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Molecular prognostication in grade 3 meningiomas and p16/MTAP immunohistochemistry for predicting *CDKN2A/B* status

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Abstract

Background. The World Health Organization 2021 classification introduces molecular grading criteria for anaplastic meningiomas, including *TERT* promoter (*TERTp*) mutations and *CDKN2A/B* homozygous deletion. Additional adverse prognostic factors include H3K27me3 and BAP1 loss. The aim of this study was to explore whether these molecular alterations stratified clinical outcomes in a single-center cohort of grade 3 meningiomas. Additionally, we examined whether p16 and MTAP immunohistochemistry can predict *CDKN2A/B* status.

Methods. Clinical and histopathological information was obtained from the electronic medical records of grade 3 meningiomas resected at a tertiary center between 2007 and 2020. Molecular testing for *TERTp* mutations and *CDKN2A/B* copy-number status, methylation profiling, and immunohistochemistry for H3K27me3, BAP1, p16, and methylthioadenosine phosphorylase (MTAP) were performed. Predictors of survival were identified by Cox regression.

Results. Eight of 15 cases demonstrated elevated mitotic index (≥20 mitoses per 10 consecutive high-power fields), 1 tumor exhibited BAP1 loss, 4 harbored *TERTp* mutations, and 3 demonstrated *CDKN2A/B* homozygous deletion. Meningiomas with *TERTp* mutations and/or *CDKN2A/B* homozygous deletion showed significantly reduced survival compared to anaplastic meningiomas with elevated mitotic index alone. Immunohistochemical loss of p16 and MTAP demonstrated high sensitivity (67% and 100%, respectively) and specificity (100% and 100%, respectively) for predicting *CDKN2A/B* status.

Conclusions. Molecular alterations of grade 3 meningiomas stratify clinical outcomes more so than histologic features alone. Immunohistochemical loss of p16 and MTAP show promise in predicting *CDKN2A/B* status.

Key Points

- *TERT* promoter mutations and CDKN2A/B homozygous deletion portend a worse prognosis for grade 3 meningioma than elevated mitotic index.
- p16/MTAP immunohistochemistry is a promising surrogate marker for CDKN2A/B status.

Importance of the Study

A growing body of evidence supports the utility of molecular markers in meningioma grading. In this study, we demonstrate that 4 established molecular markers (*CDKN2A/B* homozygous deletion, *TERT* promoter mutations, BAP1 loss, and H2K27me3 loss), and a methylome-based prediction model each stratified survival outcomes within a subset of 15 grade 3 meningiomas previously classified based on histopathologic features alone. In addition, we show that p16/MTAP immunohistochemistry may serve as a sensitive and specific surrogate marker for *CDKN2A/B* homozygous deletion in grade 3 meningiomas. Our findings support the increasing incorporation of molecular testing into the diagnostic workup of high-grade meningiomas and establish a potential cost-effective immunohistochemical-based surrogate for one such molecular prognosticator.

Although meningiomas are among the most common primary central nervous system (CNS) neoplasms, grade 3 meningiomas only comprise 1%–2% of all meningiomas, and confer substantially worse clinical outcomes.[1,2] Thus, accurate grading of meningiomas is crucial in clinical decision-making, as the prognosis and management of benign meningiomas differ markedly from that of malignant tumors.[1] Meningiomas have traditionally been assigned a histopathologic grade of 1–3 based on the presence of elevated mitotic index, presence of atypical histologic features (sheeting, hypercellularity, small cell change, prominent nucleoli, and necrosis), or predominance of certain histologic variants (papillary, rhabdoid, chordoid, or clear cell).[1] Specifically, grade 3 criteria for meningioma in the 2007 and 2016 WHO Classification of CNS Neoplasms (WHO CNS5) included the presence of anaplasia (carcinoma-, melanoma-, or sarcoma-like growth), elevated mitotic rate of ≥20 mitoses per 10 consecutive high-power fields (HPF), and papillary or rhabdoid variants. However, a subset of meningiomas progresses in manners inconsistent with histologic grading criteria alone.[3,4] While previous WHO classification schemes defined rhabdoid and papillary meningiomas as grade 3, this criterion was removed in the fifth edition of the WHO CNS5 based on its unreliable prediction of tumor behavior in the absence of other malignant features.[5]

In contrast, integration of molecular findings has been shown to substantially improve the prognostic accuracy for meningiomas across all histopathologic grades, leading to the inclusion of novel molecular criteria for grade 3 meningiomas in WHO CNS5.[5] The updated WHO CNS5 criteria for grade 3 meningioma include mitotic rate of ≥20 mitoses per 10 HPFs; frank anaplasia (sarcoma-, carcinoma- or melanoma-like appearance); *TERT* promoter (*TERTp*) mutations; and/or homozygous deletion of *CDKN2A* and/or *CDKN2B* (*CDKN2A/B*).[5–7]

The *TERT* gene encodes a telomerase reverse transcriptase protein product, which functions to lengthen telomeres and thereby facilitate cancer cell immortalization.[8] In particular, mutations in the hotspot regions of the *TERT* promoter, C228T and C250T, drive *TERT* upregulation and corresponding tumor aggression.[8] Separately, cell-cycle deregulation is a key contributor to meningioma recurrence and is commonly reflected in the high proliferative index of grade 3 meningiomas.[9] Cyclin-dependent kinase inhibitor 2A located on chromosome 9p21 encodes p16 protein, which plays a critical role in suppressing cell-cycle progression from G1 to S phase through CDK4 and CDK6 inhibition.[10,11] Utilizing p16 immunohistochemistry (IHC) as a surrogate marker for *CDKN2A* homozygous deletion has been extensively studied in a variety of neoplasms, including meningioma.[12,13] *MTAP* encodes methylthioadenosine phosphorylase (MTAP) and is located 165kb away from the *CDKN2A* locus on 9p21. *MTAP* is frequently concurrently deleted with *CDKN2A* homozygous deletion. Thus, MTAP IHC as a surrogate marker for *CDKN2A* homozygous deletion has been explored in a variety of tumors, most notably mesothelioma, and more recently in CNS neoplasms, including gliomas and meningiomas.[12,14,15]

