are IDH mutation, 1p/19q co-deletion, and ATRX mutation. Among them, IDH mutation [6] and ATRX mutation [7, 8] can be detected accurately by immunohistochemistry. Immunohistochemistry shows that ATRX mutation results in loss of protein expression, which is close to being mutually exclusive to 1p/19q co-deletion; moreover, all patients with 1p/19q co-

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deletion carry a mutation in IDH1 or IDH2 [1]. In addition, 1p/19q co-deletion is close to being mutually exclusive to p53 mutation [9, 10], which is also demonstrable by immunohistochemistry [11]. Furthermore, 1p/19q co-deletion is close to being mutually exclusive with ATRX mutation and p53 mutation with IDH mutation. Accordingly, the present study investigated the usefulness of the key molecular parameters (IDH mutation and 1p/19q co-deletion for IDH, ATRX, and p53) for the immunohistochemical diagnosis of diffuse gliomas.

Materials and methods

Patients

Fifty-three patients who underwent primary surgery between 1994 and 2011 at Tsukuba University Hospital were included. The mean patient age at the time of primary surgery was 48.7 ± 14.5 years (range 20–82 years). All tumors were diagnosed as Grade III gliomas according to the WHO classification, including 26 anaplastic astrocytomas, 8 anaplastic oligodendrogliomas, and 19 anaplastic oligoastrocytomas. Postoperative therapies were uniform. All patients were treated with maximal surgical resection, followed by 54–60 Gy radiation and ACNU-based chemotherapy. Median follow-up was 53.7 months (range 6–179 months). Informed consent was obtained from each patient or their guardian to obtain samples and use the associated data for analysis.

To extend our investigation, Grade II and IV samples were collected. All patients underwent primary surgery at Tsukuba University Hospital (Grade II between 1994 and 2004 and Grade IV between 2008 and 2010). There were 48 Grade IV samples including 37 primary glioblastomas and 11 secondary glioblastomas as well as 35 Grade II samples including 25 diffuse astrocytomas, 5 oligoastrocytomas, and 5 oligodendrogliomas. Postoperative therapies were uniform. For Grade IV tumors, the patients received 60 Gy radiation therapy, followed by ACNU and temozolomide-based chemotherapy. Patients with Grade II tumors underwent no further treatment after surgery, except for resection and radiation therapy in cases of recurrence.

Sample preparation

Tumor samples were removed intraoperatively, and the most viable part of an area devoid of macroscopically evident necrosis was taken as a specimen. The specimen was divided into 2: one part was fixed in 10 % formalin, and the other was frozen for subsequent analysis.

Immunohistochemistry

IDH mutation was determined by double immunohistochemistry using 2 mutation-specific anti-IDH antibodies (i.e., HMab-1 and MsMab-1) in gliomas in paraffin-embedded tumor specimens as described previously [6]. Briefly, IDH1-R132H protein expression was determined using HMab-1 at 5 μg/mL and an LSAB2 kit (Dako, Glostrup, Denmark), and the expressions of the other mutant proteins in paraffin sections were determined using MsMab-1 at 5 μg/mL and an Envision + kit (Dako). MsMab-1 recognizes several IDH mutations, namely IDH1-R132S, IDH1-R132G, IDH2-R172S, IDH2-R172G, and IDH2-R172M, as well as IDH1-R132H [12]. The expression of IDH mutations was determined by semi-quantitatively by assessing the proportions of positively stained tumor cells. Cases in which ≥10 % cells were stained were defined as positive.

All sections were immunostained with Ki-67 antibody (Immunotech Laboratories Inc., Monrovia, CA, USA), p53 antibody (clone DO7, Dako), and CD34 antibody (Dako) as described previously [13]. The positivity of nuclei for Ki-67 and p53 was determined by counting at least 1000 tumor cells in a homogeneously stained area. For both Ki-67 and p53, cases with ≥10 and <10 % stained cells were defined as positive and negative, respectively. The immunohistochemical cut-off of p53 mutation was defined as strong p53 immunoreactivity in >10 % of cells, which provides the most accurate prediction of mutation [11].

