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TERT promoter mutations contribute to subset prognostication of lower-grade gliomas

Aden Ka-Yin Chan[1,2,5], Yu Yao[3,5], Zhenyu Zhang[3], Nellie Yuk-Fei Chung[1,2], Joseph Shu-Ming Liu[1,2], Kay Ka-Wai Li[1,2], Zhifeng Shi[3], Danny Tat-Ming Chan[4], Wai Sang Poon[4], Liangfu Zhou[3] and Ho-Keung Ng[1,2]

[1]Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong, China; [2]The Chinese University of Hong Kong-Shenzhen Research Institute, Shenzhen, China; [3]Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai, China and [4]Neurosurgery Division, Department of Surgery, The Chinese University of Hong Kong, Hong Kong, China

Recurrent mutations in the promoter region of *telomerase reverse transcriptase* (*TERT*) have been found in various cancers including diffuse gliomas. Mutations lead to *TERT* upregulation and are associated with aggressive clinical behavior in glioblastomas. However, the clinical significance of *TERT* promoter mutations in lower-grade gliomas remains undetermined. The aim of this study is to evaluate the status of *TERT* promoter and the respective prognostic significance in a cohort of 237 lower-grade gliomas comprising grades II and III astrocytomas, oligodendrogliomas, and oligoastrocytomas. Mutually exclusive mutations in *TERT* promoter, C228T and C250T, were identified in 16/105 (15%) diffuse astrocytomas, 16/63 (25%) anaplastic astrocytomas, 13/18 (72%) oligodendrogliomas, 3/3 (100%) anaplastic oligodendrogliomas, 17/45 (38%) oligoastrocytomas, and 2/3 (67%) anaplastic oligoastrocytomas. Mutations co-occurred with 1p/19q codeletion ($P<0.001$) and are associated with oligodendroglial histology ($P<0.001$). Kaplan–Meier's survival analysis showed that *TERT* promoter mutation ($P=0.037$), *Isocitrate dehydrogenase (IDH)* mutation ($P<0.001$), and 1p/19q codeletion ($P<0.001$) were associated with favorable overall survival (OS). In the subset of 116 *IDH*-mutated lower-grade gliomas lacking 1p/19q codeletion, 19 *TERT* promoter-mutated tumors exhibited longer progression-free survival (PFS) ($P=0.027$) and OS ($P=0.004$). Consistent with this observation, in the subset of 97 *IDH*-mutated astrocytomas, 14 *TERT* promoter-mutated tumors showed longer PFS ($P=0.001$) and OS ($P=0.001$). In contrast, among the subset of 74 *IDH* wild-type lower-grade gliomas with intact 1p/19q, *TERT* promoter mutation was associated with shorter PFS ($P=0.001$) and OS ($P=0.001$). Similarly, in the subset of 65 *IDH* wild-type astrocytomas, 16 *TERT* promoter-mutated tumors exhibited unfavorable PFS ($P=0.007$) and OS ($P=0.008$). Our results indicate that when combined with *IDH* status, *TERT* promoter mutation contributes to prognostic subgroups of lower-grade astrocytic tumors or 1p/19q intact lower-grade gliomas and this may further refine future molecular classification of lower-grade gliomas.
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Diffuse gliomas, the most common primary malignant brain tumors, are classified by the World Health Organization (WHO) into astrocytoma, oligodendroglioma, and oligoastrocytoma based on histology and further graded into grade II to grade IV according to malignant features.[1] Lower-grade gliomas, comprising grade II and grade III diffuse gliomas, exhibit an infiltrative nature and intrinsic tendency to recur or progress to higher-grade lesion ie grade IV glioblastoma. Although the current classification of lower-grade gliomas is based on histology and has prognostic implication, heterogeneous clinical outcomes exist among gliomas within each group. Such histology-based classification system also leads interobserver variability in diagnosis.[2] Mounting evidence has suggested that biomarkers can aid tumor diagnosis, determine prognosis, and guide clinical management. Chromosome 1p and 19q codeletion, the genetic hallmark of

Correspondence: Dr AK-Y Chan or Professor H-K Ng, Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, 30-32 Ngan Shing Street, Shatin, Hong Kong, China or Professor L Zhou, Department of Neurosurgery, Huashan Hospital, Fudan University, Wulumuqi Road 12#, Shanghai 200040, China.
E-mail: adenchan@cuhk.edu.hk or hkng@cuhk.edu.hk or lfzhouc@126.com
[5]These authors contributed equally to this work and are co-first authors.
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oligodendrogliomas associated with long survival and chemo-radio sensitivity, represents the prototype molecular marker with unequivocal diagnostic, prognostic, and therapeutic utilities in diffuse gliomas.[3–7] *Isocitrate dehydrogenase* (*IDH*) mutation is probably the most important molecular marker discovered in diffuse gliomas with breakthrough clinical values in the recent years.[8] Apart from its use in difficult diagnostic situations,[8–10] mutation of this enzyme also stratifies diffuse gliomas prognostically.[11,12] Recently, recurrent mutations in the promoter region of *telomerase reverse transcriptase* (*TERT*), the gene encoding catalytic subunit of telomerase, were detected in ~70% of malignant melanomas.[13,14] Mutations caused a cytosine-to-thymine transition at the positions of chr5, 1 295 228 (C228T) and 1 295 250 (C250T), and generated an identical 11 base-pair nucleotide sequence (5′-CCC CTTCCGGG-3′) containing a consensus binding site (5′-TTCC-3′) for E-twenty-six transcription factors.[13–15] Importantly, this promoter mutation is associated with upregulation of *TERT* expression, suggesting it as a mechanism of telomerase activation in tumorigenesis.[14,16] Further studies demonstrated the high frequency of *TERT* promoter mutation in different histological types of diffuse gliomas, in particular oligodendrogliomas, oligoastrocytomas, and primary glioblastoma.[16–18] Although *TERT* promoter mutation was shown to be associated with poor clinical outcome in glioblastoma patients,[15,18] clinical value of this newly identified mutation in lower-grade gliomas remains elusive. In this study, we conducted mutational analysis for *TERT* promoter in 237 lower-grade gliomas with the aim to examine the clinical value of *TERT* promoter mutation in terms of classification and prognostication in lower-grade gliomas.

