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Hypermitotic meningiomas harbor DNA methylation subgroups with distinct biological and clinical features

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Abstract

Background. Meningiomas, the most common primary intracranial tumors, can be separated into 3 DNA methylation groups with distinct biological drivers, clinical outcomes, and therapeutic vulnerabilities. Alternative meningioma grouping schemes using copy number variants, gene expression profiles, somatic short variants, or integrated molecular models have been proposed. These data suggest meningioma DNA methylation groups may harbor subgroups unifying contrasting theories of meningioma biology.

Methods. A total of 565 meningioma DNA methylation profiles from patients with comprehensive clinical follow-up at independent discovery (n = 200) or validation (n = 365) institutions were reanalyzed and classified into Merlin-intact, Immune-enriched, or Hypermitotic DNA methylation groups. RNA sequencing from the discovery (n = 200) or validation (n = 302) cohort were analyzed in the context of DNA methylation groups to identify subgroups. Biological features and clinical outcomes were analyzed across meningioma grouping schemes.

Results. RNA sequencing revealed differential enrichment of FOXM1 target genes across two subgroups of Hypermitotic meningiomas. Differential expression and ontology analyses showed the subgroup of Hypermitotic meningiomas without FOXM1 target gene enrichment was distinguished by gene expression programs driving macromolecular metabolism. Analysis of genetic, epigenetic, gene expression, or cellular features revealed Hypermitotic meningioma subgroups were concordant with Proliferative or Hypermetabolic meningiomas, which were previously reported alongside Merlin-intact and Immune-enriched tumors using an integrated molecular model. The addition of DNA methylation subgroups to clinical models refined the prediction of postoperative outcomes compared to the addition of DNA methylation groups.

Conclusions. Meningiomas can be separated into three DNA methylation groups and Hypermitotic meningiomas can be subdivided into Proliferative and Hypermetabolic subgroups, each with distinct biological and clinical features.

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Key points

1. Meningiomas can be separated into 3 DNA methylation groups.
2. The Hypermitotic group is comprised of Proliferative or Hypermetabolic subgroups.
3. Meningioma DNA methylation subgrouping refined outcome prediction.

Importance of the Study

This study reports clinical, genetic, epigenetic, gene expression, and cellular features distinguishing meningioma DNA methylation subgroups within meningioma DNA methylation groups. We present these data in the context of molecular and clinical models predicting postoperative outcomes across 565 meningiomas with comprehensive clinical follow-up from independent discovery and validation institutions. By uncovering meningioma DNA methylation subgroups within meningioma DNA methylation groups, we provide an opportunity to address inconsistencies in the recent literature. In doing so, our study establishes an architecture that unifies varying biological theories about the most common primary intracranial tumor that may provide a useful foundation for biomarker-guided clinical trials for meningioma patients.

Meningiomas arise from the meningothelial lining of the central nervous system, and standard-of-care treatments for meningioma patients are limited to surgery and radiotherapy.[1–3] Biomarkers predicting meningioma outcomes are not widely implemented in clinical practice, but myriad studies investigating retrospective samples have reported genetic, epigenetic, or gene expression grouping schemes that shed light on meningioma biology, outcomes, or druggable dependencies. The number and nature of molecular groups are controversial, and contrasting studies have reported 2, 3, 4, 6, or 7 groups of meningiomas,[4–20] sometimes with overlapping biological features or clinical outcomes. Many molecular studies of meningiomas have been limited by small sample sizes, nonconsecutive tumors, imputed clinical data, a lack of independent or orthogonal bioinformatic validation, or an absence of mechanistic or functional approaches to validate bioinformatic architectures. To address these limitations, some studies have reported supervised molecular grouping schemes trained on clinical endpoints, used resampling approaches in lieu of independent validation, recombined subgroups from different groups to resolve redundant clinical outcomes, or combined multiple molecular approaches into integrated grouping schemes that may be impractical for routine clinical use. Thus, despite the abundance of studies designed to clarify meningioma outcomes in the context of meningioma biology, clinically tractable consensus biomarkers are lacking for the most common primary intracranial tumor.

DNA methylation profiling provides the robust classification of central nervous system tumors,[21] but meningiomas have an abundance of genomic copy number variants (CNVs),[6,7,9] and standard bioinformatic approaches can report inaccurate β methylation values at genomic loci with CNVs.[22] Controlling for the influence of CNVs on meningioma β methylation values and validating our bioinformatic findings from a discovery cohort (n = 200, University of California San Francisco [UCSF]) and a consecutive validation cohort (n = 365, University of Hong Kong [HKU]) using mechanistic and functional approaches in preclinical models and prospective patients, we found meningiomas are comprised of Merlin-intact, Immune-enriched, or Hypermitotic DNA methylation groups with distinct biological drivers, clinical outcomes, and therapeutic vulnerabilities.[6] In parallel, investigators from independent institutions have reported improvements in supervised or unsupervised meningioma molecular grouping by accounting for CNVs.[7,9,11] Thus, the influence of large-scale genomic instability on bioinformatic approaches may account for some variability across meningioma molecules studies,[23] but differences in sample sizes, patient demographics, or data imputation may also obscure the architecture between biological groups or subgroups of meningiomas.

Here, we test the hypothesis that meningioma DNA methylation groups may harbor subgroups that could unify contrasting theories of meningioma biology. To do so, we reanalyze DNA methylation profiles from 565 meningiomas that were resected from patients with comprehensive clinical follow-up at independent discovery (n = 200) or validation (n = 365) institutions. We use RNA sequencing from the discovery (n = 200) or validation (n = 302) cohorts to identify subgroups and analyze biological features or clinical outcomes across meningioma molecular grouping schemes. Our results reveal the Hypermitotic meningioma DNA methylation group is comprised of Proliferative and Hypermetabolic subgroups, unifying recent inconsistencies in the literature[4,6,11] and establishing a consensus architecture for the most common primary intracranial tumor.