ATRX staining

All sections were immunostained with a commercially available polyclonal antibody (HPA001906, Sigma-Aldrich, St. Louis, MO, USA) at 0.5 μg/mL and an LSAB2 kit (DAKO) with Tris–EDTA antigen retrieval buffer (pH 9.0). Preliminary staining with Tris–EDTA buffer (pH 9.0) is superior to citrate buffer (pH 6.0) as an antigen retrieval method. Physiologically, ATRX protein is ubiquitously expressed in cell nuclei. Mutations in its gene result in a loss of nuclear protein expression in tumor cells but retained expression in non-tumor cells (e.g., endothelial and pre-existing glial cells), serving as a positive internal control [14]. Cases with ≥50 % stained cells (+++) were defined as ATRX loss-negative, and cases with <50 % stained cells (++; ≥10, +; <10 %, −; 0 %) accompanied with endothelial cell-positive staining were defined as ATRX loss.

Statistical analysis

Overall survival was calculated from the time of surgery until death or the last follow-up and was compared among

groups using the Kaplan–Meier method and the log-rank test. The Cox proportional hazards model was used to test prognostic factors in univariate and multivariate analyses. The results are expressed as relative risk with 95 % confidence intervals (CIs).

Results

ATRX staining

A total of 137 gliomas including 35, 53, and 48 Grade II–IV, respectively, were stained Fig. 1). ATRX loss was frequently observed in secondary glioblastoma (53.6 %), anaplastic astrocytoma (80.8 %), anaplastic oligoastrocytoma (68.4 %), and diffuse astrocytoma (56.0 %) and less frequently in primary glioblastoma (26.3 %), anaplastic oligodendroglioma (37.5 %), oligoastrocytoma (20.0 %), and oligodendroglioma (0 %) (Table 1).

Molecular classification by immunohistochemistry

Recent investigations support IDH1/2 mutation, p53 mutation, and ATRX loss separately for the classification of biologically distinct groups of gliomas, and indicate improved outcomes using this classification [1, 2, 4, 8]. Therefore, Kaplan–Meier analysis was used to evaluate the outcomes of 53 patients with Grade III gliomas following stratification according to IDH1/2 mutation status (Fig. 2a). IDH mutation ($n = 28$) was associated with longer survival relative to wild-type IDH (IDH1/2 wild-type, $n = 25$) [median overall survival (OS) = 60.2 vs. 33.9 months; log-rank test, $P < 0.01$, hazard ratio (HR): 2.3823 (95 % CI 1.2711–5.2452)]. In addition, p53 mutation and ATRX loss status were evaluated together, and OS was compared among IDH/p53/ATRX mutants ($n = 11$), IDH mutant/others ($n = 17$) and IDH wild-type ($n = 25$); their median OS was 43.3, 94.0, and 33.9 months, respectively. The IDH mutant/other group had significantly longer survival than the other groups (log-rank test $P < 0.001$) (Fig. 2b).

There was still overlap in OS between the IDH/p53/ATRX mutant group and the IDH wild-type group. We subsequently investigated the prognostic factors in IDH wild-type Grade III gliomas. Among various genetic, clinical, and biological factors, multivariate analysis demonstrated Ki-67 positivity (≥10 %) was a significant factor [$P = 0.0111$, HR: 4.0686 (95 % CI 1.3776–12.0161)] (Table 2). Furthermore, we divided IDH wild-type into 2 groups: the IDH wild-type/high Ki-67 and IDH wild-type/low Ki-67 groups.

Prognostic analysis of Grade III gliomas using the new molecular classification by immunohistochemistry

The gliomas were classified into 4 final groups: Group A, IDH mutant/others ($n = 17$); Group B, IDH wild-type/Ki-67 low ($n = 11$); Group C, IDH/p53/ATRX mutant ($n = 11$); and Group D, IDH wild-type/Ki-67 high ($n = 14$). The Kaplan–Meier curves clearly showed prognosis differed among the 4 groups: Group A had the best prognosis (median OS: 117.0 months), followed by Group B (99.5 months), Group C (65.2 months), and Group D (28.7 months) ($P < 0.001$, Fig. 3a). Progression-free survival showed a similar trend: Group A had the best median

