Targeted gene expression profiling predicts meningioma outcomes and radiotherapy responses

A list of authors and their affiliations appears at the end of the paper

Surgery is the mainstay of treatment for meningioma, the most common primary intracranial tumor, but improvements in meningioma risk stratification are needed and indications for postoperative radiotherapy are controversial. Here we develop a targeted gene expression biomarker that predicts meningioma outcomes and radiotherapy responses. Using a discovery cohort of 173 meningiomas, we developed a 34-gene expression risk score and performed clinical and analytical validation of this biomarker on independent meningiomas from 12 institutions across 3 continents ($N = 1,856$), including 103 meningiomas from a prospective clinical trial. The gene expression biomarker improved discrimination of outcomes compared with all other systems tested ($N = 9$) in the clinical validation cohort for local recurrence (5-year area under the curve (AUC) 0.81) and overall survival (5-year AUC 0.80). The increase in AUC compared with the standard of care, World Health Organization 2021 grade, was 0.11 for local recurrence (95% confidence interval 0.07 to 0.17, $P < 0.001$). The gene expression biomarker identified meningiomas benefiting from postoperative radiotherapy (hazard ratio 0.54, 95% confidence interval 0.37 to 0.78, $P = 0.0001$) and suggested postoperative management could be refined for 29.8% of patients. In sum, our results identify a targeted gene expression biomarker that improves discrimination of meningioma outcomes, including prediction of postoperative radiotherapy responses.

Meningiomas account for 39.7% of primary intracranial tumors and are the only brain tumors that are more common in women, Black and elderly patients, who are underrepresented in brain tumor clinical trials[1,2]. Meningioma treatments are largely restricted to surgery and radiotherapy, and systemic therapies remain ineffective or experimental[3,4]. Historically, the World Health Organization (WHO) has graded meningiomas according to histological features such as mitotic count[5]. Most WHO grade 1 meningiomas can be effectively treated with surgery or radiotherapy, but many WHO grade 2 or grade 3 meningiomas are resistant to treatment and cause substantial neurological morbidity and mortality[3]. Moreover, some WHO grade 1 meningiomas develop recurrences that cannot be predicted from

histological features, and some WHO grade 2 or grade 3 meningiomas are unexpectedly well controlled with surgery and radiotherapy. In recognition of the controversies surrounding meningioma risk stratification and treatment, the NRG BN-003 and EORTC 1308 Phase III clinical trials randomize patients with primary WHO grade 2 meningiomas to postoperative surveillance or postoperative radiotherapy after gross total resection (GTR)[6]. The only multicenter prospective studies of meningioma radiotherapy that have reported data are RTOG 0539 and EORTC 22042, and these Phase II clinical trials provide safety and nonrandomized data based on clinical criteria that do not predict radiotherapy responses in most retrospective series[7-10]. Thus, there are unmet needs for improved risk stratification

