Title:

Expression of phosphoprotein enriched in astrocytes - 15kDa (PEA-15) in astrocytic tumors: a novel approach of correlating Malignancy Grade and Prognosis

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Key words:
Phosphoprotein-enriched in astrocytes -15kDa,
Astrocytic tumor,
Malignancy grade,
Prognosis,
Immunohistochemistry.

Abstract

Phosphoprotein enriched in astrocytes - 15kDa (PEA-15) is a multifunctional protein that was first identified in brain astrocytes and has subsequently been shown to be expressed in different tissues. Despite many important roles, the clinical significance of PEA-15 expression levels in astrocytic tumors has yet to be properly defined. We studied the PEA-15 expression pattern of 65 patients (diagnosed according to WHO criteria) with diffuse astrocytoma (WHO grade II), anaplastic astrocytoma (grade III) and glioblastoma (grade IV). PEA-15 expression levels were immunohistochemically measured and categorized as no, low or high expression. All tumors expressed PEA-15 in our study. 23 (35.4%) and 42 (64.6%) tumors expressed low and high PEA-15 levels, respectively. In grade II astrocytoma (diffuse astrocytoma) and grade III astrocytoma (anaplastic astrocytoma), 100% and 88.9% of patients expressed high PEA-15 levels respectively, while a smaller number (50%) of patients with grade IV astrocytoma (glioblastoma) expressed high PEA-15 levels. The PEA-15 expression levels were inversely associated with the WHO grades (P = 0.0006). Next, we evaluated the prognosis and PEA-15 expression levels in 43 patients with high-grade astrocytomas based on the following parameters: age, gender, WHO grade, surgical resection extent, MIB-1 labeling index and PEA-15 expression level. Multivariable analyses revealed that high PEA-15 expression levels displayed a significant correlation with longer overall survival (OS) in high-grade astrocytomas (P = 0.0024). Patients with total resection survived significantly longer (P = 0.0044) than those with a lower resection extent, while patients with an MIB-1 labeling index of $\leqq$ 25% indicated significant (P = 0.0434) correlation with OS as well. In conclusion, the PEA-15 expression level was inversely associated with the WHO grades, and may serve as an important prognostic factor for high-grade astrocytomas.

Introduction

Astrocytic tumors are the most malignant brain tumors. Despite multimodal treatment strategies that combine surgery, radiotherapy, and adjuvant chemotherapy, high-grade astrocytomas yield poor prognosis with a median postoperative survival time not exceeding 12 months [1, 2]. Prognosis prediction of patients with high-grade astrocytomas is critical in selecting and evaluating the effectiveness of treatment. However, prognosis prediction based on findings by light microscopy alone is controversial, as the lack of direct knowledge about the biological behavior of high-grade astrocytomas remains an issue [1, 3].

Phosphoprotein enriched in astrocytes - 15kDa (PEA-15) is a small protein that was first identified in astrocytes and subsequently shown to be extensively expressed in different tissues [4]. While the C-terminal of phosphoprotein contains two serine residues (Ser104 and Ser116), which can be phosphorylated by protein kinase C, calcium/calmodulin-dependent protein kinase II and Akt, the N-terminal contains a death-effector domain (DED) [4-6]. PEA-15 has diverse functions within the cells. Since PEA-15 contains a DED, it regulates apoptosis by competitively inhibits the binding of DED-containing proteins to initiator caspases [7, 8]. Apart from its apoptosis-related effects, PEA-15 is a potent modulator of the mitogen-activated protein kinase (MAPK)-signaling cascades. PEA-15 binds to and sequesters extracellular regulated kinase (ERK) 1/2 in the cytoplasm, thereby preventing its nuclear translocation [9-11]. It blocks subsequent phosphorylation of nuclear targets and ERK-dependent functions such as proliferation, survival, adhesion and migration of cells [12]. In addition, PEA-15 plays an important role in glucose metabolism and its overexpression contributes to development of diabetes mellitus [13].

PEA-15 is a multifunctional protein known to regulate apoptosis, proliferation, adhesion, migration and glucose transport in cells; however, the role of PEA-15 in cancers, especially astrocytic tumors, remains unresolved. There are contradicting data with regard to the relationships between PEA-15 expression levels and tumor growth: on one hand, increased PEA-15 levels inhibit apoptosis in non-small cell lung cancer [14], B cell chronic lymphocytic leukemia [15] and thyroid cancer [16] (where PEA-15 may have enhanced tumor growth by inhibiting apoptosis); but on the other hand, elevated PEA-15 levels inhibit ERK-dependent transcription and proliferation in

ovarian [17] and breast [18] cancers. To date, the only report relating PEA-15 expression to clinical outcome has been performed in patients with ovarian cancer; and the results show that high PEA-15 protein expression levels are independent of improved overall survival (OS) time in ovarian cancer patients [17].

In astrocytic tumors, PEA-15 suppresses apoptosis [19, 20] by competitively inhibiting the binding of DED-containing proteins to initiator caspases and prevents glucose-induced cell death via the ERK pathway [21]; phenomenal events similarly observed with PEA-15 in other cancers. In astrocytes, PEA-15 prevents cell migration through a PKC delta-dependent pathway [22]. PEA-15 was known to express in astrocytic tumors, but the relationship between malignancy grades in astrocytic tumors and PEA-15 expression levels is controversial [21, 23]. Moreover, the relationship between PEA-15 expression levels and prognosis in astrocytic tumors remains undefined to date. In this study, we examined the expression pattern of PEA-15 in astrocytic tumors, and analyzed the relationship between PEA-15 expression levels and clinical factors/outcome. Our findings confirmed that PEA-15 expression was inversely associated with the malignancy grade of astrocytic tumors, and this is the first study that demonstrated decreased PEA-15 expression levels were correlated with poor OS in patient with high-grade astrocytomas.

