J Neurooncol (2015) 123:289–297
DOI 10.1007/s11060-015-1797-y

CLINICAL STUDY

MRI findings and pathological features in early-stage glioblastoma

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Received: 11 August 2014 / Accepted: 20 April 2015 / Published online: 5 May 2015
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Abstract Magnetic resonance imaging (MRI) is an important diagnostic tool for glioblastoma, with almost all cases showing characteristic imaging findings such as a heterogeneous-ring enhanced pattern associated with significant edema. However, MRI findings for early-stage glioblastoma are less clear. In this study, a retrospective review of MRI findings in five patients showed slight T2WI signal changes on initial scans that developed into typical imaging findings of a ring-like or heterogeneously enhanced bulky tumor within 6 months. The diagnoses based on initial MRI were low grade glioma in three cases, venous thrombosis in one case, and uncertain in one case. Four cases were treated with gross total resection, while one case underwent biopsy. Immunohistochemical examinations showed that two cases were p53-positive, and that all cases were IDH1 R132H-negative and had overexpression of EGFR. FISH analysis showed that all cases were 1p19q LOH-negative. De novo glioblastoma was the final diagnosis in all cases. Our results show that initial MRI findings in early-stage glioblastoma of small ill-defined T2WI hyperintense lesions with poor contrast develop to bulky mass lesions with typical findings for glioblastoma in as short a period as 2.5 months. The early MRI findings are difficult to distinguish from those for non-neoplastic conditions, including ischemic, degenerative or demyelinating processes. Thus, there is a need for proactive diagnosis of glioblastoma using short-interval MRI scans over several weeks, other imaging modalities, and biopsy or resection, particularly given the extremely poor prognosis of this disease.

Keywords MRI · Early-stage · Glioblastoma · IDH1 · de novo type · Secondary type

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Introduction

Glioblastoma is usually diagnosed based on characteristic Magnetic resonance imaging (MRI) findings of strongly enhanced heterogeneous-ring mass lesions with significant peritumoral brain edema, necrosis, or hemorrhage [1]. A subset of glioblastoma is detected at an earlier stage before mass enhancement and central necrosis [2] and 4 % of glioblastoma cases show non-enhancement at initial presentation, which can be misinterpreted as a non-neoplastic condition such as infarction, neurodegenerative disease, and metabolic disease [1, 3, 4]. Delayed diagnosis of glioblastoma may lead to a poor outcome, whereas early diagnosis before progression to typical glioblastoma should give a better prognosis based on the increased potential for maximum safe resection. Therefore, definition of MRI findings in early-stage glioblastoma is important for making a correct early diagnosis.

Most cases of glioblastoma (>90 %) develop very rapidly in elderly patients without clinical or historical evidence (de novo or primary glioblastoma). Cases of secondary glioblastoma are less frequent (<10 %), affect younger patients, and progress more slowly from low-grade glioma or anaplastic astrocytoma. Primary and

glioblastomas are largely indistinguishable histologically, but develop through distinct genetic pathways [5, 6]. Primary glioblastoma is characterized by EGFR amplification, PTEN mutation, and entire loss of chromosome 10 [5–7], whereas secondary glioblastoma more commonly includes TP53 mutations and 19q loss [5, 6, 8]. The identification of an isocitrate dehydrogenase 1 (IDH1) mutation as a molecular marker of secondary glioblastoma may allow for clear definition of the two subtypes [9–11].

The combination of MRI findings and genetic analysis of biopsy specimens may be useful for early diagnosis of primary and secondary glioblastoma, and correct diagnosis at an early stage should allow appropriate and prompt treatment, including total resection. For this reason, we retrospectively reviewed MRI findings for a subset of glioblastoma cases lacking typical imaging characteristics and investigated the pathological features of these cases. We also reviewed the literature on clinicopathological features in early-stage glioblastoma.

