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Clinical Neurology and Neurosurgery 187 (2019) 105556

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

Relevance of calcification and contrast enhancement pattern for molecular diagnosis and survival prediction of gliomas based on the 2016 World Health Organization Classification

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ARTICLE INFO

Keywords:
Glioma
Molecular diagnosis
Enhancement
Calcification
Survival
World Health Organization Classification

ABSTRACT

Objectives: The significance of conventional neuroimaging features for predicting molecular diagnosis and patient survival based on the updated World Health Organization (WHO) classification remains uncertain. We assessed the relevance of neuroimaging features (ring enhancement [RE], non-ring enhancement [non-RE], overall gadolinium enhancement [GdE], and intratumoral calcification [IC]) for molecular diagnosis and survival in glioma patients.

Patients and methods: We evaluated 234 glioma patients according to the updated WHO classification. Isocitrate dehydrogenase (IDH), *H3F3A*, *BRAF* hotspot mutations, *TERT* promotor mutation, and chromosome 1p/19q co-deletion were examined. RE, non-RE, GdE, and IC were evaluated as significant neuroimaging findings. Kaplan-Meier analyses were performed to evaluate overall survival (OS) and the correlations of prognostic factors were evaluated by log-rank tests. Univariate and multivariate analyses were performed to detect prognostic factors for OS.

Results: A total of 207 patients were eligible. In 110 patients presenting RE, 102 (93%) were glioblastoma (GBM), IDH-wild type. In 97 patients without RE, presence of GdE or IC were not significantly different between IDH-mutant and -wild type tumors, whereas presence of GdE was a significant indicator of higher WHO grades. IC was the only significant finding for 1p/19q co-deleted tumors. *TERT* promoter mutation was observed in 7/17 patients with diffuse astrocytic glioma, IDH-wild type; recently-defined as "molecular GBM." IC, RE, and GdE were observed with lower prevalence in molecular GBMs. While presence of RE, GdE, and absence of IC were significant factors of OS in overall cohort, presence of GdE was not significant in OS in cases without RE, and IDH-mutant tumors. IC was a significant predictor of favorable OS in cases without RE and IDH-wild type tumors. Multivariate analysis also validated these findings.

Conclusion: GdE alone is not a significant predictor of IDH mutation status, but the pattern of enhancement is a significant predictor with RE demonstrating high sensitivity and specificity for GBM, IDH-wild type. Predicting "molecular GBM" by conventional neuroimaging is difficult. Moreover, GdE is not a significant factor of survival analyzed with pattern of enhancement or molecular stratifications. IC is an important radiographic finding for predicting molecular diagnosis and survival in glioma patients.

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https://doi.org/10.1016/j.clineuro.2019.105556
Received 28 June 2019; Received in revised form 30 September 2019; Accepted 6 October 2019
Available online 12 October 2019
0303-8467/

1. Introduction

The precise classification and diagnosis of gliomas are essential for their clinical management and outcome prediction. Conventional diagnoses for gliomas have been based primarily on histopathological findings. However, in response to the recent innovative development of molecular diagnosis of gliomas by the accumulation of molecular genetic analysis data, the updated World Health Organization Classification of Tumors of the Central Nervous System (2016 CNS WHO) includes molecular genetic diagnoses, in addition to the classical histopathological diagnosis [1–3]. Molecular characteristics of gliomas have been shown to be superior to conventional histological grading in the estimation of patient prognosis [4–6]. In the pre-molecular era, ring-shaped gadolinium enhancement (RE) on magnetic resonance imaging (MRI) was well-known as a typical feature of glioblastoma (GBM), and gadolinium enhancement (GdE) was a strong indicator of malignancy [7,8]. However, the correlation between conventional neuroimaging features and the molecular diagnosis of gliomas according to the 2016 CNS WHO has not yet been sufficiently investigated. Additionally, while the presence or degree of GdE has been reported as one of the prognostic factors of patient survival for gliomas in the pre-molecular era [8–12], the significance of radiographic features, such as RE, GdE, and intratumoral calcification (IC), for outcome prediction based on the molecular characteristics according to the 2016 CNS WHO remains uncertain. Herein, we aimed to assess the significance of the presence or pattern of GdE and IC on preoperative images for the prediction of molecular diagnosis and survival in glioma patients in the modern molecular era.

