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Brief Communication

BRIEF COMMUNICATION

TERT Promoter Mutations and Risk of Recurrence in Meningioma

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Abstract

The World Health Organization (WHO) classification and grading system attempts to predict the clinical course of meningiomas based on morphological parameters. However, because of high interobserver variation of some criteria, more reliable prognostic markers are required. Here, we assessed the *TERT* promoter for mutations in the hotspot regions C228T and C250T in meningioma samples from 252 patients. Mutations were detected in 16 samples (6.4% across the cohort, 1.7%, 5.7%, and 20.0% of WHO grade I, II, and III cases, respectively). Data were analyzed by *t* test, Fisher's exact test, log-rank test, and Cox proportional hazard model. All statistical tests were two-sided. Within a mean follow-up time in surviving patients of 68.1 months, *TERT* promoter mutations were statistically significantly associated with shorter time to progression

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($P < .001$). Median time to progression among mutant cases was 10.1 months compared with 179.0 months among wild-type cases. Our results indicate that the inclusion of molecular data (ie, analysis of *TERT* promoter status) into a histologically and genetically integrated classification and grading system for meningiomas increases prognostic power. Consequently, we propose to incorporate the assessment of *TERT* promoter status in upcoming grading schemes for meningioma.

Meningiomas are the most common primary central nervous system tumors, accounting for a third of all intracranial and spinal neoplasms. While 80% of meningioma patients can be cured by surgery alone, the tumors recur in up to 20% of patients. To identify patients at risk for recurrence, surgical specimens are subjected to histopathological evaluation and grading according to the World Health Organization (WHO) classification (1). However, some of the current grading criteria are vaguely defined and prone to a high interobserver bias (2–4). In consequence, a fraction of patients requiring adjuvant therapy are not identified while others may receive dispensable radiation therapy. Thus, meningioma classification and grading needs more powerful markers to identify the risk of recurrence, and, similar to other brain tumors, molecular parameters may contribute to this aim.

A potential biomarker for meningioma is *TERT* promoter mutation. The protein encoded by the *TERT* gene, telomerase reverse transcriptase, contributes essentially to the immortalization of cancer cells by extending their telomeres. Mutations in the promoter region at hotspots chr5:1,295,228 (C228T) or chr5:1,295,250 (C250T), result in new binding sites for members of the E-twenty-six (ETS) transcription factor family. Increased ETS-binding drives upregulation of *TERT* expression and consecutively maintains telomere length of the proliferating cancer cells (5–7). Interestingly, increased Ets-1 expression appears associated with aggressive courses of meningioma (8). After initial detection of *TERT* promoter mutations in melanoma (5,6), we have recently reported on the presence of such mutations in meningiomas, predominantly of higher grade (9). Others subsequently have described six meningioma cases with *TERT* mutations, five of which associated with recurrence (10).

Despite the reported associations with higher grade and recurrence in a few cases, no data is available on the prognostic power of *TERT* promoter mutations in meningiomas. Thus, we set out to identify its potential role and investigated the *TERT* promoter status in a cohort of 252 meningioma patients with clinical follow-up data and compared the prediction power of *TERT* status to that of the current WHO classification.

Tissue (119 meningioma WHO grade I, 88 WHO grade II, and 45 WHO grade III, mean observation time = 68.08 months, median = 66.87 months) was obtained from the Institutes and Departments of Neuropathology or Neurosurgery at the University Hospitals Berlin, Bonn, Hamburg, Heidelberg, Homburg, Magdeburg, Münster, Würzburg, Tübingen (all Germany), and the MD Anderson Cancer Center, Houston, TX, in accordance with local ethical requirements, based on respective institutional review board approval and informed consent by the patients for scientific use of tissue and data. Simpson grade was assessed by the neurosurgeon intra-operatively; grading was based on the WHO classification for brain tumors of 2007 (1).

Sanger Sequencing was performed as previously described (9). MD Anderson cases underwent targeted next-generation sequencing (Supplementary Methods, available online).

Fisher's exact test (two-tailed) was used for nominal variables, adjusted with Bonferroni correction. Univariate survival analysis was performed with the Kaplan-Meier estimator and log-rank test (for multivariable analysis and conditional tree, see Supplementary Methods, available online). Prediction power was assessed with a modified Brier score; the calculation was done with the R package pec (split method "Boot632plus" with 1000 iterations, R version 3.2). All statistical tests were two-sided, and a P value of less than .05 was considered statistically significant.