Materials and methods

Case selection

We performed an initial retrospective analysis of 63 patients treated for glioblastoma from 2004 to 2013 at Yamaguchi University Hospital. Among these patients, 5 cases with a diagnosis of glioblastoma after a second or third MRI scan following an initial scan were enrolled in this study. The patients were one man and four women and ranged in age from 63 to 82 years (mean 66.4 years). The final diagnosis of glioblastoma in all cases was confirmed by histopathological examination based on World Health Organization (WHO) criteria [12]. Immunohistochemistry for glial fibrillary acidic protein (GFAP), s100 protein, p53, IDH1-R132H, epidermal growth factor receptor (EGFR), and Ki-67, and fluorescence in situ hybridization (FISH) analyses for 1p/19q loss of heterozygosity (LOH) were performed in all cases.

Immunohistochemistry

Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded sections using the streptavidin–biotin-peroxidase complex labeling method. Primary antibodies used included anti-GFAP (ready to use, Dako Japan, Kyoto, Japan), anti-p53 (dilution 1:100, Dako Japan), anti-EGFR (1:50, Nichirei Biosciences Inc, Tokyo, Japan), anti-Ki-67 (1:50: Dako Japan), anti-IDH1 R132H (1:40: Dianova, Hamburg, Germany), and anti-S100 protein (1:600; Dako Japan) antibodies. The score for p53 was classified based on detectable staining in 10 % of the cells and ≥10 % of the cells stained. A tumor was defined as p53-positive if ≥10 % of the cells showed nuclear staining [13–15].

Fluorescent in situ hybridization

Frozen tumor tissue was pretreated with hypotonic solution followed by Carnoy fluid fixation and slide samples were obtained. FISH analysis was performed to detect major cytogenetic abnormalities including del1p and del19q (Vysis LSI 1p36/LSI 1q25 and LSI 19q13/LSI 19p13 Dual-Color Probe). Probes were hybridized to pretreated tumor sections for 3 days at 37 °C. All probe sets were from Abbott Laboratories (Abbott Molecular Inc., Abbott Park, IL, USA). Slides were washed and counterstained with DAPI. Signals were counted in 100 nuclei that had at least 1 signal for each probe under a microscope [16, 17].

Statistical analysis

Kaplan-Meyer analysis was used to assess overall survival, with a log-rank test used to evaluate differences between survival curves for the 5 patients with those for the other 58 of the original 63 cases. Statistical analyses were conducted using StatFlex software (Artech, Osaka, Japan).

Results

Clinical characteristics

Clinical characteristics and MRI findings for the 5 cases in the study and for 9 cases reported in the literature are summarized in Table 1. For the 5 cases in this study, initial MRI was performed for general symptoms such as vertigo and mild headache, and for mild hemiparesis in one case. Two cases without symptoms were detected incidentally. Initial MRI findings showed small hyperintense lesions on T2-weighted images (T2WI) with little or no gadolinium enhancement and little mass effect.

Progression leading to diagnosis of glioblastoma became evident on repeat MRI at an average of 4.1 months after the initial MRI scan, with 3 patients showing progression within 3 months. Three cases (cases 1, 2 and 4) were initially misdiagnosed as low grade glioma from MRI findings, and thus follow-up MRI was performed 2.4–5.9 months after the initial scan. The follow-up scans showed a typical gadolinium enhancement effect (Fig. 1a–f). In one case (case 5), it was hard to detect the hyperintense lesion on T2WI and fluid attenuated inversion recovery (FLAIR) images in the early stage, even retrospectively (Fig. 2a, b). However, a slightly abnormal lesion rapidly developed into a hyperintense heterogeneously enhanced-bulky mass lesion after