Materials and methods

Clinical data and patient selection

This study was approved by our institutional review board. Based on the World Health Organization (WHO) grading system, we conducted a retrospective study of 65 patients with newly diagnosed primary adult supratentorial diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III) and glioblastoma (grade IV). These patients underwent surgical interventions at Hiroshima University Hospital, Japan, between January 1998 and March 2008. Gliomas other than those mentioned above, such as oligodendroglial tumors (including oligoastrocytoma, oligodendroglioma, anaplastic oligoastrocytoma and anaplastic oligodendroglioma), ependymal tumors (including ependymoma and anaplastic ependymoma), ganglioglioma and pleomorphic

xantastrocytoma), were excluded from the study, because these tumors are pathologically and clinically distinctly different from astrocytic tumors. We also excluded brainstem astrocytic tumors, cerebellar astrocytic tumors, gliomatosis cerebri, and recurrent tumors from the study.

We evaluated the prognostic factor in high-grade (III, IV) astrocytomas to monitor the clinical outcome. Patients with diffuse astrocytoma were omitted from the prognostic study, because those with grade II tumors intrinsically indicated better prognosis than those with high-grade gliomas. Based on our institutional treatment protocol, 10 patients without postoperative radiotherapy were excluded from the prognostic study. According to the protocol, patients with high-grade astrocytomas were treated with radiotherapy (extended focal 60Gy irradiation) and administered intravenously with 1-(4-amino-2-methyl-5-pyrimidinyl) methyl-3-(2-chloroethy) -3-nytrosoureal hydrochloride (ACNU) until 2006. After 2007, patients with high-grade astrocytomas were treated with radiotherapy and oral temozolomide (TMZ).

Therefore, we could retrospectively study 43 patients with anaplastic astrocytoma and glioblastoma to evaluate the prognostic factor in high-grade astrocytomas. The follow-up data of relevant patients were retrieved from hospital records.

Tissue specimens and immunohistochemical staining

All surgically resected tumor specimens were fixed in 10% phosphate-buffered formalin before embedding in paraffin. Representative slides were stained with the hematoxylin-eosin reagent for standard histological diagnosis. Tumors were histologically classified and graded into subtypes by two authors (T.S. and T.N.) according to the WHO criteria. Paraffin-embedded tumor specimens were used for IHC staining. Diluted mouse monoclonal (dilution; 1:50) antibodies (antibody MIB-1; Immunotech, Marseille, France) for Ki-67 and rabbit polyclonal (dilution; 1:100) antibodies for PEA-15 (antibody PEA-15; Synpep, Dubin, CA) [17] were employed as primary antibodies. Pathological specimens (thickness: 4 μm) were mounted on gelatin-coated slides and deparaffinized by 15-min xylene treatment. To prevent digestion by endogenous peroxidase, the slides were immersed for 30 min in a hydrogen peroxidase (3%)/methanol mixture. Each specimen was rinsed 3 times for a total of 15 min in phosphate-buffered saline (PBS; pH 7.5) with gentle stirring before overnight

incubation with the primary antibodies at 4°C. The streptavidin-biotin procedure was next performed using the histofine SAB (R) kit (Nichirei Co., Tokyo, Japan). After washing thoroughly in PBS, the sectioned specimens were exposed to tetrahydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) in hydrogen peroxidase (0.003%)-treated PBS (pH 7.6) for 7 min. To facilitate visualization of cytoplasmic immunostaining, the slides were counterstained with Mayer hematoxylin. Each series of experiments with controls was performed at least twice on different days. We used the normal appearance of brain tissues and meningiomas as positive and negative controls, respectively.

Author FY, who had no knowledge of the pathologic diagnosis or any clinical or radiological data, determined the MIB-1 labeling index by counting the nuclei of 1,000 tumor cells. We selected the median value of the MIB-1 labeling index as the cut-off point, and classified the patients into 2 groups: i.e. ≦ median value group and > median value group.

Evaluation of PEA-15 expression

The specimens were scored by 2 other authors (K.S. and Y.K.), who had no knowledge of the pathological diagnosis or any clinical or radiological data. The count of positively identified tumor cells (%) was recorded for each specimen. Grading of PEA-15 expression levels was performed as follows: no or negative (no cells were stained); low (up to 50% of cells were stained); and high (50-100% of cells were stained).

Statistical analysis

Statistical analyses were performed using the Windows SPSS 16.0 software package (SPSS INC., Chicago, IL). Correlations between the PEA-15 expression levels and clinicopathological characteristics (including factors: age, gender, resection extent and MIB-1 labeling index) were compared using the chi-square test and Mann-Whitney U test. OS was derived by the Kaplan-Meier method from the date of surgery. To evaluate the prognostic value, multivariate survival analysis was performed using the Cox proportional hazards regression model, incorporating the following clinicopathological factors: age, gender, resection extent, WHO grade, MIB-1 labeling index and PEA-15

expression level. In all analyses, differences with $P < 0.05$ were considered statistically significant.

Results

Clinical characteristics and MIB-1 labeling index of grade II – IV astrocytic tumors

Of the 65 tumors, 12 were diffuse astrocytoma (grade II), 9 were anaplastic astrocytoma (grade III) and 44 were glioblastoma (grade IV). The female (n = 25): male (n = 40) ratio was 1: 1.6. The median age of 65 patients was 56 (range: 21-80 with standard deviation (SD) 16.7; average: 52.5) years. The median age (in years) of patients with grade II astrocytomas was 43.5 (range: 23-69 with SD 12.4; average: 43.8), while those of anaplastic astrocytomas and glioblastomas were 30 (range: 23-64 with SD 13.1; average: 35.3 and 62 (range: 21-80 with SD 14.7; average: 58.6) years, respectively. The median of MIB-1 labeling index (%) of grade II astrocytomas registered 1.05 (range: 0.5-1.7 with SD 0.58; average: 1.15), while those of anaplastic astrocytoma and glioblastoma were 7.9 (range: 3.8-62.3 with SD 18.0; average: 18.0) and 30.0 (range: 4.0-60.0 with SD 15.0; average: 28.7), respectively. The MIB-1 labeling index showed significant differences between grade II and III ($P = 0.00001$) and between grades III and grade IV ($P = 0.037$) by the Mann-Whitney U test.