Materials and methods

Patients and Tissue Samples

A total of 237 lower-grade gliomas diagnosed between 1990 and 2012 with formalin-fixed paraffin-embedded tissues available were retrieved from the tissue archive of the Department of Anatomical and Cellular Pathology, Prince of Wales Hospital (Hong Kong) and Department of Neurosurgery, Huashan hospital (Shanghai), comprising 105 diffuse astrocytomas (WHO grade II; AII), 63 anaplastic astrocytomas (WHO grade III; AAIII), 18 oligodendrogliomas (WHO grade II; OII), 3 anaplastic oligodendrogliomas (WHO grade III; AOIII), 45 oligoastrocytomas (WHO grade II; OAII), and 3 anaplastic oligoastrocytomas (WHO grade III; AOAIII). All cases were stained with haematoxylin & eosin and reviewed according to the 2007 WHO criteria.[1] Clinical and survival data of the patients were retrieved from the respective institutional medical record systems. This study was approved by the Ethics Committee of Shanghai Huashan Hospital and the New Territories East Cluster-Chinese University of Hong Kong Ethics Committee.

Mutation Analysis of *TERT* Promoter and *IDH1*/*IDH2*

Tissues from representative tumor area with tumor content >70% were scrapped off from dewaxed sections and treated with proteinase K at a final concentration of 2 μg/μl in 10 mM Tris-HCl buffer (pH 8.5) at 55 °C for 2–18 h and then at 98 °C for 10 min. The crude cell lysate was centrifuged and supernatant was used for subsequent PCR analysis. The forward primer TERT-F (5′-GTCCTGCCCCTTCA CCTT-3′) and reverse primer TERT-R (5′-CAGCGCTG CCTGAAACTC-3′) were used to amplify a 163 bp fragment spanning the two mutational hotspots (chr5, 1 295 228 (C228T) and 1 295 250 (C250T)) in *TERT* promoter region (Figure 1, Supplementary Figure S1 and S2). PCR was performed in 10 μl reaction mixture containing 1 μl of cell lysate, 0.3 mM of each dNTP, 2.5 mM $MgCl_2$, 0.3 μM of each primer, and 0.2 U of KAPA HiFi HotStart DNA Polymerase (Kapa Biosystems Wilmington, DE, USA), and was initiated at 95 °C for 5 min, followed by 40–45 cycles of 98 °C for 20 s, 68 °C for 15 s and 72 °C for 30 s, and a final extension of 72 °C for 1 min. Products were then treated with exonuclease I and alkaline phosphatase (TakaRa, Japan). Sequencing was performed using BigDye Terminator Cycle Sequencing kit v1.1 (Life Technologies). The products were resolved in Genetic Analyzer 3130xl and analyzed by Sequencing Analysis software. Mutational hotspots of *IDH1* at R132 and *IDH2* at R172 were evaluated by direct sequencing as previously described.[19]

Chromosome 1p/19q Status by Fluorescence *in situ* Hybridization

Chromosome 1p/19q status was evaluated by fluorescence *in situ* hybridization as reported previously.[20] In brief, 5-μm thick formalin-fixed, paraffin-embedded sections were deparaffinized in xylene, treated with 1 M sodium thiocyanate at 80 °C for 10 min, digested in pepsin solution at 37 °C for 20–30 min, rinsed in milli-Q water and dehydrated.

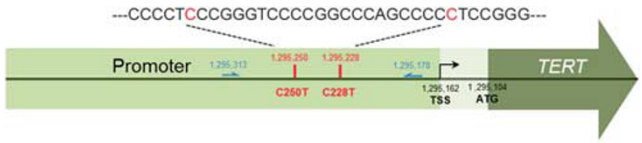

Figure 1 Schematic diagram showing the positions of the two mutation hotspots (red), the primers for PCR (blue), the transcription start site (TSS) (black) and translation start site (ATG) (black) in the promoter region of *TERT*. Numbers denote the nucleotide positions on chromosome 5. The sequence at the top shows the wild-type sequence of the promoter region spanning the two hotspots (red).