2. Materials and methods

2.1. Patients and genetic analyses

We retrospectively reviewed the clinical, radiographic, and molecular-pathological data of our case series of 234 consecutive patients who were treated at our university hospital (Fukuoka, Japan), between January 2002 and February 2017, and whose tumors had been diagnosed as “diffuse astrocytic and oligodendroglial tumors” according to the 2016 CNS WHO [1]. Snap-frozen tissue samples of the tumors of enrolled cases were used to detect isocitrate dehydrogenase (IDH), *BRAF*, and *H3-G34R* hot spot mutations, *TERT* promoter mutation, and chromosome 1p/19q co-deletion as described previously [13–16]. The flow diagram of molecular work-up is described in Fig. 1. Subsequently, molecular diagnoses were categorized as diffuse astrocytoma IDH-mutant (DA_{mut}); diffuse astrocytoma IDH-wild type (DA_{wt}); anaplastic astrocytoma IDH-mutant (AA_{mut}); anaplastic astrocytoma IDH-wild type (AA_{wt}); oligodendroglioma IDH-mutant and 1p/19q co-deleted ($OD_{1p/19q}$); anaplastic oligodendroglioma IDH-mutant and 1p/19q-co-deleted ($AO_{1p/19q}$); GBM IDH-mutant (GBM_{mut}); and GBM IDH-wild type (GBM_{wt}). Patients without sufficient neuroimaging examinations via computed tomography (CT) and MRI were excluded. In addition, patients diagnosed with diffuse midline gliomas, gliosarcomas, or aged < 18 years old were also excluded. We only included patients with newly-diagnosed gliomas, and recurrent or re-treatment cases were excluded.

2.2. Neuroimaging findings

Preoperative neuroimaging examination was performed via contrast-enhanced, thin-slice CT and gadolinium-enhanced MRI for all patients. GdE was evaluated in a post-contrast enhanced T1-weighted image and categorized into RE and non-ring enhancement (non-RE). RE was defined as the presence of gadolinium enhancement at the tumor margin and a lack of enhancement in the center of the tumor. Cases that presented with GdE but lacked RE were defined as non-RE. IC was also defined as intratumoral lesions presenting a very high-density level, similar to that presented by the skull in pre-contrast-enhanced, thin-slice CT, including both coarse and minute calcification. Fig. 2 shows typical cases with each neuroimaging finding. All neuroimages were evaluated and reported by experienced neuroradiologists before surgeries. In this study, neuroimaging findings were evaluated based on the radiology reports and confirmed by at least one neurosurgeon.

All CT scans were performed by single detector (X Vigor; Toshiba, Tokyo, Japan), or multi-detector row CT (Aquilion; Toshiba). Typical imaging parameters were as follows: collimation, 4 mm; tube voltage, 120 kVp; tube current, 150–200 mA; field-of-view (FOV), 240 x 270 mm; and matrix, 512 × 512. All MRI studies were performed using 1.5 T (Vision or Symphony; Erlangen, Germany or Achieva; Philips, Best, the Netherlands) or 3 T system (Achieva or Ingenia; Philips). For 1.5 T system, the typical imaging parameters were as follows: axial T1-weighted spin-echo imaging using repetition time (TR)/echo time (TE) = 522–612/12–14 ms, field-of-view (FOV) = 240 x 240 mm, matrix = 256 × 256, and slice thickness/gap = 5/1–2.5 mm. The contrast material was injected at 0.1 mmol/kg (gadopentetate dimeglumine, (Magnevist) Bayer, Osaka, Japan). Axial postcontrast T1-weighted spin-echo imaging using TR/TE = 558–593/17 ms, FOV = 240 x 240 mm, matrix = 256 × 256, and slice thickness/gap = 5/1-2.5. For 3 T system, the typical imaging parameters for the brain were as follows: axial T1-weighted spin-echo imaging using TR/TE = 450/9 ms, FOV = 240 x 240 mm, matrix = 256 × 256, slice thickness/gap = 5/1 mm, and acquisition time = 2 min 39 s. The contrast material was injected at 0.1 mmol/kg (gadopentetate dimeglumine, (Magnevist) Bayer, Osaka, Japan). Axial postcontrast T1-weighted spin-echo imaging using TR/TE = 465/21 ms, FOV = 256 x 256 mm, matrix = 256 × 256, and slice thickness/gap = 5/1 mm.

2.3. Statistical analysis

Chi-square tests were assessed to investigate the relationship between each neuroimaging finding and molecular characteristics or WHO grades. Sensitivity and specificity were also evaluated for each radiographic finding. Kaplan-Meier analysis was performed to evaluate overall survival (OS), and a log-rank test was used to compare the survival distributions. Univariate and multivariate Cox proportional hazards regression models were performed to evaluate putative prognostic factors for OS. The level of statistical significance was set at $p < 0.05$. JMP Pro version 13 (SAS Institute Inc., NC, USA) was used for all statistical analyses.

3. Results

A total of 207 patients were eligible for evaluation based on the criteria of the present study (Fig. 1). The patient characteristics, molecular diagnoses, and neuroimaging findings of participants are summarized in Table 1. Correlations between radiographic findings and molecular characteristics or WHO grades are described in Table 2.