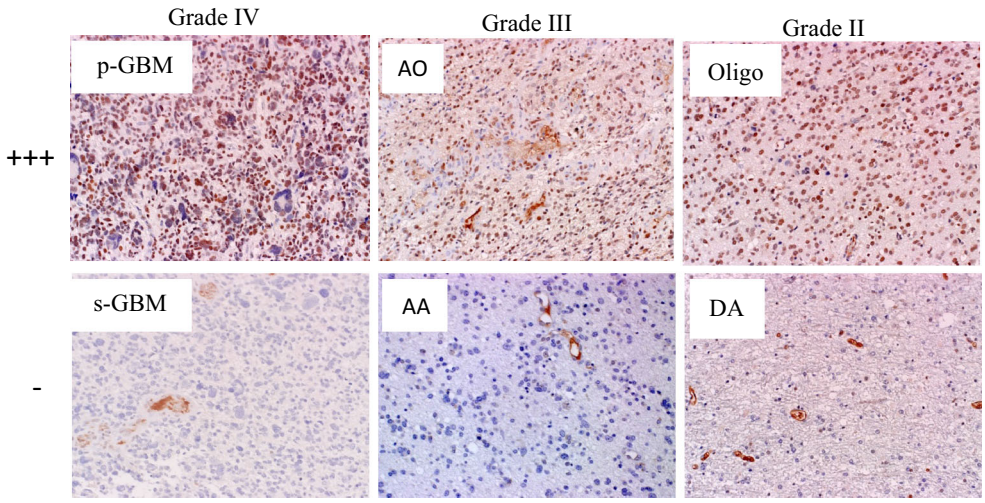

Fig. 1 ATRX staining with diffuse gliomas. +++ positive, − negative, *p-GBM* primary glioblastoma, *AO* anaplastic oligodendroglioma, *oligo* oligodendroglioma, *s-GBM* secondary glioblastoma, *AA* anaplastic astrocytoma, *DA* diffuse astrocytoma. Please note endothelial cells were positive in all sections

Table 1 ATRX staining pattern in Grade II, III and IV glioma

	Number	+++	++	+, −	Mut %
GBM					
Primary	38	28	9	1	26.3
Secondary	11	4	5	2	53.6
Grade III					
AA	26	5	14	7	80.8
AOA	19	6	7	6	68.4
AO	8	5	2	1	37.5
Grade II					
DA	25	11	10	4	56.0
OA	5	4	1	0	20.0
Oligo	5	5	0	0	0

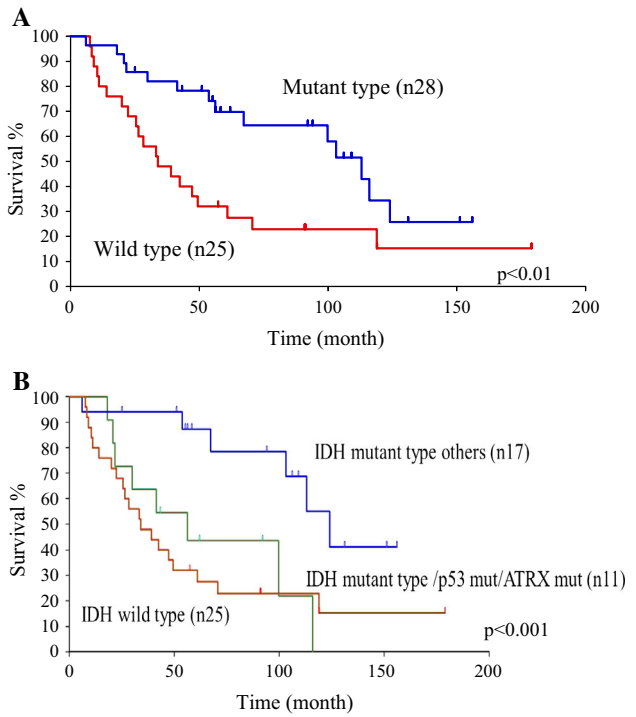

Fig. 2 **a** Kaplan–Meier curves of overall survival in 53 patients with Grade III glioma following stratification by IDH1/2 mutation status. **b** Kaplan–Meier curves following stratification by IDH1/2, p53, and ATRX mutation status