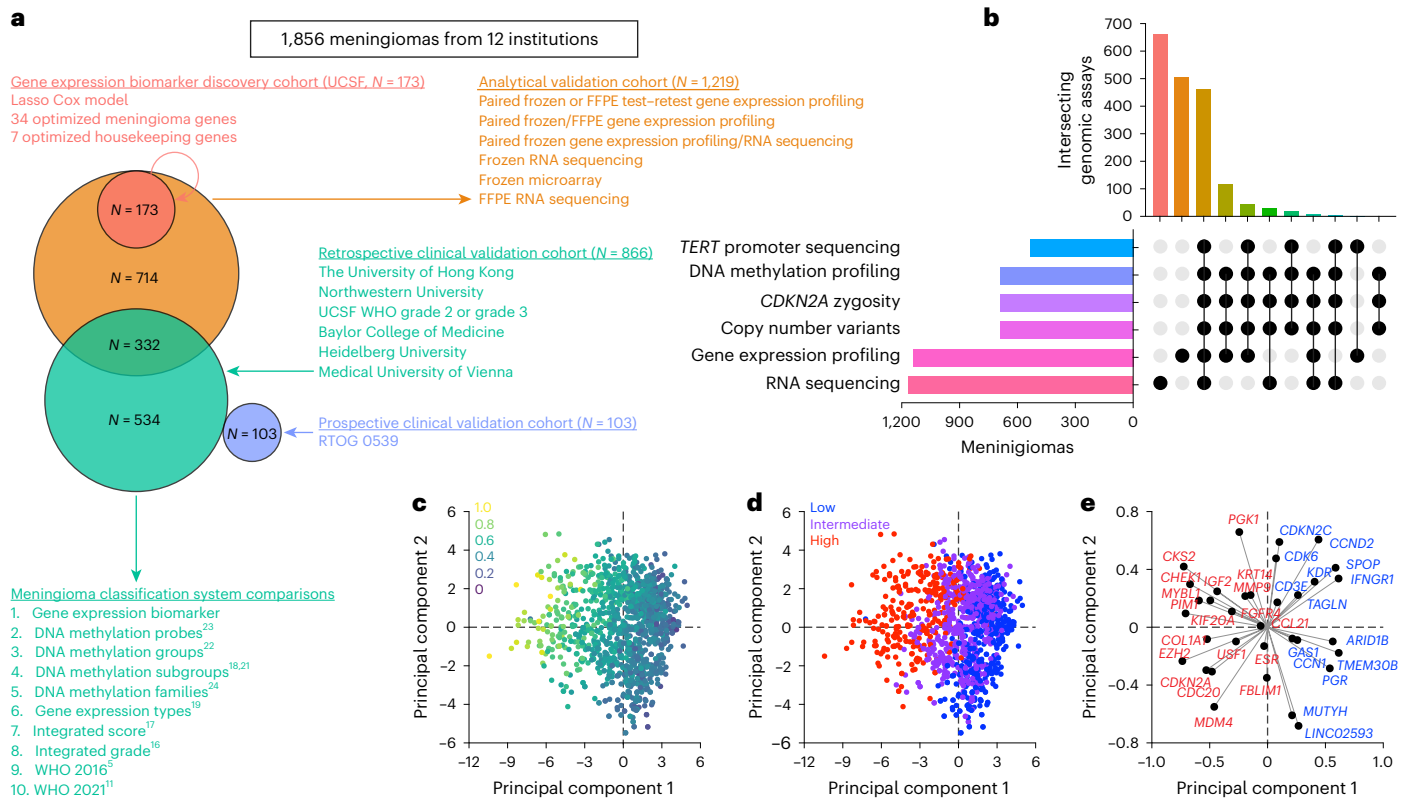✉e-mail: william.chen@ucsf.edu; stephen.magill@northwestern.edu; david.raleigh@ucsf.edu

Fig. 1 | Study design and gene expression biomarker characteristics. a, Study design and numbers of meningiomas used for gene expression biomarker development, analytical validation (Extended Data Fig. 4 and Supplementary Data Table 4), clinical validation and comparison across classification systems. For additional details, see Table 1, Supplementary Methods and Supplementary Data Tables 1–9. Numbers in black text denote intersecting (for example, 173 and 332) or non-intersecting (for example, 714, 534 and 103) meningiomas used for each analysis. Color-matched numbers in adjacent text annotations denote the total number of meningiomas (whether intersecting or non-intersecting) that were used for each analysis. Superscript numbers correspond to manuscripts reporting comparator meningioma classification systems in the references.

b, Upset plot of 4,898 bioinformatic assays (horizontal) performed across 1,856 unique meningiomas (vertical) to define and compare molecular classification systems in this study. **c,d**, Distribution of continuous (**c**) or discrete (**d**) gene expression risk scores in principal component space. Dots represents individual meningiomas from the training, multicenter retrospective and prospective clinical validation cohorts ($N = 1,142$). **e**, Loading scores for the 34 genes composing the gene expression biomarker. A simplified color scheme shows genes associated with higher risk in red and genes associated with lower risk in blue in the first two principal components. Further information regarding the biological function of these genes can be found in Supplementary Methods and Supplementary Table 3.

and prediction of postoperative radiotherapy responses for patients with meningiomas.

