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CDKN2A deletion associated with poor prognosis in patients with TERT promoter wild-type, IDH wild-type glioblastoma

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Research Article

Keywords: glioblastoma, TERT promoter, MGMT promoter, CDKN2A deletion

Posted Date: May 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9350156/v1

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Abstract

Background

The most common alterations in *IDH* wild-type primary glioblastoma (GBM) are mutations in the promoter region of the telomerase reverse transcriptase gene (*TERT*p). This retrospective study investigated whether the impact of clinicopathological factors on prognosis in patients with GBM depends on *TERT*p status.

Methods

Among 112 cases of *IDH* wild-type GBM, 70 patients (62.5%) had the *TERT*p mutation. Clinical, immunohistochemical, and genetic factors (ATRX expression, and O^6-methylguanine-DNA methyltransferase promoter [*MGMT*p] methylation), and copy number alterations (CNAs) were investigated.

Results

Loss of ATRX, *PDGFR* amplification/gain, *MDM2* amplification/gain, and *TP53* hemizygous deletion were more frequent in patients with *TERT*p wild-type GBM ($P = 0.005$, $P = 0.009$, $P = 0.033$, $P = 0.009$, respectively). *EGFR* amplification/gain and *PTEN* deletion were more frequent in those with *TERT*p mutant GBM (both $P < 0.001$). Postoperative residual tumor volume and *MGMT*p methylation were significantly correlated with PFS and OS in patients with *TERT*p mutant GBM. Only *CDKN2A* deletion was strongly correlated with poor OS in patients with *TERT*p wild-type GBM ($P < 0.001$).

Conclusions

CDKN2A deletion alone was associated with the prognosis of *TERT*p wild-type GBM. Therefore, *TERT*p wild-type GBM may be a distinct clinical subtype of *IDH* wild-type GBM.

Introduction

Glioblastoma (GBM) is the most common primary malignant tumor of the central nervous system in adults [1]. Despite radical surgery plus concomitant temozolomide-based chemoradiation therapy, patients have a median survival of approximately 18 months [2]. There are many known prognostic factors for GBM, which can be classified as patient factors (i.e., age, gender, and preoperative KPS), treatment factors (i.e., degree of surgical resection and presence of adjuvant treatment), and molecular biological factors (i.e., methylation of the O6-methylguanine-DNA methyltransferase promoter [*MGMT*p], *EGFR* amplification, and *PTEN* deletion) [3–5].

Mutations in the promoter region of the telomerase reverse transcriptase (*TERT*p) gene are most frequently found in GBM, but their prognostic significance remains unclear [6–9]. Several recent reports have shown that *MGMT*p methylation, which is a prognostic factor for GBM, did not have prognostic significance in *TERT*p wild-type GBM [10, 11]. In contrast, *CDKN2A* homozygous deletion was reported to be a poor prognostic factor in *IDH*-mutant gliomas, but the prognostic significance of *CDKN2A* deletion in *IDH* wild-type GBM remains unclear [12, 13].

This study analyzed the relationship between *TERT*p status with other clinical and molecular factors, specifically *MGMT*p and *CDKN2A* status, in patients with GBM.

Materials and methods

Patients and samples

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

Between January 2014 and October 2024, a total of 112 patients were enrolled in this study. The inclusion criteria were as follows: 1) diagnosis of *IDH* wild-type GBM according to the World Health Organization classification of central nervous system tumors (WHO CNS) 5th edition; 2) no history of lower-grade tumors; 3) availability of genomic DNA; and 4) available outcome data (i.e., recurrence or death during follow-up) or absence of such events for ≥ 12 months of follow-up. Patients with previous biopsies were excluded from the study. Tumor specimens were obtained from lesions that exhibited enhancement on gadolinium-enhanced MRI and were immediately stored at − 80°C until DNA extraction.