Table 2 Prognostic factors for IDH wild type Grade III glioma

	P value	HR	95 % CI
Univariate			
p53			
>10 %: 1	0.7595	0.8418	0.2795–2.5356
<10 %: 0			
ATRX			
Mut: 1	0.2821	0.5843	0.2195–1.5555
WT: 0			
Age			
>55: 1	0.1985	1.8058	0.7336–4.4455
<55: 0			
Sex			
Male: 1	0.8214	0.9015	0.3663–2.2184
Female: 0			
Removal			
Non-total: 1	0.2577	3.2180	0.4253–24.3511
Total: 0			
Location			
Others: 1	0.4726	0.7181	0.2908–1.7729
Frontal: 0			
MGMT			
>10 %: 1	0.5790	1.2964	0.5181–3.2439
<10 %: 0			
Ki-67			
>10 %: 1	0.0057	4.4656	1.546–12.902
<10 %: 0			
Density			
>30: 1	0.0491	2.5638	1.0038–6.5484
<30: 0			
VEGF			
+: 1	0.2271	1.7326	0.7101–4.2171
−: 0			
Multivariate			
Ki-67			
>10 %: 1	0.0111	4.0686	1.3776–12.0161
<10 %: 0			
Density			
>30: 1	0.1117	2.1826	0.8341–5.7108
<30: 0			

progression-free survival (67.2 months), whereas Group D had the worst (12.2 months) ($P < 0.001$, Fig. 3b; Table 3).

Kaplan–Meier curves based on classical morphological diagnosis, which included anaplastic oligodendroglioma ($n = 9$), anaplastic oligoastrocytoma ($n = 18$), and anaplastic astrocytoma ($n = 26$), demonstrated significant differences in prognosis; however, differences in survival were unclear compared to the curve based on the proposed molecular diagnosis by immunohistochemistry, especially between anaplastic oligoastrocytoma and anaplastic astrocytoma, ($P < 0.001$, Fig. 3c).

The initial morphological diagnosis and immunohistochemical molecular diagnosis were compared (Fig. 4a). Most anaplastic oligodendrogliomas were classified into Group A, and anaplastic oligoastrocytomas and anaplastic astrocytomas were classified equally into the 4 groups.

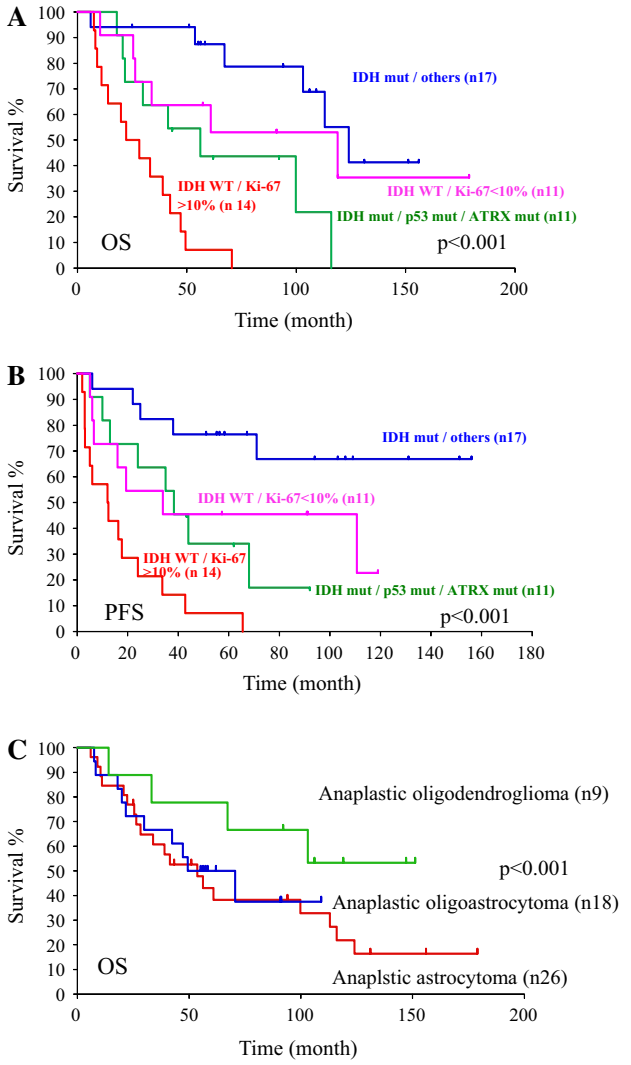

Fig. 3 **a** Kaplan–Meier curves of overall survival in 53 patients with Grade III gliomas following stratification by IDH1/2, p53, ATRX mutation status, and Ki-67 positivity. **b** Kaplan–Meier curves of progression-free survival. **c** Kaplan–Meier curves of overall survival according to the three classic histological groups