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Materials and Methods

Compliance With Ethical Standards

This study complied with all relevant ethical regulations and was approved by the UCSF Institutional Review Board (IRB #13-12587, #17-22324, #17-23196, and #18-24633), and by the HKU Institutional Review Board (UW 07-273 and UW 21-112). As part of routine clinical practice at both institutions, all patients who were included in this study signed a waiver of informed consent to contribute de-identified data to research projects. Meningiomas and de-identified clinical information were transferred from HKU to UCSF under the protection of a Material Transfer Agreement that was certified by both institutions. Detailed methods are provided in the original report of these patients,[6] and brief descriptions of relevant approaches are provided below.

Meningiomas and Clinical Data

Meningiomas for the discovery cohort were selected from the UCSF Brain Tumor Center Biorepository and Pathology Core in 2017, with an emphasis on high-grade meningiomas or low-grade meningiomas with long clinical follow-ups. All World Health Organization (WHO) grade 2 and grade 3 meningiomas with available frozen samples were included. For WHO grade 1 meningiomas, frozen samples were cross-referenced for clinical follow-up data from a retrospective institutional meningioma clinical outcomes database, and all cases with available frozen tissue and clinical follow-up greater than 10 years (n = 40) were included. To achieve a discovery cohort of 200 cases, additional WHO grade 1 meningiomas with available frozen tissue and the longest possible clinical follow-up (albeit less than 10 years, n = 47) were included. The electronic medical record was reviewed for all patients in late 2018, and paper charts were reviewed in early 2019 for patients treated prior to the advent of the electronic medical record. All available clinical pathology material was reviewed for diagnostic accuracy by a board-certified neuropathologist. Histologic WHO grading was performed using contemporary criteria outlined in the WHO classification of tumors of the central nervous system.[2] Cases for which other tumors remained in the differential diagnosis (such as schwannoma or solitary fibrous tumor) were excluded. All magnetic resonance imaging studies in the discovery cohort were reviewed for accuracy and meningioma location by a board-certified radiologist with a Certificate of Added Qualification in Neuroradiology. The validation cohort was comprised of 365 consecutive meningiomas from patients who were treated at the University of Hong Kong (HKU) from 2000 to 2019 that had frozen tissue suitable for DNA methylation profiling. The medical record was reviewed for all patients in late 2019. For the discovery and validation cohorts, meningioma recurrence was defined as a new radiographic tumor on magnetic resonance imaging after gross total resection, or enlargement/progression/growth of a residual tumor on magnetic resonance imaging after subtotal resection. Inconsistent Simpson grading across neurological surgeons and institutions precluded statistical analysis.

DNA Methylation Profiling and Analysis

DNA was extracted from all 565 meningiomas included in this study. Genomic DNA was processed on the Illumina Methylation EPIC Beadchip (#WG-317-1003, Illumina) according to manufacturer's instructions at the Molecular Genomics Core at the University of Southern California. Downstream analysis was performed in R (v3.5.3 and v3.6.1) with SeSAMe (Bioconductor v3.10). Probes were filtered and analyzed using the standard SeSAMe preprocessing pipeline, including normal-exponential out-of-band background correction, nonlinear dye bias correction, P-value with out-of-band array hybridization masking, and β value calculation (β = methylated/[methylated+unmethylated]). A total of 272 041 probes were masked in at least one sample by the SeSAMe preprocessing pipeline, and 593 877 probes were retained for subsequent analysis.

Preprocessing and β value calculation was repeated using the minfi R package with functional normalization for comparison (Bioconductor v3.10). Probes were filtered based on the following criteria: (1) removal of probes containing common single nucleotide polymorphisms (SNPs) within the targeted CpG sites or on an adjacent base pair (n = 30 435), (2) removal of probes targeting the X and Y chromosomes (n = 19 298), (3) removal of cross-reactive probes previously reported in the literature (n = 39 605), and (4) removal of probes with a detection P > .05 in any samples (n = 12 572).

Principal component analysis was performed independently on the β methylation values from the SeSAMe preprocessing pipeline in R using the base command "prcomp" with the parameters "center = TRUE, scale. = FALSE." Variable probes were identified from the first 3 principal components (PCs), which were chosen for analysis. The elbow method identified 3–4 PCs as the optimal number, but PC4 was excluded from analysis as it contributed to <5% of β value variance and PCs 1–3 contributed to >5% of β value variance. The top 700 probes from PC 1–3 (2100 total probes) were selected for analysis by ranking the absolute gene loading score values within PCs. A cutoff of 700 probes for each PC was chosen based on the distribution of loading scores to balance signal and noise from probes minimally contributing to β value variance. Using 500, 1000, or 1500 probes only regrouped 1%–4% of meningiomas, suggesting the precise number of probes across the top 3 PCs did not significantly affect unsupervised hierarchical clustering. Duplicate probes were removed, and probes with the lowest gene loading scores were culled until 2000 variable probes remained, which were used for unsupervised hierarchical clustering (Pearson correlation distance, Ward's method). Using all 2094 unique probes did not affect unsupervised hierarchical clustering results and using as few as 1900 probes only reclustered 1% of meningiomas, suggesting the precise number of probes from the union set across the top 3 PCs did not significantly affect unsupervised hierarchical clustering.

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Copy Number Variant Analysis

CNV profiles from DNA methylation data were generated with the "cnSegmentation" command within the SeSAMe R package, using the "EPIC.5.normal" dataset from the sesameData package as a copy number-normal control. CNV profiles were generated independent of meningioma DNA methylation groups, and sample-level DNA methylation group identities were unblinded for integrated analyses in the context of other genetic data only after CNVs were defined. CNV intensity value distributions were manually inspected for local minima and maxima, and nadirs separating copy number losses, gains, and neutral events were identified. Segments with mean intensity values less than –0.1 were defined as copy number losses, segments with mean intensity values greater than 0.15 were defined as copy number gains, and segments with intensity values between –0.1 and 0.15 were defined as neutral copy number events. Chromosome arms with at least 80% of their length meeting these criteria were considered losses or gains of the chromosomal arm, respectively. This analysis excluded sex chromosomes and the p arms of acrocentric chromosomes, which had insufficient methylation probes for robust CNV quantification (13p, 14p, 15p, 21p, and 22p). The percentage of the genome with copy number variation was determined by calculating the average number of segments per sample with mean intensity values less than –0.1 or greater than 0.15, weighted by segment length. Genome-wide CNV plots were generated using the R package karyoplotR (Bioconductor v3.10).