Volumetric image analysis

Magnetic resonance imaging (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. Pre- and postoperative MRI (obtained ≤ 72 h after surgery) were used to quantify tumor volumes, which were delineated using preferred institutional software (BrainLab Smartbrush). The total contrast-enhanced (CE) tumor volume was measured on contrast-enhanced T1-weighted sequences, while the non-CE (nCE) tumor volume (i.e., signal alterations beyond the enhancing tumor borders) was measured on FLAIR sequences. The extent of resection was evaluated according to the classification system proposed by the *RANO* resect group [14], described as follows:

Class 1: supramaximal resection of the CE tumor (0 cm^3 CE + ≤ 5 cm^3 nCE)

Class 2A: maximal CE resection (complete CE resection) (0 cm^3 CE + > 5 cm^3 nCE)

Class 2B: maximal CE resection (near-total CE resection) (≤ 1 cm^3 CE)

Class 3A: submaximal resection (subtotal CE resection) (≤ 5 cm^3 CE)

Class 3B: submaximal resection (partial CE resection) (> 5 cm^3.CE)

Class 4: biopsy (no reduction of tumor volume)

Clinical parameters

The patients' clinical profiles were obtained from the medical records. All patients underwent radical surgery followed by temozolomide and radiotherapy. If the primary tumor recurred, patients underwent salvage surgery, second-line chemotherapy, radiotherapy, or palliative therapy.

Prognosis

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

Molecular analysis

Genomic DNA was extracted using the QIAamp DNA mini kit (Qiagen) as per the manufacturer's instructions. The isocitrate dehydrogenase1 (*IDH1*), H3.3 histone A (*H3F3A*), *BRAF*, and *TERTp* genes were amplified via polymerase chain reaction (PCR), and sequencing was conducted as previously described [15, 16]. In the *MGMT* promoter methylation analysis, quantitative methylation-specific PCR was performed following the bisulfite modification of tumor DNA [17]. To assess copy number alteration (CNA)s, multiplex ligation-dependent probe amplification (MLPA) was performed using the SALSA MLPA KIT P105 (version D2) following the manufacturer's protocol (MRC Holland, Amsterdam, Netherlands). The P105 kit is designed to detect CNAs typical in gliomas, including probes against *PDGFRA, EGFR, CDKN2A, PTEN*, and *CDK4.* Each category was classified by the following thresholds: homozygous deletion ($x \leq 0.4$), hemizygous deletion ($0.4 < x \leq 0.7$), gain ($1.3 \leq x < 2.0$), amplification ($x \geq 2.0$) [17–19]. The CNAs in 89 patients were described previously [19]. The OncoPrinter, a tool provided by the cBioPortal for Cancer Genomics (cbioportal.org/oncoprinter), was used to visualize and analyze the data with some modifications [20, 21].

Immunohistochemistry

Immunohistochemistry was conducted on 4-μm formalin-fixed, paraffin-embedded tissue sections. The sections were immunostained using an anti-human ATRX antibody (Abcam) or an anti-human Ki-67

protein antibody (Dako). ATRX loss was defined as the absence of staining in more than 90% of tumor cells [22]. The Ki67 labeling index was determined as described previously [23].

Statistical analysis

Statistical analyses were performed using 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 using the Kaplan–Meier method. The log-rank (Mantel–Cox) test was used to evaluate differences between groups. Cox regression was used for multivariate analysis. Statistical significance was set at $P < 0.05$.

Results

Population and tumor characteristics on MRI

The study cohort included 112 patients (60 males, 52 females) with a median age of 66 years (range: 29–89) and a median preoperative Karnofsky Performance Status of 80 (range: 20–100) (Table 1). Genomic DNA was obtained from all patients. The median follow-up duration was 18 months (range: 4–124 months), and 88 patients (78.5%) died. Patients were stratified using the classification system proposed by the *RANO* resect group, as follows: class 1 (2/112 patients, 1.8%), class 2A (49/112 patients, 43.8%), class 2B (27/112 patients, 24.1%), class 3A (20/112 patients, 17.9%), and class 3B (14/112 patients, 12.5%).