3.1. Correlations between neuroimaging findings and molecular diagnoses or WHO grades

Among 110 tumors presenting with RE, 102 (93%) were diagnosed as GBM_{wt} and 106 (96%) were categorized as IDH-wild type tumors (Table 1). In all patients, the presence of RE, overall GdE, and lack of IC (non-IC) were significant features of GBM_{wt} compared to other glioma subgroups ($p < 0.0001$) (Table 2). As most cases of GBM_{wt} presented with RE, non-RE was significant in tumors other than GBM_{wt} ($p < 0.0001$). In 97 tumors without RE, the presence of GdE (= non-RE) and IC were not significant findings between IDH-mutant and -wild type tumors. However, the presence of GdE was a significant feature in WHO grade IV tumors compared to grade II/III tumors ($p < 0.0001$). Similarly, in the lower-grade glioma (grade II/III) subgroup, the presence of GdE was a significant feature in WHO grade III gliomas ($p = 0.0344$). Therefore, GdE was a significant indicator of WHO

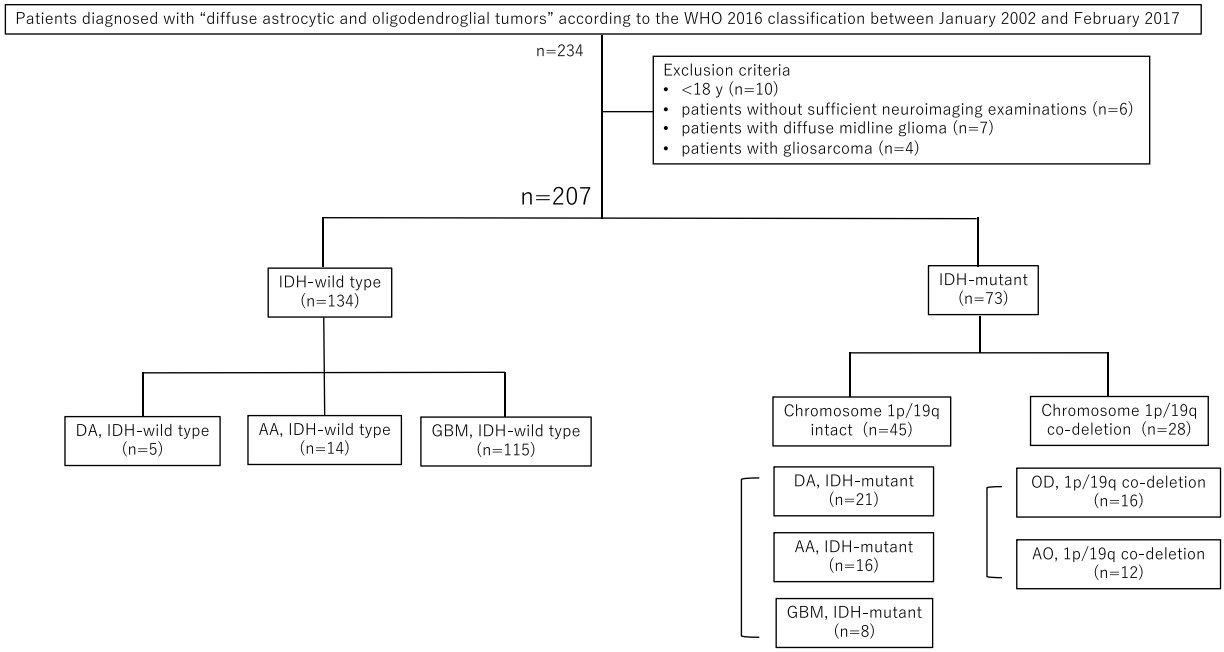

Fig. 1. Patient flow diagram according to molecular diagnosis. DA, diffuse astrocytoma; AA, anaplastic astrocytoma; OD, oligodendroglioma; AO, anaplastic oligodendroglioma; GBM, glioblastoma; IDH, isocitrate dehydrogenase.

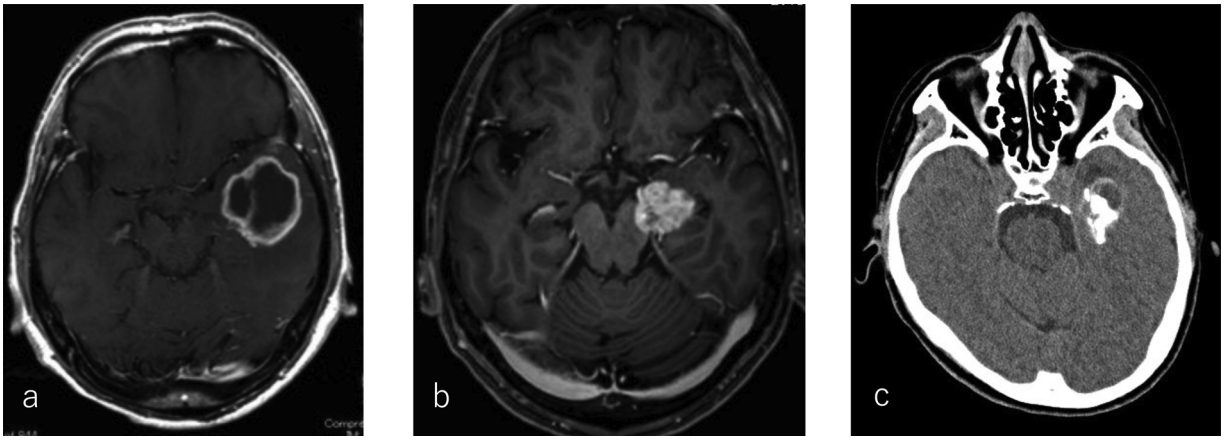

Fig. 2. Typical cases with ring enhancement (a), non-ring enhancement (b), and intratumoral calcification (c).

grades, but was not a significant predictor of IDH mutation status. Presence of IC was the only significant feature of 1p/19q co-deleted tumors in both the lower-grade glioma ($p = 0.0008$) and IDH-mutant tumor subgroups ($p = 0.0002$).