Among the groups, Group D had the poorest prognosis, close to that of glioblastomas; the profile of Group D was further investigated. First, a central review of all samples was performed by another neuropathologist using other sections of the sample. Second, other molecular parameters related to tumor biology were evaluated immunohistochemically, including VEGF, HIF-1α, podoplanin, phosphorylated Akt staining [15, 16], and microvascular vascular density stained by CD34. Among Group D ($n = 14$, Table 4), in addition to IDH mutation, the following other mutations were observed: p53 mutation in 3 cases and ATRX loss in 7 cases. The central review demonstrated necrosis and microvascular proliferation in 2 and 4 cases, respectively, (Table 4; cases 1–4), were diagnosed as 2 glioblastomas and 2 glioblastoma with an oligodendroglioma component, respectively. Among these 4 cases, 3 were ATRX wild-type and 1 was 1p/19q co-deletion–negative according to FISH analysis and other molecular profile expression (i.e., VEGF, HIF-1α, podoplanin, and phosphorylated Akt positivity), suggesting the possibility of Grade IV at the initial diagnosis.

Prognostic analysis of Grade II and IV gliomas using the molecular classification by immunohistochemistry

The same molecular classification by immunohistochemistry was adapted for Grade II and IV gliomas, and its effectiveness for prognosis analysis was evaluated. There were 20, 9, 6, and 0 Grade II gliomas classified into Groups A–D, respectively, ($n = 35$, Fig. 4b). All histological oligodendrogliomas were in Group A. Oligoastrocytomas were divided equally into Groups A and B. Astrocytomas were divided equally among Groups A–C. The prognosis of Group C tended to be shorter than that of other groups (Fig. 5a, b).

Table 3 Detail molecule profile in molecular diagnosis group

	n	IDH mut	P53 mut	ATRX loss	Ki67 >10 %	Med OS (mo)
Grade III						
Group A	17	17	3	8	9	117.0
Group B	11	0	3	10	0	99.5
Group C	11	11	11	11	5	65.2
Group D	14	0	3	7	14	28.7
Total	53	28	20	36	28	
Grade II + III						
Group A	37	37	4	14	11	84.0
Group B	20	20	4	13	0	104.9
Group C	17	17	17	17	5	52.0
Group D	14	14	3	7	14	25.3
Total	88	88	28	51	30	

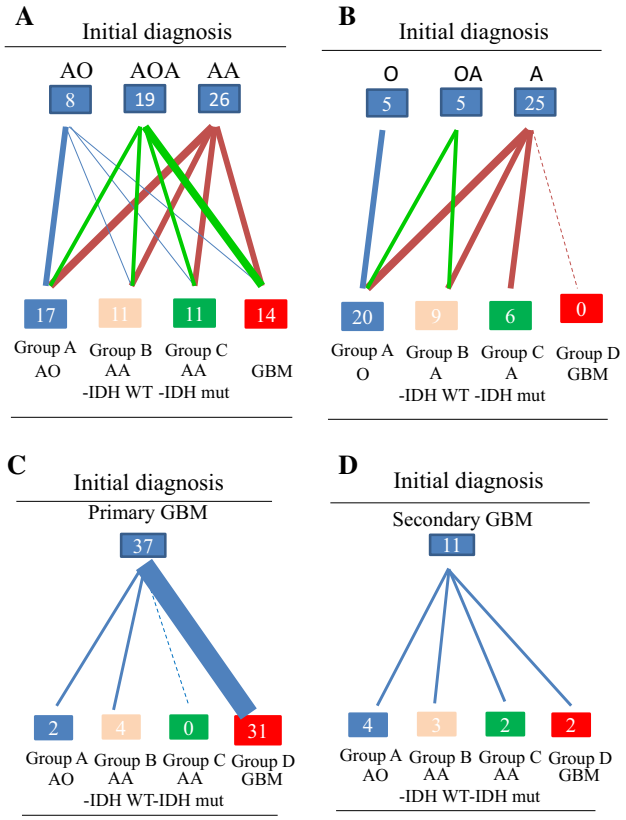

Fig. 4 Comparison of initial morphological diagnosis and immunohistochemical molecular diagnosis. **a** Grade III gliomas; **b** Grade II gliomas; **c** Grade IV primary glioblastomas; **d** Grade IV secondary glioblastomas; *AO* anaplastic oligodendroglioma, *AOA* anaplastic oligoastrocytoma, *AA* anaplastic astrocytoma, *O* oligodendroglioma, *OA* oligoastrocytoma, *A* astrocytoma, *GBM* glioblastoma