Regarding genetic mutations, no *IDH1* or *H3F3A* gene mutations were detected in this cohort. *TERT*p gene mutations were detected in 70 patients (62.5%), among whom 52 (74.3%) and 18 (25.7%) patients had C228T and C250T mutations, respectively. Loss of ATRX was found in 21 patients (18.8%). *MGMT* gene promoter methylation was found in 53 patients (47.3%). Figure 1 and Table 1 also describe other specific mutations, such as *PDGFR* amp/gain (n = 20, 17.8%), *EGFR* amp/gain (n = 69, 61.6%), *CDKN2A* (n = 75, 70.0%) deletion, *PTEN* deletion (n = 48, 42.9%), *CDK4* amp/gain (n = 18, 16.1%), *MDM2* amp/gain (n = 18, 16.1%), *NFKBIA* deletion (n = 18, 16.1%), and *TP53* hemizygous deletion (n = 10, 10.7%).

Regarding postoperative treatments, all patients received combined radiation and chemotherapy with temozolomide. Bevacizumab was administered as a second-line therapy in 67 patients. Tumor-treating therapy was administered in 6 patients after radiation.

Correlation analyses between the TERTp status and other prognostic factors

The preoperative KPS was higher in patients with *TERT*p mutant versus *TERT*p wild-type GBM ($P = 0.009$) (Table 1). Loss of ATRX, *PDGFR* amplification/gain, *MDM2* amplification/gain, and *TP53* hemizygous deletion were more frequent in *TERT*p wild-type GBM ($P = 0.005$, $P = 0.009$, $P = 0.033$, $P = 0.009$, respectively), whereas *EGFR* amplification/gain and *PTEN* deletion were more frequent in *TERT*p mutant GBM (both $P < 0.001$) (Table 1). No correlations were observed for other factors (Table 1).

Univariate analysis for the prediction of PFS and OS

Patients with *IDH* wild-type GBM had a median PFS of 7 months and a median OS of 20 months (Table 2). Based on the Kaplan–Meier analysis, longer PFS and OS correlated with RANO class 1/2A (complete resection of the CE tumor) (P = 0.025 and P = 0.012, respectively) (Supplementary Fig. 1A and B). *CDKN2A* deletion was correlated with OS (P = 0.015) (Supplementary Fig. 1C and D).

Among patients with *TERT*p mutant GBM, there were significant differences in PFS (P = 0.012) and OS (P = 0.003) between RANO class 1/2A versus RANO class 2B/3 (Fig. 2A and B, Table 3). Moreover, *MGMT*p methylation was significantly correlated with PFS and OS (P = 0.010 and P = 0.010, respectively) (Fig. 2C and D, Table 3), whereas *CDKN2*A deletion had no correlation with either (Fig. 2E and F, Table 3).

Among patients with *TERT*p wild-type GBM, there were no significant differences in PFS and OS among RANO class 1/2A versus RANO class 2B/3 (P = 0.265 and P = 0.391, respectively) (Fig. 3A and B, Table 3) and among patients with versus without *MGMT*p methylation (P = 0.906 and P = 0.371, respectively) (Fig. 3C and D, Table 3). *CDKN2A* deletion was strongly correlated with poor OS (P < 0.001) (Fig. 3E and F, Table 3).

Multivariate analysis of prognostic factors

The multivariate analysis for PFS and OS included the following factors: age, extent of resection (RANO class), *MGMT* gene promoter methylation, and *CDKN2A* deletion (Supplementary Table 1). The absence of total CE tumor resection (RANO class 1/2A) was an independent predictor of unfavorable prognosis in terms of both PFS (hazard ratio [HR]: 1.6, 95% confidence interval [CI]: 1.1–2.4, P = 0.024) and OS (HR: 1.9, 95% CI: 1.2–2.7, P = 0.008). Meanwhile, *CDKN2A* deletion independently predicted poor OS (HR: 1.3, 95% CI: 1.1–3.0, P < 0.001).