Although rhabdoid histologic variants are no longer designated as grade 3 tumors as per WHO CNS5 due to the heterogeneous clinical behavior of this meningioma subtype, a subset of rhabdoid meningiomas with mutations in the breast cancer (BRCA)1-associated protein-1 tumor suppressor gene (*BAP1*) have been shown to exhibit a particularly aggressive tumor phenotype, and show similar survival outcomes to grade 3 meningiomas.[11,16] *BAP1* mutations in rhabdoid meningioma can occur as either somatic or germline mutations, with the latter cases representing manifestations of *BAP1* cancer predisposition syndrome.[16] Additionally, loss of trimethylation on lysine 27 of histone 3 (H3K27me3) is associated with more rapid progression of meningiomas and has been shown to predict reduced recurrence-free survival (RFS) among grade 2 meningiomas.[17,18] This is reflected in the finding that the proportion of H3K27me3 loss in meningiomas significantly increases with higher WHO grade (eg, 37% of grade 3 tumors compared to 20% of grade 2 tumors).[19] BAP1 loss and loss of H3K27me3 can both be investigated in the pathology laboratory with IHC.

In addition to the genetic markers, recent work has highlighted the utility of epigenetic classifiers in predicting meningioma progression.[20–22] Perturbations in DNA methylation, in particular, have been found to occur early in the development of meningiomas,[21] and are predictive of recurrence risk independent of established prognostic factors.[20] Nassiri et al.[20] present both a methylome-based score for predicting 5-year recurrence risk in meningiomas of all grades, and an integrated score combining methylome-based predictors with clinical prognostic factors, which outperformed models based on clinical factors alone.[20]

In the study herein, we perform a retrospective genomic, epigenomic, and immunohistochemical analysis of grade

3 meningiomas diagnosed between 2007 and 2020 initially utilizing 2007/2016 WHO grading criteria in an effort to (1) determine the clinical significance of molecular characterization of grade 3 meningiomas using WHO CNS5 criteria (CDKN2A/B homozygous deletion and TERTp mutations), (2) investigate the role of p16 and MTAP IHC as surrogate markers for predicting CDKN2A/B status, (3) validate the WHO CNS5 decision to remove rhabdoid/papillary variants from anaplastic grading criteria, while investigating the prognostic role of BAP1 loss in these morphologic variants, (4) apply the global DNA methylome-based risk stratification scheme presented by Nassiri et al.[20] to our cohort of anaplastic meningiomas in an effort to validate the model, and (5) assess the feasibility and inform the design of larger multicenter studies powered to conclusively address the relationship between individual molecular alterations and clinical outcomes in grade 3 meningiomas.[20]

Materials and Methods

Clinical Data

Demographic, clinical, and histopathological information were retrospectively reviewed from the electronic medical records of 15 patients diagnosed with WHO grade 3 meningioma at Vancouver General Hospital between 2007 and 2020. This cohort of patients was previously included in a multi-institutional series of grade 3 meningioma cases.[23] Their formalin-fixed paraffin-embedded (FFPE) tissue blocks were reviewed for morphologic features by a neuropathologist before molecular testing was performed. Gross tumor volume was estimated using the ellipsoid formula ½(L × W × H).

TERTp Sequencing

TERTp sequencing was performed as described previously.[24] Briefly, tumor DNA was extracted from FFPE specimens and genotyping of the *hTERT* promoter was performed using Sanger sequencing. Small (163 base pair) and large (193 base pairs spanning the hotspot mutations, C228T and C250T on chromosome 5) amplicons were amplified using primers tagged with T7 forward and M13 reverse tags. DNA was first quantified using NanoDrop prior to PCR performed on Tetrad (Bio-Rad). Post-PCR products were treated with ExoSAP-IT (Thermo Fisher Scientific, #78201.1.ML). Finally, BigDye Terminator v3.1 (Thermo Fisher Scientific, #4337455) was utilized for cycle sequencing on the Applied Biosystems (ABI) capillary electrophoresis platform.

CDKN2A/B copy-number profiling:

CDKN2A/B copy-number status was determined as described previously.[25] In brief, *CDKN2A/B* deletion status was evaluated by inspection of genome-wide copy-number variation (CNV) plots for each sample. "Heterozygous deletion" was called where the depth of *CDKN2A/B* deletion was comparable to the depth of single copy chromosomal losses in the same sample (eg, of chr 1p, 10, 22q, etc.). "Homozygous deletion" was called where the deletion depth of the *CDKN2A/B* locus was approximately twice that of other chromosomal losses in the same sample.

Immunohistochemistry

Standardized protocol using the DAKO platform was used for IHC on FFPE tissue for BAP1 (1:50 primary, Clone C4; Santa Cruz Biotech, TX), p16 (1:400 primary, Clone JC2; Cell Marque, CA), and MTAP (1:100 primary, Clone 2G4; Abnova, Taipei, Taiwan). IHC for H3 K27me3 (1:600 primary, Clone C36B11; Cell Signaling, MA) was performed on the Leica BOND platform.

DNA Methylation Profiling

DNA methylation data were obtained as previously published using the Illumina Infinium MethylationEPIC BeadChip Array (Illumina, San Diego, CA).[20] Raw data files (*.idat) were imported, processed, and normalized. General quality control measures were performed as per the manufacturer's instructions. Methylation probe annotation was performed using the University of California Santa Cruz Genome Browser (GRCh38/hg38 assembly). Copy-number alterations were inferred from the DNA methylation data using conumee.