RNA Sequencing and Analysis

RNA sequencing was performed on the 200-meningioma discovery cohort and on 302 meningiomas meeting quality metrics from the 365-meningioma validation cohort.

For RNA sequencing of the discovery cohort, library preparation was performed using the TruSeq RNA Library Prep Kit v2 (#RS-122-2001, Illumina) and 50 bp single-end reads were sequenced on an Illumina HiSeq 4000 to a mean of 42 million reads per sample at the UCSF IHG Genomics Core. Quality control of FASTQ files was performed with FASTQC (v0.11.9). Reads were trimmed with Trimmomatic to remove Illumina adapter sequences, leading and trailing bases with quality scores below 20, and any bases that did not have an average quality score of 20 within a sliding window of 4 bases. Any reads shorter than 36 bases after trimming were removed. Reads were mapped to the human reference genome GRCh38 using HISAT2 (v2.1.0) with default parameters. For downstream expression analysis, exon-level count data were extracted from the mapped HISAT2 output using featureCounts (v2.0.1). The FOXM1 target genes analyzed across meningioma DNA methylation groups or subgroups were *TOP2S, CENPF, NEK2, KIF14, MKI67, HJURP, FAM83D, KIF11, NUF2, HMMR, KIFC1, KIF18B, TROAP, CDC20, FAM64A, NCAPH, SPAG5, PTTG1, CDKN3, CCNB1, GAS2L3, ODF2, CCNF, FKBP2, KLHDC4, PGP, TACO1, CDC25B, ZBTB5, ZNF785, HYLS1, RNFT2, CNIH4,* and *METTL13*.[6,18] Pathway analyses and network maps were generated using the gene set file Human_GOBP_AllPathways_no_GO_iea_January_01_2022_symbol.gmt that is maintained and updated regularly by the Bader laboratory (http://download.baderlab.org/EM_Genesets/). GeneSet size was limited to the range between 10 and 200, and 2000 permutations were carried out. The results of the pathway analysis were visualized using the EnrichmentMap App (v.1.2.0) in Cytoscape (v.3.7.2). Network maps were generated and nodes sharing gene overlaps with Jaccard coefficient >0.25 were connected. Clusters of related pathways were identified and annotated using a Cytoscape app that uses a Markov Cluster algorithm connecting pathways by shared keywords in the description of each pathway (AutoAnnotate, v.1.2).

For RNA sequencing of the validation cohort, library preparation was performed using the TruSeq Standard mRNA Kit (#20020595, Illumina) and 150 bp paired-end reads were sequenced on an Illumina NovaSeq 6000 to a mean of 20 million reads per sample at MedGenome Inc. Analysis was performed using a pipeline comprised of FastQC for quality control, and Kallisto for reading pseudo alignment and transcript abundance quantification using the default settings (v0.46.2). Enrichr gene ontology analyses of the validation cohort were used for orthogonal validation of gene expression programs underlying Hypermetabolic or Proliferative meningiomas revealed using EnrichmentMap and Cytoscape in the discovery cohort.

Clinical Analyses

Multivariate Cox regression was performed using the Survival R package. Time-dependent area under the receiver operant conditioning curves (AUC) was calculated for local freedom from recurrence (LFFR) and OS for histologic WHO grade, DNA methylation groups with or without subgroups, or other meningioma molecular grouping schemes using the survivalROC R package, which uses a Kaplan–Meier estimation method. Confidence intervals were estimated using a bootstrap resampling approach with 10 000 iterations. Brier error scores with confidence intervals were estimated using the pec R package, using the "Boot632" split method, with 1000 iterations. Propensity matching with the nearest neighbor approach was performed using the MatchIt R package using a caliper value of 0.2.

Meningioma Molecular Reclassification

The meningiomas in this study were reclassified according to independent molecular grouping schemes based on gene expression profiles,[15] orthogonal DNA methylation analysis techniques,[17] CNVs integrated with histologic features,[7] or DNA methylation profiling integrated with CNVs and histologic features.[9] Reclassification was performed independently by the authors of these studies,[9,15,17] or by integrating histologic features with redefined CNVs based on chromosome arms with at least 30% of their length being lost or gained.[7]

Statistics

No statistical methods were used to predetermine sample sizes, but our discovery and validation cohort sizes are

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similar or larger to those reported in previous publications. Data distribution was assumed to be normal, but this was not formally tested. Investigators were blinded to conditions during clinical data collection and analysis. Bioinformatic analyses were performed blind to clinical features, outcomes, or molecular characteristics. The clinical samples used in this study were retrospective and nonrandomized with no intervention, and all samples were interrogated equally. Thus, controlling for covariates among clinical samples was not relevant. Unless specified otherwise, lines represent means, and error bars represent the standard error of the means. Results were compared using log-rank tests, ANOVA, and Chi-squared tests, which are indicated in the text, methods, and figure legends alongside approaches used to adjust for multiple comparisons. Statistical significance is shown by $*P \leq .05$, $**P \leq .01$, or $***P \leq .0001$.

Data Availability

DNA methylation (n = 565) and RNA sequencing (n = 502) of the meningiomas analyzed in this manuscript have been deposited in the NCBI Gene Expression Omnibus under the accessions GSE183656 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE183656), GSE101638 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE101638), and GSE212666 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE212666). The publicly available GRCh38 (hg38, https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.39/), CRCh37.p13 (hg19, https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.25/), and Kallisto index v10 (https://github.com/pachterlab/kallisto-transcriptome-indices/releases) datasets were used in this study.

Code Availability

The open-source software, tools, and packages used for data analysis in this study, as well as the version of each program, were R (v3.5.3 and v3.6.1), FASTQC (v0.11.9), HISAT2 (v2.1.0), featureCounts (v2.0.1), Kallisto (v0.46.2), caret R package (v6.0–90), PAMES R package (v2.6.2), survival R package (v3.2–13), rms R package (v6.2–0), rpart R package (v4.1.16), DESeq2 (Bioconductor v3.10), SeSAMe (Bioconductor v3.10), karyoplotR (Bioconductor v3.10), ConsensusClusterPlus (Bioconductor v3.10), pec R package(v2021.10.11), survivalROC R package (v1.0.3), and MatchIt R package (v4.3.3). No software was used for data collection.