PEA-15 expression and WHO grade of astrocytic tumors

We used normal intact brain tissues obtained from an epileptic patient by temporal lobectomy as the positive controls because normal astrocytes express PEA-15. We also used meningiomas for negative control because these tumors were not stained by PEA-15. In normal brain tissues, PEA-15 was stained in the cytoplasm with perinuclear and nuclear distribution. In our study, all astrocytic tumors expressed either low or high PEA-15 expression. Of these astrocytic tumors, 23 (35.4%) and 42 (64.6%) tumors expressed low (Fig. 1) and high (Fig. 2, 3, 4) PEA-15 levels, respectively, and PEA-15 was stained in the cytoplasm with perinuclear and nuclear distribution like normal brain tissues.

We examined the relationship between PEA-15 expression and the WHO grades based on the result of immunostaining studies. In diffuse astrocytoma (Fig. 2) and anaplastic astrocytoma (Fig. 3), 100% and 88.9% of all patients expressed high PEA-15 levels, respectively. In glioblastoma, 22 (50%) and 22 patients (50%) expressed low (Fig. 1) and high (Fig. 4) PEA-15 levels, respectively. The numbers of patients with high PEA-15 expression levels registered significant differences between grade II, III and IV by the Fisher's exact test (P = 0.0006). The PEA-15 expression level was inversely correlated with the WHO grade in astrocytic tumors (Fig. 5).

PEA-15 expression and clinicopathological factors of high-grade astrocytic tumors

We evaluated the relationships between the PEA-15 expression levels and certain relevant clinicopathological factors in high-grade astrocytomas such as the gender, age, and MIB-1 labeling index.

PEA-15 expression correlated with neither the gender (P = 0.46) when verified by the chi-square test nor the age (P = 0.19) and MIB-1 labeling index (P = 0.41) when compared by the Mann-Whitney U test. In fact, none of the clinicopathological factors in high-grade astrocytomas were associated with the PEA-15 expression level.

Prognostic factors of high-grade astrocytoma

Next, we evaluated the prognostic factor in 43 patients with high-grade astrocytomas consisting grade III astrocytoma (anaplastic astrocytoma) and grade IV astrocytoma (glioblastoma). The median overall survival of grade III astrocytomas was 1151 (range: 626-2052) days, while that of grade IV astrocytomas was 537 (range: 93-3548) days. The characteristics of patients (including the age, gender, resection extent, MIB-1 labeling index, histopathological diagnosis and PEA-15 expression levels) are summarized in Table 1. Of the 43 patients enrolled in this study, the female: male ratio was 1: 1.9. All patients received postoperative radiotherapy based on our institutional protocol. Patients were treated with TMZ (n = 9) or ACNU-based (n = 29) chemotherapy (see Materials and methods for details), while 5 cases did not receive chemotherapy. There is no difference in the clinical outcome with relation to post-operative chemotherapy when the verified with the Kaplan-Meier method. Of the

43 cases with high-grade astrocytoma, 19 (41.2%) and 24 (58.8%) tumors expressed low and high PEA-15 expression levels, respectively (Table 1). Patients with grade III astrocytomas expressed high (n=6) and low (n=1) PEA-15 levels, while those with grade IV astrocytomas expressed high (n=18) and low (n=18) PEA-15 levels.

We conducted a multivariable analysis with the Cox proportional hazards model to assess the independent predictive value of PEA-15 expression along with other prognostic factors such as the age, gender, resection extent, WHO grade and MIB-1 labeling index in patients with high-grade astrocytomas. We selected the median value of the MIB-1 labeling index as the cut-off point, and classified the patients into 2 groups accordingly: patients with MIB-1 labeling index of either $\leqq$ 25% or > 25%. Based on our results, patients with high PEA-15 expressing tumors survived significantly (P = 0.0024) longer than those with low PEA-15 expressing tumors. The Kaplan-Meier overall survival curves with respect to the PEA-15 status also showed that the group with high PEA-15 expression had significantly (P = 0.0034, log-rank test) longer OS than that with low PEA-15 expression (Fig. 6). In a similar tendency, patients with total resection survived significantly (P = 0.0044) longer than the patient without total resection, and those with an MIB-1 labeling index $\leqq$ 25% significantly (P = 0.0434) correlated well with OS. Other factors such as the age, gender and WHO grade were not correlated with prognosis (Table 2).

Moreover, we analyzed the relationships between PEA-15 expression and OS of grade IV astrocytomas (glioblastomas). High PEA-15 expression levels were statistically ($P < 0.05$, log-rank test) associated with good prognosis of glioblastoma (data not shown).

Discussion

This study is novel because PEA-15 may provide a new approach for predicting prognosis of patients with high-grade astrocytomas, a method which has conventionally relied on the WHO classification. Previous studies of astrocytic tumors have not investigated PEA-15 as a possible prognostic factor; however, the present study not

only focused on the importance of PEA-15 expression as a prognosis predictor, it also confirmed that PEA-15 expression was inversely correlated with the WHO grading of astrocytic tumors. Previous findings on PEA-15 expression and WHO grading of astrocytic tumors have been controversial [21, 23]. Petalidis et al. [23] have demonstrated that PEA-15 expression is inversely associated with WHO grade when comparing diffuse astrocytoma - glioblastomas and diffuse astrocytoma - anaplastic astrocytomas. However, Eckert et al. [21] have revealed intense PEA-15 expression in diffuse astrocytomas, anaplastic astrocytomas and glioblastomas. In this study, we showed that PEA-15 expression is inversely associated with histological grade. Apart from the fact that PEA-15 regulates the nuclear localization of ERK 1/2 and, consequently, transcription of ERK-dependent targets and cell proliferation in normal astrocytes [9], it blocks ERK-dependent functions in astrocytic tumors as well [21]. These studies imply that the inverse association between PEA-15 expression and the WHO grade may depend on the ERK-dependent function. While activation of ERK 1/2 is observed in cancers [12], positive immunostaining ERK 1/2 is manifested in approximately 90% of glioblastomas (i.e. displaying mainly nuclear immunolocalization) [24]. Furthermore, phosphorylated-ERK (p-ERK)-positive tumor nuclei are significantly associated with lower OS [24]. These results suggest that nuclear translocation of p-ERK may be increased by degradation of PEA-15 expression, and sequentially results in poor prognosis. Therefore, PEA-15 could functionally be considered as an anti-oncogene. In fact, another study on PEA-15 and astrocyte migration [22] also indicates PEA-15 functions as an anti-oncogene in astrocytes. In their study, loss of PEA-15 expression results in increased astrocyte migration, probably via a PKC delta-dependent mechanism [22].