The methylome-recurrence risk score was derived from applying a generalized boosted regression model using 9529 probes found to be significantly associated with PFS (on univariate analysis with $P < .001$) in Nassiri et al.'s original training cohort.[20]

Statistical Analysis:

The experimental protocol was approved by local clinical research ethics boards and conformed to the Declaration of Helsinki. All data analyses were conducted in R (version 4.2.1, The R Foundation for Statistical Computing). Statistical significance was set a priori at $P < .05$. Group differences between de novo and malignant transformation were compared using the Wilcoxon rank-sum test, Pearson's chi-square test, and Fisher's exact test dependent on data type. The Kaplan–Meier method was used to generate overall survival (OS) and progression-free survival (PFS) curves for the entire cohort. Kaplan–Meier curves and associated hazard ratios were compared using the log-rank test between the following groups: (1) *TERTp* WT versus mutated, (2) *CDKN2A/B* WT versus heterozygous loss versus homozygous loss, 3) high versus low methylation risk score, 4) H3K27me3 retained versus lost. We additionally explored the relationship between molecular and histopathological features, and OS and PFS using univariate Cox regression models.

Results

Clinical, Histopathologic, and Molecular Characteristics of Cohort

Fifteen patients (53% female; median age of 64 [IQR: 48, 73.5]) were followed for a median of 40 [IQR: 28, 100] months from the time of the first surgery. A descriptive

summary of the demographic and histologic features for these 15 cases can be found in Table 1 and Figure 1, respectively. Twelve (80%) patients presented symptomatically, including motor deficits (10, 67%), headache (7, 47%), seizures (7, 47%), altered levels of consciousness (4, 27%), language deficits (5, 33%), visual dysfunction (6, 40%), and sensory changes (5, 33%). The mean Karnofsky Performance Status score at the time of diagnosis was 70

Table 1. Demographic, clinical, and histopathologic characteristics of 15 WHO 2007/2016 grade 3 meningioma cases

Characteristic	Anaplastic, *n* = 8[a]	Papillary, *n* = 1[a]	Rhabdoid, *n* = 6[a]	*P*-value[b]
Sex				.067
Female	2 (25%)	1 (100%)	5 (83%)	
Male	6 (75%)	0 (0%)	1 (17%)	
Age at diagnosis	68 (50, 73)	59 (59, 59)	60 (47, 78)	>.9
KPS at diagnosis	70 (58, 70)	80 (80, 80)	80 (73, 80)	.10
Transformation status				>.9
De novo	7 (88%)	1 (100%)	6 (100%)	
Recurrent	1 (13%)	0 (0%)	0 (0%)	
Postoperative RT[c]				>.9
No RT	2 (33%)	0 (0%)	1 (17%)	
RT	4 (67%)	1 (100%)	5 (83%)	
Unknown	2	0	0	
Simpson resection grade				.3
I	4 (50%)	0 (0%)	0 (0%)	
II	1 (13%)	1 (100%)	2 (33%)	
III	1 (13%)	0 (0%)	1 (17%)	
IV	2 (25%)	0 (0%)	3 (50%)	
TERTp mutation[d]				.4
228C >T	2 (25%)	0 (0%)	0 (0%)	
250C >T	2 (25%)	0 (0%)	0 (0%)	
WT	4 (50%)	1 (100%)	6 (100%)	
CDNK2A/B Status				.6
WT	4 (50%)	1 (100%)	5 (83%)	
Heterozygous loss	1 (13%)	0 (0%)	1 (17%)	
Homozygous loss	3 (38%)	0 (0%)	0 (0%)	
BAP1				.5
Loss	0 (0%)	0 (0%)	1 (17%)	
Retained	8 (100%)	1 (100%)	5 (83%)	
H3K27me3 loss	2 (29%)	0 (0%)	0 (0%)	.6
Meth 5-y RR[e]	0.93 (0.87, 0.98)	0.58 (0.58, 0.58)	0.53 (0.19, 0.79)	.14
Mitoses (per 10hpf)	22 (20, 28)	0 (0,0)	3 (1, 8)	.13
Prominent nucleoli	3 (38%)	0 (0%)	2 (33%)	>.9
Hypercellularity	4 (50%)	0 (0%)	2 (33%)	.8
Sheeting	5 (63%)	0 (0%)	0 (0%)	**.026**
Small cells	3 (38%)	0 (0%)	1 (17%)	.7
High N/C ratio[f]	3 (38%)	0 (0%)	1 (17%)	.7
Brain invasion	3 (38%)	0 (0%)	3 (50%)	>.9

a*n* (%); Median (IQR)
bFisher's exact test; Kruskal–Wallis rank-sum test.
cRadiotherapy.
dTERT promoter mutation.
eMethylation 5-y recurrence risk.
fHigh nuclear/cytoplasmic ratio.

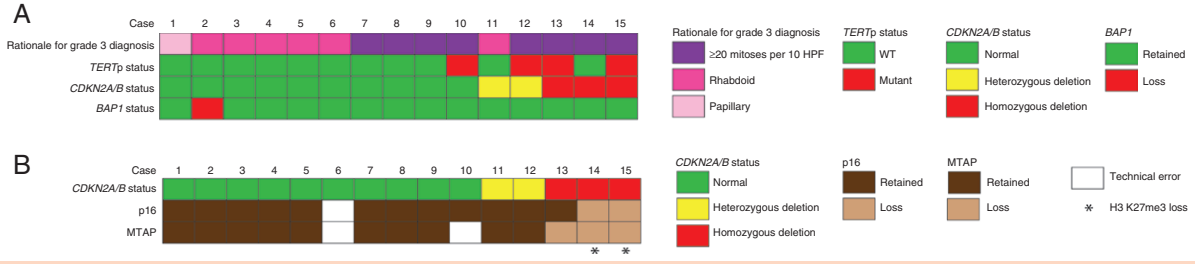

Figure 1. Rationale for grade 3 diagnosis and identified genomic alterations (A). Correlation of CDKN2A homozygous deletion and immunohistochemical loss of p16 and MTAP staining (B).