DNA methylation profile multi-class support vector machine (SVM) classifiers were generated using the caret R package and deposited in the github repository abrarc/meningioma-svm (DOI:10.5281/zenodo.6353877). In brief, a linear kernel SVM was constructed using training data comprising 75% of randomly selected samples from the discovery cohort (n = 150) with 10-fold cross-validation. 2000 probes from each preprocessing pipeline were used as variables. The remaining 25% of samples from the discovery cohort (n = 50) were used to test the model, which performed with 97.9% accuracy when classifying samples into 3 groups (95% CI 89.2%–99.9%, $P < 2.2 \times 10^{-16}$). SVM classifiers for 4, 5, or 6 groups plus subgroups were generated using the same approach and performed with 93.9% (95% CI 83.1–98.7, $P < 2.2 \times 10^{-16}$), 95.8% (95% CI 85.8–99.5, $P < 2.2 \times 10^{-16}$), or 85.4% (95% CI 72.2–93.9, $P = 1.04 \times 10^{-11}$) accuracy, respectively.

Results

Subgroup heterogeneity confounds analyses of central nervous system tumors,[24,25] but rigorous definitions of molecular groups or subgroups have elucidated biological drivers and clarified clinical outcomes.[26,27] Thus, we hypothesized meningioma DNA methylation groups may harbor subgroups unifying contrasting theories of meningioma biology or revealing biomarkers to improve the prediction of postoperative meningioma outcomes. To test this, we reanalyzed meningioma DNA methylation profiling (n = 565), RNA sequencing (n = 502), CNVs (n = 565), or retrospective clinical data (n = 565) from patients with comprehensive follow-up who underwent surgery for meningiomas at 2 independent, international institutions from 1991 to 2019.[6] Unsupervised hierarchical clustering of DNA methylation profiles suggested subgroups may exist within meningioma DNA methylation groups (Figure 1A). In support of this hypothesis, RNA sequencing from the discovery cohort (n = 200) revealed differential enrichment of FOXM1 target genes across two subgroups of Hypermitotic meningiomas (Figure 1A). FOXM1 target genes are biomarkers for meningioma recurrence that drive cell proliferation and radioresistance.[5,6,8,14,18] Differential expression and ontology analyses on the discovery cohort showed the subgroup of Hypermitotic meningiomas without FOXM1 target gene enrichment were distinguished by gene expression programs driving macromolecular metabolism (Figure 1B and Supplementary Table 1). These subgroups of Hypermitotic meningiomas are concordant with Proliferative or Hypermetabolic meningiomas reported alongside Merlin-intact and Immune-enriched tumors after the integration of multiple molecular approaches across 201 tumors.[11] Thus, meningioma DNA methylation subgrouping may unify contrasting theories of meningioma biology into a consensus architecture.

There are no prospective meningioma molecular grouping studies. Indeed, most bioinformatic reports are comprised of nonconsecutive, retrospective cohorts and the natural incidence of molecular groups or subgroups of meningiomas is unknown. To address this limitation, support vector machine (SVM) classifiers assigning meningiomas into 3 groups or 2 groups plus 2 subgroups were constructed using the discovery cohort and performed with 97.9% (95% CI 89.2%–99.9%, $P < 2.2 \times 10^{-16}$) or 93.9% accuracy (95% CI 83.1–98.7, $P < 2.2 \times 10^{-16}$), respectively. SVM classification of DNA methylation profiles revealed the consecutive validation cohort was comprised of 33% Merlin-intact, 41% Immune-enriched, and 26% Hypermitotic meningiomas, the latter of which could be further subdivided into 19% Hypermetabolic and 7% Proliferative meningiomas (Figure 1C). Additional SVM classifiers assigning meningiomas into 5 or 6 groups or subgroups failed to identify tumors meeting the criteria for classification in the discovery cohort. These data

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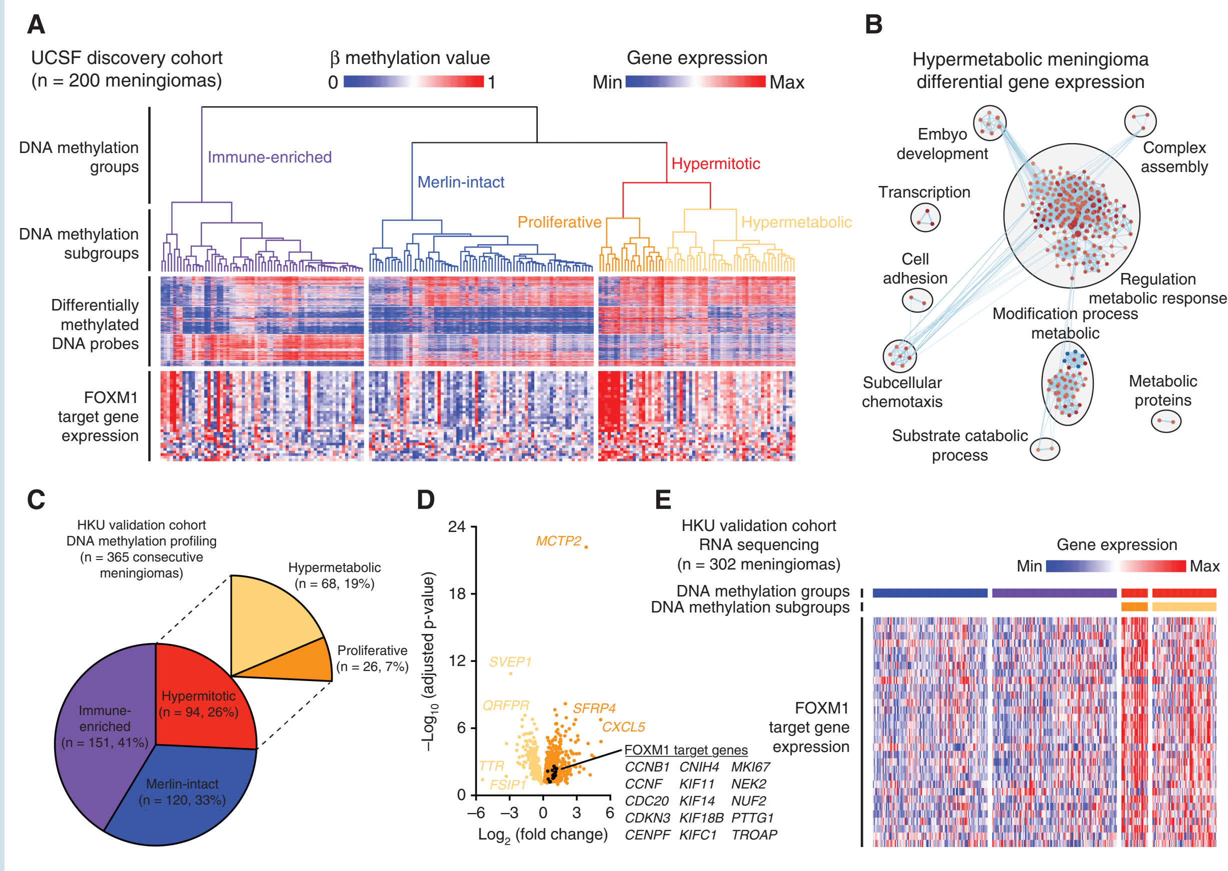