Clinical studies of PEA-15 and malignant conditions support the fact that PEA-15 functionally serves as anti-oncogene. In breast [18] and ovarian [17] cancers, PEA-15 binds to and sequesters ERK 1/2 in the cytoplasm, thereby inhibiting ERK-dependent transcription and proliferation. Moreover, PEA-15 expression is inversely correlated with the invasive behavior of tumor cells by preventing ERK-signaling of integrins in breast cancer [18]. In a previous immunohistochemical study investigating the effect of PEA-15 expression on OS in 395 women with primary ovarian cancer, patients with high PEA-15 expression survived significantly ($P < 0.05$) longer than those with low PEA-15 expression [17]. These results coincide well with our present results.

Furthermore, their results show that the presence of PEA-15 expression in ovarian cancer cells inhibits tumor cell proliferation and induces autophagy as well as upregulation of PEA-15 expression to prevent nuclear translocation of ERK 1/2 and subsequent ERK-stimulated transcription and proliferation.

PEA-15 not only prevents proliferation, adhesion and migration of cells, but this multifunctional protein also regulates apoptosis and glucose transport. Increased PEA-15 levels inhibit apoptosis in B cell chronic lymphocytic leukemia [15] and thyroid cancer [16]. In these tumors, PEA-15 inhibits Fas, tumor necrosis factor-α (TNF-α) and TNF-related apoptosis-inducing ligand (TRAIL)-activated apoptosis by localizing the death-inducing signaling complex (DISC) via binding to the Fas-associated death domain protein (FADD), thereby preventing recruitment of death-initiating caspase-8. These in-vitro studies suggest that PEA-15 may enhance tumor growth by preventing apoptosis in some cancer cell lines. In a mouse model for chemically induced skin carcinogenesis, PEA-15 has also been shown to promote skin tumors and is overexpressed in transformed and metastatic murine squamous carcinoma cells, suggesting that PEA-15 probably displays both anti-apoptotic and tumorigenic functions [25]. However, findings on the association of PEA-15 expression with either malignancy grade of cancers or poor prognosis have yet to be documented in a clinical setting. PEA-15 expression in non-small cell lung cancers has previously been implied in a clinical setting [14]. However, detailed analysis of TNM-staging revealed that PEA-15 expression is more intensive during early (T1 and N0) than subsequent (T2 and N1 lesions) stages of the disease [14], implying that PEA-15 was not functioning as an oncogene, probably because decreased PEA-15 expression in high-grade tumors.

Certain differences in PEA-15 expression in malignant tumors could be due to the phosphorylation status of PEA-15, which may be important in determining whether PEA-15 regulates cell proliferation or apoptosis [10]. PEA-15 is phosphorylated at position Ser104 by protein kinase C and Ser116 by calcium/calmodulin-dependent protein kinase II (CaMK2) and Akt, thus producing three isoforms: non-phosphorylated, monophosphorylated, and diphosphorylated forms of the PEA-15 protein. Non-phosphorylated PEA15 tends to block cellular proliferation by inhibiting the nuclear translocaton of p-ERK1/2, whereas phosphorylated PEA-15 has lost the ability to bind ERK1/2, followed by the initiation of antiapoptotic and pro-survival transcriptional signaling [4-6]. Furthermore, Renganathan et al. [26] have demonstrated

that phosphorylation at Ser104 triggers inhibition of ERK binding to PEA-15 and facilitates ERK translocation to the nucleus, while phosphorylation at Ser116 promotes PEA-15 binding to FADD to subsequently inhibit apoptosis. Further studies on phosphorylation of PEA-15 are warranted to clarify the role of PEA-15 in cancers.

All in all, this study has the following limitations and future strategies: (i) the sample-size comprised only 43 patients with high-grade astrocytic tumors, and the study therefore warrants further confirmatory investigations with larger sample-size patient populations; (ii) post-operative chemotherapy was varied, and the relation between PEA15 expression and outcomes with a common treatment modality for brain tumor needs to be explored; (iii) the phosphorylation status of PEA-15 in the enrolled high-grade astrocytoma patients was unknown (PEA-15 expression may depend on phosphorylation status), and thus the mechanism will be better clarified with an identified phosphorylation status; (iv) only the PEA-15 expression levels and MIB-1 labeling index were examined, and other relevant molecules (such as p16, p53, ERK, epidermal growth factor receptor etc.) which may serve as important prognostic factors in astrocytic tumors should be measured; (v) ERK localization was not examined (although we have reviewed these molecules sequentially), future investigations should incorporate this cellular event; (vi) PEA-15 could functionally be considered as an anti-oncogene (albeit the function of PEA-15 is not fully resolved), and therefore examining siRNA of PEA-15 with respect to the phosphorylation site may help monitor malignant transformation in malignant gliomas; and (vii) this study was based on a retrospective design, and therefore a prospective study is essential to confirm our present findings.

In conclusion, we have demonstrated that the PEA-15 expression level is inversely correlated with the WHO grading of astrocytic tumors. Low PEA-15 expression is associated with a poor prognosis in patients with high-grade astrocytomas. Our findings may provide a foundation for predicting clinical outcomes in patients with high-grade astrocytomas, and encourage possible incorporation of new therapeutic options in the treatment strategy.

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Figure legends

Fig. 1
a) MRI, T2 weighted image
b) MRI, T1 weighted gadolininum-enhanced images
c) Hematoxylin-eosin (H&E) staining (original magnification: x 100)
d) Immunohistochemical staining of PEA-15 (original magnification: x 200)

A 47-year-old male with glioblastoma in the left frontal lobe indicated low PEA-15 expression with immunohistochemical staining. The tumor was totally removed. Thirteen months later, he died for the local recurrence of tumor.

Fig. 2
a) Hematoxylin-eosin (H&E) staining (original magnification: x 200)
b) Immunohistochemical staining of PEA-15 (original magnification: x 200)

A 46-year-old female with diffuse astrocytoma indicated high PEA-15 expression by immunohistochemical staining.