Locus-specific probes were generated from bacterial artificial chromosome clones using nick translation, in the presence of Spectrum Orange deoxyuridine triphosphate (dUTP) or Spectrum Green dUTP. The labeled probes were mixed with Cot-1 DNA (Life Technologies) in Hybrisol VI solution (Appligene Oncor, Graffenstaden, France), applied to the section and denatured. Hybridization was carried out at 37 °C overnight for 16 h. Sections were washed in 1.5 M urea in 0.1 × saline sodium citrate at 48 °C for 30 min and then in 2 × saline sodium citrate at 48 °C for 5 min. After washing, sections were stained with Vectashield mounting medium containing 4′,6-diamidino-2-phenylindole (Vector Laboratories) and viewed under a Zeiss Axioplan fluorescence microscope (Carl Zeiss Microscopy LLC, NY, USA). Hybridizing signals in at least 100 non-overlapping nuclei were counted. The loci interrogated were 1p36.3 (RP11-62M23 labeled red)/1q25.3-q31.1 (RP11-162L13 labeled green) and 19q13.3 (CTD-2571L23 labeled red)/19p12 (RP11-420K14 labeled green). A sample was considered 1p or 19q deleted when >50% of counted nuclei exhibited one target (red) signal and two reference (green) signals.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 20 (IBM Corporation, NY, USA). Correlation between molecular markers and clinical parameters were examined by χ^2-test or Fisher's exact test, whichever was appropriate. Comparison between two groups was performed by Student's *t*-test or Mann–Whitney *U*-test. Comparison between three or more groups used one-way analysis of variance (ANOVA) and *post hoc* analysis with Bonferroni correction. Overall survival (OS) was defined as the time between diagnosis and death or last follow-up. Progression-free survival (PFS) was defined as the time between diagnosis and tumor recurrence or progression. Survival curves were plotted by Kaplan–Meier method and analyzed by Log-rank test. Multivariate analysis was performed by Cox proportional hazards model. *P*-value of <0.05 (two sided) was considered statistically significant.

Results

Cohort Characteristic

The male to female ratio of the cohort was 1:0.63. The mean and median age of the patients were 40.5 years and 40 years (range 3–9), respectively. There were 15 pediatric patients (at or below 18 years) (five diffuse astrocytomas, five anaplastic astrocytomas, one oligodendroglioma and four oligoastrocytomas) in the cohort. Among the 224 cases with known tumor location, 89 (40%), 49 (22%), 25 (11%), and 3 (1%) tumors located in frontal, temporal, parietal, and occipital lobe, respectively. Twelve (5%) cases involved infratentorial region, 24 (11%) cases involved more than one lobe and 22 (10%) cases involved other regions including corpus callosum, thalamus, and basal ganglia. Treatment data in operation, radiotherapy, and chemotherapy was available in 214 of 237 (90%), 201 of 237 (85%), and 200 of 237 (84%) patients, respectively. In total,123 of 214 (58%) patients received total resection, 145 of 201 (72%) patients received radiotherapy and 114 of 200 (57%) patients received chemotherapy. In total, 101 of 200 (51%) patients received concomitant chemoradiotherapy.

TERT Promoter Mutation

Mutation in *TERT* promoter was found in 67 of 237 (28%) lower-grade gliomas examined, including 16 of 105 (15%) diffuse astrocytomas, 16 of 63 (25%) anaplastic astrocytomas, 13 of 18 (72%) oligodendrogliomas, 3 of 3 (100%) anaplastic oligodendrogliomas, 17 of 45 (38%) oligoastrocytomas and, 2 of 3 (67%) anaplastic oligoastrocytomas. Two pediatric astrocytomas (one diffuse astrocytoma and one anaplastic astrocytoma) harbored *TERT* promoter mutation. Among the 67 mutated tumors, C228T and C250T mutations were mutually exclusive and were observed in 44 (19%) cases (12/105 diffuse astrocytomas, 12/63 anaplastic astrocytomas, 7/18 oligodendrogliomas, 2/3 anaplastic oligodendrogliomas, 10/45 oligoastrocytomas and 1/3 anaplastic oligoastrocytomas) and 23 (10%) cases (4/105 diffuse astrocytomas, 4/63 anaplastic astrocytomas, 6/18 oligodendrogliomas, 1/3 anaplastic oligodendrogliomas, 7/45 oligoastrocytomas and 1/3 anaplastic oligoastrocytomas), respectively (Table 1, Figure 2a and Supplementary Figure S2).

Correlating *TERT* promoter mutation with clinicopathological variables, patients with *TERT* promoter mutation were older than those without the mutation (mean age 44.3 *vs* 39, $P=0.007$). No association was observed between *TERT* promoter mutation and gender. Tumors with oligodendroglial histology showed high frequency of *TERT* promoter mutation (16/21, 76%) compared with tumors with mixed oligoastrocytic histology (19/48, 40%) and tumors with astrocytic histology (32/168, 19%) ($P<0.001$) (Figure 2b).

Forty-nine of 67 (73%) *TERT*-mutated tumors harbored *IDH* mutation and 109 of 170 (64%) *TERT* wild-type tumors had *IDH* mutation (Figure 2c). Among the *IDH*-mutated tumors, *TERT* promoter mutation was found in 14/98 (14%), 16/20 (80%), and 19/40 (48%) gliomas with astrocytic, oligodendroglial, and oligoastrocytic histology, respectively ($P<0.001$). Among tumors with wild-type *IDH*, *TERT* promoter mutation was detected in 18/70 (22%) gliomas with astrocytic histology exclusively (6 diffuse astrocytomas and 12 anaplastic astrocytomas). Wild-type *TERT* was found in 52/70 (74%), 1/1, and 8/8 tumors with astrocytic, oligodendroglial,

Table 1 Summary of clinical and molecular data of lower-grade gliomas in the study