Figure 1. Meningioma DNA methylation groups harbor biologically distinct subgroups. **(A)** Unsupervised hierarchical clustering of meningiomas from the University of California San Francisco (UCSF) discovery cohort (n = 200) using 2094 differentially methylated DNA probes. Dendrogram colors represent previously reported DNA methylation groups or novel DNA methylation subgroups. Heatmaps for DNA methylation probes and FOXM1 target genes from RNA sequencing are shown below the DNA methylation dendrogram. **(B)** Network of gene circuits distinguishing the subgroups of Hypermitotic meningiomas without enrichment of FOXM1 target genes (Hypermetabolic meningiomas) from the subgroup of Hypermitotic meningiomas with enrichment of FOXM1 target genes (Proliferative meningiomas) from RNA sequencing of the UCSF discovery cohort. Nodes represent pathways and edges represent shared genes between pathways. Nodes colored in red or blue are enriched or suppressed pathways in Hypermetabolic compared to Proliferative meningiomas, respectively. **(C)** Incidence of meningioma DNA methylation groups (Merlin-intact, Immune-enriched, or Hypermitotic) or subgroups (Hypermetabolic or Proliferative) after SVM classification of consecutive meningiomas from The University of Hong Kong (HKU) validation cohort. **(D)** Volcano plot from RNA sequencing differential expression analysis of Hypermetabolic versus Proliferative meningiomas from the validation cohort. **(E)** Heatmap for FOXM1 target genes from RNA sequencing of the validation cohort (n = 302) is shown below the DNA methylation group or subgroup.

suggest larger sample sizes, integration of multiple molecular approaches, or technical innovations may be required to accurately classify meningiomas beyond Merlin-intact, Immune-enriched, and Hypermitotic groups, or Hypermetabolic and Proliferative subgroups.

To validate and generalize the gene expression programs distinguishing DNA methylation groups and subgroups of meningiomas, RNA sequencing was performed on the validation cohort (n = 302). Differential expression analysis of RNA sequencing data across subgroups of Hypermitotic meningiomas corroborated the enrichment of cellular metabolism pathways in Hypermetabolic compared to Proliferative meningiomas (Figure 1D and Supplementary Table 2). Differentially expressed genes in Hypermetabolic compared to Proliferative meningiomas from the validation or discovery cohort were conserved (35%), and gene ontology analyses of conserved Hypermetabolic genes showed enrichment of pantothenate and CoA biosynthesis, sphingolipid metabolism, carbohydrate metabolism, and protein transport pathways. Differential expression analysis of Proliferative meningiomas from the validation cohort revealed enrichment of genes underlying adverse meningioma outcomes (*SFRP4*)[5] and FOXM1 target genes that were also enriched in Proliferative meningiomas from the discovery cohort (Figure 1A, D, Supplementary Table 1, 2). FOXM1 target genes were also enriched in Proliferative meningiomas compared to meningiomas from other DNA methylation groups in the validation cohort (Figure 1E). These data suggest a conserved gene expression architecture distinguishes DNA methylation groups and subgroups of meningiomas.

We next investigated the genetic, epigenetic, gene expression, and cellular features distinguishing meningioma DNA methylation groups[6] across meningioma DNA methylation subgroups. Merlin-intact meningiomas were enriched in *NF2* expression, and Immune-enriched, Hypermetabolic, and Proliferative meningiomas were enriched in CNVs deleting *NF2* (Supplementary Figure 1A). Cell-type deconvolution of DNA methylation profiles showed immune cell enrichment in Immune-enriched meningiomas (Supplementary Figure 1B), which also had fewer CNVs deleting the *HLA* locus (Supplementary Figure 1C) and hypomethylation plus increased expression of meningeal lymphatic genes compared to other DNA methylation groups or subgroups (Supplementary Figure 1D). Ki-67 labeling index, genomic instability, CNVs deleting the cell cycle inhibitor *CDKN2A/B,* CNVs amplifying the cell cycle activator *USF1*, or epigenetic silencing of *CDKN2A/B* were enriched in Proliferative meningiomas compared to other DNA methylation groups or subgroups (Supplementary Figure 1E–H). Thus, genetic, epigenetic, gene expression, and cellular features distinguishing meningioma DNA methylation groups are conserved across meningioma DNA methylation subgroups.