In 2021, the WHO revised meningioma grading criteria to incorporate rare hotspot *TERT* promoter mutations and homozygous deletion of *CDKN2A/CDKN2B* alongside traditional histological features[11]. The WHO 2021 update reflects a growing understanding of the molecular landscape of meningiomas from diverse bioinformatic studies. DNA sequencing[12–15], copy number variant (CNV) analyses[16–18], RNA sequencing[19,20] or DNA methylation profiling[21–24] have been used to classify meningiomas based on recurring somatic short variants[12–15], chromosome gains or losses[16–18], differentially expressed genes[19,20] or DNA methylation probes[23], families[24], groups[22] or subgroups[21]. Integrated systems have been proposed based on (1) CNVs, *CDKN2A/CDKN2B* status and histological features (integrated grade)[16], (2) CNVs, DNA methylation families and histological features (integrated score)[17], or (3) CNVs, DNA methylation profiling, RNA sequencing and DNA sequencing, which reveal biological groups and subgroups of meningiomas that are concordant with results from DNA methylation profiling or RNA sequencing alone[18,21,22]. It is unknown which of these diverse classification system(s) may optimize risk stratification or predict postoperative radiotherapy responses for patients with meningiomas.

Knowledge of biological pathways underlying diverse cancers has generated robust targeted gene expression biomarkers that

are recommended for risk stratification and prediction of treatment response by the National Comprehensive Cancer Network (NCCN)[25–30]. A small pilot study suggested that targeted gene expression profiling may be useful for meningioma risk stratification[31], but an optimized gene expression biomarker as well as the analytical validity, clinical validity, generalizability and potential impact of this approach on postoperative meningioma management were unknown.

In this Article, we use knowledge of biological pathways underlying meningiomas from bioinformatic studies[11–22,31] to develop a 34-gene expression biomarker that is prognostic for clinical outcomes in a single-institution discovery cohort. We perform clinical and analytical validation of the gene expression biomarker using independent meningiomas from a large multicenter retrospective cohort, and compare biomarker performance across contemporary meningioma classification systems and clinical contexts using a total of 4,898 bioinformatic assays (Fig. 1a,b). We provide investigator-blinded, independent validation of the gene expression biomarker using a multicenter prospective cohort of meningiomas from patients enrolled on RTOG 0539. In sum, our results reveal the gene expression biomarker provides additional information for meningioma outcomes compared with recent classification systems, including prediction of postoperative radiotherapy responses.

Table 1 | Discovery and clinical validation cohort characteristics

	Discovery	Retrospective clinical validation	Prospective clinical validation
Meningiomas, no.	173	866	103
Patients, no.	166	801	103
Females, no. (%)	112 (67.5)	543 (68.7)	68 (66.0)
Median age (IQR), years	57.0 (45–65.1)	58.9 (48.6–67.6)	57 (49–65)
Setting, no. (%)			
Primary	143 (82.7)	635 (80.1)	81 (78.6)
Recurrent	30 (17.3)	153 (19.4)	22 (21.4)
Not available	0 (0.0)	78 (9.0)	0 (0.0)
Extent of resection, no. (%)			
Gross total	110 (63.6)	541 (69.8)	70 (68.0)
Subtotal	63 (36.4)	234 (30.2)	17 (16.5)
Not available	0 (0.0)	91 (10.5)	16 (15.5)*
WHO grade, no. (%)**			
1	83 (50.0)	499 (57.6)	51 (49.5)
2	65 (37.6)	240 (27.7)	37 (35.9)
3	25 (14.4)	127 (14.7)	15 (14.6)
Gene expression risk score, no. (%)			
Low	63 (36.4)	252 (29.1)	39 (37.9)
Intermediate	72 (41.6)	406 (46.9)	46 (44.7)
High	38 (22.0)	208 (24.1)	18 (17.5)
Postoperative radiotherapy, no. (%)	33 (19.1)	147 (17.3)	63 (61.1)
Median follow up (IQR), years	8.1 (3.9–11.9)	5.2 (2.3–8.7)	8.4 (5.1–9.3)
Local recurrence, no. (%)	61 (35.3)	253 (29.2)	29 (28.2)***
Death, no. (%)	46 (26.6)	190 (21.9)	21 (20.4)

The discovery cohort was composed of frozen meningiomas from a single institution (UCSF) (Supplementary Data Table 2). The non-overlapping retrospective clinical validation cohort was composed of frozen (N=572) and FFPE meningiomas (N=294) from six institutions: consecutive meningiomas from The University of Hong Kong (Supplementary Data Table 5), and nonconsecutive meningiomas from Northwestern University (Supplementary Data Table 6), UCSF (Supplementary Data Table 7), Baylor College of Medicine (Supplementary Data Table 8), Heidelberg University and Medical University of Vienna (Supplementary Data Table 9). The non-overlapping prospective clinical validation cohort was composed of FFPE meningiomas from RTOG 0539 (Supplementary Data Table 13), a completed prospective clinical trial of postoperative radiotherapy or postoperative observation for patients with meningioma. *Some recurrent meningiomas from patients enrolled on RTOG 0539 received radiotherapy without repeat surgery. **WHO 2016 grade based on histological criteria. ***The events from RTOG 0539 were defined as progression or death, and outcomes for this cohort are reported as PFS or OS.