(IQR: 65, 80). Tumor location included anterior fossa (1, 7%), convexity (4, 27%), parafalcine (1, 7%), parasagittal (2, 13%), posterior fossa (2, 13%), and spheno-orbital/orbital regions (5, 33%). The median gross tumor volume was 25.8 (IQR: 8.7, 47.2) cc. Radiologic features included vasogenic edema (5, 33%), mass effect (5, 33%), hyperdensity (3, 20%), and enhancement (5, 33%), with 3 (20%) cases exhibiting heterogeneous enhancement. Four patients underwent Simpson Grade I resection (27%), 4 underwent Simpson Grade II (27%), 2 (13%) underwent Simpson Grade III, and 5 (33%) underwent Simpson Grade IV resection.

Brain invasion was noted in 6 (40%) of cases. Eight (53.3%) tumors were histologically characterized as malignant morphologic variants, 6 (40%) as rhabdoid, and 1 (7%) as papillary (Figure 1A). One case involved a malignant transformation of a previously resected lower-grade meningioma, while all other cases represented primary grade 3 meningiomas. Atypical histologic features included necrosis in 11 (73%) cases, hypercellularity in 6 (40%) cases, sheeting and prominent nucleoli were each noted in 5 (33%) cases, while small cell change and high nuclear-to-cytoplasmic ratio were each noted in 4 (27%) cases. Elevated mitotic index (≥20/10 HPFs) was noted in 8 cases. Genetic abnormalities (*BAP1* loss, homozygous *CDKN2A/B* deletion, and/or *TERTp* mutations) were present in 6 (40%) cases. One (7%) of these cases involved genetically and immunohistochemically confirmed BAP1 loss, 4 (27%) harbored *TERTp* mutations, of which 2 also demonstrated *CDKN2A/B* homozygous deletion. *CDKN2A/B* homozygous deletion was also noted in a third case without coincident TERT*p* mutations, and heterozygous *CDKN2A/B* loss was noted in 2 additional cases, one of which also had a TERT*p* mutation. No significant associations were identified between the presence of genetic alterations and histologic subtype or malignant histologic features, though our study was likely underpowered to detect such relationships (Table 1).

Ten patients (67%) received adjuvant radiotherapy (RT) for the primary tumor, starting a median of 94 (72, 130) days post-resection. Tumor recurrence occurred in 7 (47%) of patients, with 5 of these cases harboring 1 or more molecular alterations (*CDKN2A* homozygous deletion, *TERTp* mutations, or *BAP1* loss), and the remaining 2 having no such molecular alterations ($P = .04$). Median PFS time, defined as the time from index OR to the first evidence of radiographic progression (in the case of GTR), progression (in the case of STR), or death, was 39 [IQR: 18, 47] months. Median OS time was 40 [IQR: 28, 100] months.

Immunohistochemistry for p16 and MTAP for Predicting *CDKN2A/B* Status

All 3 cases harboring homozygous deletion of *CDKN2A/B* showed loss of MTAP cytoplasmic immunoreactivity by IHC (sensitivity 100%; specificity 100%) (Figure 1B). Two of the cases with *CDKN2A/B* homozygous deletion showed loss of p16 nuclear immunoreactivity, defined as <1% of immunoreactive cells (sensitivity 67%, specificity 100%), while focal loss (defined as <50% of immunoreactive cells) was seen in the third case. However, focal loss of p16 immunoreactivity was also seen in 4 cases with an intact *CDKN2A/B* locus, and a fifth case with heterozygous *CDKN2A/B* loss. Figure 2 provides a representative case showing homozygous deletion of *CDKN2A/B* (A) with loss of p16 (B) and MTAP (C) staining and a second case showing an intact *CDKN2A/B* gene locus (D) with retained staining of p16 (E) and MTAP (F).

Molecular Predictors of PFS and OS:

In univariate Cox regression, *TERTp* mutations and *CDKN2A/B* status were the only 2 significant predictors of both OS and PFS, while H3K27me3 was a significant predictor of PFS alone (Supplementary Table 1). Of note, 2 of the 4 cases harboring *TERTp* mutations were also found to have *CDKN2A/B* homozygous deletion, and a third case had *CDKN2A/B* heterozygous deletion. Two cases with *CDKN2A/B* homozygous deletions also showed loss of H3K27me3, with one of these cases also harboring a *TERTp* mutation. Given the small sample size and consequent low number of events per variable, the relative effects of these 2 molecular characteristics could not be disentangled using a multivariate model.[26,27]

In our cohort of 15 grade 3 meningiomas diagnosed based on WHO 2007/2016 criteria, we compared survival outcomes between patients with tumors harboring 1 or more genetic alterations (*TERTp* mutation, *CDKN2A/B* homozygous deletion, and/or BAP1 loss) to wild-type cases diagnosed as grade 3 based on histologic criteria alone using the log-rank test (Figure 3A). Patients with 1 or more genetic abnormalities ($n = 6$) survived for a median of 22 months post-resection, while those with no genetic abnormalities ($n = 9$) survived for a median of 123 months ($P = .0011$). Likewise, median PFS was 11 months for those with genetic abnormalities versus 101 months for those with no such abnormalities ($P = .0029$).