						TERT promoter mutation			*IDH mutation*			
Diagnosis	N	*Male*	*Mean age (years)*	*Median PFS (months)*	*Median OS (months)*	*C228T*	*C250T*	*Combined %*	*IDH1*	*IDH2*	*Combined %*	*1p/19q codeletion (%)*
Diffuse astrocytoma	105	60%	39.9	65.3	101.2	12	4	15%	69	2	68%	7%
Anaplastic astrocytoma	63	76%	43.3	16	22	12	4	25%	26	1	43%	0%
Oligodendroglioma	18	44%	41.2	108	NR	7	6	72%	17	0	94%	59%
Anaplastic oligodendroglioma	3	33%	50.7	24	24	2	1	100%	3	0	100%	67%
Oligoastrocytoma	45	51%	37.2	NR	120.6	10	7	38%	34	3	82%	41%
Anaplastic oligoastrocytoma	3	67%	38	19.6	37.2	1	1	67%	2	1	100%	0%

Abbreviations: N, number of case; NR, median survival not yet reached; OS, overall survival; PFS, progression-free survival.

and oligoastrocytic histology, respectively. Opposite correlations between *IDH* mutation and *TERT* promoter mutation were observed in subsets of astrocytic tumors with intact 1p/19q and oligodendroglial tumors. Among the astrocytic tumors with intact 1p/19q, 9 of 27 (33%) *TERT*-mutated tumors harbored *IDH* mutation and 80 of 131 (61%) *TERT* wild-type tumors had *IDH* mutation ($P=0.008$) (Figure 2d). In contrast, among the subsets of oligodendroglial tumors, all 35 *TERT*-mutated tumors harbored *IDH* mutation and 25 of 34 (74%) *TERT* wild-type tumors had *IDH* mutation ($P=0.001$) (Figure 2e). Notably, Patients with *IDH* wild-type-*TERT*-mutated tumors were older (mean age = 51 years) than those with *IDH* wild-type-*TERT* wild-type tumors (mean age = 36.3 years, $P<0.001$), *IDH*-mutated-*TERT* wild-type tumors (mean age = 40.6 years, $P=0.012$) and trended to be older than *IDH*-mutated-*TERT*-mutated tumors (mean age = 41.8 years, $P=0.068$) (Figure 2g).

Chromosome 1p/19q codeletion was detected in 28 of 65 (43%) *TERT*-mutated tumors and 9 of 167 (5%) *TERT* wild-type tumors ($P<0.001$) (Figure 2f). Among the 1p/19q codeleted tumors, *TERT* promoter mutation was identified in 5/7 (71%) gliomas with astrocytic histology, 10/12 (83%) gliomas with oligodendroglial histology, and 13/18 (72%) gliomas with oligoastrocytic histology. Among tumors lacking 1p/19q codeletion, 27/158 (17%) gliomas with astrocytic histology, 5/8 (63%) gliomas with oligodendroglial histology and 5/29 (17%) gliomas with oligoastrocytic histology harbored *TERT* promoter mutation ($P=0.006$).

1p/19q Codeletion

Chromosome 1p/19q codeletion was detected in 37/232 (16%) cases including 7/102 (7%) diffuse astrocytomas, 10/17 (59%) oligodendrogliomas, 2/3 (67%) anaplastic oligodendrogliomas, and 18/44 (41%) oligoastrocytomas (Table 1, Figure 2a). Five cases (three diffuse astrocytomas, one oligodendroglioma and one oligoastrocytoma) were not examined for 1p/19q FISH owing to the lack of tissue section. None of the 15 pediatric gliomas had 1p/19q codeletion. All 37 1p/19q codeleted tumors harbored *IDH* mutation and 117/195 (60%) tumors lacking 1p/19q codeletion harbored *IDH* mutation ($P<0.001$).

IDH Mutation

IDH mutation was found in 71/105 (68%) diffuse astrocytomas, 27/63 (43%) anaplastic astrocytomas, 17/18 (94%) oligodendrogliomas, 3/3 (100%) anaplastic oligodendrogliomas, 37/45 (82%) oligoastrocytomas, and 3/3 (100%) anaplastic oligoastrocytomas, with an overall mutation frequency of 67% (158/237) (Table 1, Figure 2a). In the 158 *IDH*-mutated gliomas, 151 tumors harbored *IDH1* mutation and 7 tumors harbored *IDH2* mutation. One 18-year-old patient with anaplastic astrocytoma had mutation in *IDH1*.

Survival Analysis

Follow-up data were available in 231 patients. The median follow-up, median PFS and median OS of the cohort were 113 months, 56 months, and 83.2 months, respectively.

Univariate analysis showed age ≤ 35 years ($P=0.02$), WHO grade II ($P<0.001$), oligodendroglial histology ($P=0.006$), *IDH* mutation ($P<0.001$), and 1p/19q codeletion ($P<0.001$) were associated with longer PFS. Age ≤ 35 years ($P=0.005$), WHO grade II ($P<0.001$), oligodendroglial histology ($P<0.001$), *TERT* promoter mutation ($P=0.037$), *IDH* mutation ($P<0.001$), and 1p/19q codeletion ($P<0.001$) were associated with longer OS (Table 2, Figure 3a–f). Prognostic value of *TERT* promoter mutation was further evaluated in subset analysis (Table 3, Figure 3g–r). Among the 157 *IDH*-mutated lower-grade gliomas, 49 *TERT* promoter-mutated tumors showed longer progression-free survival