Meningioma care is comprised of surgery, grading according to WHO criteria, and consideration of postoperative radiotherapy.[1,2] Genetic, epigenetic, or gene expression grouping appears to improve prediction of meningioma LFFR compared to histologic grading in some studies,[5,7,9–11,14,17] but robust validation cohorts and overall survival (OS) analyses are lacking. To determine if DNA methylation grouping or subgrouping sheds light on postoperative meningioma outcomes, we analyzed extent of resection, histologic WHO grades, LFFR, or OS in the context of postoperative surveillance or postoperative radiotherapy for the 565 meningiomas in our study (Figure 2). There was no difference in the extent of resection across meningioma DNA methylation groups or subgroups (Figure 3A), but Hypermetabolic and Proliferative DNA methylation subgroups were enriched in WHO grade 3 meningiomas that have adverse clinical outcomes[2] (Figure 3B). Indeed, 5-year LFFR was 87.2%, 75.8%, or 41.6% for Merlin-intact, Immune-enriched, or Hypermitotic meningiomas, and 54.1% or 5.9% for Hypermetabolic or Proliferative meningiomas, respectively (Figure 3C). Moreover, 5-year OS was 90.9%, 87.8%, or 64.5% for Merlin-intact, Immune-enriched, or Hypermitotic meningiomas, and 82.2% or 21.8% for Hypermetabolic or Proliferative meningiomas, respectively (Figure 3C). Multivariate Cox regressions including DNA methylation groups with or without subgroups, WHO grade, the extent of resection, postoperative radiotherapy, and primary versus recurrent presentation found that the addition of DNA methylation subgroups independently predicted postoperative LFFR and OS. DNA methylation groups without subgroups were only predictive for postoperative LFFR (Supplementary Table 2). Thus, the addition of DNA methylation subgroups to multivariate clinical models refines the prediction of postoperative LFFR or OS compared to DNA methylation groups.

The performance of meningioma DNA methylation profiling in predicting postoperative LFFR or OS was compared to histologic WHO grading using the time-dependent area under the receiver operating characteristic curves (AUC) and Brier error scores. Models predicting LFFR were marginally refined using DNA methylation profiling compared to WHO grade, but WHO grade was superior in predicting OS with higher true positive, lower false positive, and lower predicted error rates compared to DNA methylation profiling (Figure 3D, E). Hazard ratios for multivariate Cox regression models predicting OS were also higher for WHO grade compared to DNA methylation profiling (Supplementary Table 3).

Biomarkers predicting meningioma radiotherapy responses are lacking, and analysis of LFFR after postoperative radiotherapy in the context of DNA methylation profiling suggested Immune-enriched or Hypermetabolic meningiomas may have adverse responses to ionizing radiation (Supplementary Figure 2A, B). Propensity matching by patient age, histologic WHO grade, extent of resection, and primary versus recurrent presentation revealed

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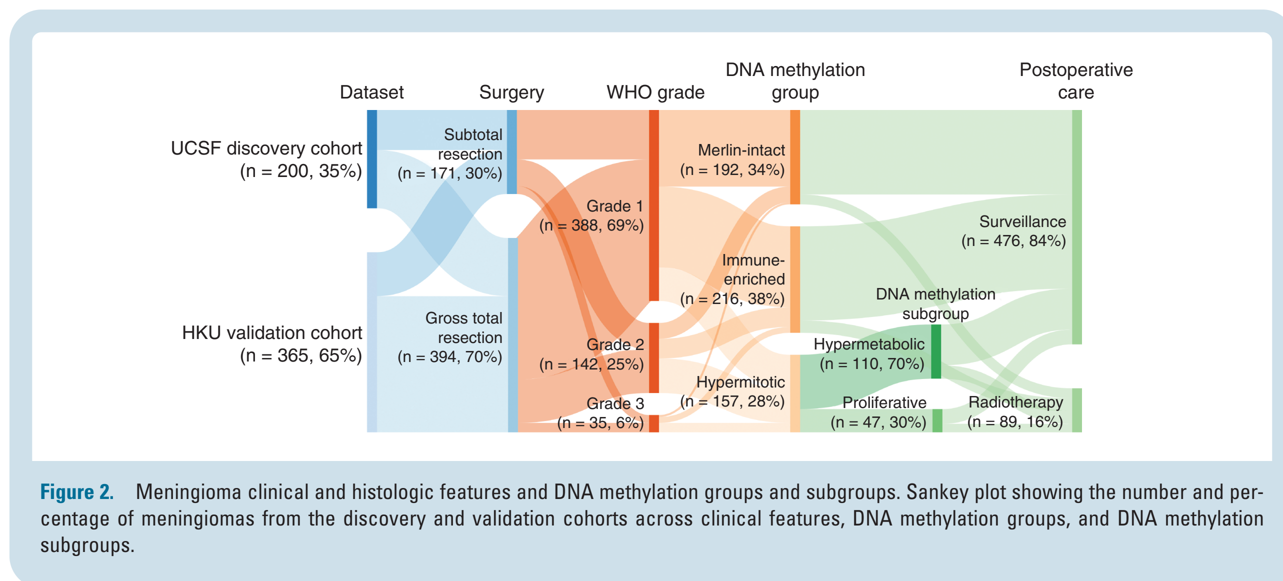

Figure 2. Meningioma clinical and histologic features and DNA methylation groups and subgroups. Sankey plot showing the number and percentage of meningiomas from the discovery and validation cohorts across clinical features, DNA methylation groups, and DNA methylation subgroups.

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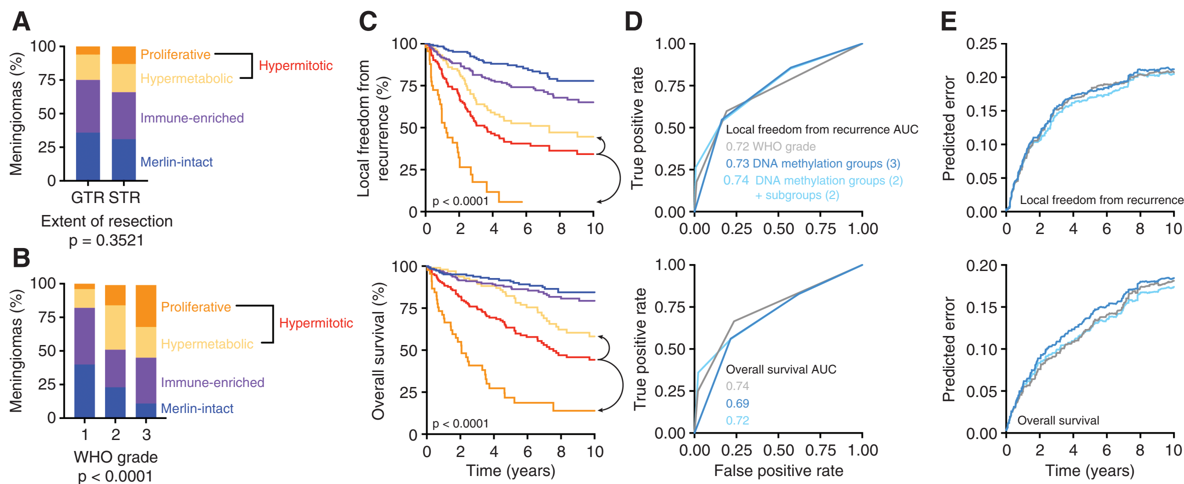