Among patients with *TERT*p wild-type GBM, *CDKN2A* deletion was the sole factor that independently predicted poor OS (HR: 5.0, 95% CI: 1.8–13.6, P = 0.002) (Supplementary Table 2).

Discussion

The *TERT*p mutation has been found in approximately 70%–80% of patients with *IDH* wild-type GBM [6, 7]. In our previous report, only 50%–70% of Japanese patients with *IDH* wild-type GBM were found to have the *TERT*p mutation. The frequency of *TERT*p mutation (62.5%) found in the present study is in line with previous reports from Japan [18, 24].

Aside from *TERT*p mutations, *ATRX* or *SMARCAL*1 gene mutations are also known mechanisms of tumor cell immortalization [25]. In fact, ATRX loss is more common in patients with *TERT*p wild-type GBM. However, since the frequency of ATRX loss was not very high, other immortalization mechanisms are likely involved as well. Furthermore, unlike in *IDH*-mutant gliomas, *TERT*p mutation and loss of ATRX

expression are not mutually exclusive. Since it may be somewhat difficult to interpret the loss of ATRX expression on immunohistochemistry, *ATRX* mutation analysis may be necessary in the future.

The clinical and prognostic impacts of *TERT*p mutation remain controversial in patients with GBM. In other cancers, several studies have demonstrated its correlations with poor prognosis, aggressive clinicopathological characteristics, and metastasis [26, 27]. In our previous report, *TERT*p mutation was significantly associated with shortened PFS and OS on univariate and multivariate analyses [19]. However, in this cohort, patients with *TERT*p mutations tended to have shorter OS than patients without mutations, although the difference was not statistically significant. Thus, the prognostic significance of *TERT*p mutations will require further investigation in a larger number of cases.

In a previous study, prognosis remained similar regardless of the presence of *MGMT*p methylation, despite it being a well-known prognostic factor for GBM [4]. Similarly, several studies have shown that *MGMT*p status has little prognostic impact among cases of *TERT*p wild-type GBM [10, 11, 19]. In fact, in our study, *MGMT*p methylation was a favorable prognostic factor for both PFS and OS in *TERT*p mutant GBM.

In *IDH*-mutant astrocytoma, homozygous *CDKN2A* deletion is associated with poor prognosis; such cases are classified as WHO CNS grade 4. However, the prognostic impact of *CDKN2A* deletion remains unclear in GBM [12, 13]. One recent report demonstrated that *TERTp* mutations and *CDKN2A* deletions occur in the early stage of GBM development [28]. Thus, the mechanism of development of *TERT*p wild-type GBM may be completely different from that of *TERT*p mutant GBM. In the present analysis, *CDKN2A* deletion did not affect prognosis in *TERT*p mutant GBM, but it was a statistically significant factor for poor prognosis in *TERT*p wild-type cases. Thus, the development of *TERT*p wild-type GBM may follow a similar mechanism as *IDH*-mutant astrocytoma.

The limitations of the present study must be acknowledged. First, this was a retrospective study with a small sample size. Second, it remains unclear why *MGMT*p methylation was not identified as a prognostic factor. Lastly, the mechanism of temozolomide resistance in *TERT*p wild-type GBM warrants further investigation.

Conclusion

This retrospective study investigated whether *TERT*p status influences the prognostic impact of certain clinicopathological factors in patients with GBM. *CDKN2A* deletion was strongly associated with PFS and OS in *TERT*p wild-type GBM.

Abbreviations

CE
contrast-enhanced
GBM

glioblastoma
H3F3A
H3.3 histone A
IDH
isocitrate dehydrogenase
IDH1
isocitrate dehydrogenase 1
MGMT
O^6-methylguanine-DNA methyltransferase
MRI
magnetic resonance imaging
OS
overall survival
PCR
polymerase chain reaction
PFS
progression-free survival
*TERT*p
promoter region of telomerase reverse transcriptase

Declarations

Acknowledgments

The authors thank Enago (https://www.enago.jp/)for the English-language review.