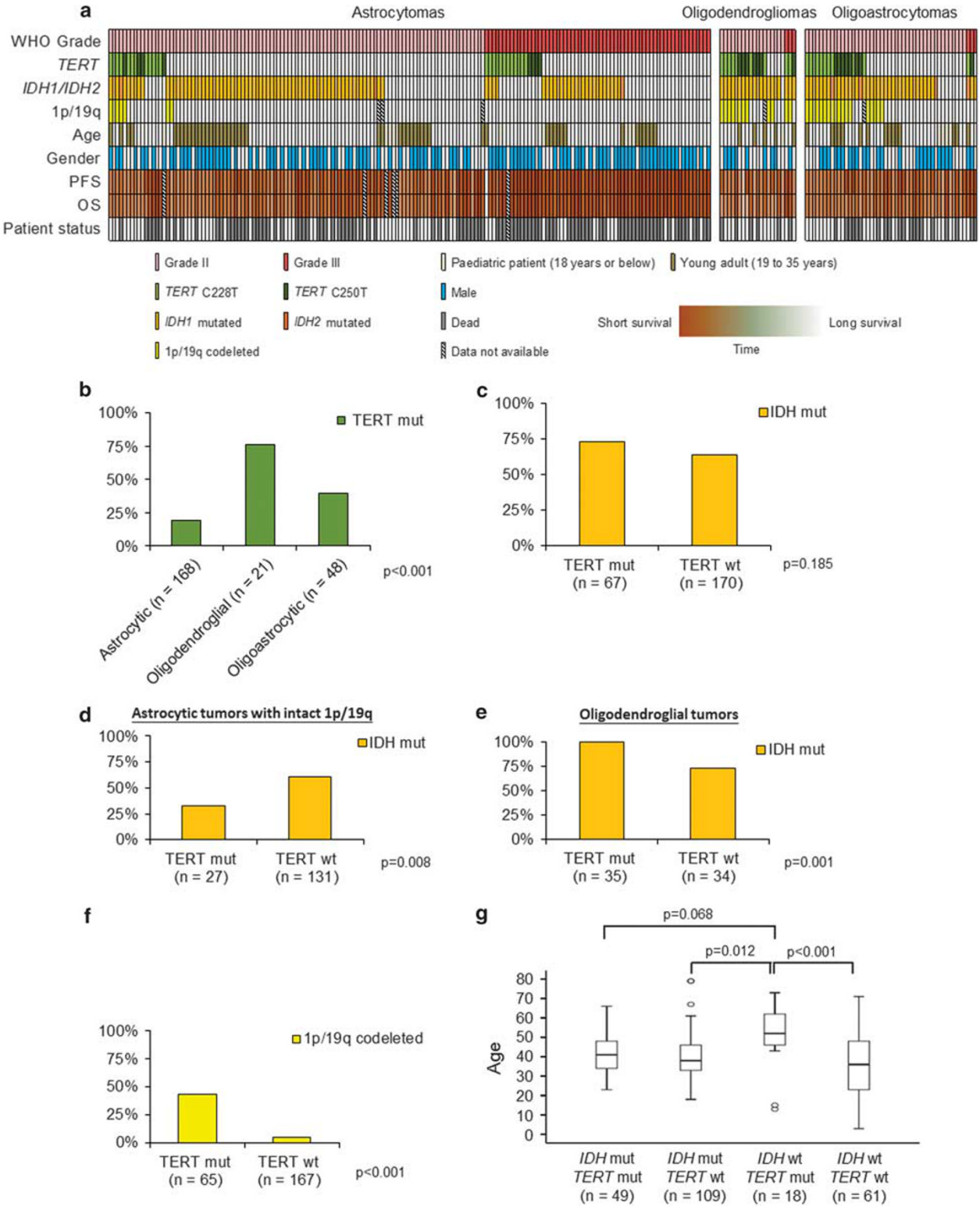

Figure 2 Correlations between clinicopathological and molecular variables of lower-grade gliomas. Clinical and molecular data of 237 lower-grade gliomas (**a**). *TERT* promoter mutation is associated with oligodendroglial histology ($P<0.001$, χ^2-test) (**b**). *TERT* promoter mutation was not associated with *IDH* mutation in the whole cohort ($P=0.185$, χ^2-test) (**c**). *TERT* promoter mutation was inversely associated with *IDH* mutation among astrocytic tumors with intact 1p/19q ($P=0.008$, χ^2-test) (**d**), co-occurred with *IDH* mutation among oligodendroglial tumors ($P=0.001$, Fisher's exact test) (**e**), and co-occurred with 1p/19q codeletion in the whole cohort ($P<0.001$, χ^2-test) (**f**). Patients with *IDH* wild-type-*TERT*-mutated gliomas are older than those with *IDH* wild-type-*TERT* wild-type tumors, *IDH*-mutated-*TERT*-wild-type tumors and trended to be older than *IDH*-mutated-*TERT*-mutated tumors, (One-way ANOVA) (**g**). Mut, mutated; wt, wild type.

Table 2 Univariate analysis of clinical and molecular variables

Variables	N	*Median PFS (months)*	P	*Median OS (months)*	P
Age					
≤35 years	81	78	0.02	141.5	0.005
>35 years	150	48		65.6	
WHO Grade					
Grade II	163	84	<0.001	122.5	<0.001
Grade III	68	19.6		24	
Histology					
Astrocytic	162	47	0.006	59.1	<0.001
Oligodendroglial	21	108		NR	
Oligoastrocytic	48	108.4		120.6	
TERT promoter mutation					
Mutant	65	108	0.103	126	0.037
Wild type	166	48		67	
IDH mutation					
Mutant	157	71	<0.001	120	<0.001
Wild-type	74	20		24	
1p/19q codeletion					
Yes	37	NR	<0.001	142.1	<0.001
No	189	48		60	

Abbreviations: N, number of case; NR, median survival not yet reached; OS, overall survival; PFS, progression-free survival.