Fig. 3
a) Hematoxylin-eosin (H&E) staining (original magnification: x 200)
b) Immunohistochemical staining of PEA-15 (original magnification: x 200)

A 30-year-old female with anaplastic astrocytoma indicated high PEA-15 expression by immunohistochemical staining.

Fig. 4
a) MRI, T2 weighted image
b) MRI, T1 weighted gadolininum-enhanced images
c) Hematoxylin-eosin (H&E) staining (original magnification: x 100)
d) Immunohistochemical staining of PEA-15 (original magnification: x 200)

A 67-year-old male with glioblastoma in the left frontal lobe indicated high PEA-15

expression by immunohistochemical staining. Tumor recurrence has not been observed in this case till now (for 6 years after surgery).

Fig. 5

PEA-15 expression in diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III) and glioblastoma (grade IV). Bar graphs show the numbers of cases with high and low PEA-15 expression levels, while the linear plot shows cases with high PEA-15 expression levels (%).

Fig. 6

Kaplan Meier survival curves showing the correlations between PEA-15 expression and survival ($P = 0.0034$)

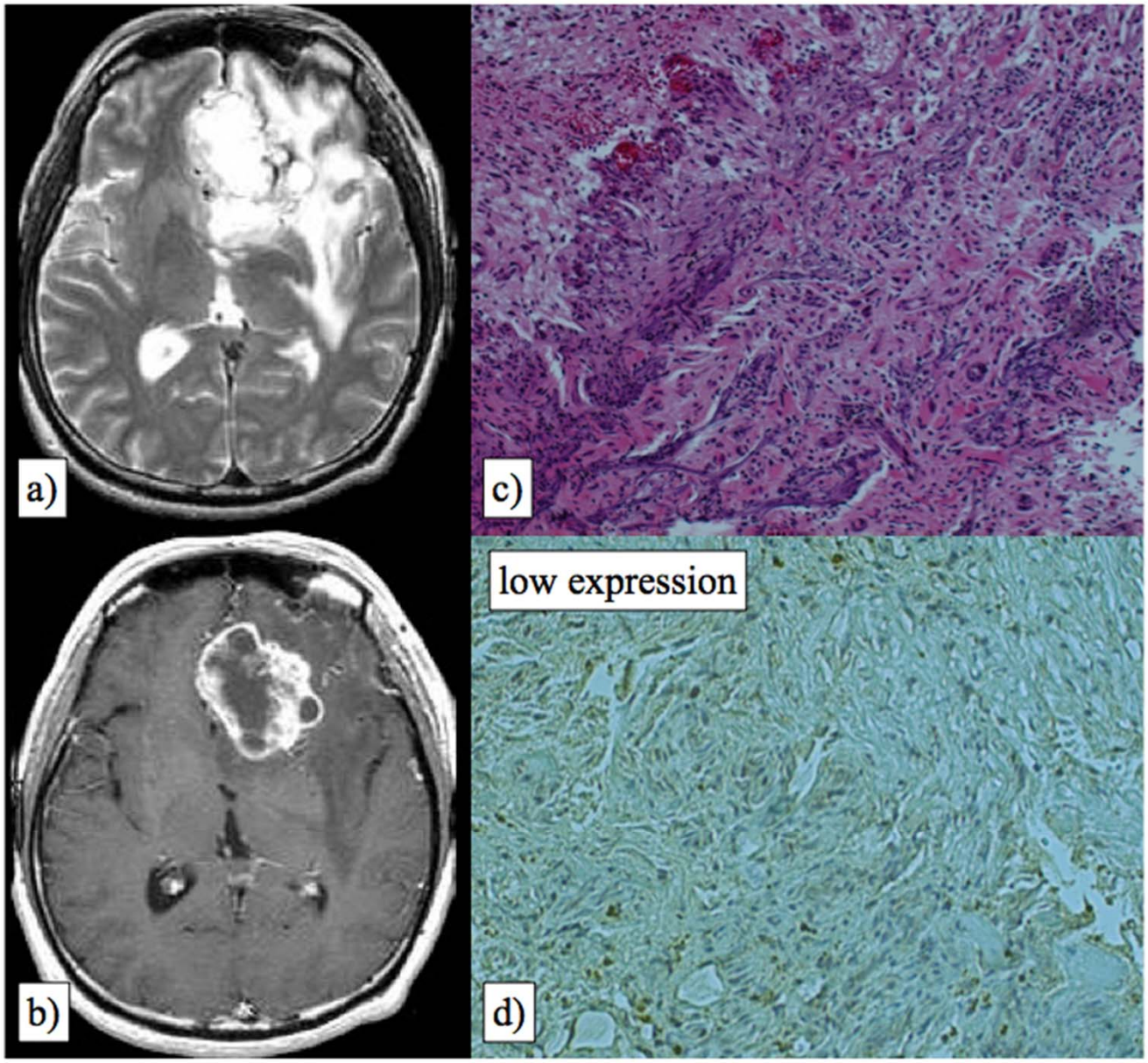

Fig. 1

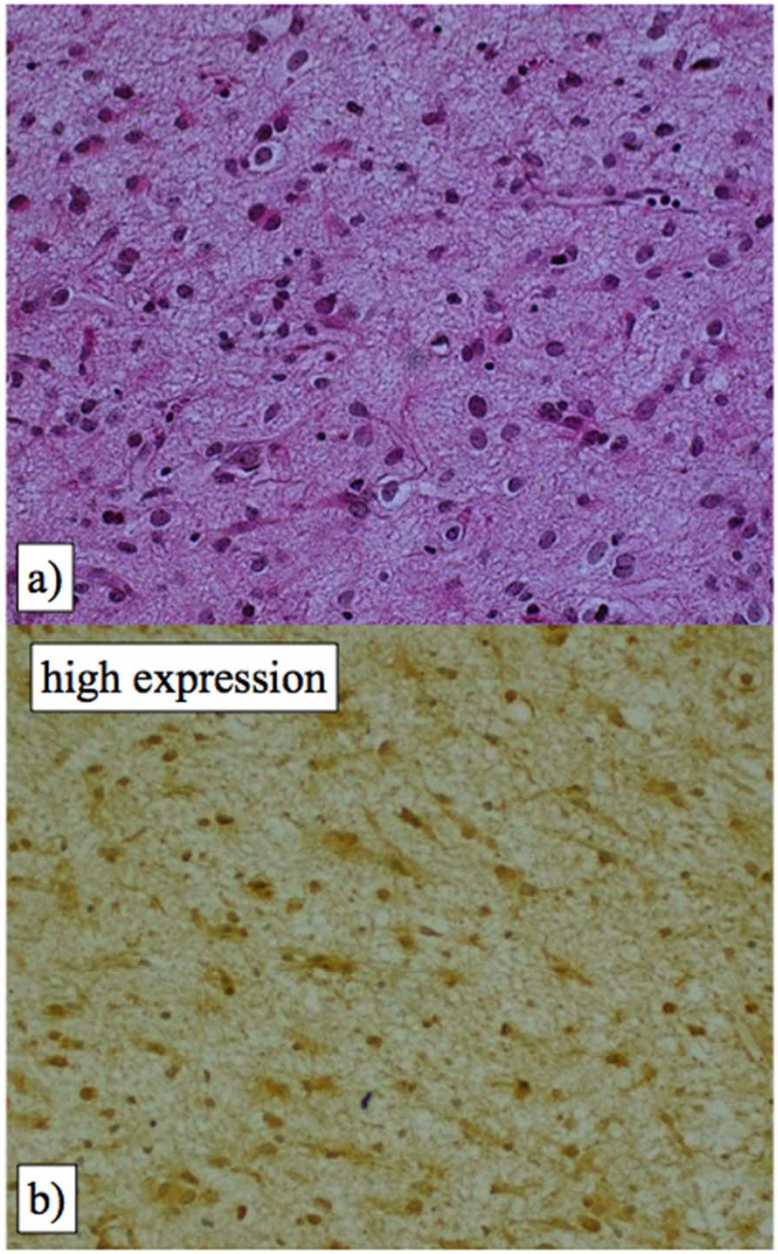

Fig. 2

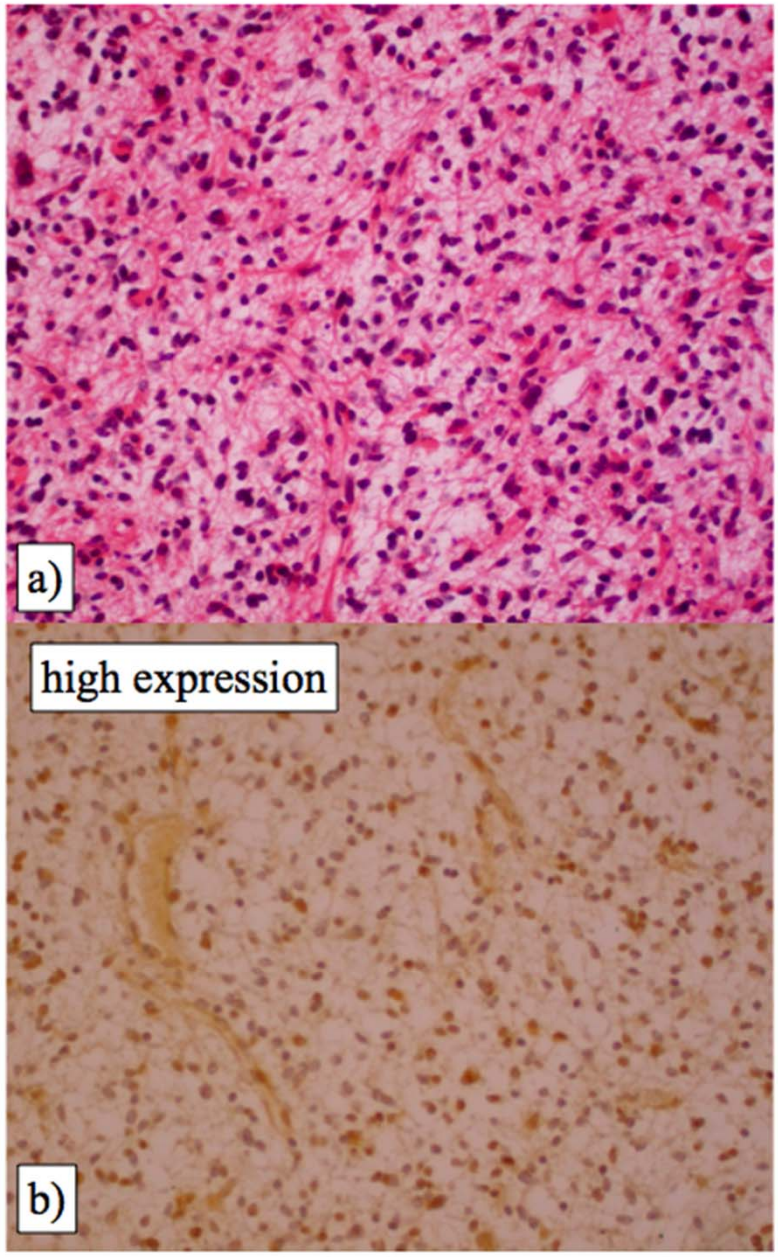

Fig. 3

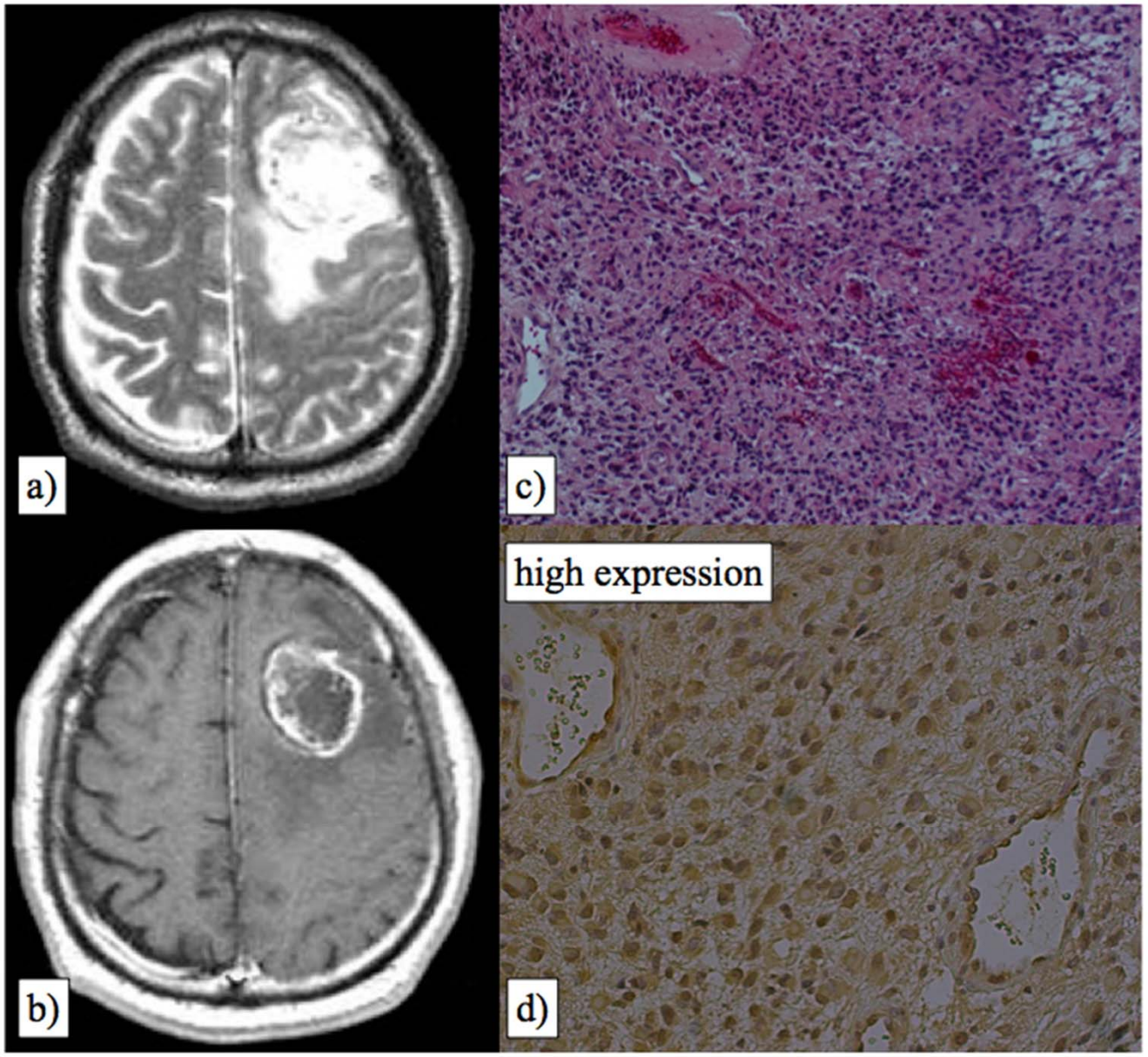

Fig. 4

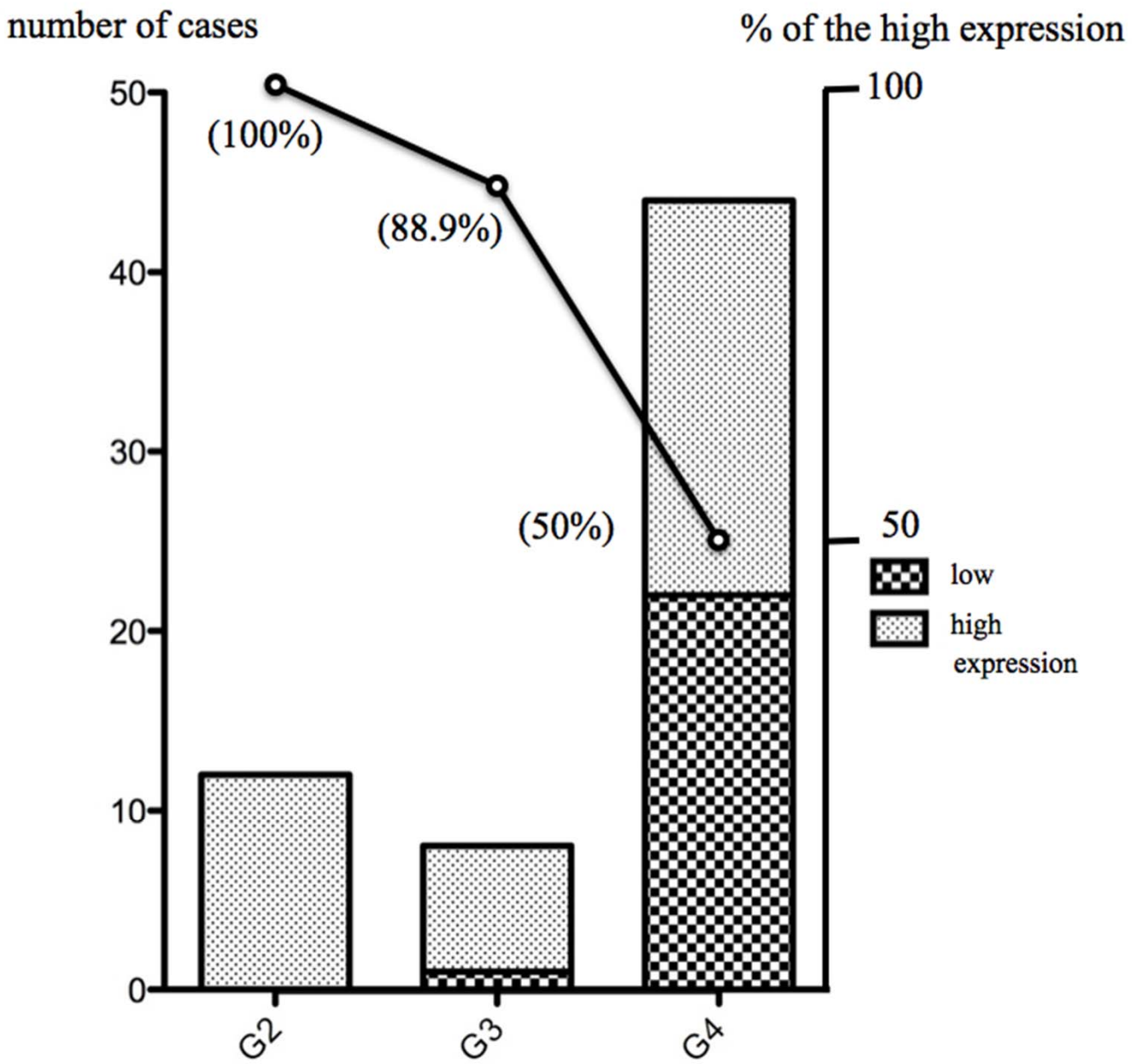
number of cases
% of the high expression
50
40
30
20
10
0
100
50
(100%)
(88.9%)
(50%)
low
high expression
G2
G3
G4