Table 1 Clinical characteristics of all reported cases

Author and Year	Age, sex	Symptom	Location	Surgical manner	Initial MR study	Initial radiol. diag.	Second MR study	MR study at diag.	Interval	Survival after diag.
Oyama et al. [2]	73, M	Rt. hemiparesis	Lt frontal	Removal	F, H on T2WI	Cerebral infarction	Enlargement of focal lesion	Homogeneous enhancing	1.25 months	12 months
Cohen-Gadol et al. [19]	40, M	Partial seizure	Rt frontal	Biopsy	F, H on T2WI NE	Neoplastic lesion		Ring-enhancing	4 months	NA
	68, M	General seizure	Rt medial temporal	NA	F, H on T2WI NE	Tumor or encephalitis		Ring-enhancing	4 months	NA
Okamoto et al. [20]	77, M	Vertigo	Rt frontal	Biopsy	ND	Diagnostic challenge		Multiple focal lesion, heterogeneous-enhancing	4 months	4 months
	38, F	Dysesthesia	Lt. parietal	Removal	F, H on T2WI NE	Demyelinating process	Small cyst no enhancing	Heterogeneous-enhancing	10 months	20 months
Landy et al. [21]	58, M	Seizure	NA	Removal	No neoplasm	NA		NA	8 months	NA
	40, F	Seizure	NA	Removal	No neoplasm	NA		NA	3.25 months	NA
	71, F	Seizure	NA	Removal	No neoplasm	NA		NA	2 months	NA
Ono et al. 2000	57, M	Loss of consciousness	Lt temporal-parietal	Removal	F, H on T2WI no enhancing	NA	Small enhancing	Ring-irregular enhancing	4 months	NA
Present series										
Case 1	63, F	No symptom	Lt occipital	Removal	F, H on T2WI no enhancing	LGG and diag. challenge	Small enhancing	Heterogeneous-enhancing	2.9 months	49 months (alive)
Case 2	82, F	Vertigo	Rt basal ganglia	Biopsy	F, H on T2WI slightly enhancing	LGG		Ring-enhancing	2.4 months	8.3 months
Case 3	63, F	Lt hemiparesis	Rt parietal	Removal	F, H on T2WI no enhancing	Venous thrombosis		Heterogeneous-enhancing	2.5 months	16 months
Case 4	70, M	Headache	Lt frontal	Removal	F, H on T2WI no enhancing	LGG		Ring-enhancing	5.9 months	13 months
Case 5	54, F	No symptom	Rt temporal	Removal	F, H on T2WI	Diag. challenge		Ring-irregular enhancing	3.4 months	5 months (alive)
Overall	63* F(50 %)	Seizure (36 %)	Rt. side (55 %) frontal (34 %)		F (100 %) no enhancing (89 %)			Ring-enhancing (55 %)	4.1 months	15.9 months

Interval between the initial MR study and the diagnosis, *NA* not available, *ND* not detectable, *NE* no enhancing, *F* focal lesion, *H* hyperintense, *LGG* low grade glioma, *Radiol.* radiological, *Diag.* diagnose