Author contributions

SK, YS: conception and design of the work. SK, YM, KN, KS, TK: data curation and writing of the original draft. YS: funding acquisition. MF, YK: writing, review, and editing.

Funding

This work was supported by JSPS KAKENHI (Grant Number 23K08493 and 26K12030).

Conflict of interest:

All authors declare that they have no conflicts of interest.

Ethical approval:

Thisstudy was approved by the Ethics Committee of Yamagata University Hospital (approval number: 2018-498).

Informed consent:

Informed consent was provided by all participants included in the study.

References

1. Louis DN, Perry A, Brat DJ et al (2021) WHO Classification of Tumours of the Central Nervous System in The WHO Classification of Tumours Editorial Board (ed): World Health Organization Classification of Tumours (5th Edition), Lyon, IARC Press, 2021, pp 516–552
2. Stupp R, Mason WP, van den Bent MJ et al (2015) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987–996
3. Curran WJ Jr., Scott CB, Horton J et al (1993) Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. J Natl Cancer Inst 85:704–710
4. Hegi ME, Diserens AC, Gorlia T et al (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl Med 352:997–1003
5. Aldape K, Zadeh G, Mansouri S et al (2015) Glioblastoma: pathology, molecular mechanisms and markers. Acta Neuropathol 129:829–848
6. Killela PJ, Reitman ZJ, Jiao Y et al (2013) TERT promoter mutations occur frequently in gliomas. Proc Natl Acad Sci U S A 110:6021–6026
7. Labussière M, Di Stefano AL, Gleize V et al (2014) TERT promoter mutations in gliomas: genetic associations and clinical outcome. Acta Neuropathol 128:219–231
8. Spiegl-Kreinecker S, Lötsch D, Ghanim B et al (2015) Prognostic quality of TERT promoter mutations in glioblastoma. Neuro Oncol 17:1231–1240
9. Nencha U, Rahimian A, Giry M et al (2016) TERT promoter mutations and polymorphism in glioblastoma. J Neurooncol 126:441–446
10. Nguyen HN, Lie A, Li T et al (2017) TERT promoter mutation enables survival advantage from MGMT methylation. Neuro Oncol 19:394–404
11. Arita H, Yamasaki K, Matsushita Y et al (2016) TERT promoter mutation and MGMT methylation define subgroups. Acta Neuropathol Commun 4:79
12. Brat DJ, Aldape K, Colman H et al (2020) cIMPACT-NOW update 5. Acta Neuropathol 139:603–608
13. Louis DN, Perry A, Wesseling P et al (2021) The 2021 who classification of tumors of the CNS. Neuro Oncol 23:1231–1251
14. Karschnia P, Vogelbaum MA, van den Bent M et al (2023) Prognostic validation of RANO resection classification. Neuro Oncol 25:940–954
15. Sonoda Y, Kumabe T, Nakamura T et al (2009) IDH mutation analysis in gliomas. Cancer Sci 100:1996–1998
16. Sasaki T, Fukai J, Kodama Y et al (2018) Molecular and clinical characteristics of gliomas. J Neurooncol 140:329–339