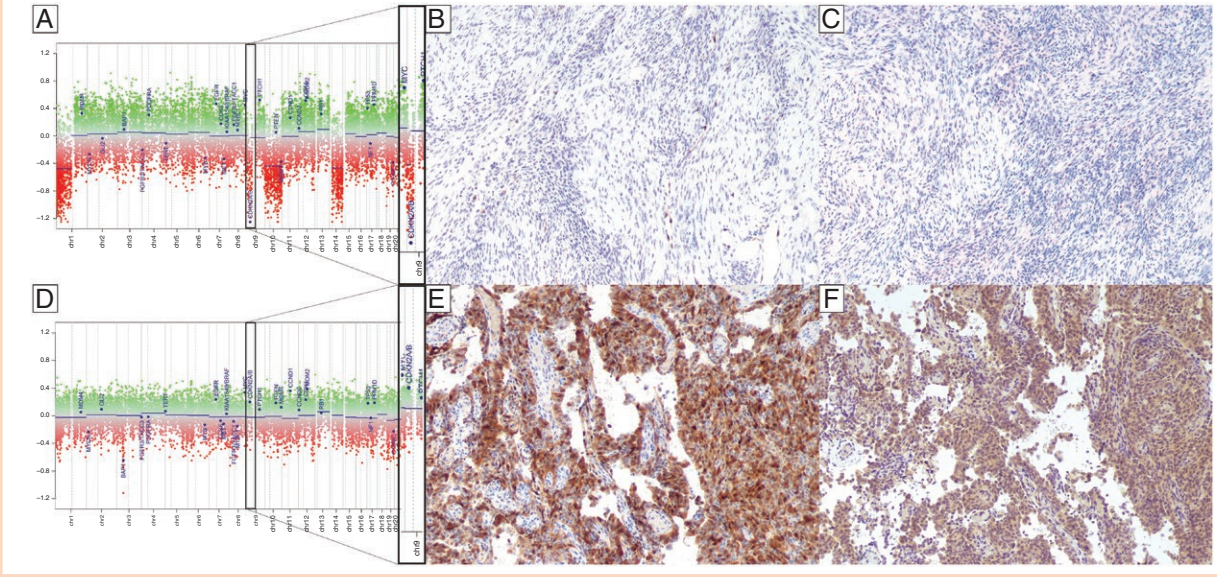

Figure 2. Copy-number analysis of Case 15 showing homozygous deletion of CDKN2A/B (A) with loss of p16 (B) and MTAP (C) staining. Copy-number analysis of Case 2 showing intact CDKN2A/B gene locus (D) with retained staining of p16 (E) and MTAP (F).

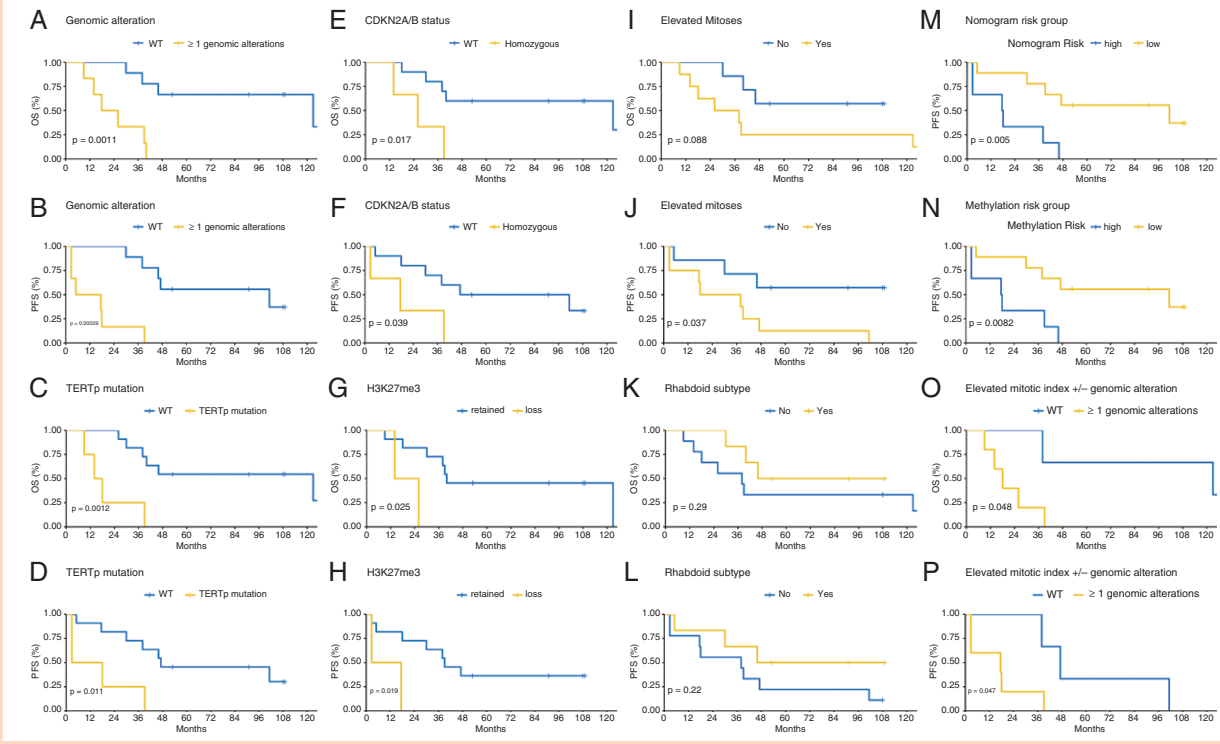

Figure 3. Comparison of OS (A) and PFS (B) for patients with and without genomic alterations. Comparison of OS (E) and PFS (F) for patients with and without TERT*p* mutations. Comparison of OS (E) and PFS (F) for patients with and without CDKN2A/B homozygous deletion. Comparison of OS (G) and PFS (H) for patients with and without H2K27me3 loss. Comparison of OS (I) and PFS (J) for patients with and without elevated mitoses. Comparison of OS (K) and PFS (L) for patients initially diagnosed based on rhabdoid versus all other grade 3 meningiomas. Comparison of OS (M) and PFS (N) for patients with high versus low methylation risk scores. Comparison of OS (O) and PFS (P) in the subgroup of patients with elevated mitotic index, with or without additional genomic alterations.