3.2. GBM subtypes, DA_{wt}, and AA_{wt} with TERT promoter mutation

Among 8 patients with GBM_{mut}, 5 (63%) lacked RE and 1 presented with IC. In addition, 1 and 2 patients with GBM_{wt} presented with *H3-G34R* (GBM_{H3G34R}) and *BRAF* (GBM_{BRAF}) hotspot mutations, respectively. In these tumors, 1 GBM_{H3G34R} lacked RE, and 1 GBM_{BRAF} lacked RE and presented with IC. Accordingly, GBM_{mut}, GBM_{H3G34R}, and GBM_{BRAF} could demonstrate different radiographic features from those of a typical GBM_{wt}. Among the 17 patients with DA_{wt} and AA_{wt} (excluding 2 cases with radiation induced glioma), *TERT* promoter mutation was observed in 7 patients, who can be diagnosed as having the recently-defined "molecular GBM" [17]. Absence of IC was significant in such molecular GBM cases ($p = 0.0241$), and presence of RE and GdE was observed with lower prevalence in patients with molecular GBMs (Table 3).

3.3. Survival analyses

Kaplan-Meier analyses revealed that the presence of RE ($p < 0.0001$), GdE ($p < 0.0001$), and non-IC ($p = 0.0003$) were significantly associated with unfavorable OS in the overall patient cohort (Fig. 3a–c). However, presence or absence of GdE was not a significant factor of OS in cases excluding ring enhanced tumors ($p = 0.3779$, Fig. 4a) and IDH-mutant tumors ($p = 0.5472$, Fig. 4b). On the other hand, presence of IC was significantly associated with favorable OS in cases excluding those with ring enhanced tumors ($p = 0.0431$, Fig. 4d) and IDH-wild type tumors ($p = 0.0195$, Fig. 4f), whereas IC was not significant for OS among IDH-mutant tumors ($p = 0.2343$ Fig. 4e). Multivariate Cox regression analysis revealed poor preoperative Karnofsky Performance Status (KPS) score, IDH-wild type tumors, and lack of calcification were significant prognostic factors for unfavorable OS, though GdE was not significant (Table 4).

4. Discussion

Since the 2016 CNS WHO was recently enacted, accurate correlation

Table 1
Summary of neuroimaging features and glioma classification and characteristics according to the 2016 WHO classification.

		WHO grade II		WHO grade III		WHO grade II	WHO grade III	WHO grade IV	
	Total	DA, ID-wild type	DA, IDH-mutant	AA, IDH-wild type	AA, IDH-mutant	OD, IDH-mutant & 1p19q-codeleted	AO, IDH-mutant & 1p19q-codeleted	GBM, IDH-wild type	GBM, IDH-mutant
n	207	5	21	14	16	16	12	115	8
Median age (range) (y.o.)	55 (19-85)	33 (24-66)	30 (20-57)	56.5 (28-74)	37 (19-52)	44 (20-73)	46 (27-74)	65 (26-85)	38 (23-62)
Sex (male/female)	114/93	1/4	16/5	10/4	12/4	9/7	4/8	60/55	2/6
Overall gadolinium enhancement (presence/absence)	164/43	1/4	5/16	7/7	11/5	8/8	9/3	115/0	8/0
Ring enhancement (presence/absence)	110/97	0/5	0/21	4/10	0/16	0/16	1/11	102/13	3/5
Non-ring enhancement (presence/absence)	54/153	1/4	5/16	3/11	11/5	8/8	8/4	13/102	5/3
Intratumoral calcification (presence/absence)	24/183	2/3	0/21	2/12	3/13	6/10	7/5	3/112	1/7

Abbreviations: WHO, World Health Organization; IDH, isocitrate dehydrogenase; DA, diffuse astrocytoma; AA, anaplastic astrocytoma; OD, oligodendroglioma; AO, anaplastic oligodendroglioma; GBM, glioblastoma.

between conventional neuroimaging features and molecular diagnosis, and survival prediction based on molecular stratifications of gliomas, has not been thoroughly determined. The present study revealed the correlations between neuroimaging features and molecular stratification or WHO grades based on the 2016 CNS WHO. In addition, we discovered the relevance of RE, GdE, and IC as prognostic factors of survival in glioma patients in the modern molecular era.

The presence of contrast-enhancement, derived from either the destruction or permeability of the blood-brain barrier, is known to be a representative radiographic finding of malignant gliomas in the pre-molecular era [7,8]. RE, which reflects internal tumor necrosis due to increased tumor volume, is a distinctive feature of GBM [8]. IC, which reflects high focal differentiation of the tumor components, is occasionally observed in certain lower-grade tumors, such as oligodendroglioma or ganglioglioma [18–22]. Therefore, we hypothesized that the presence or pattern of GdE and IC are correlated with the molecular characteristics of gliomas and are also associated with patient prognosis in the molecular era.