Of 252 meningiomas, 16 carried a *TERT* promoter mutation (6.4%) (Table 1). Of 119 WHO grade I tumors, two harbored the mutation (1.7%, both C228T). Of 88 WHO grade II tumors, five carried the mutations (5.7%, thereof 2 with C228T and three with C250T variant), and of 45 WHO grade III tumors, nine presented with *TERT* promoter mutations (20.0%, thereof 5 with C228T and 4 with C250T variant). Mutational variants were not associated with sex, localization, or distinct outcome, nor was *TERT* mutation associated with certain localizations (8 tumors were located at the skull base and 8 at the convexity). WHO grade, time to progression (TTP), age at diagnosis, sex, localization, and mutational variant are given in Supplementary Table 1 (available online).

Among grade I tumors, *TERT* promoter mutation was statistically significantly associated with morphologically progressive recurrence, ie, recurrence presenting as tumor of higher WHO grade (Fisher's exact test $P = .01$). Among grade II and grade III tumors, *TERT* promoter mutations were also associated with recurrence ($P = .02$ and $P = .009$, respectively) (Table 1). Importantly, *TERT* promoter mutation was a prognostic marker of unfavorable TTP over the entire cohort (Figure 1), with a mean observation time of 68.1 months. And here, *TERT* mutated meningiomas WHO grade III recurred statistically significantly earlier than those without mutation ($P < .001$, median = TTP 10.1 months compared with 179.0 months among wild-type cases). This observation also holds true when analyzing only cases with at least 60 ($P < .001$) or 96 ($P < .004$) months of follow-up (80% of patients with relapse developed recurrence in 60 months, 90% in 96 months). In line with the literature, WHO grade ($P = .005$, Student's t test) and sex ($P < .001$, Student's t test, unfavorable for male patients) were associated with recurrence across our cohort. We also found strong association between recurrence and *TERT* promoter mutation ($P < .01$, Student's t test). Distribution of age, sex, Simpson grade, recurrent cases, and *TERT* mutation among WHO grades are given in Supplementary Table 2 (available online), hazard

Table 1. TERT promoter status in WHO grade I-III meningioma and recurrence status*

WHO Grade	All (mut/n)	Recurred (mut/n)	Higher grade at recurrence (mut/n)
I	2/119	2/32	2/4
II	5/88	5/39	2/11
III	9/45	8/26	NA
All grades	16/252	15/97	4/15

* Occurrence of *TERT* promoter mutations among meningioma of WHO grades I, II, and III ("all"), stratification for *TERT* promoter mutations among cases that resulted in recurrence ("recurred") and cases that recurred with histologically higher grade ("higher grade at recurrence"). mut = mutant; WHO = World Health Organization.

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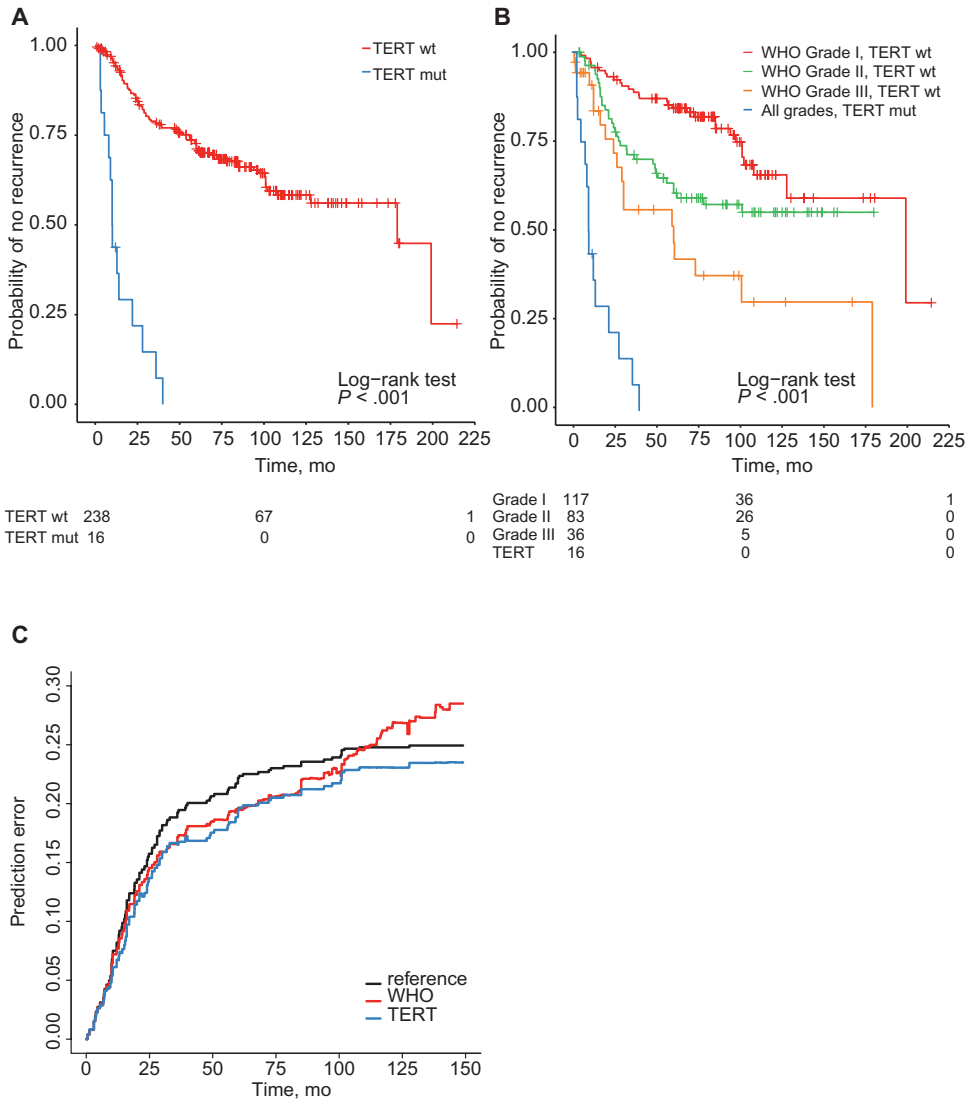

