TERT promoter mutation associated with multifocal phenotype and poor prognosis in patients with IDH wild-type glioblastoma

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Abbreviations:

ACNU: nimustine hydrochloride; CEL: contrast-enhanced lesions; GBM: glioblastoma; H3F3A: H3.3 histone A; IDH: isocitrate dehydrogenase; IDH1: isocitrate dehydrogenase 1; MGMT: O6-methylguanine-DNA methyltransferase; MRI: magnetic resonance imaging; PCR: polymerase chain reaction; CNA: copy number alterations; MLPA: Multiplex Ligation-dependent Probe Amplification; OS: overall survival; PFS: progression-free survival; TERTp: promoter region of telomerase reverse transcriptase
Abstract

**Background:** Although mutations in the promoter region of the telomerase reverse transcriptase gene (TERTp) are the most common alterations in glioblastoma (GBM), their clinical significance remains unclear. Therefore, we investigated the impact of TERTp status on patient outcome and clinicopathological features in patients with GBM over a long period of follow-up.

**Methods:** We retrospectively analyzed 153 cases of GBM. Six patients with isocitrate dehydrogenase 1 (IDH1) or H3F3A gene mutations were excluded from this study. Among the 147 cases of IDH wild-type GBM, 92 (62.6%) had the TERTp mutation. Clinical, immunohistochemical, and genetic factors (BRAF, TP53 gene mutation, CD133, ATRX expression, O6-methylguanine-DNA methyltransferase [MGMT] promoter methylation) and copy number alterations (CNAs) were investigated.

**Results:** GBM patients with the TERTp mutation were older at first diagnosis versus those with TERTp wild type (66.0 vs. 60.0 years, respectively, \(P=0.034\)), and had shorter progression-free survival (7 vs. 10 months, respectively, \(P=0.015\)) and overall survival (16 vs. 24 months, respectively, \(P=0.017\)). Notably, magnetic resonance imaging performed showed that TERTp-mutant GBM was strongly associated with multifocal/distant lesions \((P=0.004)\).

According to the CNA analysis, TERTp mutations were positively correlated with EGFR amp/gain, CDKN2A deletion, and PTEN deletion; however, these mutations were negatively correlated with PDGFR amp/gain, CDK4 gain and TP53 deletion.

**Conclusions:** TERTp mutations were strongly correlated with multifocal/distant lesions and poor prognosis in patients with IDH wild-type GBM. Less aggressive GBM with TERTp wild type may be a distinct clinical and molecular subtype of IDH wild type GBM.

**Keywords:** glioblastoma; multifocal; distant; TERT promoter; IDH wild type
Key Points

- TERTp mutations strongly correlated with multifocal/distant lesions and poor prognosis in patients with IDH wild-type GBM.
- The IDH wild-type GBM with and without TERTp mutations may be a distinct clinical and molecular subtype.

Importance of study

Mutations in the promoter region of the telomerase reverse transcriptase gene (TERTp) are the most common mutations in isocitrate dehydrogenase (IDH) wild-type glioblastoma (GBM). While TERTp mutations are correlated with poor prognosis, aggressive clinicopathological characteristics, and metastasis in other cancers, their clinical significance in GBM remains unclear. Here, we analyzed GBMs to determine whether the TERTp status is associated with other clinical and molecular factors. Particularly, this study focused on whether multifocal/distant lesions were observed during the clinical course. In this study, we demonstrated that TERTp mutant GBMs are strongly associated with the prognosis and multifocal/distant lesions during a long follow-up period. In addition, TERTp mutation was positively correlated with EGFR amp/gain, CDKN2A deletion, and PTEN deletion; however, it negatively correlated with PDGFR amp/gain, CDK4 gain and TP53 deletion. Less aggressive GBM with TERTp wild type could be distinct clinical and molecular subtype of IDH wild-type GBM.
Introduction

Glioblastoma (GBM) is the most common primary malignant tumor affecting the central nervous system in adults.\textsuperscript{1} Despite of radical surgery combined with concomitant chemoradiation therapy based on temozolomide, the median survival of patients is approximately 18 months.\textsuperscript{2}

According to the World Health Organization revised neuropathological criteria, these tumors are divided into two categories, namely isocitrate dehydrogenase (IDH)-wild type and IDH-mutant GBMs. In addition, recent reports indicated that 70–80% of GBM genomes harbor either C228T or C250T mutations in the promoter region of the telomerase reverse transcriptase (TERTp) gene.\textsuperscript{3,4} These mutations are associated with enhanced telomere maintenance.\textsuperscript{5,6,7} Although several studies reported the prognostic significance of TERTp mutation in patients with GBM, its clinical and pathological roles remain unclear.\textsuperscript{3-6}

Recently, GBM patients with unmethylated O\textsuperscript{6}-methylguanine-DNA methyltransferase (MGMT) and TERTp mutation have a worse prognosis than those with TERTp wild type.\textsuperscript{3,8} However, the mechanism of interaction of TERTp mutation and MGMT promoter methylation is not well established.