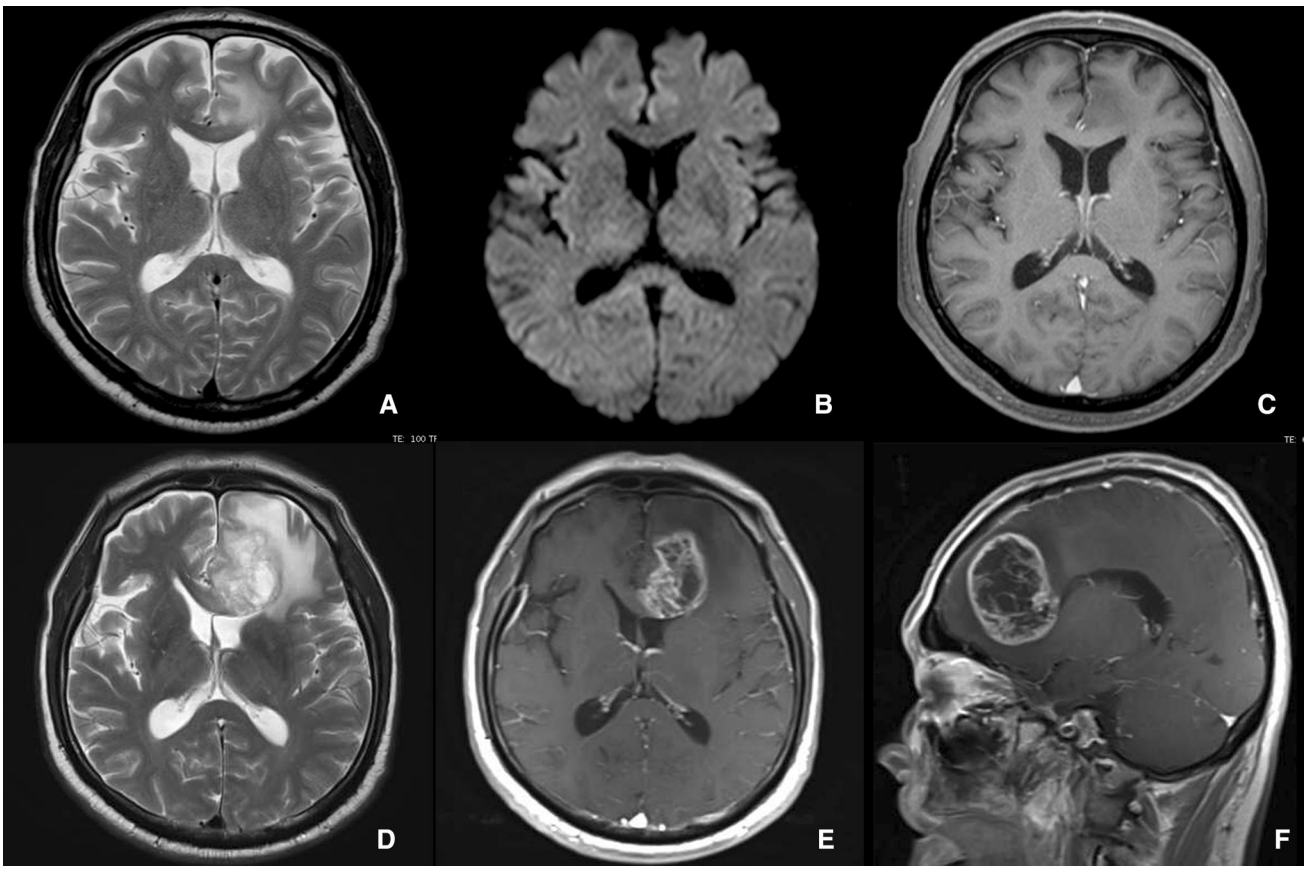

Fig. 1 Case 4. **a** Axial T2-weighted MRI showed a poorly demarcated hyperintense lesion in the left medial frontal lobe. **b** There was no abnormal signal intensity on a diffusion-weighted image. **c** There was no significant mass effect or associated enhancement on a contrast-enhanced T1-weighted image. **d** At 5.9 months follow-up, T2-weighted MRI showed a hyperintense mass lesion with a strong mass effect and peritumoral edema in the left medial frontal lobe. **e** A contrast-enhanced T1-weighted image showed a ring and heterogeneous enhanced mass lesion. **f** A coronal image with an extended mass lesion through the medial aspect

3.4 months (Fig. 2c, d). Another case (case 3) was misinterpreted as venous thrombosis with hemorrhagic infarction based on initial MRI and on angiography findings showing arteriovenous shunt with no sinus occlusion (data not shown). It was hard to distinguish a neoplasm from a vascular event based on these findings.

Four patients underwent gross total resection, and a biopsy in one case confirmed glioblastoma histopathologically. All patients received external beam radiotherapy (a total dose of 60 Gy in 30 fractions) and temozolomide chemotherapy, and median survival was 15.9 months (5–49 months). The localization of the tumor showed no particular tendency (Table 1). There was no significant difference by log-rank test in median survival compared to that of 13.1 months in a control group of patients among the 58 cases with typical MRI findings characterized by heterogeneous enhanced bulky mass lesions.