Figure 3. Meningioma DNA methylation groups and subgroups predict postoperative outcomes. **(A)** Meningioma extent of resection (n = 565) across DNA methylation groups and subgroups (Chi-squared test). **(B)** Meningioma WHO grades (n = 565) across DNA methylation groups and subgroups (Chi-squared test). **(C)** Kaplan–Meier curves for meningioma local freedom from recurrence (LFFR) or overall survival (n = 565) across DNA methylation groups and subgroups (Log-rank tests). **(D)** Time-dependent area under the receiver operating characteristic curves (AUC) for meningioma LFFR or overall survival across histologic or DNA methylation classifications. **(E)** Brier error scores for meningioma LFFR or overall survival across histologic or DNA methylation classifications.

equivalent LFFR in the overall cohort (Supplementary Figure 2C and Supplementary Table 4) and in Immune-enriched or Hypermetabolic meningiomas with or without postoperative radiotherapy (Supplementary Figure 2C, D and Supplementary Table 4). Adjusting for meningioma location did not enhance prediction of postoperative radiotherapy responses, although there were significant differences in the convexity versus skull base location of Merlin-intact (39% versus 61%), Immune-enriched (74% versus 26%), Hypermitotic (75% versus 25%), Hypermetabolic (71% versus 29%), or Proliferative (81% versus 19%) meningiomas (P < .0001, Chi-squared test). Thus, unsupervised meningioma DNA methylation profiling may not predict radiotherapy responses, providing a rationale to explore alternate genetic, epigenetic, or gene expression biomarkers to resolve this pervasive limitation in postoperative treatment recommendations for meningioma patients.

Discussion

In summary, meningioma DNA methylation profiling reveals groups and subgroups that are distinguished by biological features and demonstrate divergent clinical outcomes. Integrating genetic, epigenetic, gene expression, cellular, and clinical analyses, we find unsupervised meningioma DNA methylation profiling predicts LFFR and OS, but not radiotherapy responses. These results reveal an architecture unifying contrasting theories of meningioma biology that may provide a useful foundation for future prospective trials investigating molecular therapies or molecular grouping schemes for meningioma patients. To that end, we developed an online public resource to perform meningioma DNA methylation grouping (https://william-c-chen.shinyapps.io/MeninMethylClassApp/).

Our approach and the approach previously used to identify Hypermetabolic and Proliferative meningiomas have significant differences.[11] Here, we analyzed 565 meningioma DNA methylation profiles from independent discovery (n = 200) or validation (n = 365) institutions using a bioinformatic pipeline that controls for inaccurate β methylation values at genomic loci with CNVs.[6,22] We trained SVM classifiers to assign meningioma DNA methylation profiles from our validation cohort into groups or subgroups identified in our discovery cohort, and we used RNA sequencing of the discovery (n = 200) or validation (n = 302) cohort to understand gene expression programs distinguishing DNA methylation groups or subgroups of meningiomas. The prior study reporting Hypermetabolic and Proliferative meningiomas alongside Merlin-intact and Immune-enriched tumors used a cluster-of-clusters approach to integrate DNA methylation profiles, DNA sequencing, RNA sequencing, and CNVs from discovery (n = 121) or validation (n = 80) cohorts.[11] Broadly, we are encouraged such different approaches have converged on such similar biology, suggesting the findings of our studies are robust and, hopefully, clinically tractable. Nevertheless, intratumor and informatic heterogeneity can influence meningioma molecular classification, and although infrequent, paired primary and recurrent meningiomas can classify in different DNA methylation groups.[23] Thus, rigorous, prospective interrogation of meningioma molecular classification systems will be necessary to establish the clinical utility of these approaches for meningioma patients.

Alternate meningioma molecular grouping schemes have been proposed based on unsupervised hierarchical

clustering of RNA sequencing (gene expression types),[15] recombinations of DNA methylation classes according to clinical outcomes (DNA methylation families),[17] CNVs integrated with histologic features training on clinical outcomes (integrated grade),[7] or DNA methylation families integrated with CNVs and histologic features (integrated score).[9] Reclassifying the meningiomas in our study according to these independent molecular grouping schemes revealed high concordance across unsupervised systems shedding light on meningioma biology,[6,11,15] but poor concordance across supervised systems incorporating or trained on clinical endpoints[7,9,17] (Figure 4). Thus, diverse meningioma molecular grouping schemes appear to provide complementary information about meningioma clinical outcomes, but the biologically driven groups and subgroups we report may be particularly useful for designing biomarker-guided clinical trials for meningioma patients. As each of these systems can be derived or closely approximated from DNA methylation profiles (Figure 4), future clinical trials may wish to incorporate this technique to facilitate prospective comparisons across prognostic systems.