Grade IV gliomas ($n = 48$) included 37 and 11 primary and secondary glioblastomas, respectively. The majority of primary glioblastomas (31/37 cases) were classified into Group D; 2 and 4 were classified into Groups A and B, respectively, (Fig. 4c). Because of the few cases in Group D, the molecular classification by immunohistochemistry did not demonstrate significant superiority for determining the prognosis of primary glioblastoma (Fig. 5c). In contrast, secondary glioblastomas were distributed equally among groups: 4, 3, 2, and 2 in Groups A–D, respectively, (Fig. 4d); there was a significant difference in the prognosis among groups (Fig. 5d).

As it was recently reported that there is no genetic difference between Grade II and III gliomas [17–19], we evaluated our classification by combining Grade II and III gliomas as lower-grade gliomas. Among the 88 lower-grade gliomas, 37, 20, 17, and 14 were classified into Groups A–D, respectively. Median OS in Groups A–D was 84.0, 104.9, 52.0, and 25.3 months, respectively; OS was significantly longer in Groups A and B than Groups C and D (Fig. 6; Table 3) despite different initial treatments between Grade II and III gliomas.

Discussion

The results of the present study indicate that combined immunohistochemistry including IDH1/2, ATRX, p53, and Ki-67 index is useful for the molecular classification of diffuse gliomas, which is useful for the evaluation of prognosis, especially Grade III and lower-grade gliomas (i.e., Grade II + III). Although our immunohistochemical approach does not replace the genetic testing completely, it is a practical and a powerful means of assessing molecular genetic changes.

Table 4 Molecular profile of Group D

IDH	Status	Ki-67 %	P53 mut	ATRX loss	Pathol 1	Pathol 2	Necrosis	MVP	Density	VEGF	HIF1	Podoplanin	PAkt
1	WT	17.1	+	−	AOA	GBMO	+	+	31	−	+	+	+
2	WT	30.8	−	−	AOA	GBMO	+	+	107	+	+	+	−
3	WT	20.5	−	−	AO	GBM	+	−	81	−	+	+	−
4	WT	23.8	−	+	AA	GBM	+	−	32	+	+	+	+
5	WT	29	+	+	AOA	GBMO	+	−	45	−	+	+	+
6	WT	10.6	−	+	AOA	AOA	−	−	11	−	−	−	+
7	WT	36.4	−	+	AA	AA	−	−	96	+	+	+	+
8	WT	14.2	−	+	AOA	AOA	−	−	24	+	+	+	+
9	WT	10.6	−	+	AOA	AOA	−	−	65	−	+	+	+
10	WT	20.1	−	+	AA	AA	−	−	18	−	+	−	+
11	WT	12.1	+	−	AA	AA	−	−	15	+	+	+	+
12	WT	12.2	−	−	AA	AA	−	−	8	+	−	−	−
13	WT	26.1	−	−	AO	AO	−	−	32	−	+	+	−
14	WT	52.1	−	−	AOA	AOA	−	−	28	+	+	−	−