Regarding imaging analysis, necrosis detected through magnetic resonance imaging (MRI) has been reported to indicate the presence of TERTp mutation.\textsuperscript{9} However, predicting the TERTp status by preoperative imaging study alone remains difficult.

A recent systematic review and meta-analyses stated that the incidence of solitary GBM is 83%.\textsuperscript{10} Other previous studies showed that 20% of patients with GBM had multiple lesions and their prognosis was worse than that recorded in patients with a single lesion.\textsuperscript{11}

In this study, we analyzed GBMs to determine whether the TERTp status was associated with other clinical and molecular factors. Particularly, this study utilized MRI to determine the development of multifocal/distant lesions during the clinical course.

Materials and methods

Patients and samples

This retrospective study was conducted with the approval of the Ethics Committees of the Tohoku University School of Medicine and Yamagata University School of Medicine. Written informed consent was provided by all patients prior to their participation in the study.

Between January 2009 and October 2019, a total of 153 patients (89 treated at Yamagata University Hospital [Yamagata cohort] and 64 treated at Tohoku University Hospital [Tohoku cohort]) were analyzed. All patients met the following inclusion criteria: 1) diagnosis of GBM, World Health Organization grade IV; 2) no history of lower-grade tumors; 3) availability of genomic DNA; and 4) availability of information regarding events, such as recurrence or death during the follow-up period, or absence of such events for ≥12 months of follow-up. Patients who had previously undergone biopsies were excluded from the study. Tumor specimens were obtained from a lesion that exhibited enhancement on gadolinium-enhanced MRI and immediately stored at −80°C until DNA extraction.
**Classification of GBM according to preoperative MRI**

MRI sequences were acquired on a 1.5-T or 3.0-T scanner and typically included axial T1-weighted, T2-weighted fast spin-echo, and fluid-attenuated inversion-recovery sequences as well as a post-contrast three-dimensional spoiled gradient-recalled acquisition in the steady state T1-weighted sequence. Contrast-enhanced lesions (CEL) were assessed to clarify whether they were in contact with the subventricular zone, as previously described.\(^\text{12}\)

**Definition of multifocal/distant lesions**

One or more enhancing non-contiguous lesions >1 cm distant from the original tumor on preoperative MRI were defined as multifocal/distant lesions at diagnosis.\(^\text{13}\)

In addition, as previously reported, “multifocal/distant lesions at recurrence” were defined as distant or multifocal recurrence. Recurrence was characterized by the development of new CEL centered >3 cm distant from the primary resection cavity or at the margins of the primary residual tumor, or at more than one site, with each lesion having a well-defined border and the patient exhibiting normal brain signals.\(^\text{14,15}\)

**Clinical parameters**

The clinical profiles of patients were obtained from their medical records. The majority of patients underwent radical surgery followed by chemotherapy (nimustine hydrochloride [ACNU] or temozolomide) and radiotherapy. Total surgical resection was defined as the disappearance of CEL according to pre- and postoperative gadolinium-enhanced MRI studies. In cases in which the primary tumor recurred, patients underwent salvage surgery, second-line chemotherapy, radiotherapy, or palliative therapy. The Ki67 labeling index was determined by immunohistochemical staining of resected specimens with the Ki-67 antigen (Dako, Agilent Technologies). We also analyzed the expression of CD133 (Miltenyi Biotec), p53(Dako, Agilent Technologies), and ATRX (Abcam) by immunohistochemical staining. The expression of CD133 in 144 patients among the Yamagata and Tohoku cohorts was previously reported.\(^\text{16,17}\)

**Prognosis**

Progression-free survival (PFS) was defined as the interval between the day of first surgery and the day of recurrence detection on MRI scans. Overall survival (OS) was defined as the time between the day of the first operation and the day of death or final follow-up.
Molecular analysis