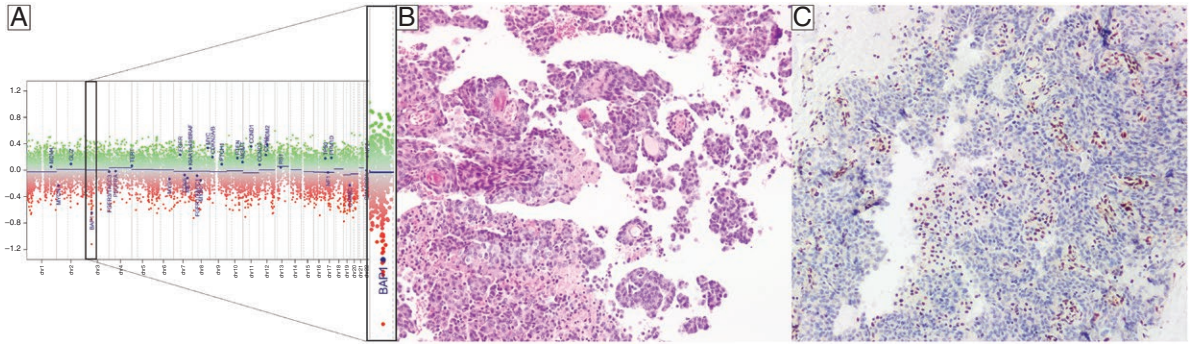

Figure 4. Copy-number analysis (A) with H&E (B) and BAP1-stained (C) images from a grade 3 meningioma exhibiting BAP1 loss.

When we assessed survival outcomes based on histologic features alone, there was no significant difference in PFS ($P = .22$) or OS ($P = .29$) in tumors classified as possessing rhabdoid versus non-rhabdoid morphologies (Figure 3K, L). However, a significant difference in PFS ($P = .037$) was noted in tumors demonstrating elevated mitotic index defined as ≥20 mitoses per 10 HPF compared to tumors classified as grade 3 based on other morphological features (Figure 3J).

Furthermore, when we removed the rhabdoid and papillary tumors from our survival analyses and only included cases that fulfill current CNS WHO5 criteria for grade 3 meningiomas, we found that tumors harboring *TERTp* mutations and/or *CDKN2A/B* homozygous deletion demonstrated significantly decreased PFS ($P = .047$) and OS ($P = .048$) compared to those neoplasms classified as grade 3 based on elevated mitotic index alone (Figure 3O, P). Cases with *TERTp* mutations had a median OS of 16 months versus 123 months for cases with wild-type *TERT* promoters ($P = .0012$; Figure 3C). Similarly, cases with *TERTp* mutations had a median PFS of 10 months versus 47 months for wild-type cases ($P = .011$; Figure 3D).

Homozygous loss of *CDKN2A/B* was associated with reduced PFS ($P = .039$) and OS ($P = .017$)) in our cohort (Figure 3E). Nonsignificant trends toward reduced PFS ($P = .11$) and OS ($P = .11$) were observed among cases with heterozygous loss of CDKN2A/B relative to wild-type (Supplementary Figure 1A, B). Loss of p16 itself was associated with significantly reduced PFS ($P = .009$) and OS ($P = 0.01$; Supplementary Figure 2A, B). Individuals with wild-type *CDKN2A/B* status showed a median survival of 123 months post-resection. When compared to wild-type tumors, cases with *CDKN2A/B* homozygous deletion showed a median OS of 26 months ($P = .032$), while those with heterozygous deletions showed a median OS of 28 months ($P = .13$; Figure 3E). Similarly, a median PFS for individuals with intact *CDKN2A/B* was 74 months versus 3 months for those with both heterozygous ($P = .14$) and homozygous ($P = .044$) *CDKN2A/B* deletion (Figure 3F).

Only 1 of the 7 tumors initially classified as rhabdoid showed BAP1 loss both by immunohistochemistry (Figure 4) and CNV analysis. The initial pathological diagnosis of this tumor was malignant neuroendocrine carcinoma. Histologic features of tumor necrosis, pseudopapillary patterns, increased mitotic activity, elevated Ki67 index, and local invasion were noted. At the time of recurrence, the diagnosis was amended to that of rhabdoid meningioma with focal papillary features (WHO grade 3). The PFS and OS for this case were 5 and 40 months, respectively.

Loss of H3K27me3 was associated with worse OS ($P = .025$) and PFS ($P = .019$) in our cohort (Figure 3G, H). However, of note, the 2 cases in our cohort with H3K27me3 loss also harbored *CDKN2A/B* homozygous deletions.

Methylation Risk Scores

We assessed the performance of methylome-based predictions of 5-year recurrence risk.[20] The AUC for the methylome-based prediction model was 0.83, indicating good discriminatory ability. When stratifying by low versus high recurrence risk scores, we found a significant difference in PFS based on the methylation risk score ($P = .0082$; Figure 3M; Supplementary Figure 3). Rhabdoid histology was associated with a significantly lower methylation risk score ($P = .047$). However, there were no significant differences in methylation risk scores based on any other histologic characteristics (Supplementary Table 2).