($P=0.001$) and longer OS ($P<0.001$). As *TERT* promoter mutation was highly associated with 1p/19q codeletion, which may account for the favorable prognostic effect, we evaluated the subset of 116 *IDH*-mutated tumors lacking 1p/19q codeletion and found that 19 *TERT* promoter-mutated tumors exhibited favorable PFS ($P=0.027$) and OS ($P=0.004$). Consistent with this observation, subset analysis in 97 *IDH*-mutated astrocytomas also revealed that *TERT* promoter mutation in 14 astrocytomas was associated with good prognosis in both PFS ($P=0.001$) and OS ($P=0.001$). In contrast, among the subset of 74 *IDH* wild-type lower-grade gliomas, *TERT* promoter mutation was associated with shorter PFS ($P=0.001$) and OS ($P=0.001$). In the subset of 65 *IDH* wild-type astrocytomas, 16 *TERT* promoter-mutated astrocytomas exhibited unfavorable PFS ($P=0.007$) and OS ($P=0.008$).

Multivariate analysis was performed by Cox proportional hazards model to evaluate the independent prognostic values of the clinical and molecular variables (Table 4). Variables evaluated in multivariate analysis included age, WHO grade, tumor histology, *TERT* promoter mutation, *IDH* mutation, and 1p/19q codeletion. Age≤35 years ($P=0.001$), WHO grade II ($P<0.001$), *IDH* mutation ($P=0.013$) and 1p/19q codeletion ($P=0.036$) were favorable prognostic factors for PFS. Age≤35 years ($P=0.001$), WHO grade II ($P<0.001$), and *IDH* mutation ($P=0.009$) were favorable prognostic factors for OS. 1p/19q codeletion showed a strong trend as good prognostic factor for OS ($P=0.067$). As *TERT* promoter mutation showed opposite prognostic value in *IDH*-mutated and *IDH* wild-type tumors in univariate analysis, multivariate analysis was conducted separately in the two subsets to further define the prognostic implication of *TERT* promoter mutation. Among *IDH*-mutated tumors, Age≤35 years ($P=0.015$), WHO grade II ($P=0.001$), *TERT* promoter mutation ($P=0.007$), and 1p/19q codeletion ($P=0.047$) were favorable prognostic factors for PFS. Age≤35 years ($P=0.013$), WHO grade II ($P<0.001$), and *TERT* promoter mutation ($P=0.002$) were good prognostic factors for OS. 1p/19q codeletion showed a strong trend as good prognostic factor for OS ($P=0.057$). Among *IDH* wild-type tumors, WHO grade II was a favorable prognostic factor for PFS ($P=0.002$) and OS ($P<0.001$). *TERT* promoter mutation exhibited as a poor prognostic factor for PFS ($P=0.027$) and a trend as a poor prognostic factor for OS ($P=0.07$).

Discussion

Our study evaluated the frequency of *TERT* promoter mutation and its clinical significance in lower-grade gliomas. We demonstrated that *TERT* promoter mutation was frequently detected in lower-grade gliomas, particularly in oligodendroglial tumors. The strong association of *TERT* promoter mutation with oligodendroglial histology and 1p/19q codeletion suggested that this activating mutation was involved in oligodendroglial oncogenesis, which was also demonstrated by other groups.[15–17] Koelsche *et al*[17] analyzed over 1500 tumors of nervous system and found that *TERT* promoter mutation was inversely associated with *IDH* mutation. Similar inverse correlation was shown by Nonoguchi *et al*[18] examining over 350 glioblastomas. In our cohort of lower-grade gliomas, no correlation was found between *IDH* mutation and *TERT* promoter mutation. Interestingly, we observed opposite correlations between the two molecular markers in different subsets of tumors—inverse correlation in the subset of astrocytomas with intact 1p/19q and co-occurring correlation in the subset of oligodendroglial tumors. These observations were consistent with the current literature[15–18] and we speculated that the co-occurring association between 1p/19q codeletion and *IDH* mutation as well as 1p/19q codeletion and *TERT* mutation 'neutralized' the inverse correlation between *IDH* mutation and *TERT* mutation in the whole cohort. Nevertheless, *IDH* mutation and 1p/19q codeletion have been suggested as early event in gliomagenesis.[21,22] With the high frequency of *TERT* promoter mutation identified in oligodendrogliomas and its co-occurrence with 1p/19q codeletion, it will be interesting to examine these molecular markers in paired primary and recurrent oligodendrogliomas to determine their temporal relationship.