17. Umehara T, Arita H, Yoshioka E et al (2019) Copy number alteration profiles in glioblastoma. Acta Neuropathol Commun 7:99
18. Jeuken JW, Sijben A, Alenda C et al (2009) Detection of EGFR copy number alterations. Brain Pathol 19:661–671
19. Kikuchi Z, Shibahara I, Yamaki T et al (2020) TERT promoter mutation associated with poor prognosis. Neurooncol Adv 2:vdaa114
20. Gao J, Aksoy BA, Dogrusoz U et al (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6:pl1
21. Cerami E, Gao J, Dogrusoz U et al (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2:401–404
22. Wiestler B, Capper D, Holland-Letz T et al (2013) ATRX loss refines glioma classification. Acta Neuropathol 126:443–451
23. Takano S, Kato Y, Yamamoto T et al (2016) Ki-67 labeling index in gliomas. Brain Tumor Pathol 33:83–88
24. Arita H, Narita Y, Fukushima S et al (2013) TERT promoter mutations in gliomas. Acta Neuropathol 126:267–276
25. Diplas BH, He X, Brosnan-Cashman JA et al (2018) Genomic landscape of TERT wild-type glioblastoma. Nat Commun 9:2087
26. Yuan P, Cao JL, Abuduwufuer A et al (2016) TERT promoter mutations in cancer: meta-analysis. PLoS ONE 11:e0146803
27. Campos MA, Macedo S, Fernandes M et al (2019) TERT promoter mutations and prognosis in carcinoma. J Am Acad Dermatol 80:660–669
28. Barthel FP, Johnson KC, Varn FS et al (2019) Molecular trajectories of diffuse glioma. Nature 576:112–120

Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

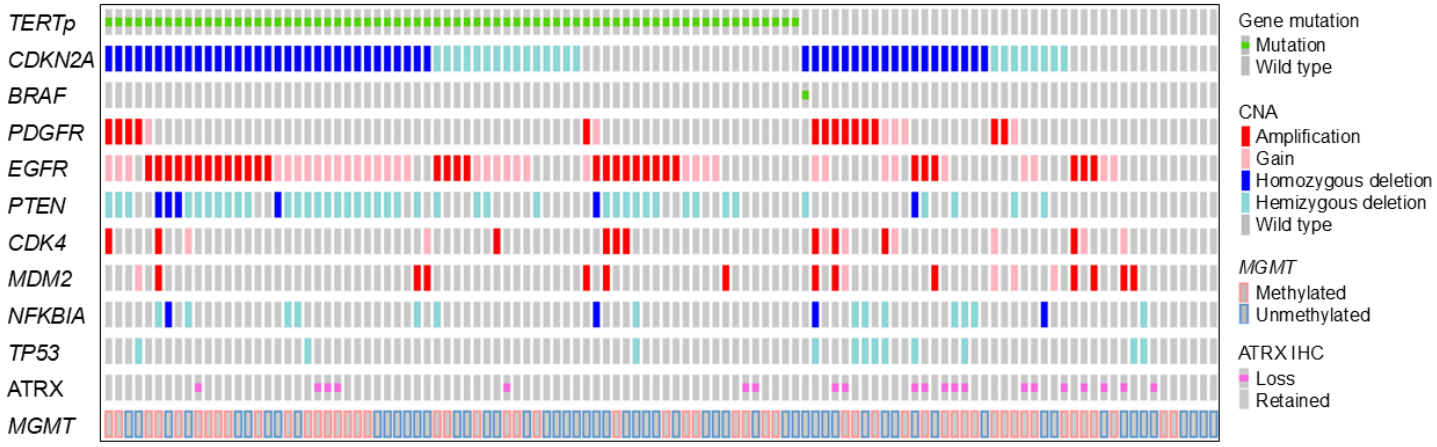

Figure 1

Genetic distribution of mutations across 112 cases of glioblastoma. IHC, Mutations, CNAs, and methylation were generated and visualized by OncoPrinter via the cBioPortal for Cancer Genomics (cbioportal.org/oncoprinter) with some modifications [20,21]. The diagram shows the landscape of the molecular characteristics of GBMs, which are sorted by *TERT*p mutations and CDKN2A deletion. Abbreviations: IHC, immunohistochemistry; CNA, copy number alteration; GBM, glioblastoma; N/A, not available.