Fig. 5

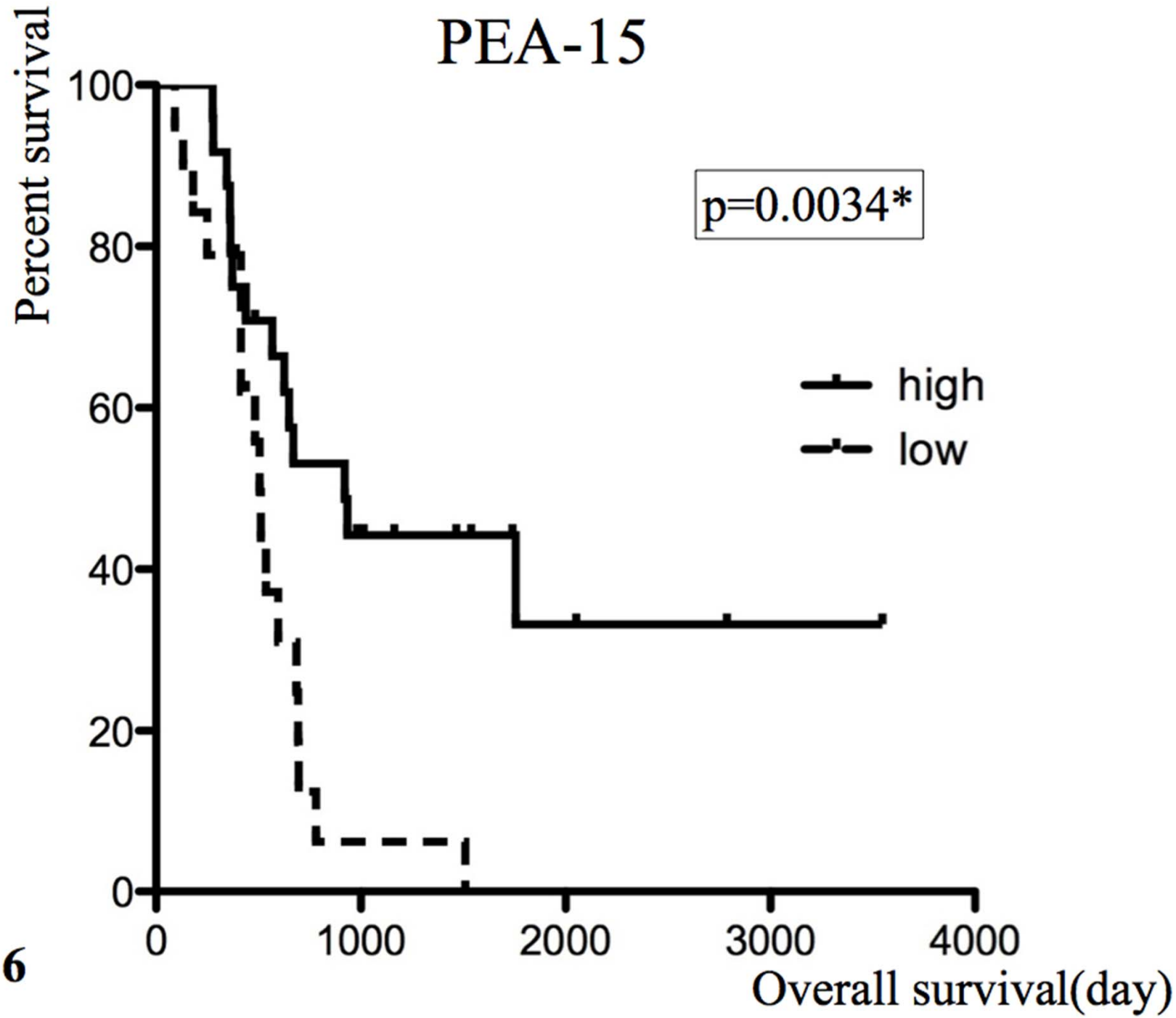
PEA-15
Percent survival
100
80
60
40
20
0
p=0.0034*
high
low
0
1000
2000
3000
4000
Overall survival(day)

Fig. 6

Table. 1 Clinical characteristics of 43 patients with high-grade astrocytic tumors.

Characteristic	No. of patients(%)
Age (yrs)	
Range	21 - 76
Mean ± SD	60 ± 17.4
Gender	
Male	28 (65.1%)
Female	15 (34.9%)
Resection extent	
Total	15 (34.9%)
Subtotal	4 (9.3%)
Partial resection	21 (48.8%)
Biopsy	3 (7.0%)
MIB-1 L.I. (%)	
Range	3.6 - 60
Mean ± SD	25 ± 16.0
Histopathologic diagnosis	
Anaplastic astrocytoma	7 (16.3%)
Glioblastoma	36 (83.7%)
PEA-15 expression	
Low	19 (44.2%)
High	24 (55.8%)

Table. 2 Cox regression multivariate analysis of survival

Factor	Survival		
	Hazard ratio	**P value**	**95% CI**
Age	1.007	0.6531	0.978-1.036
Gender (Female vs Male)	0.497	0.1232	0.204-1.209
Resection extent (Other vs Total)	3.715	0.0044	1.506-9.162
Histopathologic diagnosis (Anaplastic astrocytoma vs Glioblastoma)	0.358	0.1264	0.096-1.336
MIB-1 L.I. (≦ 25% vs > 25%)	0.401	0.0434	0.165-0.973
PEA-15 (Low vs High expression)	4.478	0.0024	1.704-11.768