4.1. Significance of RE

In this study cohort, 93% of ring enhanced tumors were GBM_{wt} and 96% were categorized as IDH-wild type tumors. Moreover, ring enhanced tumors exhibited unfavorable OS (Fig. 3a). Thus, gliomas with RE can be predicted as GBM_{wt} with high sensitivity (0.89) and specificity (0.91), and ring enhanced tumors present an unfavorable course, even in the modern molecular era. Therefore, it is considered clinically beneficial to treat non-ring enhanced tumors separately from ring enhanced tumors to predict their diagnosis and survival.

4.2. Significance of GdE and IC

Among tumors without RE, there were no significant differences between the presence or absence of GdE or IC and IDH mutation status, whereas presence of GdE was a strong indicator of higher WHO grades. Although previous studies showed higher prevalence of GdE in IDH-wild type gliomas than in IDH-mutant gliomas [23–25], our study revealed that the presence of GdE could no longer be considered a predictor of IDH-wild type tumors when ring enhanced tumors, which were most cases of GBM_{wt}, were excluded.

IC is a well-known characteristic of oligodendroglial tumors, especially in those with 1p/19q co-deletion [18–22]. Our study also revealed that IC was the only significant neuroimaging feature of 1p/19q co-deleted tumors. In our cohort, the presence or absence of GdE was not significant in 1p/19q co-deleted tumors, supporting previous reports that have described the insufficient specificity of GdE for the prediction of $OD_{1p/19q}$ [23,26]. However, contradictory reports have been published. While Reyes-Botero et al. reported that $OD_{1p/19q}$ was associated with no or non-measurable enhancement [27], Sonoda et al. reported that OD was associated with the presence of GdE [28]. These results, along with our findings, suggest that the significance of GdE in 1p/19q co-deleted tumors is yet to be clarified.

4.3. Neuroimaging features of GBM subtypes

This study also identified that patients with GBM_{mut}, GBM_{H3G34R}, and GBM_{BRAF} frequently presented with non-RE or IC, which are non-typical features of GBM_{wt}. We previously reported that GBM_{mut} demonstrates multiple heterogeneous, non-ring, or non-markedly enhancing lesions [29], GBM_{H3G34R} shows non-markedly enhancing or calcified masses [13], and that GBM_{BRAF} occasionally presents with IC [14]. Absence of RE and presence of IC have been identified as findings of lower-grade gliomas or oligodendroglial tumors. Therefore, the results of this study caution against judging the tumor as a lower-grade glioma by conventional neuroimaging findings alone, because GBM might be hidden in cases presenting with radiographic features similar

Table 2
Correlations between neuroimaging findings and molecular stratifications or WHO grades.

		Ring enhancement		Non-ring enhancement		Overall gadolinium enhancement		Intratumoral calcification	
	n	presence	absence	presence	absence	presence	absence	presence	absence
GBM, IDH-wild type	115	102	13	13	102	115	0	3	112
others	92	8	84	41	51	49	43	21	71
p value		< **0.0001**		< **0.0001**		< **0.0001**		< **0.0001**	
sensitivity (GBM, IDH-wild type)		0.89		0.11		1.0		0.03	
specificity (GBM, IDH-wild type)		0.91		0.55		0.47		0.77	
Tumors without ring enhancement (n = 97)									
IDH-wild type tumors	28					17	11	4	24
IDH-mutant tumors	69					37	32	15	54
p value						0.5229		0.3905	
sensitivity (IDH-wild type tumors)						0.61		0.14	
sensitivity (IDH-wild type tumors)						0.46		0.78	
WHO grade IV	18					18	0	2	16
WHO grade II/III	79					36	43	17	62
p value						< **0.0001**		0.2895	
sensitivity (WHO grade IV)						1.0		0.11	
specificity (WHO grade IV)						0.54		0.78	
WHO grade II/III tumors without ring enhancement (n = 79)									
WHO grade III	38					22	16	9	29
WHO grade II	41					14	27	8	33
p value						**0.0344**		0.6522	
sensitivity (WHO grade III)						0.58		0.24	
specificity (WHO grade III)						0.66		0.80	
WHO grade II/III tumors (n = 84) / IDH-mutant tumors (n = 73)									
1p/19q co-deleted	28/28	1/1	27/27	16/16	12/12	17/17	11/11	13/13	15/15
1p/19q intact	56/45	4/3	52/42	20/21	36/24	24/24	32/21	7/4	49/41
p value		0.4966/0.5610		0.0618/0.3834		0.1217/0.5358		**0.0008/0.0002**	
sensitivity (1p/19q co-deleted)		0.04/0.04		0.57/0.57		0.61/0.61		0.46/0.46	
specificity (1p/19q co-deleted)		0.93/0.93		0.64/0.53		0.57/0.47		0.88/0.91	

Abbreviations: IDH, isocitrate dehydrogenase; GBM, glioblastoma, WHO, World Health Organization.

to those of lower-grade gliomas. In addition, other driver gene mutations, such as *H3* or *BRAF* mutations, should be examined in cases of GBM with non-typical radiographic features.