Results

Gene expression biomarker development

Targeted gene expression profiling of 173 meningiomas in the discovery cohort (Table 1 and Supplementary Tables 1 and 2) resulted in a 34-gene expression biomarker and continuous risk score between 0 and 1 that was converted into discrete low-, intermediate- and high-risk groups for Kaplan–Meier analyses (Fig. 1c–e, Extended Data Fig. 1 and Supplementary Table 3). The gene expression biomarker was well distributed across intracranial meningioma locations and recurring somatic short variants, and was prognostic for local freedom from recurrence (LFFR) and overall survival (OS) (Extended Data Figs. 2 and 3). The gene expression biomarker model, risk score and cutoffs were locked and applied without alteration to multicenter retrospective

and prospective validation cohorts from 12 institutions (Table 1 and Supplementary Tables 4–15).

Gene expression biomarker analytical validation

Analytical validity, including reproducibility over time and across laboratories, paired frozen and formalin-fixed paraffin-embedded (FFPE) meningioma samples, and different approaches for gene expression quantification was established using the multicenter analytical validation cohort (N = 1,219 meningiomas, 8 institutions) (Fig. 1a, Extended Data Fig. 4 and Supplementary Table 4). Test–retest conditions, different centers and paired frozen/FFPE meningiomas generated concordant gene expression risk scores (Extended Data Fig. 4a,b) that were tractable and discriminatory for meningioma outcomes when RNA sequencing or microarray approaches were used to assess the 34-gene signature (Extended Data Fig. 4c–g). With regard to cross-platform interoperability, we found DNA methylation profiling was not able to reliably reproduce the gene expression risk score (Supplementary Methods)

Gene expression biomarker clinical validation

In the multicenter retrospective clinical validation cohort (N = 866 meningiomas, 6 institutions, N = 572 frozen, N = 294 FFPE) (Fig. 1a and Extended Data Figs. 4–9), the gene expression biomarker achieved a concordance index (c-index) of 0.78 for LFFR and 0.78 for OS (Extended Data Fig. 5). The gene expression biomarker delineated clinically meaningful low-, intermediate- or high-risk groups with 5-year LFFR of 92.2% (95% confidence interval (CI) 88.3% to 96.2%), 72.6% (95% CI 67.8% to 77.8%) and 19.4% (95% CI 13.5% to 28.0%), respectively (Fig. 2a), and remained well calibrated in meningiomas from individual clinical validation institutions (Extended Data Fig. 5a). The gene expression biomarker was prognostic for LFFR and OS among meningiomas presenting in primary or recurrent settings, after GTR or subtotal resection (STR), across WHO grades using histological (WHO 2016)[5] or histological and molecular criteria (WHO 2021)[11], and remained independently prognostic on multivariate analysis incorporating meningioma setting (primary or recurrent), extent of resection, and WHO grade (Fig. 2b, Extended Data Fig. 5b and Supplementary Tables 10 and 11). The gene expression biomarker was prognostic for LFFR and OS within strata from other meningioma molecular classification systems based on DNA methylation probes[23], groups[22], subgroups[18,21] or families[24], or based on gene expression types[19], integrated score[17] or integrated grade[16] (Extended Data Fig. 5c), and remained independently prognostic on multivariate analyses incorporating each of the nine other meningioma classification systems (Supplementary Tables 10 and 11).