Genomic DNA was extracted with the QIAamp DNA mini kit (Qiagen), according to the instructions provided by the manufacturer. The isocitrate dehydrogenase1/2 (IDH1/2), H3F3A, HIST1H3B, TP53, BRAF, and TERTp genes were amplified via polymerase chain reaction (PCR), and sequencing was conducted as previously described. In the MGMT promoter methylation analysis, we performed methylation-specific PCR or quantitative methylation-specific PCR following the bisulfite modification of tumor DNA. To assess copy number alterations (CNAs), we performed Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA MLPA KIT P105 (version D2), in accordance with the manufacturer’s protocol (MRC Holland, Amsterdam, the Netherlands). The P105 kit is designed to detect CNAs typically found in gliomas and includes probes against the PDGFRα, EGFR, CDKN2A, PTEN, TP53, CDK4, MDM2, and NFKBIA genes. Based on previous publications, the CNA categories were classified according to the following thresholds: homozygous deletion (x ≤ 0.4), hemizygous deletion (0.4 < x ≤ 0.7), gain (1.3 ≤ x < 2.0), and amplification (x ≥ 2.0). We used OncoPrinter, a tool provided by the cBioPortal for Cancer Genomics (cbioportal.org/oncoprinter), to visualize and analyze our data with some modifications.

Statistical analysis

Statistical analyses were performed using the SPSS (IBM Japan, Tokyo, Japan) software. The relationship between two variables was evaluated using the Mann–Whitney U test and Fisher’s exact test. Estimates of PFS and OS were calculated with the Kaplan–Meier method, and the Log-rank (Mantel–Cox) test was used to evaluate differences between the groups. Cox regression was used for the multivariate analysis. The significance level was set at P<0.05.

Results

Population and tumor characteristics on MRI

A total of 153 patients, including 82 males and 71 females with a median age of 63 years (range: 27–86 years) and median preoperative Karnofsky Performance Status of 80 (range: 30–100), were included in the present study. Patients in the Yamagata cohort were older than those in the Tohoku cohort (P<0.001) (Supplementary Table 1). Genomic DNA and paraffin-embedded samples were obtained from all patients. The median duration of the follow-up period was 17 months (range: 1–152 months), and 119 patients (77.8%) expired. Total surgical resection was achieved in 96 patients (62.7%). In this group, IDH1, H3F3A, and BRAF gene mutations were detected in four (2.6%), two (1.3%), and one patient (0.65%), respectively; however, neither IDH2 nor HIST1H3B gene mutations were detected. TERTp gene mutations were detected in 92 patients (60.1%), including 65 (42.5%) and 27 (17.6%) with C228T and C250T mutations, respectively. Although the frequency of TERTp gene mutations in the Yamagata cohort was higher than that in the Tohoku cohort (P=0.019, Supplementary Table 1), there was no significant difference in the mutation frequency in older patients (age ≥ 60) between the two cohorts (P=0.348) (data not shown). MGMT gene promoter methylation was found in 62 patients (40.5%). Postoperative treatments consisted of radiation alone
for six patients, while the remaining 147 patients received combined radiation and chemotherapy with temozolomide (n=123), ACNU (n=14), or other agents (n=10). Bevacizumab was administered as first- and second-line therapy in one and 50 patients, respectively. There were no significant differences observed in PFS and OS between patients treated with ACNU and temozolomide (data not shown). TP53 gene mutations and/or strong immunoreactivity of p53 were found in 63 patients (43.4%) (Fig.2). Eleven of 88 patients (12.5%) displayed the loss of ATRX expression. The major CNAs frequently observed in 139 GBMs included EGFR amp/gain (66.2%), CDKN2A deletion (60.4%), and PTEN deletion (51.8%) (Fig.2, Table 1).

Correlation analyses between the TERTp status and other prognostic factors

Six patients with IDH1 or H3F3A mutations were excluded from this study. Therefore, we analyzed 147 GBM patients with IDH wild type to determine the factors correlated with the TERTp mutation. The median age was higher in GBM patients with TERTp mutation than those with TERTp wild type (P = 0.034) (Table 1).

In terms of MRI characteristics, 21 of the 147 patients (14.3%) had multifocal/distant lesions at diagnosis (Table 1). During the follow-up, 129 patients (87.7%) experienced the first recurrence, which included local recurrence and multifocal/distant recurrence in 99 (67.3%) and 30 (20.4%) patients, respectively. Among the patients with a well-controlled first recurrent lesion, 15 patients (10.2%) had new multifocal/distant lesions at second recurrence. Neither local nor distal recurrence were observed at the time of the last observation in the remaining 18 patients (12.3%).

Although multifocal/distant lesions at diagnosis or recurrence were weakly correlated with TERTp mutations (P=0.087, P=0.096, respectively), these lesions were significantly more common in patients with TERTp-mutant GBM than in patients with TERTp wild-type GBM during the entire follow-up period (P=0.004, Table 1).