4.4. DA_{wt} and AA_{wt} with TERT promoter mutation; molecular GBM

"Diffuse astrocytic glioma, IDH-wild type, with molecular features of GBM, WHO grade IV" is a recently described new classification of GBM [17]. This "molecular GBM" is defined as DA_{wt} or AA_{wt} with epidermal growth factor receptor (EGFR) amplification, combined whole chromosome 7 and whole chromosome 10 loss, or *TERT* promoter mutation. As these tumors exhibit poor survival, predicting molecular GBM by neuroimaging is clinically important. In the present study, 17 patients (excluding those with radiation induced glioma) were diagnosed with diffuse astrocytic glioma, IDH-wild type, and *TERT* promoter mutation was observed in 7 patients. Absence of IC was significant in *TERT*-mutant cases (molecular GBMs), and the presence of RE and GdE was observed at a lower prevalence in patients with molecular GBM. Hence, diffuse astrocytic glioma, IDH-wild type with *TERT* promoter mutation, were likely to show absence of IC, RE, and GdE, but it was difficult to predict molecular GBM by conventional neuroimaging findings. These paradoxical results (the clinical course and neuroimaging findings were inconsistent) suggest that molecular examinations should be evaluated even in cases with "non-malignant" features on neuroimaging in IDH-wild type gliomas.

4.5. Survival analyses

In this study, although ring enhanced gliomas were associated with unfavorable survival, there were no significant differences in OS between patients with and without GdE, when ring enhanced gliomas were excluded. Since the majority of ring enhanced tumors were categorized as GBM_{wt}, it is reasonable to assume that these tumors tend to have a poor prognosis, as demonstrated in this study. In addition, multivariate analysis revealed GdE was not a significant factor of OS. While some previous studies have indicated that the presence or degree of GdE in gliomas is a predictor of poor prognosis in the pre-molecular era [8–12], other recent reports have reported that GdE was not a significant survival predictor when analyzed with various factors [23,25]. In their recent report with detailed molecular evaluation, Hempel et al. demonstrated that the presence of GdE was not a significant survival factor when the analysis included molecular stratification [23]. Considering these reasons, it was considered acceptable in our cohort that there were significant differences in survival associated with both RE and GdE when overall patients were included, but the presence of GdE was not a significant factor for OS among cases without RE and in IDH-mutant tumors. Together with the result that GdE was not a significant indicator of molecular stratifications, its importance for prognosis prediction has also declined in the modern molecular era.

A unique finding of this study is that the presence of IC was recognized as a significant predictor of favorable OS among overall cases, IDH-wild type tumors, and tumors without RE. Among patients without RE, multivariate analysis also revealed presence of IC was a significant factor of favorable OS. This is probably because IC was not a significant survival predictor in a small number of patients with IC in IDH-mutant tumors. While IC was a significant finding in 1p/19q co-deleted tumors, it can be indicated as a favorable survival predictor even in IDH-wild type tumors. While IC is widely known to be a favorable prognostic factor, our study revealed IC can be a significant survival predictor even when analyzed based on molecular stratifications and pattern of enhancement. Therefore, the significance of IC for patient prognosis appears to be important in the molecular era.

Table 3
Correlations between neuroimaging findings and *TERT* promoter mutation in patients with diffuse astrocytoma, IDH-wild type and anaplastic astrocytoma, IDH-wild type, excluding radiation induced glioma.

		Ring enhancement		Non-ring enhancement		Overall gadolinium enhancement		Intratumoral calcification	
	n	presence	absence	presence	absence	presence	absence	presence	absence
DA/AA IDH-wild type *TERT* promoter mutation (+)	7	0	7	2	5	2	5	0	7
DA/AA IDH-wild type *TERT* promoter mutation (-)	10	3	7	2	8	5	5	4	6
p value		0.0569		0.6833		0.3723		**0.0241**	
sensitivity (TERT promoter mutation)		0		0.29		0.29		0	
specificity (TERT promoter mutation)		0.70		0.80		0.50		0.60	

Abbreviations: DA, diffuse astrocytoma; AA, anaplastic astrocytoma; IDH, isocitrate dehydrogenase.

4.6. Study limitations

Our study had several limitations. The limitation in the neuroimaging was that various types of CT/MRI had been used for patients participating in this study. In our institute, we used a 1.5 T MRI unit before 2006, and a 3 T MRI unit thereafter. Radiographic findings may be different between 1.5 T and 3 T MRI. Additional limitations include the selection of a limited number of patients, sample size differences among molecular diagnoses, and the incomplete exclusion of potential selection biases due to the non-randomized, non-blinded study design. We also could not evaluate T2WI/fluid attenuated inversion recovery (FLAIR) sequences in this study. As previous studies reported that T2/FLAIR features can be associated with 1p/19q status [30,31], T2/FLAIR features are also important findings to predict molecular diagnosis. In addition, we could not evaluate EGFR amplification, or combined whole chromosome 7 and whole chromosome 10 loss. Due to this reason, there were limited cases of molecular GBMs presenting with *TERT* promoter mutation in this study.