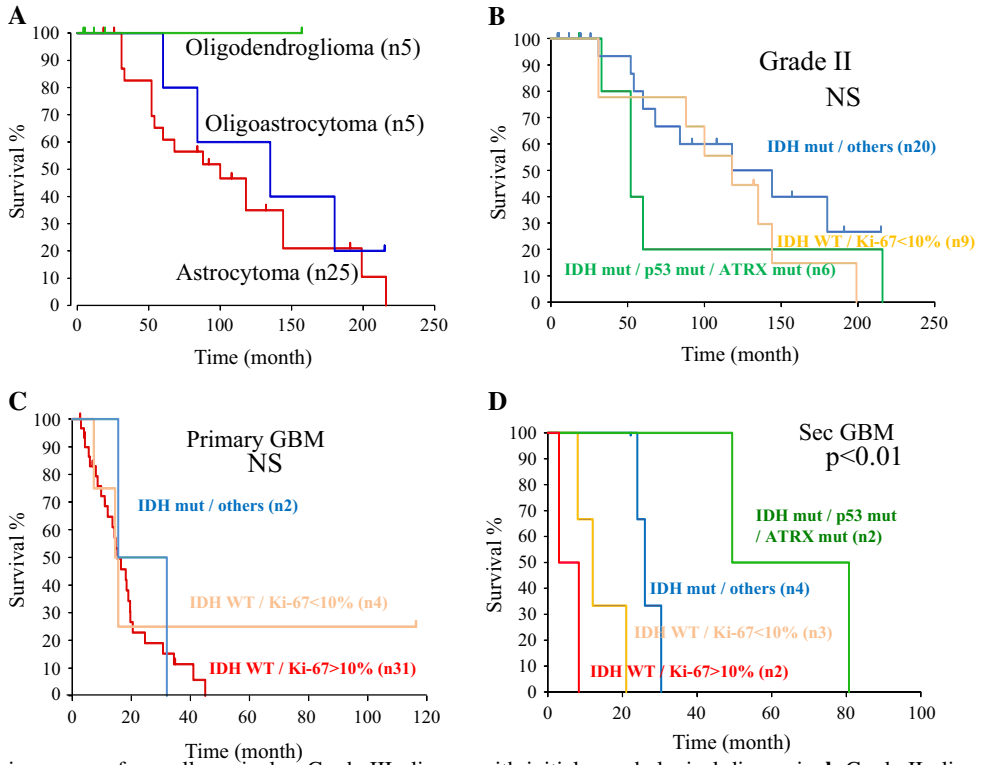

Fig. 5 Kaplan–Meier curves of overall survival. **a** Grade III gliomas with initial morphological diagnosis; **b** Grade II gliomas with molecular diagnosis; **c** Grade IV primary glioblastomas with molecular diagnosis; **d** Grade IV secondary glioblastomas with molecular diagnosis

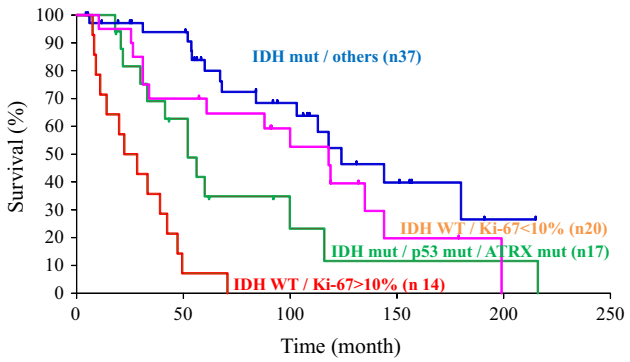

Fig. 6 Kaplan–Meier curves of overall survival in 88 lower-grade (i.e., Grade II + Grade III) gliomas following stratification according to IDH1/2, p53, ATRX mutation status, and Ki-67 positivity

Immunohistochemistry-based molecular diagnosis

The molecular diagnosis for diffuse gliomas has definitive treatment and prognostic importance [1, 2, 4, 8]. The original molecular markers were IDH mutation status and 1p/19q LOH status, following by ATRX status [1].

The present study revealed the frequency of immunohistochemical ATRX loss (i.e., ATRX mutation); it occurred frequently in Grade IV secondary glioblastoma; in the majority of Grade III anaplastic astrocytomas (80.8 %), Grade III anaplastic oligoastrocytomas (68.4 %), and Grade II astrocytomas (56 %); infrequently in primary glioblastomas (26.3 %), Grade III anaplastic oligodendrogliomas (37.5 %), and Grade II oligodendrogliomas (20 %); and in 0 % of oligoastrocytomas. These results are similar to those of previous studies [7, 8] reporting ATRX loss in 45–73 % of Grade III anaplastic astrocytomas, 4 % of primary glioblastomas, 27–68 % of Grade III anaplastic oligodendrogliomas, and 14 % of Grade II oligodendrogliomas.

With regard to the immunohistochemical cut-off for p53 mutation, strong p53 immunoreactivity in $\geq$10 % of cells is reported to provide the most accurate prediction of mutation [11]. Using this cut-off, it was found that 52 of 55 immunopositive cases harbored a mutation, whereas only 14 of 102 immunonegative cases showed mutations; the sensitivity and specificity of this cut-off were 78.8 and 96.7 %, respectively. Therefore, p53 immunohistochemistry was proposed as a moderately sensitive and highly specific marker for the prediction of p53 mutation. Because p53 mutation is almost invariably associated with IDH1/2 mutations in Grade II astrocytomas and Grade III anaplastic astrocytomas [20, 21] and mutually exclusive 1p/19q co-deletion [9, 10], we selected p53 as a molecular marker.