The loss of ATRX expression occurred more frequently in TERTp wild-type GBM; however, this difference was not significant (P=0.085, Table 1). EGFR amp/gain, CDKN2A deletion, and PTEN deletion were significantly associated with TERTp mutations (P<0.0001, P=0.048, P<0.0001, respectively, Fig.2, Table 1). Conversely, PDGFR amp/gain, CDK4 gain, and TP53 hemizygous deletion were more frequently observed in TERTp wild-type GBM (P=0.001, P=0.012, P=0.001, respectively, Fig.2, Table 1).

Univariate analysis for the prediction of PFS and OS

The median PFS and OS for the patients with IDH wild-type GBM were 8 and 18 months, respectively (Table 2). Based on the Kaplan–Meier analysis, longer PFS and OS were correlated with TERTp wild-type (P=0.015 and P=0.017, respectively) (Figs. 3A, B; Table 2), gross total resection (P<0.001 and P<0.001, respectively) (Table 2), MGMT gene promoter methylation (P=0.037 and P=0.015, respectively) (Table 2), CDK4 amp/gain (P=0.015 and P=0.042, respectively), and local lesions (P=0.006 and P=0.001, respectively) (Table 2). The female sex was associated with longer PFS (P=0.047) (Table 2).
To determine whether the TERTp mutation was negatively correlated with PFS and OS in the non-multifocal/distant group, we analyzed the survival of the 81 patients in the non-multifocal/distant group. The median PFS and OS were 9 and 23 months, respectively, with no significant correlation of PFS and OS with the TERTp mutation (P=0.129 and P=0.148, respectively) (data not shown).

We also investigated the prognostic value of TERTp mutation in combination with MGMT promoter methylation. Among patients with TERTp mutation, unmethylated MGMT was significantly associated with poor PFS and OS (P<0.0001, P<0.0001, respectively) (Fig. 3C, D). However, among patients with TERTp wildtype, there was no significant difference of PFS and OS between patients with and without MGMT promoter methylation (P=0.938 and P=0.699, respectively) (Fig.3C, D).

Factors associated with multifocal/distant lesions

We investigated several factors to determine whether they correlated with multifocal/distant lesions. As shown in Supplementary Table 2, TERTp mutations, the expression of CD133, and PTEN deletion were significantly associated with multifocal lesions (P=0.004, P=0.004, P=0.004, respectively).

Multivariate analysis of prognostic factors

The factors included in the multivariate analysis for PFS and OS were TERTp status, sex, age, extent of resection, Ki-67 labeling index, MGMT gene promoter methylation, CDK4 amp/gain, number of lesions, and cohort site. We found that TERTp mutation, absence of gross total resection, and MGMT gene promoter unmethylation were independent unfavorable prognostic factors for PFS (hazard ratio [HR]: 2.0, 95% confidence interval [CI]: 1.2–3.3, P=0.006; HR: 2.2, 95% CI: 1.3–3.5, P=0.002; and HR: 2.0, 95% CI: 1.3–3.0, P=0.002, respectively) (Table 3). TERTp mutations (HR: 2.0, 95% CI: 1.2–3.3, P=0.010), absence of total resection (HR: 2.9, 95% CI: 1.7–4.8, P<0.001), and MGMT gene promoter unmethylation (HR: 2.2, 95% CI: 1.4–3.5, P=0.001) were independent unfavorable prognostic factors for OS.

Discussion

TERTp mutation is the most common alteration in GBM; however, the clinical impact of TERTp mutations in GBM remains unclear. To understand the poor prognosis of GBM with TERTp mutations, we hypothesized that malignant clinical features exist in this group. Long-term follow-up revealed that the cumulative incidence of multiple/distant lesions was significantly higher in GBM with TERTp mutations than in patients with TERTp wild-type GBM. Conversely, the non-multifocal/distant group did not show any differences in PFS and OS based on TERTp status. Therefore, we, for the first time, demonstrated that GBM with TERTp mutations has a poor prognosis because of its clinically aggressive behavior. In accordance with this finding, several studies regarding other cancers demonstrated that these mutations were correlated with a poor
prognosis, aggressive clinicopathological characteristics, and metastasis.\textsuperscript{24-28} Xing et al. found that \textit{TERT} mutation strongly correlated with vascular invasion in patients with papillary thyroid cancer.\textsuperscript{25} Yuan et al. reported that thyroid cancer patients with the \textit{TERT} mutation have a four-fold higher risk of distant metastasis than those with \textit{TERT} wild type\textsuperscript{27}