Although the meningioma DNA methylation groups and subgroups we report shed light on biological drivers and therapeutic vulnerabilities informing new treatments for meningioma patients,[6] in the context of current surgical or radiotherapy treatments, histologic WHO grade provides superior prediction of postoperative OS (Supplementary Table 3). The recent addition of *TERT* promoter mutation or *CDKN2A/B* deletion to WHO grading criteria for meningiomas will likely enhance the performance of this enduring system,[2] and could serve as a roadmap for the addition of other meningioma molecular data such as DNA methylation groups or subgroups to future revisions of the WHO classification of central nervous system tumors. To that end, the 6 meningioma molecular grouping schemes we investigate across the 565 meningiomas in our study each provide robust estimates of 5-year local freedom from progression and overall survival using time-dependent area under the receiver operating characteristic curves

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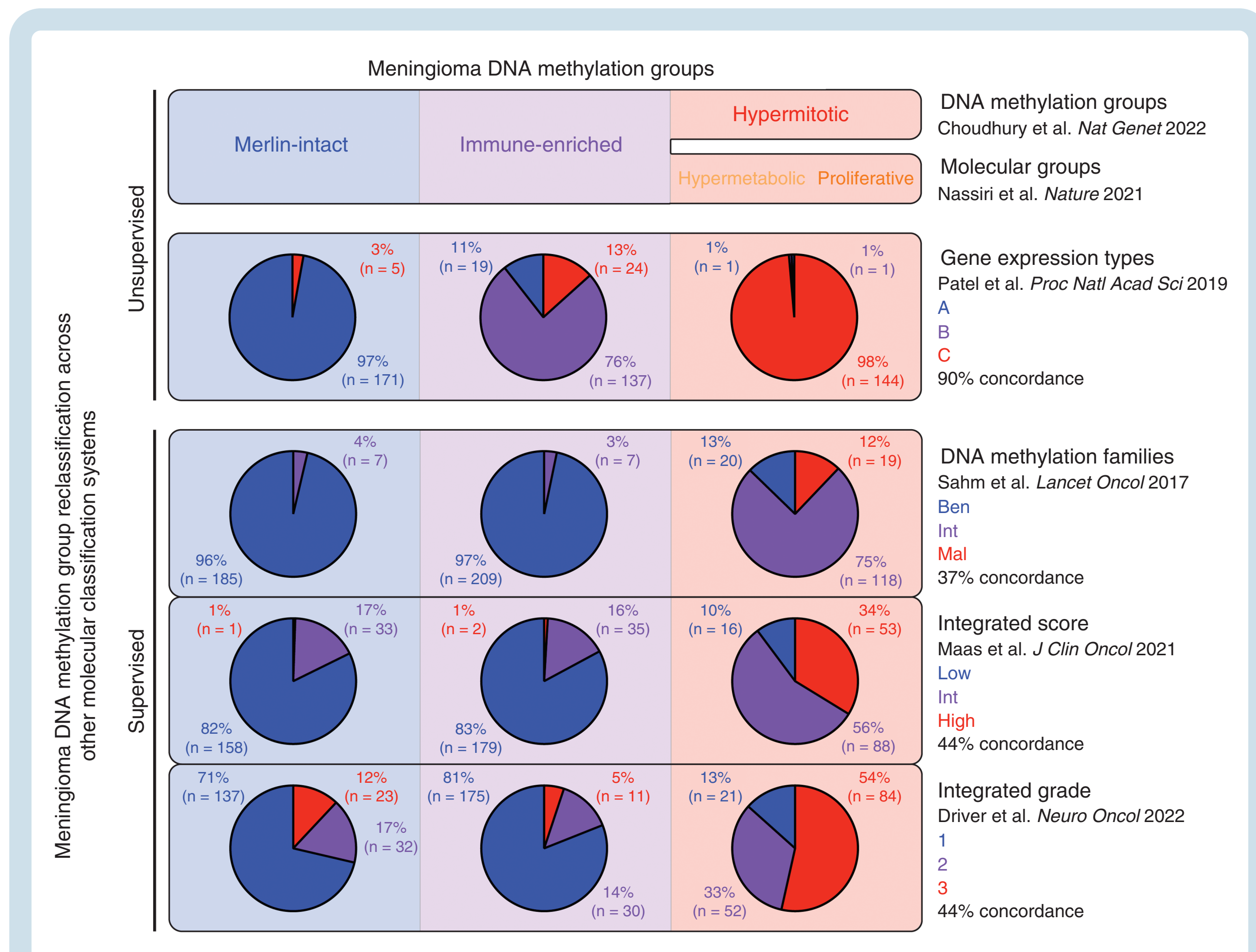

Figure 4. Meningioma DNA methylation groups and subgroups across alternate molecular grouping schemes. Reclassification of the meningioma in this study according to alternate molecular grouping schemes based on gene expression profiles[15] (gene expression types, n = 502), orthogonal DNA methylation analysis techniques[17] (DNA methylation families, n = 565), copy number variants (CNVs) integrated with histologic features[7] (integrated grade, n = 565), or DNA methylation profiling integrated with CNVs and histologic features[9] (integrated score n = 565). Each of these molecular schemes proposes 3 groups of meningiomas, and concordance across schemes was calculated by dividing the observed distribution of low/intermediate/high-risk cases by the expected distribution from meningioma DNA methylation grouping.[6].

(DNA methylation groups: 0.73 and 0.69, DNA methylation subgroups: 0.74 and 0.72, gene expression types: 0.73 and 0.68, DNA methylation families: 0.70 and 0.71, integrated grade: 0.79 and 0.79, integrated score: 0.75 and 0.75). Determining which of these systems may be "best" will likely require integration of many thousands of retrospective meningiomas plus prospective validation. Even if a "winner" can be identified, regional or institutional logistic, technical, or financial barriers may encumber conformity in clinical practice. In the interim, the unifying biological architecture we report may be a useful foundation for biomarker-guided clinical trials for meningioma patients.

Supplementary Material

Supplementary material is available online at *Neuro-Oncology* (http://neuro-oncology.oxfordjournals.org/).

Keywords

cancer | central nervous system | DNA methylation | meningioma | tumor

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Authorship

All authors made substantial contributions to the conception or design of the study, or to the acquisition, analysis, or interpretation of data. All authors approved the manuscript. All authors agree to be personally accountable for individual contributions and to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated, resolved, and the resolution documented in the literature. A.C. developed, designed, performed, and analyzed bioinformatic data. W.C.C. developed, designed, performed, and analyzed clinical data. C.H.G.L. analyzed meningioma histology. J.C.B. and T.K. reclassified meningiomas according to alternate molecular grouping schemes. S.S., A.J.P., W.L.B., F.S., and A.P. supervised the study. S.T.M. extracted nucleic acids from meningiomas, assembled clinical data, and supervised the study. D.R.R. conceived, designed, and supervised the study.

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