Routine immunohistochemistry can be used to evaluate ATRX loss, p53 protein accumulation, and IDH1R132H mutation but not 1p/19q co-deletion; this may enable the classification of diffuse glioma outcomes. There is strong evidence of relationships among ATRX loss, mutually

exclusive 1p/19q co-deletion [8, 22], and p53 mutually exclusive 1p/19q co-deletion [9, 10], suggesting the usefulness of ATRX and p53 immunohistochemistry without 1p/19q co-deletion analysis. In addition, we focused on Ki-67, which is a classic and reliable indicator of cancer cell proliferation activity used in routine clinical investigation; it predicts poorer prognosis in patients with gliomas [23, 24]. It was recently reported that high Ki-67 expression is dominant in IDH1/2 wild-type gliomas [25] and that low Ki-67 expression is associated with IDH1 mutations in primary glioblastomas [26]. Ki-67 expression in combination with IDH1/2 mutation status quickly indicates prognosis in gliomas [27]. Olar et al. [28] report that the mitotic index is significantly associated with outcome in IDH wild-type tumors but not in IDH mutant tumors. These findings demonstrate the relationship between Ki-67 expression and IDH1/2 mutations in gliomas. On the basis of these results, Ki-67 expression was applied as a molecular marker of IDH wild-type gliomas. Application of Ki-67 labeling index to separate IDH-wild type gliomas is important, because IDH-wild-type gliomas are composed of heterogeneous group [4, 20].

Molecular classification by immunohistochemistry predicts glioma prognosis

Finally, we selected IDH, p53, ATRX, and Ki-67 as immunohistochemical molecular markers. The 53 histological Grade III cases were divided into 4 groups. First, Group C, characterized by IDH positivity (mutant type), p53 positivity (mutant type), and ATRX loss (mutant type), suggesting molecular astrocytomas, exhibited intermediate prognosis. Group A, characterized by IDH positivity (mutant type) other than Group C, suggesting molecular oligodendroglioma, exhibited the best prognosis. Third, Group B, characterized by IDH negativity (wild type) and Ki-67 <10 % demonstrated intermediate prognosis; 10 of 11 cases in this group demonstrated ATRX loss (mutant type), suggesting molecular astrocytomas. Finally Group D, which was characterized by IDH negativity (wild type) and Ki-67 ≥ 10 %, suggesting molecular glioblastoma, demonstrated the poorest prognosis.

A similar molecular classification by immunohistochemistry was applied for Grade II and IV gliomas. There were no Grade II gliomas in Group D. Group C tended to have poorer prognosis than the other groups. Compared to classical histological diagnosis, tumors with molecular oligodendroglioma (Group A) were predominant. In addition, this molecular classification could not predict prognostic group for primary glioblastomas. Regarding the prediction of the prognosis of secondary glioblastoma, which mainly depends on IDH status, Groups A and C (IDH mutant) tended to have good prognosis, whereas Groups B and D (IDH wild-type) tended to have poorer prognosis.

However, molecular classification by immunohistochemistry for lower-grade gliomas again demonstrated the usefulness of the present classification for the prediction of prognosis. Group A tumors suggesting molecular oligodendroglioma exhibited best prognosis, Group C tumors suggesting molecular astrocytoma exhibited intermediate prognosis, and Group D tumors suggesting molecular glioblastoma exhibited the poorest prognosis despite different initial treatments (e.g., radiation and chemotherapy). These results suggest our molecular classification based on immunohistochemical analysis is useful for predicting cases with good prognosis among lower-grade gliomas. Accordingly, lower-grade gliomas with poor prognosis according to the molecular classification should receive more intensive treatment. The group of lower-grade gliomas with the best prognosis had a median OS of 117 months with current treatment strategies. Meanwhile, the median OS of the group with intermediate prognosis in Group C was only 55 months; this group possessed 3 molecular mutations of IDH, p53, and ATRX, suggesting a new molecular target for the tumor [29–32]. Group D exhibited the poorest prognosis, with a median OS of only 25.3 months and should, therefore, be treated as glioblastoma.

Conclusion

The molecular immunohistochemical classification including IDH, p53, ATRX, and Ki-67 showed that the group of molecular oligodendrogliomas had the best prognosis, followed by the molecular group of astrocytomas; meanwhile, the molecular glioblastoma group had the poorest outcome among Grade III and lower-grade gliomas.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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