The frequency of \textit{TERT} mutations in our study was 62.6\%, which is lower than that of previous reports from North America and European countries, which reported mutation frequencies of 73—75\% in \textit{IDH} wild-type GBMs.\textsuperscript{3,5} Other reports from Japan also showed relatively low frequencies of \textit{TERT} mutations among \textit{IDH} wild-type GBM, ranging from 50—70\%.\textsuperscript{6,9,29} Thus, racial differences in the frequency of \textit{TERT} mutations may exist. One possible explanation for the low frequency of \textit{TERT} mutations in the Japanese cohort is that other mechanisms involved with replicative immortality in \textit{TERT} wild-type GBM. One such mechanism is \textit{TERT} hypermethylation, and the other is \textit{ATRX} or \textit{SMARCAL1} gene mutation. \textit{TERT} hypermethylation can aberrantly activate telomerase in cancer,\textsuperscript{30} and the \textit{ATRX} or \textit{SMARCAL1} gene mutations are strongly associated with the maintenance of telomere length, referred to as alternative lengthening of telomeres (ALT).\textsuperscript{31} Indeed, our results indicated the frequent loss of \textit{ATRX} expression in \textit{TERT} wild-type GBM. The other explanation is potential inclusion of other \textit{IDH} wild-type high grade gliomas such as anaplastic astrocytoma with piloid features.\textsuperscript{32} Although our cases were histologically confirmed as GBM, further molecular testing may be required to classify into novel entities.

The prognostic significance of the \textit{TERT} mutation remains controversial in patients with GBM.\textsuperscript{3,33-36} In the present study, univariate and multivariate analyses showed that the \textit{TERT} mutation was significantly associated with both PFS and OS. In accordance with previous reports, we also found that unmethylated GBM with \textit{TERT} mutations presented a poor prognosis.\textsuperscript{3,8} However, among patients with \textit{TERT} wildtype, there was no significant difference of PFS and OS between patients with and without \textit{MGMT} promoter methylation. The reason may be that GBM tumors with the \textit{TERT} mutation form multifocal/distant lesions by invading various directions. Nevertheless, those with methylated \textit{MGMT} were sensitive to treatment with alkylating agents, such as temozolomide. Therefore, \textit{TERT} mutated GBM patients with methylated \textit{MGMT} may survive longer than those with unmethylated \textit{MGMT}.

Recently, GBMs were divided into two groups according to the \textit{IDH} mutation status. Although \textit{IDH} mutation is frequently found in lower-grade diffuse glioma, only 5—10\% of patients with GBM had this mutation.\textsuperscript{35,37} In addition, GBM patients with the \textit{IDH} mutation are usually young and diagnosed with progression from a lower grade of diffuse astrocytoma. Thus, \textit{TERT} mutation, frequently found in GBM is more useful for predicting survival and clinical behavior, such as the pattern of invasion.

Our data showed that \textit{TERT} mutations were significantly associated with \textit{EGFR} amp/gain, \textit{CDKN2A} deletion, and \textit{PTEN} deletion and were typically found in \textit{IDH} wild-type GBM; conversely, the \textit{TERT} wild-type was associated with \textit{PDGFR} amp/gain, \textit{CDK4} gain, and \textit{TP53} deletion. Recently, Williams et al. reported \textit{TERT} wild-type glioblastomas showed frequent \textit{PI3K} pathway and \textit{BAF} complex gene family (\textit{ATRX}, \textit{SMARCA4}, \textit{SMARC1}, and \textit{ARID1A}) mutations.\textsuperscript{38} Our data also suggest that \textit{TERT} wild-type GBMs are genetically distinct from \textit{TERT} mutant GBMs.

The present study had some limitations. First, since this was a retrospective study, patients were not treated in the same manner. Although we performed a multivariate analysis, differences in
treatment may have affected the pattern of recurrence. Second, we demonstrated the malignant features of GBM with the TERTp mutation based on clinicopathological characteristics, but patients with oligodendroglia (the most benign diffuse glioma) also had the TERTp mutation. Third, it has been reported that PTEN, PI3K3A mutation and the expression of CD133 are associated with distant recurrence in patients with GBM. In the present study, there was no significant association between CD133 expression and the TERTp mutation, but PTEN deletion was significantly correlated with TERTp mutations and multifocal/distant lesions. The mechanism of invasiveness based on the TERTp mutation warrants further investigation.