5. Conclusions

GdE (excluding RE) alone was not a significant predictor of IDH mutation status, but the pattern of enhancement is a significant predictor with RE demonstrating high sensitivity and specificity for GBM, IDH-wild type which were associated with unfavorable prognosis. Moreover, presence of GdE was not a significant factor of survival prognosis analyzed with molecular stratifications and pattern of enhancement. In addition, GBM_{mut}, GBM_{H3G34R}, and GBM_{BRAF} occasionally presented with non-RE or IC, which are findings of classical lower-grade gliomas. Moreover, “molecular GBM” were likely to lack IC, RE,

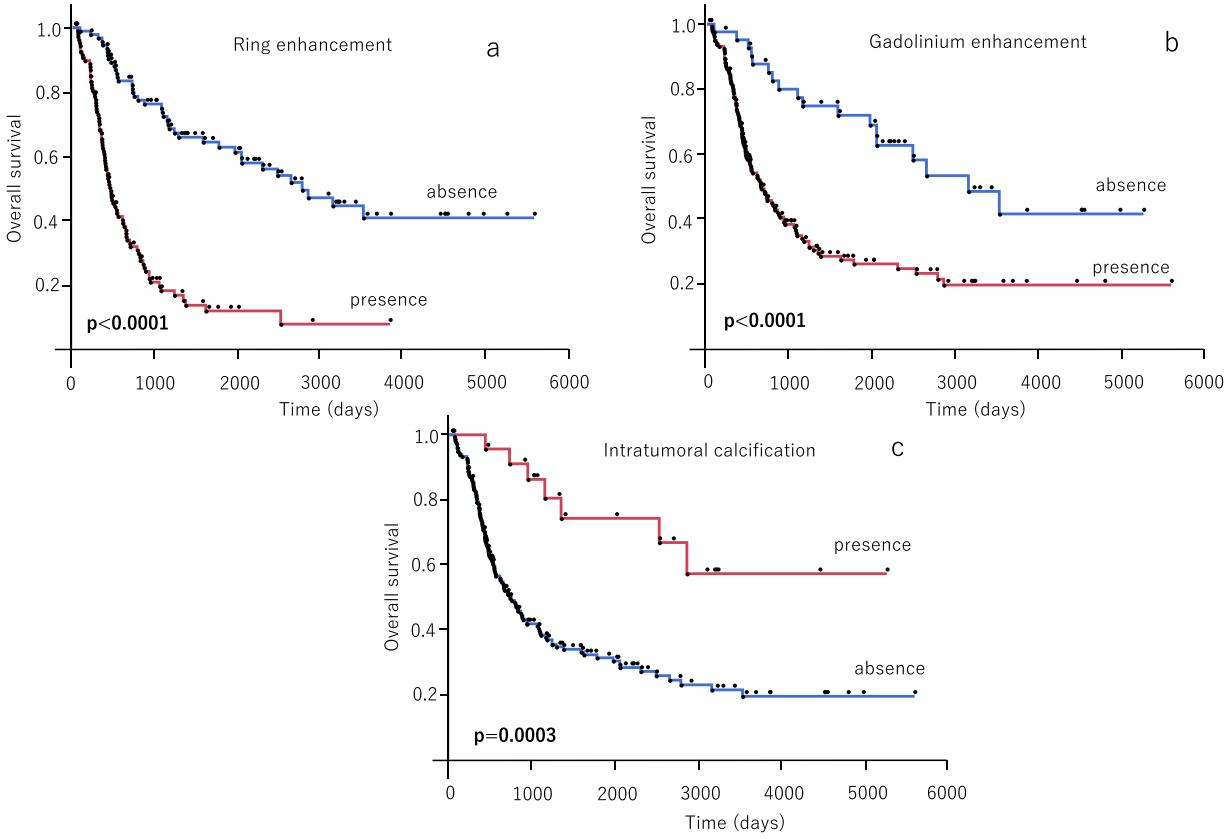

Fig. 3. Survival analyses between neuroimaging features and overall survival. Kaplan-Meier analyses between presence or absence of ring enhancement (a), gadolinium enhancement (b), intratumoral calcification (c) and overall survival.