Discussion

In this series of 15 grade 3 meningiomas, we identified 6 cases bearing 1 or more previously established molecular markers of clinical aggressiveness (*TERTp* mutations, *CDKN2A/B* homozygous deletion, *BAP1* loss, and H2K27me3 loss),[3] which were collectively and individually associated with reduced RFS and OS. Furthermore, when excluding meningioma cases that were designated as grade 3 based on morphologic characteristics (rhabdoid and papillary histologic subtypes), and only including those cases that satisfy current WHO 2021 grade 3 meningioma criteria ($n = 8$), our results indicate that tumors that harbor *CDKN2A/B* and/or *TERTp* mutations show significantly reduced OS and PFS compared to cases that are diagnosed as grade 3 based on elevated mitotic index (≥20 mitoses/10 HPF) alone. Despite the small size of our cohort, reaching statistical significance in a small cohort speaks to vastly different survival outcomes between molecularly

altered and wild-type tumors, which supports these molecular alterations as prognostic markers of high clinical importance. Additionally, we found that the accuracy of an established methylome-based prognostic model for 5-year recurrence outperformed that of a combined clinical and methylome-based prognostic model.[20] The clinical correlates of molecular findings in our study bolster support for the incorporation of molecular information into prognostic stratification for grade 3 meningioma patients.

A large previous study involving 850 high-grade/progressive meningiomas identified 3 distinct genomic subgroups of clinically aggressive tumors: a group of *NF2*-mutant tumors associated with *CDKN2A/B* mutations and male sex, an *NF2*-agnostic subgroup involving *TERTp* mutations, and a third group generally lacking *NF2* mutations, but displaying alterations in *BAP1* or other chromatin regulators.[28] In keeping with this proposed framework, all 5 of the patients exhibiting heterozygous (2) or homozygous (3) *CDKN2A/B* loss in our study were male. While *TERTp* mutations were seen in patients with or without *CDKN2A/B* alterations, *BAP1* loss was found in a separate case, representing an aggressive meningioma subtype driven by distinct mechanisms. Our finding of *TERTp* mutations in 27% of grade 3 tumors is consistent with previous reports of *TERTp* mutations in 15%[29] and 20% of primary grade 3 meningiomas.[8] The significantly reduced RFS and OS seen in our cases with *TERT* promoter mutations adds to the current body of evidence supporting the incorporation of this molecular marker into prognostic stratification schemes for grade 3 meningioma.[8,29]

The prognostic relevance of rhabdoid histology in meningiomas has been the source of considerable debate. Rhabdoid histology was added to the classification criteria for grade 3 meningioma in the WHO 2000 Tumors of the Central Nervous System, in response to evidence linking this rare histologic subtype with a particularly aggressive clinical course.[30] However, later work suggested that the association between rhabdoid histology and poor clinical outcomes applied primarily to a small subset of rhabdoid cases, leading to the removal of this criterion from the WHO 2021 updated classification scheme.[31] Efforts to identify genetic features defining the subset of clinically aggressive rhabdoid meningiomas have suggested inactivating *BAP1* mutations as an important prognostic marker, with these mutations being present in approximately 10% of tumors exhibiting predominantly rhabdoid morphology and confer significantly worse clinical outcomes, akin to grade 3 meningiomas.[16] Although rhabdoid and papillary morphologic variants do not fulfill WHO CNS5 anaplastic grading criteria in the absence of other high-grade histologic or molecular features, the goal of our study was to explore molecular alterations of meningiomas that predict survival. Since *BAP1* loss is currently not a molecular feature that upgrades meningiomas to grade 3, rhabdoid and papillary morphologic variants were included in our cohort to determine the frequency of BAP1 loss in these cases and their prognostic implications.

In keeping with this previously reported prevalence, *BAP1* mutations were found in only 1 of 7 rhabdoid cases in our study, in an adolescent patient who was diagnosed with local recurrence 1-year post-resection with adjuvant RT and who ultimately died of metastatic meningioma 40 months following initial diagnosis, demonstrating the most aggressive clinical course in our cohort. This finding has both diagnostic and clinical implications. Albeit a small sample size, it adds to the body of literature that supports that meningiomas with *BAP1* loss show worse prognostic outcomes, and as such, including BAP1 loss as a grade 3 defining molecular alteration in future renditions of WHO classification of CNS tumors can be considered.

Previous work has suggested a roughly equivalent proportion of somatic and germline *BAP1* mutations in *BAP1*-negative rhabdoid meningioma, with the latter predisposing carriers to several other cancer types.[32] The young age of the patient in our study raises the possibility of a germline *BAP1* mutation suggestive of a hereditary tumor predisposition syndrome (BAP1-TPDS), which has previously been identified in cases of pediatric rhabdoid meningioma.[16,33] However, germline analysis was not performed on this patient, and neither personal nor family histories of BAP1-TPDS-associated tumors were indicated in the available reports within our electronic medical records. In either case, concordance between genetic and immunohistochemical findings in our study validates previous evidence that *BAP1* loss can be detected via routine laboratory IHC.[16] Given the relationship between *BAP1* mutations and aggressive meningioma behavior as well as the potential predisposition to other cancers, our findings lend support for the routine immunochemical testing of BAP1 in meningiomas with rhabdoid features.[32]

While incompletely understood, H3K27 trimethylation is thought to inhibit tumorigenesis by regulating DNA repair and silencing proximate genes via chromatin compaction.[18] Loss of immunoreactivity for H3K27 trimethylation is associated with increased recurrence risk in WHO grade 1 and 2 meningioma.[17,18] Additionally, H3K27me3 loss is found in up to 37% of grade 3 meningiomas compared to 20% of grade 2, and H3K27me3 loss has been independently associated with reduced survival in grade 3 tumors.[19,34] Although our results share these findings, as loss of H3K27me3 was associated with worse OS and PFS in our series, both cases with H3K27me3 loss also harbored concomitant *CDKN2A/B* homozygous deletion. Given this confounding molecular alteration, our cohort cannot provide clarity on the contributing role H3K27me3 loss independently plays on OS and PFS in grade 3 meningiomas. There has been growing interest in the use of DNA methylation-based profiling for prognostication in meningioma. Nassiri et al. demonstrated that global methylation profiling predicts recurrence risk in meningioma independent of histopathologic grade, resection extent, and copy-number alterations.[20] Sahm et al. likewise clustered 497 meningioma samples into 6 clinically distinct groups based on methylation profiling, and methylation classes better predicted disease outcomes than WHO classification. Our study further demonstrates the utility of methylation-based scores within the class of WHO grade 3 meningioma.