Conclusion

We retrospectively investigated whether the TERTp mutation was associated with multifocal/distant lesions in GBM. The results suggested that the TERTp mutations strongly correlated with the multifocal phenotype and poor prognosis in patients with IDH wild-type GBM. We further demonstrated that TERTp mutations were significantly associated with EGFR amp/gain, CDKN2A deletion, and PTEN deletion, whereas the TERTp wild-type was correlated with PDGFR amp/gain, CDK4 gain, and TP53 deletion. The IDH wild-type GBM with and without TERTp mutations may be a distinct clinical and molecular subtype.
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Figure legends

Fig. 1 Definition of multifocal lesion. Representative gadolinium-enhanced MRI scans of patients treated at Yamagata University Hospital. The scans were obtained at diagnosis and after surgery, and at the first and second recurrence. (A) Multifocal lesions at diagnosis. (B) Multifocal/distant lesion at first recurrence. Eight months after surgery, an enhanced lesion was observed at a location distant from the initial lesion (arrow). (C) Local recurrence during the entire follow-up period. (D) Multifocal/distant lesion at second recurrence. Seven months after surgery, local recurrence was observed adjacent to the resection cavity. Ten months later, an enhanced lesion was detected at a distant location (arrow).

Fig. 2 Genetic distribution in 153 GBMs. Mutations, CNAs, and methylation were generated and visualized by OncoPrinter via the cBioPortal for Cancer Genomics (cbioportal.org/oncoprinter) with some modifications.\textsuperscript{22,23} The diagram shows the landscape of the molecular characteristics of GBMs, which are sorted by IDH, H3F3A, and TERTp mutations. Abbreviations: CNA, copy number alteration; N/A, not available

Fig. 3 (A, B) Kaplan–Meier curves based on the TERTp mutation in patients with IDH wild-type GBM. (A) Progression-free survival (PFS). (B) Overall survival (OS). (C, D) Kaplan–Meier curves based on the combination of TERTp mutation and MGMT promoter methylation in patients with IDH wild-type GBM. (C) Progression-free survival (PFS). (D) Overall survival (OS).
Table 1. Relationships between TERTp status and other prognostic factors

|                                | Total (n=147) | TERTp wild-type (n=55) | TERTp mutant (n=92) | P       |
|--------------------------------|---------------|------------------------|---------------------|---------|
| Sex, female, n (%)             | 66 (44.8)     | 27 (49.1)              | 39 (42.4)           | 0.494*  |
| Age, y, median (range)         | 64 (27.86)    | 60 (27.82)             | 66 (32.86)          | 0.034*  |
| Preoperative KPS ≥80, n (%)    | 84 (60.0)     | 27 (51.9)              | 57 (64.8)           | 0.155#  |
| Gross total resection, n (%)   | 93 (63.2)     | 33 (60.0)              | 60 (65.2)           | 0.597#  |
| CD133 expression, mean (%)     | 12.7±12.9     | 12.1±11.1              | 12.9±13.8           | 0.729a  |
| Ki-67 labeling index, mean (%) | 33.8±17.9     | 34.8±17.9              | 33.2±18.0           | 0.477a  |
| Multifocal/distant lesions     |               |                        |                     |         |
| at diagnosis, n (%)            | 21 (14.3)     | 4 (7.3)                | 17 (18.5)           | 0.087b  |
| at recurrence, n (%)           | 45 (30.6)     | 12 (21.8)              | 33 (35.9)           | 0.096b  |
| at the first recurrence, n (%) | 30 (20.4)     | 8 (14.5)               | 22 (23.9)           | 0.208b  |
| at the second recurrence, n (%)| 15 (10.2)     | 4 (7.3)                | 11 (12.0)           | 0.415b  |
| Total, n (%)                   | 66 (44.9)     | 16 (29.1)              | 50 (54.3)           | 0.004b  |
| MGMT gene promoter methylation, n (%) | 57 (38.8) | 19 (34.5)          | 38 (41.3)           | 0.485b  |
| TP53 gene mutation, n (%)      | 57 (40.7)     | 19 (37.3)              | 38 (42.7)           | 0.477b  |
| Loss of ATRX expression, n (%) | 11 (12.5)     | 6 (22.2)               | 5 (8.2)             | 0.085b  |
| CNA PDGFR Amp, n (%)           | 11 (7.9)      | 9 (17.6)               | 2 (2.3)             | 0.002b  |
| Gain, n (%)                    | 8 (5.8)       | 5 (9.8)                | 3 (3.4)             | 0.143b  |
| Amp/Gain, n (%)                | 19 (13.7)     | 14 (27.5)              | 5 (5.7)             | 0.001b  |
| EGFR Amp, n (%)                | 46 (33.1)     | 8 (15.7)               | 38 (43.2)           | 0.001b  |
| Gain, n (%)                    | 46 (33.1)     | 7 (13.7)               | 39 (44.3)           | <0.0001b|
| Amp/Gain, n (%)                | 92 (66.2)     | 15 (29.4)              | 77 (87.5)           | <0.0001b|
| CDKN2A Homo, n (%)             | 61 (43.9)     | 20 (39.2)              | 41 (46.6)           | 0.479b  |
| Hemi, n (%)                    | 23 (16.5)     | 5 (9.8)                | 18 (20.5)           | 0.154b  |
| Deletion, n (%)                | 84 (60.4)     | 25 (49.0)              | 59 (67.0)           | 0.048b  |
| PTEN Homo, n (%)               | 8 (5.8)       | 1 (2.0)                | 7 (8.0)             | 0.258b  |
| Hemi, n (%)                    | 64 (46.0)     | 8 (15.7)               | 56 (63.6)           | <0.0001b|
| Deletion, n (%)                | 72 (51.8)     | 9 (17.6)               | 63 (71.6)           | <0.0001b|
| CDK4 Amp, n (%)                | 13 (9.4)      | 4 (7.8)                | 9 (10.2)            | 0.768b  |
| Gain, n (%)                    | 9 (6.5)       | 7 (13.7)               | 2 (2.3)             | 0.012b  |
| Gene   | Category | n (%)       | Group 1 | Group 2 | Group 3 | P value |
|--------|----------|-------------|---------|---------|---------|---------|
|        | Amp/Gain, n (%) | 22 (15.8) | 11 (21.6) | 11 (12.5) | 0.227\textsuperscript{b} |
| MDM2   | Amp, n (%)       | 16 (11.5)  | 6 (11.8)  | 10 (11.4) | 1.000\textsuperscript{b} |
|        | Gain, n (%)       | 5 (3.6)    | 4 (7.8)   | 1 (1.1)   | 0.061\textsuperscript{b} |
|        | Amp/Gain, n (%)   | 21 (15.1)  | 10 (19.6) | 11 (12.5) | 0.327\textsuperscript{b} |
| NFKBIA | Hemi, n (%)       | 20 (14.4)  | 8 (15.7)  | 12 (13.6) | 0.804\textsuperscript{b} |
| TP53   | Hemi, n (%)       | 23 (16.5)  | 16 (31.4) | 7 (8.1)   | 0.001\textsuperscript{b} |
|        | Mut/Hemi, n (%)   | 12 (8.6)   | 7 (13.7)  | 5 (5.7)   | 0.124\textsuperscript{b} |
|        | SVZ-positive, n (%) | 66 (44.9) | 25 (45.5) | 41 (44.6) | 1.000\textsuperscript{b} |