Figure 2

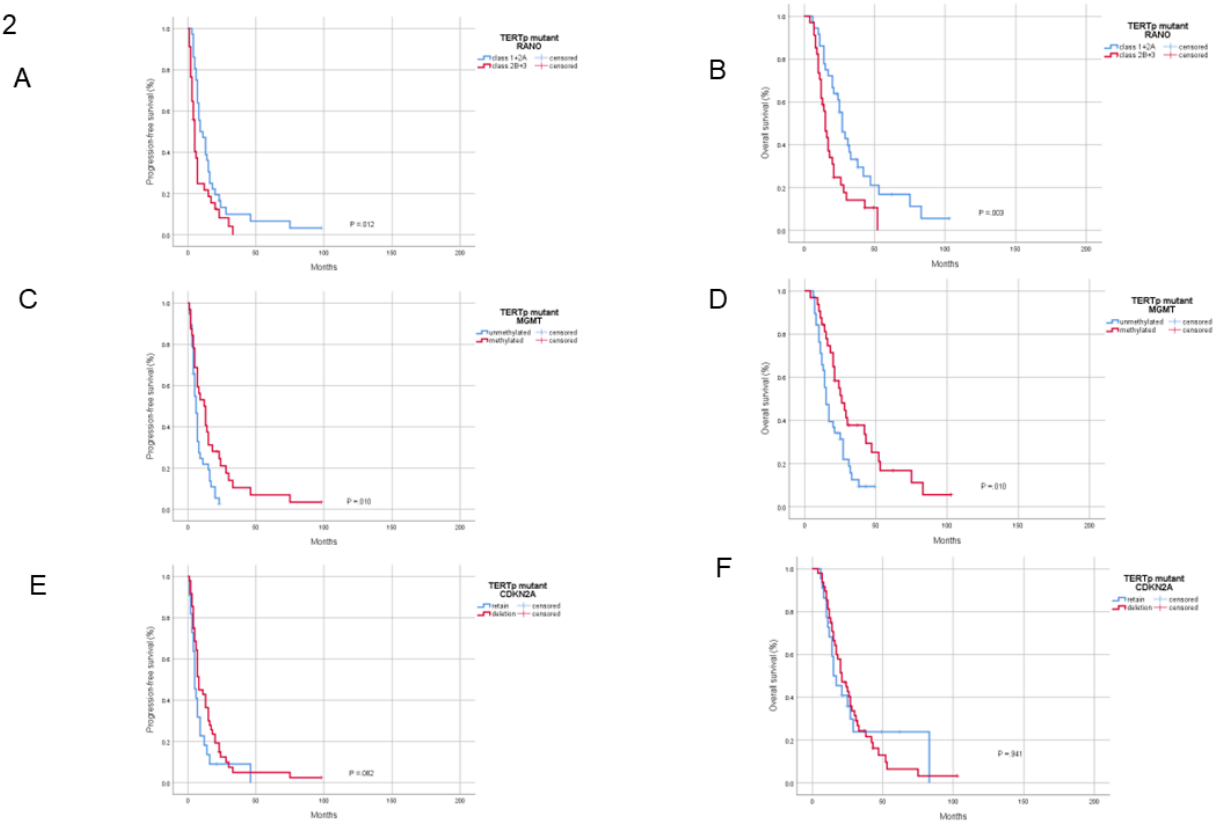

Figure 2

Kaplan–Meier survival curves in TERT promoter–mutant glioblastoma.
(A, B) Kaplan–Meier curves for PFS and OS according to the RANO classification (class 1/2A vs. 2B/3).
(C, D) Kaplan–Meier curves for PFS and OS according to MGMT promoter methylation status (methylated vs. unmethylated).
(E, F) Kaplan–Meier curves for PFS and OS according to CDKN2A status (deleted vs. intact).
In the TERT promoter–mutant subgroup, RANO classification and MGMT promoter methylation were significantly associated with survival, whereas CDKN2A status was not. Abbreviations: PFS, progression-free survival; OS, overall survival.