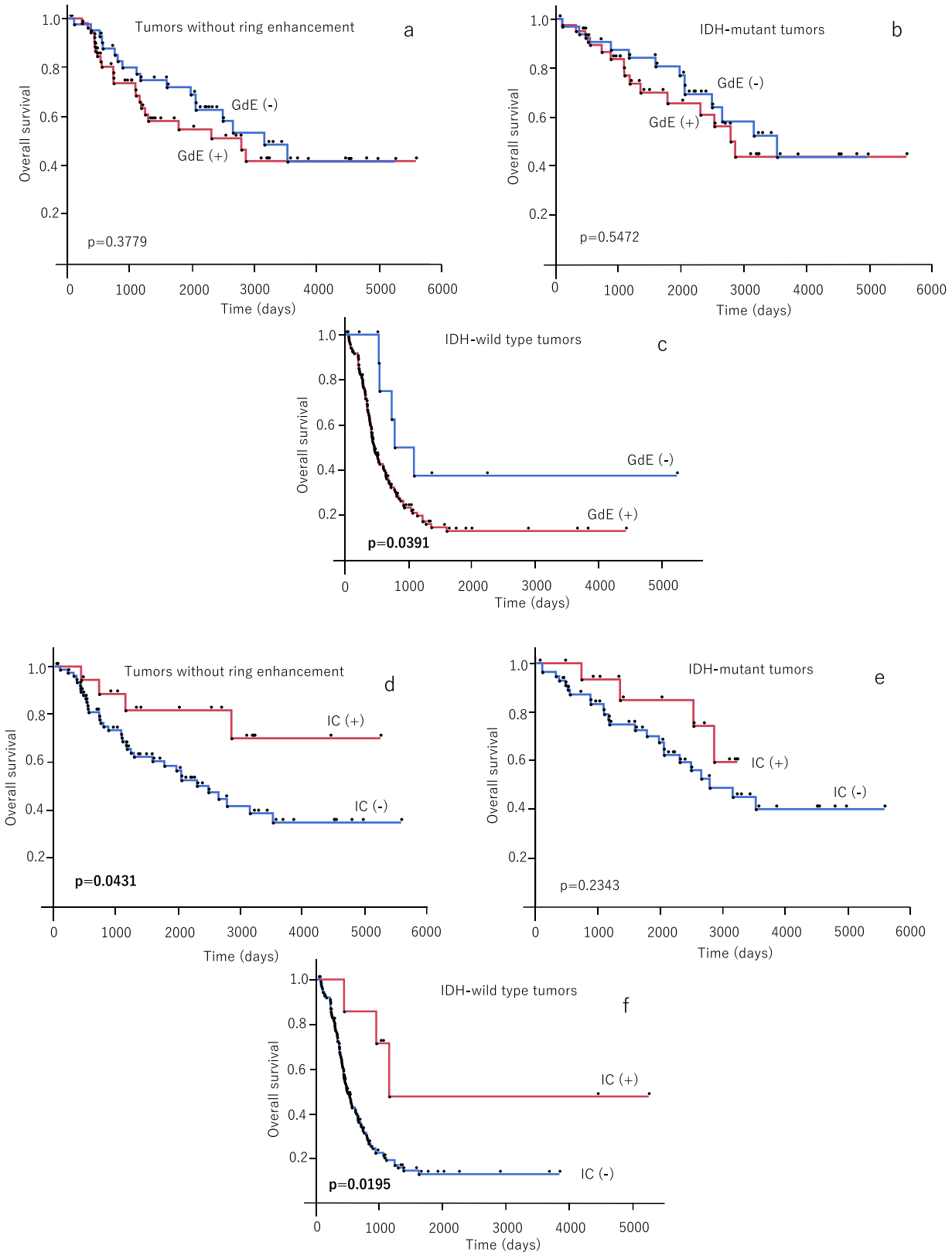

Fig. 4. Survival analyses between neuroimaging features and overall survival. Kaplan-Meier analyses between presence or absence of gadolinium enhancement among tumors without ring enhancement (a), IDH-mutant (b), and -wild type tumors (c). Kaplan-Meier analyses between presence or absence of intratumoral calcification among tumors without ring enhancement (d), IDH-mutant (e), and -wild type tumors (f). GdE; gadolinium enhancement, IC; intratumoral calcification.

Table 4
Univariate and multivariate Cox regression analyses of prognostic factors of the overall survival in patients without ring enhancement.

	Univariate			Multivariate		
	Hazard ratio	95% CI	P value	Hazard ratio	95% CI	P value
Age (y.o.)	1.00	0.97-1.02	0.8626	0.98	0.95-1.00	0.0902
Preoperative KPS score 80-100 (Ref.) vs. 10-70	6.99	3.13-15.62	< **0.0001**	4.58	1.80-11.65	**0.0014**
IDH status IDH-mutant (Ref.) vs. IDH-wild type	3.19	1.69-6.02	**0.0003**	4.04	1.84-8.86	**0.0005**
Extent of resection gross total ≦ (Ref.) vs. < gross total	1.62	0.75-3.51	0.2217	1.49	0.67-3.34	0.3286
Gadolinium enhancement absence (Ref.) vs. presence	1.32	0.71-2.45	0.3794	1.24	0.59-2.61	0.5628
Intratumoral calcificationn presence (Ref.) vs. absence	2.28	0.99-7.82	0.0526	3.38	1.07-10.70	**0.0382**

and GdE, but it was difficult to predict "molecular GBM" by conventional neuroimaging findings. Therefore, GdE has only limited significance in evaluating molecular diagnosis and patient survival in the modern molecular era. According to the results of this study, we may have to dispel the stereotype that the tumor exhibits malignant behavior simply because of the presence of GdE. On the other hand, IC is an important finding for predicting molecular diagnosis and patient prognosis.

Funding

This research was supported by the Japanese Society for the Promotion of Science Grants-in-Aid for Scientific Research (KAKENHI) Award (Grant No. 16K10779).

Ethical standards

The present study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. Informed consent from patients was acquired to investigate the genetic analyses of tumors preoperatively.

Acknowledgments

None.

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