Abbreviations: \textsuperscript{a}Mann-Whitney test. \textsuperscript{b}Fisher's exact test. \textit{P} values <0.05 are in bold. Amp, Amplification. Homo, homozygous deletion, Hemi, hemizygous deletion. Mut, mutation
Table 2. Clinical and genetic parameters affecting PFS and OS in primary glioblastoma

| Parameters                          | No. of patients | PFS | OS |
|------------------------------------|----------------|-----|----|
|                                    | (n=147)        | Median (months) | $P^*$ | Median (months) | $P^*$ |
| **TERT**p status                   |                |     |    |
| Mutated                            | 92             | 7   | 16 |
| Wild type                          | 55             | 10  | 24 | 0.017 |
| **Sex**                            |                |     |    |
| Female                             | 66             | 9   | 22 |
| Male                               | 81             | 7   | 16 | 0.055 |
| **Age at diagnosis**               |                |     |    |
| <60 years                          | 52             | 8   | 18 |
| ≥60 years                          | 95             | 7   | 19 | 0.115 |
| **Preoperative KPS**               |                |     |    |
| ≥80                                | 84             | 8   | 21 |
| <80                                | 56             | 7   | 15 | 0.294 |
| **Surgery**                        |                |     |    |
| Gross total resection              | 93             | 11  | 23 |
| Absence of gross total resection   | 54             | 4   | 11 | <0.001 |
| **Ki-67 labeling index**           |                |     |    |
| Low (<30%)                         | 55             | 8   | 20 |
| High (>30%)                        | 68             | 7   | 16 | 0.061 |
| **CD133 expression**              |                |     |    |
| Low (<15%)                         | 97             | 8   | 21 |
| High (≥15%)                        | 47             | 7   | 17 | 0.146 |
| **MGMT**                           |                |     |    |
| Methylated                         | 57             | 13  | 24 |
| Unmethylated                       | 90             | 7   | 16 | 0.015 |
| **PDGFR**                          |                |     |    |
| Amp/Gain                           | 19             | 10  | 17 |
| Retain                             | 120            | 8   | 20 | 0.669 |
| **EGFR**                           |                |     |    |
| Amp/Gain                           | 92             | 7   | 17 |
| Genes     | CDK4N2A | PTEN | CDK4 | MDM2 | NFKBIA | TP53 | SVZ |
|-----------|---------|------|------|------|--------|------|-----|
| Deletion  | 47      | 84   | 72   | 22   | 20     | 68   | 66  |
| Retain    | 10      | 9    | 9    | 19   | 13     | 10   | 7   |
|           | 0.060   | 0.522| 0.281| 0.015| 0.054  | 0.802| 0.795|
|           | 24      | 17   | 19   | 34   | 21     | 18   | 18  |
|           | 0.142   | 0.350| 0.497| 0.042| 0.662  | 0.042| 0.368|
| Amp/Gain  | 117     | 21   | 118  | 21   | 119    | 119  | 119 |
| Retain    | 7       | 10   | 8    | 10   | 8      | 8    | 8   |
|           | 0.015   | 0.795| 0.802| 0.952| 0.054  | 0.802| 0.802|
|           | 18      | 24   | 18   | 21   | 19     | 18   | 18  |
|           | 0.042   | 0.368| 0.662| 0.580| 0.580  | 0.662| 0.580|