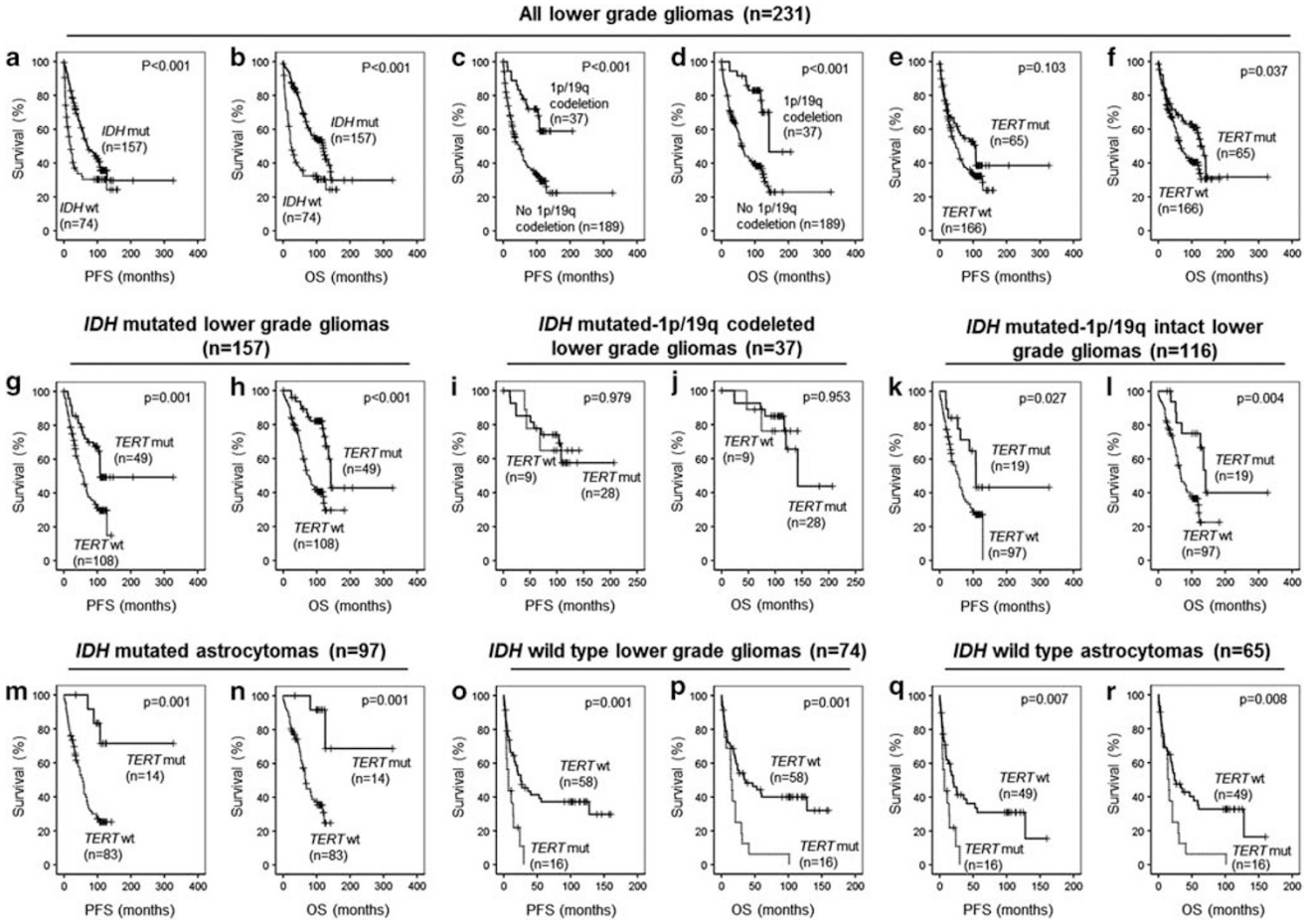

Figure 3 Kaplan–Meier survival analysis of *IDH* mutation, 1p/19q codeletion and *TERT* promoter mutation in lower-grade gliomas. Among all lower-grade gliomas analyzed, *IDH* mutation was associated with longer PFS (**a**) and OS (**b**), 1p/19q codeletion was associated with longer PFS (**c**) and OS (**d**), *TERT* promoter mutation was associated with longer OS (**f**) but not PFS (**e**). Among all *IDH*-mutated tumors, *TERT* promoter mutation was associated with longer PFS (**g**) and OS (**h**). Among all *IDH*-mutated-1p/19q codeleted tumors, *TERT* promoter mutation was not associated with PFS (**i**) nor OS (**j**). Among all *IDH*-mutated-1p/19q intact tumors, *TERT* promoter mutation was associated with longer PFS (**k**) and OS (**l**). Among all *IDH*-mutated astrocytomas, *TERT* promoter mutation was associated with longer PFS (**m**) and OS (**n**). In *IDH* wild-type tumors, *TERT* promoter mutation was associated with shorter PFS (**o**) and OS (**p**). Among *IDH* wild-type astrocytomas, *TERT* promoter mutation was associated with shorter PFS (**q**) and OS (**r**).

Apart from the subgroup of lower-grade gliomas harboring all the three genetic alterations, there existed *TERT* promoter-mutated lower-grade gliomas lacking 1p/19q codeletion and *IDH* mutation. Importantly, prognostic difference was found between these molecular subgroups. *TERT* promoter mutation had opposite prognostic values in *IDH*-mutated and *IDH* wild-type tumors in both univariate analysis and multivariate analysis. In *IDH* wild-type lower-grade gliomas, *TERT* promoter mutation identified aggressive tumors. *TERT* promoter mutation was frequently detected in primary glioblastoma lacking *IDH* mutation and was associated with poor prognosis in glioblastoma patients.[15,17] The *IDH* wild-type-*TERT*-mutated lower-grade gliomas identified in our series, with a similarly worsened outcome compared with the *IDH*-mutated lower-grade gliomas, may require more treatment and follow-up. In contrast, among *IDH*-mutated lower-grade gliomas, particularly in astrocytomas, *TERT* promoter mutation exhibited favorable prognosis. Existence of this good prognostic subgroup in *IDH*-mutated lower-grade gliomas was further evidenced by the association between *TERT* promoter mutation and good prognosis in *IDH*-mutated tumors lacking 1p/19q codeletion as 1p/19q codeletion is a group with good prognosis anyway. These findings suggested that *TERT* promoter mutation could potentially aid the molecular stratification of lower-grade gliomas in addition to *IDH* mutation and 1p/19q codeletion, especially in patients with astrocytic tumors.