Immunohistochemical and molecular evaluation

Histological examination revealed glioblastoma with typical findings such as pseudopalisading necrosis formation and microvascular proliferation in all cases (Table 2). Typical histopathological findings of glioblastoma in representative case 5 are shown in Fig. 3a, b. GFAP, S-100 and EGFR immunostaining were positive in all 5 cases (Fig. 3d; Table 2). IDH1-R132H immunostaining and 1p19q LOH in FISH were negative in all 5 cases (Fig. 3c, h; Table 2). Two cases were p53-positive and three were p53-negative (Fig. 3e; Table 2). The average Ki-67 labeling index was 25.8 % (range 3–38 %; Fig. 3g; Table 2). These immunohistochemical results showing IDH1 wild type, EGFR overexpression, and normal 1p/19q status are consistent with a diagnosis of de novo type (primary) glioblastoma in all 5 cases [18].*Diag.*

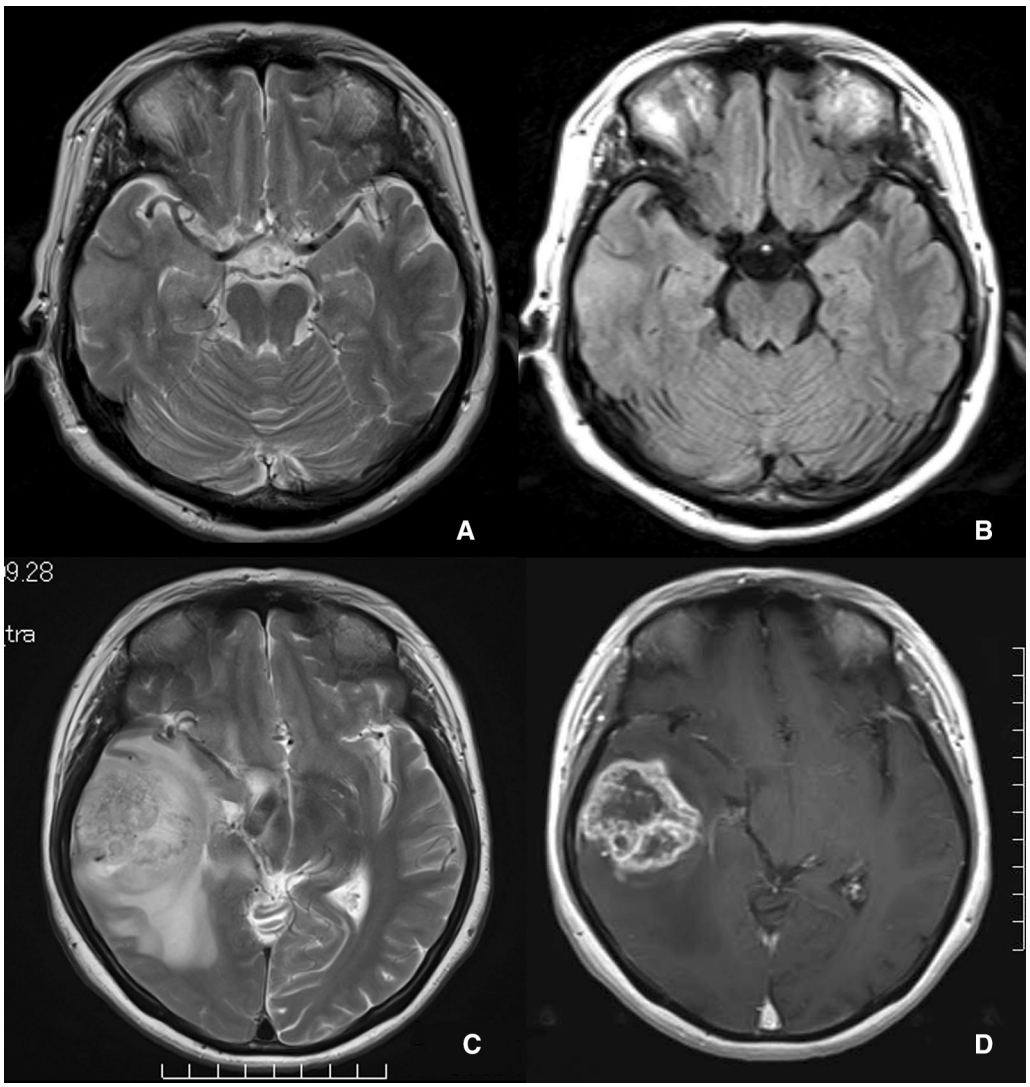

Fig. 2 Case 5. **a** Axial T2-weighted MRI showed no abnormal signal intensity. **b** An axial fluid-attenuated inversion recovery (FLAIR) image showed a very slightly hyperintense and poorly demarcated lesion without a mass effect in the right temporal lobe. **c** At 3.4 months after the initial scan, a T2-weighted image showed a hyperintense bulky mass lesion with severe peritumoral edema in the right temporal lobe. **d** A contrast enhanced T1-weighted image showed a strong contrast-enhanced mass lesion with uncal herniation