Abbreviations: PFS, progression-free survival. OS, overall survival. *Log-rank test. *P* values <0.05 are in bold.
Table 3. Multivariate analysis of independent prognostic factors associated with PFS and OS

| Parameters                      | PFS         |          |          | OS         |          |          |
|--------------------------------|-------------|----------|----------|------------|----------|----------|
|                                | HR          | 95%CI    | *P*      | HR         | 95%CI    | *P*      |
| TERTp status                   |             |          |          |            |          |          |
| Mutant vs Wild-type            | 2.0         | 1.2-3.3  | **0.006**| 2.0         | 1.2-3.3  | **0.010**|
| Sex                            | 1.3         | 0.9-2.0  | 0.218    | 1.4         | 0.9-2.2  | 0.157    |
| Age                            | 1.2         | 0.8-2.0  | 0.364    | 1.1         | 0.7-1.9  | 0.600    |
| Gross total resection          |             |          |          |            |          |          |
| No vs Yes                      | 2.2         | 1.3-3.5  | **0.002**| 2.9         | 1.7-4.8  | **<0.001**|
| Ki-67 labeling index           |             |          |          |            |          |          |
| ≥30 vs <30                     | 1.4         | 0.9-2.1  | 0.129    | 1.5         | 1.0-2.4  | 0.069    |
| MGMT                           |             |          |          |            |          |          |
| Unmethylated vs Methylated     | 2.0         | 1.3-3.0  | 0.002    | 2.2         | 1.4-3.5  | **0.001**|
| CDK4                           |             |          |          |            |          |          |
| Amp/Gain vs Retain             | 1.5         | 0.8-2.8  | 0.261    | 1.5         | 0.7-2.9  | 0.284    |
| Number of lesions              |             |          |          |            |          |          |
| Multifocal/distant vs Local    | 1.3         | 0.8-2.0  | 0.327    | 1.3         | 0.8-2.2  | 0.241    |
| Cohort site                    |             |          |          |            |          |          |
| Yamagata vs Tohoku             | 1.1         | 0.7-1.7  | 0.656    | 1.1         | 0.7-1.8  | 0.589    |

P values <0.05 are in bold
### Table 1: Genomic Alterations in GBM Tumors

| Gene   | MGMT | IDH1/2 | H3F3A | BRAF | TERTp | TPS3 | PDGFR | EGFR | CDKN2A | PTEN | CDK4 | MDM2 | NFkBIA | TP53 | CD133 |
|--------|------|--------|-------|------|-------|------|-------|------|--------|------|------|------|--------|------|-------|
|        |      |        |       |      |       |      |       |      |        |      |      |      |        |      |       |

| Alteration Type | Methylation | Unmethylated | Gene Mutation | Mutation | Wildtype | NA | CNA | Amplification | Gain | Homozygous deletion | Hemizygous deletion | Wildtype | NA | CD133 |
|-----------------|-------------|--------------|---------------|----------|-----------|----|-----|----------------|------|---------------------|---------------------|-----------|----|-------|
| MGMT            | Blue        |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| IDH1/2          | Green       |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| H3F3A           | Green       |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| BRAF            |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| TERTp           | Green       |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| TPS3            |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| PDGFR           |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| EGFR            |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| CDKN2A          |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| PTEN            |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| CDK4            |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| MDM2            |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| NFkBIA          |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| TP53            |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
|                |             |              |               |          |           |    |     |                |      |                     |                      |            |    |       |
| CD133           |              |              |               |          |           |    |     |                |      |                     |                      |            |    |       |

*Fig. 2*