Figure 1. Kaplan-Meier **plots** of time to progression in *TERT* promoter mutant vs wild-type samples **(A)** and World Health Organization (WHO) grade I-III vs *TERT* mutant **(B)**. **Tables below plots** indicate patients at risk at time points 0, 100, and 225 months. All statistical tests were two-sided Brier prediction plot of WHO grading vs *TERT* status. The reference group is the marginal Kaplan-Meier prediction model **(C)**. mut = mutant; WHO = World Health Organization; wt = wild-type.

ratios and *P* values for Cox univariate and multivariable analyses in Supplementary Tables 3 and 4 (available online). Of note, the majority of *TERT* mutant cases were male (10/16), with 4% of all female and 11% of all male patients in the cohort harboring *TERT* mutations. Mean age at diagnosis was 63 years (SD = 10 years) for mutant and 56 years (SD = 14 years) for wild-type cases.

Thus, the prediction power of *TERT* promoter status essentially supports current WHO grading in identifying patients at risk for early recurrence. In a conditional inference tree model with the five predictors Simpson grade, age at diagnosis, sex, *TERT* status, and WHO grade, *TERT* status was the dominant criterion for TTP prediction ($P < .001$) (data not shown). In line, *TERT* status was superior to WHO grade in predicting TTP in a modified prediction error model ($P < .001$) (Figure 1C).

Thus, *TERT* promoter status proved to be a valuable tool in identification of a subset with shorter TTP compared with meningiomas with *TERT* wild-type status. Of note, mutant *TERT* promoter status also identified cases with early recurrence among samples histologically attributed to WHO grades I or II.

Previous studies striving for more reliable predictors of meningioma progression focused on cytogenetics and expression profiles (2,3,7,8,11,12). However, none of the proposals have been implemented in diagnostic routine. The data here paradigmatically demonstrate that genes recently found mutated in meningiomas such as *TERT*, *KLF4*, *AKT1*, *TRAF7*, and *SMO* (9,13–16) may be translated into novel markers of prognostic power. Application of such markers will assist in overcoming the current limitations of histological classification. Applying molecular genetics for tumor stratification is in line with the recommendations of the "ISN-Haarlem" guidelines proposed by the International Society of Neuropathology as basis for an updated WHO classification of brain tumors (17). Based on this concept, meningioma diagnostics may also soon be "layered" as proposed for parenchymal brain tumors (18), taking into account histological classification (eg, meningothelial meningioma), histological WHO grade (eg, grade I), and molecular information (eg, *TERT* promoter mutant). Information on *TERT* promoter mutation will also allow the clinician to identify cases that are even more aggressive than other meningiomas with anaplastic

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grade III histology. However, consideration of assigning grade IV to this subgroup would require comparison of overall survival with other entities with grade IV variants. Lack of those overall survival data is a limitation of this study, along with the lack of external validation data and functional characterizations. Further, the number of mutant cases (n = 16) does not allow for robust biological conclusions on the effect of sex and age among *TERT* mutant cases, although statistically significant in this dataset (Supplementary Tables 3 and 4, available online).

In conclusion, *TERT* promoter mutations are associated with higher meningioma grades and with early recurrence and may be a useful tool assisting in the grading of meningioma.

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Notes

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