Comparison across meningioma classification systems based on molecular[18,19,21–24], molecular and histological[16,17], or WHO criteria[5,11] using pairwise model combinations[32] revealed the gene expression biomarker provided additional prognostic information for LFFR and OS in combination with each of the nine other systems tested (Fig. 3a and Extended Data Fig. 6a). No other meningioma classification system provided additional prognostic information for LFFR in combination with the gene expression biomarker (Fig. 3a and Extended Data Fig. 6b,c), and only WHO 2021 grade provided additional prognostic information for OS (Fig. 3a). The gene expression biomarker achieved the lowest Brier error score over time for LFFR across meningioma classification systems and had an error score that was comparable to WHO 2021 grade and integrated grade over time for OS (Fig. 3b). The gene expression biomarker achieved the highest 5-year area under the curve (AUC) for LFFR (0.81) and OS (0.80) across meningioma classification systems, with a delta-AUC for LFFR of +0.07 (95% CI 0.02 to 0.12, P < 0.001) compared with the next best performing system (integrated grade), and a delta-AUC for LFFR of +0.11 (95% CI 0.07 to 0.17, P < 0.001) compared with the current standard of care (WHO 2021 grade) (Fig. 3c). To translate these findings into clinical practice, nomograms were generated for prediction of 5-year LFFR or OS based on
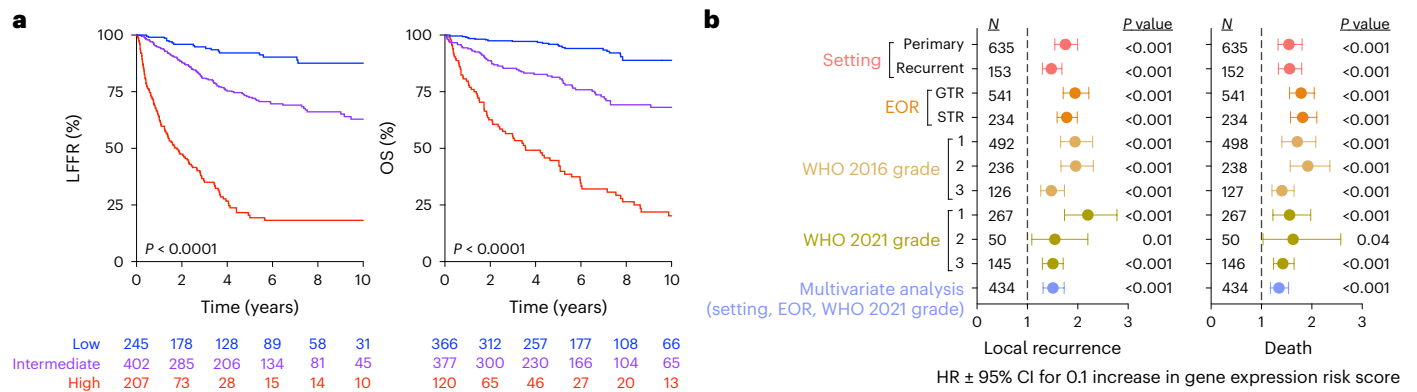
Fig. 2 | Gene expression biomarker discrimination of meningioma outcomes. **a**, Kaplan–Meier curves for LFFR or OS in the multicenter retrospective clinical validation cohort stratified by gene expression risk score ($N = 866$ meningiomas, 6 institutions, $N = 854$ with LFFR data, $N = 863$ with OS data). P values shown are from a two-sided log-rank test. Low, intermediate and high gene expression risk scores were associated with 5-year LFFR of 92.2% (95% CI, 88.3% to 96.2%), 72.6% (95% CI 67.8% to 77.8%) and 19.4% (95% CI 13.5% to 28.0%), and 5-year OS of 95.3% (95% CI 92.9% to 97.8%), 83.3% (95% CI 79.3% to 87.5%) and 44.3% (95% CI 35.6% to 55.1%), respectively. **b**, Forest plot of HRs with 95% CI for local recurrence or

death in the multicenter retrospective clinical validation cohort for each 0.1 increase in gene expression risk score across meningioma settings (primary or recurrent), extent of resection (EOR), WHO grades or multivariate analysis. N refers to the number of meningiomas included within each strata for which the HR is reported. For multivariate analysis, the variables shown in parentheses were included as covariates, and N refers to the number of meningiomas included in multivariate analysis. P values are from a two-sided Wald's test without multiple comparisons adjustment.

meningioma gene expression risk score, setting (primary or recurrent), extent of resection, and WHO grade (Fig. 4 and Extended Data Fig. 7).