Recent work has suggested p16 and MTAP immunohistochemistry as viable surrogate markers for *CDKN2A/B* CNV analysis for prognostication in high-grade gliomas,[15,35–41] and high-grade meningiomas.[12,13] Our results corroborate the high concordance rates between *CDKN2A/B* copy-number status and p16/MTAP

labeling in grade 3 meningiomas. Specifically, our results demonstrate a sensitivity and specificity of 100% when using loss of MTAP immunohistochemistry (defined as loss of cytoplasmic staining with or without nuclear immunoreactivity) to predict *CDKN2A/B* homozygous deletion, and a sensitivity of 67% and specificity of 100% when using loss of p16 immunohistochemistry as a predictor of *CDKN2A/B* homozygous deletion (defined as less than 1% of cells demonstrating immunoreactivity). Some of the tumors in our cohort demonstrated focal loss of p16 immunoreactivity (<50% of immunoreactive cells); however, utilizing this cutoff value for scoring p16 led to poor specificity for predicting *CDKN2A/B* homozygous deletion and speaks to potential post-translational mechanisms of reduced p16 expression that can be encountered in neoplasms.[42]

Although the sample size of our cohort is small, with only 3/8 grade 3 tumors demonstrating CDKN2A/B homozygous deletion, it reflects that grade 3 meningiomas are rare entities, comprising 1%–2% of all meningiomas. To our knowledge, the only other study in the literature that showed the high sensitivity and specificity of MTAP IHC in predicting CDKN2A status in meningiomas includes a sample size of 3 anaplastic meningiomas,[12] 2 of which were found to have CDKN2A/B homozygous deletion (67%). Furthermore, one other study in the literature showed high sensitivity and specificity of p16 IHC for predicting CDKN2A/B status from a cohort of 12 grade 3 meningiomas, 7 of which demonstrated CDKN2A/B homozygous deletion (58%).[13] Our cohort of WHO CNS5 defined anaplastic meningiomas includes 8 tumors, 3 of which showed CDKN2A/B deletion (38%). Our study includes the assessment of both p16 and MTAP, which has not been previously done on a single cohort, and validates both immunohistochemical stains as viable surrogate markers. Importantly, our findings corroborate the utility of p16 and MTAP IHC for predicting CDKN2A/B status in anaplastic meningiomas, and show similar frequency of these molecular alterations to previous cohorts.

Additionally, recent studies have shown that loss of p16 expression alone is not a reliable marker for *CDKN2A/B* loss in histologically WHO grade 1 meningioma.[13,25] More work is needed to determine whether the combination of p16 and MTAP IHC could be used as a surrogate for *CDKN2A/B* status in WHO grade 1 and 2 meningioma. Overall, while interrogation of the *CDKN2A/B* locus via CNV analysis or FISH continues to be the gold standard for grading meningiomas, these molecular analyses are often unavailable and/or costly in resource-limited neuropathology centers. Utilizing p16 and MTAP IHC can be considered as informative screening studies to determine specific cases of high-grade meningioma with lower-grade morphology which may require further molecular analysis for *CDKN2A/B* status confirmation to help inform final tumor grade.

Limitations

Interpretation of our results is primarily constrained by our small sample size of 15 cases. In particular, the effects of BAP1 loss on survival outcomes could not be assessed quantitatively, given that this genetic alteration was found in only one case. Similarly, survival outcomes related to H3 K27me3 loss could not be adequately assessed in our cohort due to the confounding finding of *CDKN2A/B* homozygous deletion in both of these cases. Our findings regarding the utility of p16 and MTAP as surrogate markers of *CDKN2A/B* status require further validation in larger cohorts that are representative of all WHO grades of meningioma.

Conclusions

Despite our small sample size, we found statistically significant reductions in PFS and OS for patients whose tumors harbored a variety of previously established genetic alterations. We demonstrate that p16 and MTAP immunohistochemistry are promising surrogate markers for *CDKN2A/B* homozygous deletion. Our results support the increasing trend towards molecular-based prognostication in meningioma.

Supplementary material

Supplementary material is available online at *Neuro-Oncology* (https://academic.oup.com/neuro-oncology).

Keywords

meningioma | molecular grading | MTAP | p16

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Conflict of interest statement

S.Y. is a member of advisory boards of Amgen, AstraZeneca, Bayer, Janssen, Pfizer, Roche, and Servier. G.Z. is the Editor-in-Chief of Neuro-Oncology Advances. The other authors have no conflicts to report.

Authorship statement

Conception and design: S.Y., K.C.M. Data acquisition, analysis, and interpretation: K.T., K.C.M., A.D.R., J.Z.W., F.N., A.L. Drafting the manuscript: K.T., K.C.M., A.D.R. Reviewing and approving the final version of the manuscript: K.T., K.C.M., A.D.R., J.Z.W., F.N.,

A.L., G.Z., S.M., S.Y. Supervision and administrative support: G.Z., S.M., S.Y.

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