Discussion

In this retrospective study, we found that early-stage glioblastoma is characterized by MRI findings of T2WI hyperintense ill-defined small lesions, little or no mass effect, and no or subtle contrast enhancement. Within several months, these lesions develop typical MRI findings such as a heterogeneous enhanced bulky mass with central necrosis. In previous reports, the average period from the initial to final scan in diagnosis of glioblastoma has been 4.5 months (1.25–10 months) (Table 1) [2, 19–22]. At a population level, most patients with primary glioblastoma (68 %) had a clinical history of <3 months and the mean period was 6.3 months [5, 6]. These histories were similar to those in our cases. Thus, there is a need to interpret abnormal findings on MRI in early-stage glioblastoma, but it is hard to diagnose glioblastoma from initial MRI findings such as a T2WI small hyperintense lesion with little or no enhancement. If complete resection is achieved before a bulky mass lesion forms and prior to too much spread of tumor cells, some patients with glioblastoma may obtain an extended survival benefit.

Three of our cases were identified as low grade glioma from initial MRI findings, but others (including in previous reports) were not even identified as neoplasms, and instead were misinterpreted as conditions such as cerebral infarction, encephalitis, demyelinating process and venous thrombosis (Table 1). For example, although multiple sclerosis may be indistinguishable from early stage glioblastoma, a solitary ill-defined lesion (as seen in cases

Table 2 Summary of immunohistochemistery and molecular background

Author & Year	Age	Sex	Immunohistochemistery						FISH	Final diag.
			GFAP	S100	p53	IDH1R132H	EGFR	Ki-67 LI	1p19qLOH	
Oyama H et al. [2]	73	M	NA	NA	NA	NA	NA	26 %	NA	NA
Coehn-Gadol et al. [19]	40	M	NA	NA	Negative	NA	NA	35 %	NA	Primary
	68	M	NA	NA	Negative	NA	NA	NA	NA	Primary
Present series										
Case 1	63	F	+	+	Positive	–	55 %	30 %	–	Primary
Case 2	82	F	+	+	Negative	–	78 %	3 %	–	Primary
Case 3	63	F	+	+	Negative	–	65 %	30 %	–	Primary
Case 4	70	M	+	+	Negative	–	63 %	28 %	–	Primary
Case 5	54	F	+	+	Negative	–	81 %	38 %	–	Primary

NA not available, *Exp.* expression, diagnose, *LI* labeling index

2, 4 and 5 in this study) suggests a brain neoplasm, rather than multiple sclerosis [23–25]. Landy et al. reported 3 cases with MRI findings of no abnormal intensity on an initial scan in which a new bulky lesion with a typical enhancement effect was found on MRI 2–8 months later [21]. Initial MRI findings on T2WI in two of our cases (cases 1 and 5) were also too slight to be detected (Table 1). Thus, some cases of very early-stage glioblastoma might be particularly difficult to detect using MRI, even with careful interpretation. Evaluation of symptoms at the time of the initial MRI scan may be useful, since 5 (35.7 %) of 14 reported cases (including our cases) had seizure, 3 (21.4 %) had a focal sign such as hemiparesis and dysesthesia, and the remaining cases had indefinite complaints such as headache and vertigo, or no symptoms (Table 1). Thus, cases with seizure onset and abnormal MRI signal intensity might be suspected as early-stage glioblastoma.