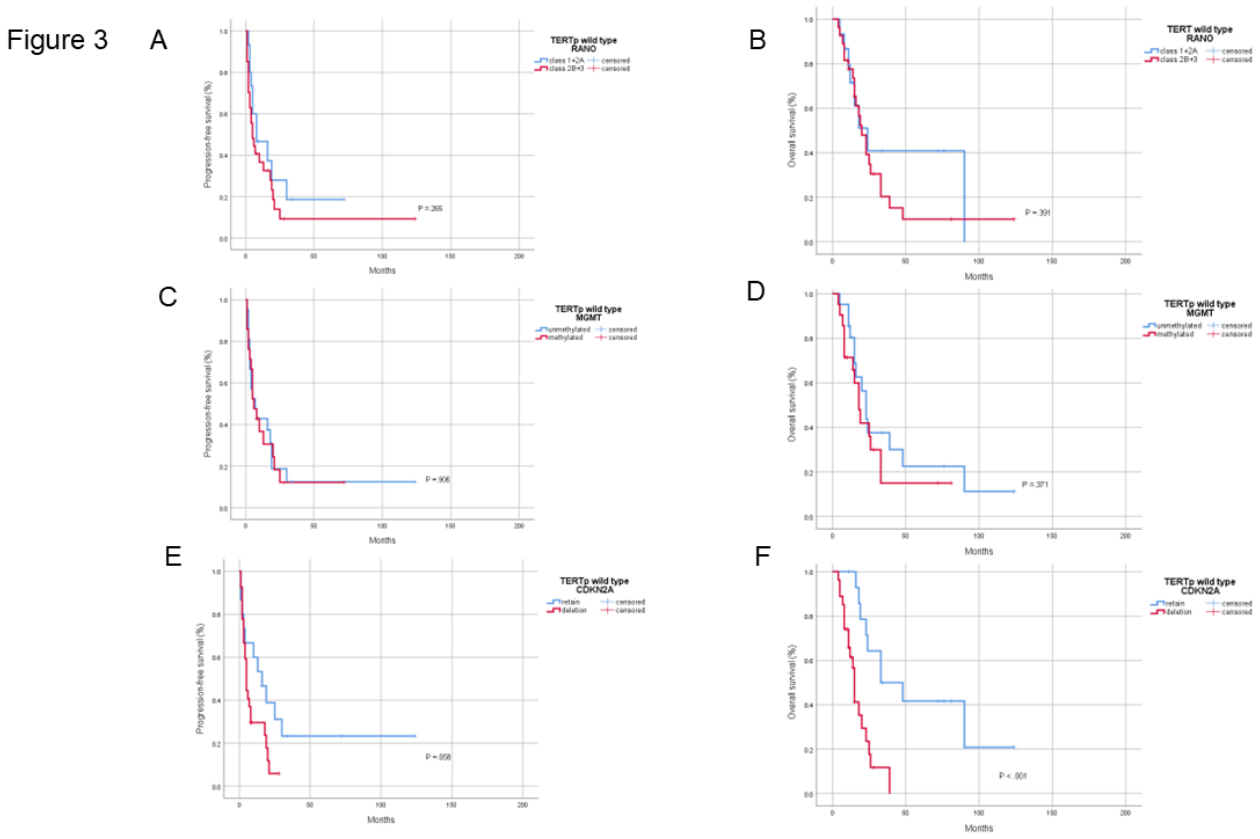

Figure 3

Kaplan–Meier survival curves in TERT promoter–wild-type glioblastoma.
(A, B) Kaplan–Meier curves for PFS and OS according to RANO classification (class 1/2A vs. 2B/3).
(C, D) Kaplan–Meier curves for PFS and OS according to MGMT promoter methylation status (methylated vs. unmethylated).
(E, F) Kaplan–Meier curves for PFS and OS according to CDKN2A status (deleted vs. intact).
In the TERT promoter–wild-type subgroup, CDKN2A deletion was significantly associated with worse OS. RANO classification and MGMT promoter methylation were not significantly associated with survival.

Abbreviations: PFS, progression-free survival; OS, overall survival.

Supplementary Files

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