Contrary to the concept of promoter methylation leading to gene silencing, *TERT* promoter methylation was found to be associated with *TERT* expression in other cancers including pediatric brain tumors.[23–25] Together with the exceedingly low frequency of *TERT* promoter mutation identified in

Table 3 Univariate analysis in different subsets of lower-grade gliomas

Lower-grade glioma subset	*TERT promoter*	N	*Median PFS (months)*	P	*Median OS (months)*	P
IDH mutated						
	Mutated	49	108.4	0.001	142.1	<0.001
	Wild type	108	58		74.4	
IDH-mutated-1p/19q codeleted						
	Mutated	28	NR	0.979	142.1	0.953
	Wild type	9	NR		NR	
IDH-mutated-1p/19q intact						
	Mutated	19	108.4	0.027	141.5	0.004
	Wild type	97	56		67	
IDH-mutated astrocytomas						
	Mutated	14	NR	0.001	NR	0.001
	Wild type	83	56		67	
IDH wild type						
	Mutated	16	7	0.001	14.1	0.001
	Wild type	58	25.6		36	
IDH wild-type astrocytomas						
	Mutated	16	7	0.007	14.1	0.008
	Wild type	49	20.3		24	

Abbreviations: N, number of case; NR, median survival not yet reached; OS, overall survival; PFS, progression-free survival.

Table 4 Multivariate analysis in different subsets of lower-grade gliomas

Tumor subset	*Variables*	*PFS*		*OS*	
		HR (95% CI)[a]	P	*HR (95% CI)*[a]	P
All lower-grade gliomas	Age ≤35 years	0.54 (0.37–0.79)	0.001	0.52 (0.34–0.78)	0.001
	WHO grade II	0.36 (0.24–0.54)	<0.001	0.28 (0.18–0.43)	<0.001
	Oligodendroglial	1	0.991	0.54	0.183
	Oligoastrocytic	0.87	0.579	0.82	0.464
	TERT promoter mutation	0.91	0.695	0.83	0.433
	IDH mutation	0.63 (0.43–0.91)	0.013	0.6 (0.41–0.88)	0.009
	1p/19q codeletion	0.49 (0.25–0.96)	0.036	0.47	0.06
IDH-mutated lower-grade gliomas	Age ≤35 years	0.55 (0.34–0.89)	0.015	0.51 (0.3–0.87)	0.013
	WHO grade II	0.39 (0.22–0.69)	0.001	0.3 (0.16–0.55)	<0.001
	Oligodendroglial	1.6	0.215	0.8	0.65
	Oligoastrocytic	1.13	0.675	1.15	0.645
	TERT promoter mutation	0.44 (0.24–0.8)	0.007	0.34 (0.18–0.66)	0.002
	1p/19q codeletion	0.5 (0.25–0.99)	0.047	0.46	0.057
IDH wild-type lower-grade gliomas	Age ≤35 years	0.91	0.762	0.93	0.829
	WHO grade II	0.36 (0.19–0.69)	0.002	0.29 (0.15–0.58)	<0.001
	Oligodendroglial	<0.001	0.974	<0.001	0.975
	Oligoastrocytic	0.54	0.29	0.37	0.186
	TERT promoter mutation	2.16 (1.09–4.28)	0.027	1.83	0.07

Abbreviations: 95%CI, 95% confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.
[a]95% confidence intervals are shown for variables with significant *P*-value.

over 350 pediatric brain tumors by Koelsche *et al*,[17] *TERT* promoter methylation probably represents a major mechanism for telomerase activation in pediatric brain tumors. Intriguingly, a recent study by Arita *et al*[26] demonstrated that *TERT* promoter mutation rather than methylation was the main mechanism for *TERT* upregulation in adult gliomas. Such results not only illustrated the diverse mechanisms of telomerase activation in different cancer types, but also demonstrated the distinct pathogenesis between pediatric and adult brain tumors.

One of the crucial findings in this study was the opposite prognostic values of *TERT* promoter mutation in *IDH*-mutated and *IDH* wild-type lower-grade

gliomas. Interestingly, subgroup-specific prognostic implication of *TERT* promoter mutation was also demonstrated in medulloblastoma by Remke *et al*[27], with the mutation identified patients with good prognosis in SHH subgroup and patients with bad prognosis in Group 4 subgroup. Such subgroup-specific prognostic values, together with its high frequency in certain groups of brain tumors and the relatively easy assay method (PCR followed by direct sequencing), made *TERT* promoter mutation as an emerging molecular marker for patient stratification in neuro-oncology.

In conclusion, *TERT* promoter is frequently mutated in lower-grade gliomas, with a particularly high incidence in oligodendroglial tumors. The mutations identify a favorable prognostic subset of *IDH*-mutated-1p/19q intact or astrocytic tumors and an aggressive subset of *IDH* wild-type tumors. Our study suggests the potential values of *TERT* promoter mutational analysis in molecular classification and prognostic evaluation in lower-grade gliomas in the era of personalized medicine.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)