Previous reports have shown that 14–45 % of non-enhancing gliomas are malignant and that about 4 % of glioblastoma cases lack contrast enhancement at initial presentation [1, 3, 26]. The risk for anaplasia in non-enhancing gliomas increases with age [27]. In other words, glioblastoma showing untypical MRI findings can be a challenging diagnosis. However, early diagnosis and initiation of treatment including gross total resection are likely to prolong progression-free and overall survival [28]. Thus, a new diagnostic modality is required to interpret these untypical signal lesions. As mentioned above, some lesions are hard to detect even on T2-weighted images and may be overlooked. Lesions including gliomas may be evaluated more effectively with FLAIR imaging (Fig. 2a, b) [29].

Baehring et al. found that diffusion-weighted imaging (DWI) was useful for diagnosis of malignant glioma with slightly enhanced lesions without central necrosis [30]. In all cases, DWI had increased intensity and apparent diffusion coefficient maps had low-signal intensity in corresponding areas, which indicates decreased water diffusion indicating high cellularity [28]. One of our cases (case 4) underwent DWI, but showed no abnormal signal intensity (Fig. 1b), and thus the utility of this modality is unclear for detecting early-stage glioblastoma. In such non-enhancing glioma, positron emission tomography (PET) findings that typically show hypermetabolism are often used as additional diagnostic information. F-18-fluorodeoxyglucose (FDG)-PET findings have a general correlation with the histopathological grade of high-grade malignant tumors [31–33]. Because uptake of L-methyl-^{11}methionine (^{11}C-methionine) is greater in glioma than in intact tissue, methionine PET may be useful for diagnosis of glioma [34].

The difficulty of early diagnosis using various imaging modalities emphasizes the continued importance of pathological diagnoses including biopsy or open surgery, which permit differential diagnoses including neoplasms or other diseases and low or high grade glioma. As mentioned above, most glioblastoma are the primary type, the median survival time of which is 14.6 months with radiotherapy plus temozolomide [35]. In contrast, secondary glioblastomas are less common (<10 %) and develop more slowly through progression from low-grade disease over an average of 5.3 years or from anaplastic astrocytoma over 1.4 years [36]. Thus, if early-stage primary glioblastoma is suspected, more aggressive treatment including gross total resection is required because the clinical course differs from that of non-enhanced low-grade glioma. Genetic background can also be used to distinguish primary and secondary glioblastoma since the diseases develop through different genetic pathways [5, 6], despite being indistinguishable histologically. IDH1 mutations have recently been identified as a very early and frequent genetic

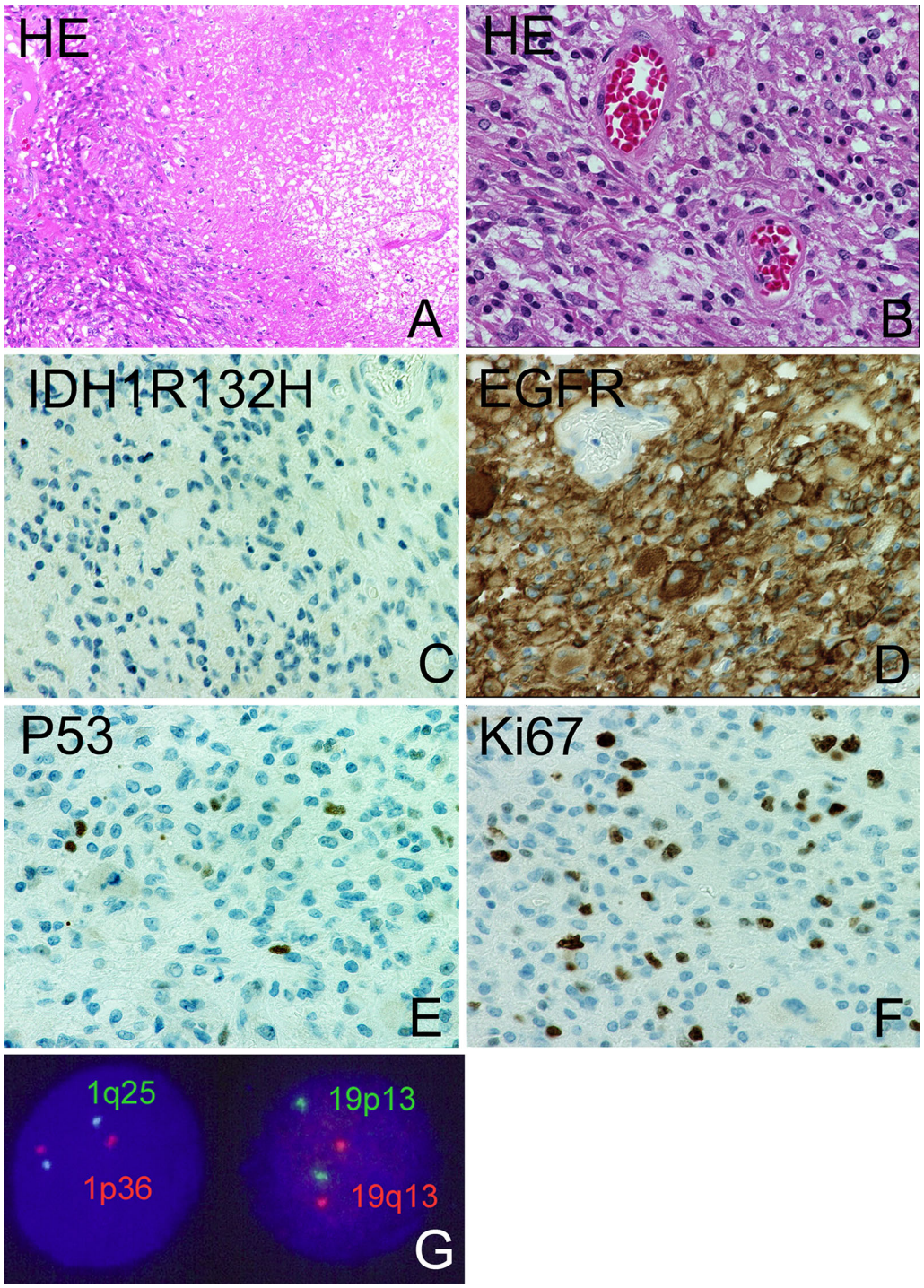

Fig. 3 Photomicrographs showing histological findings of tumor tissue in case 5. **a** Hematoxylin and eosin staining showed proliferation of atypical cells with irregular cytoplasm and chromatin-condensed heterogeneous nuclei, and necrotic changes. **b** Microvascular proliferation was marked among the tumor cells. **c** IDH1-R132H immunostaining showed no reactive cells. **d** EGFR immunostaining resulted in a large number of labeled cells. **e** A small number of P53-positive cells were present. **f** 38 % of total cells were Ki-67-positive. **a** ×200, **b**–**f** ×600. **g** FISH showed no 1p19q loss of heterozygosity

alteration in the pathway to secondary glioblastomas, as well as in oligodendroglial tumors [9–11]; in contrast, primary glioblastomas very rarely contain IDH1 mutations [9–11]. Use of a combination of these genetic changes permitted diagnosis of primary glioblastoma in all of our cases (Table 2).

In conclusion, MRI findings in developing early-stage glioblastoma include a small ill-defined T2WI hyperintense lesion, with little or no mass effect and poor contrast enhancement. These findings can develop into a bulky mass lesion with typical enhancement of glioblastoma within several months. In the current study, cases showing these findings were diagnosed as primary glioblastoma clinically and pathologically. As discussed above, it is difficult to distinguish early-stage glioblastoma from non-neoplastic diseases such as ischemic, degenerative or demyelinating processes using MRI. Therefore, definite diagnosis is important in cases with suspected early-stage glioblastoma, using a proactive approach with short-interval MRI scans over several weeks, PET scans, and biopsy or resection. This is particularly important because of the extremely poor prognosis, especially for de novo type glioblastoma. This is particularly important because of the extremely poor prognosis, especially for de novo type glioblastoma. This approach does provide a survival benefit based on the increased potential for total resection when the lesion is still small.

Conflict of interest The authors declare that they have no conflict of interest.

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