Biomarker prediction of radiotherapy responses

To incorporate the gene expression biomarker into a clinical framework consistent with contemporary NCCN and European Association of Neuro-Oncology (EANO) guidelines[4,33], meningiomas treated with surgical monotherapy in the multicenter retrospective clinical validation cohort were stratified by extent of resection and gene expression risk score, resulting in a range of clinical subgroups spanning the spectrum of recurrence risk from 5-year LFFR of 96.1% for gene expression low-risk meningiomas with GTR, to 9.8% for gene expression high-risk meningiomas with STR (Fig. 5a). Based on these combined biomarker/surgical strata, favorable and unfavorable meningiomas were distinguished using (1) gene expression low risk with any resection, or gene expression intermediate risk with GTR (favorable), versus (2) gene expression intermediate risk with STR, or gene expression high risk with any resection (unfavorable) (Fig. 5a).

In clinical practice, meningiomas with unfavorable histological features or STR are often treated with postoperative radiotherapy on the basis of retrospective data[4,6,33]. NRG BN-003 and EORTC 1308 represent important prospective studies of radiotherapy for meningioma, but these trials were initiated before the development of biomarkers for meningioma risk stratification and do not incorporate biomarkers potentially elucidating postoperative radiotherapy responses, as defined by a reduced risk of recurrence. In the multicenter retrospective clinical validation cohort, the gene expression biomarker remained prognostic for primary meningioma outcomes among patients receiving fractionated postoperative radiotherapy (Extended Data Fig. 8a), and also among patients with primary WHO grade 2 meningiomas with GTR who may have been eligible for NRG BN-003 or EORTC 1308 (Extended Data Fig. 8b). However, in the absence of biomarker stratification, primary WHO grade 2 meningiomas with GTR did not benefit from postoperative radiotherapy in the multicenter retrospective clinical validation cohort (Extended Data Fig. 8c). Thus, to determine if the gene expression biomarker could predict meningioma radiotherapy responses, primary WHO grade 2 meningiomas were stratified based on favorable versus unfavorable biomarker/surgical criteria (Fig. 5a), which revealed that unfavorable primary WHO grade 2 meningiomas benefited from postoperative radiotherapy (hazard ratio (HR) 0.33,

95% CI 0.14–0.76, $P = 0.009$) but favorable primary WHO grade 2 meningiomas did not ($P = 0.88$) (Fig. 5b). Applying the same biomarker/surgical strata across all WHO grades in the multicenter retrospective clinical validation cohort with propensity matching based on gene expression risk score, extent of resection, and WHO grade revealed that unfavorable meningiomas benefited from postoperative radiotherapy (HR 0.54, 95% CI 0.37 to 0.78, $P = 0.0001$) but favorable meningiomas did not ($P = 0.42$) (Fig. 5c and Supplementary Table 12).

RTOG 0539 was a Phase II multicenter prospective trial that enrolled patients with meningiomas from 78 institutions into 3 clinical risk groups: (1) low clinical risk composed of primary WHO grade 1 meningiomas after any resection, (2) intermediate clinical risk composed of recurrent WHO grade 1 meningiomas after any resection, or primary WHO grade 2 meningiomas after GTR, and (3) high clinical risk composed of WHO grade 3 meningiomas after any resection, recurrent WHO grade 2 meningiomas after any resection, and primary WHO grade 2 meningiomas after STR. Intermediate and high clinical risk patients enrolled on RTOG 0539 received postoperative radiotherapy[8,9], and low clinical risk patients underwent postoperative surveillance[7]. To determine how the gene expression biomarker could potentially refine postoperative management, meningiomas in the multicenter retrospective clinical validation cohort were assigned to RTOG 0539 clinical risk groups and compared across assignments with gene expression biomarker risk groups. The gene expression biomarker improved discrimination of meningioma outcomes across clinical groups used for postoperative radiotherapy stratification in RTOG 0539 (Extended Data Fig. 8d) and reclassified 52.0% (Supplementary Table 15) of meningiomas compared with clinical criteria, including downstaging 21.3% of intermediate clinical risk patients who would have received postoperative radiotherapy on RTOG 0539 (Fig. 5d). Using favorable versus unfavorable biomarker/surgical strata that predict radiotherapy responses (Fig. 5a–c), these data suggest that postoperative management could have been refined for 29.8% of patients in the multicenter retrospective clinical validation cohort compared with clinical criteria from RTOG 0539.

Biomarker validation in meningiomas from a prospective clinical trial

Investigator-blinded, independent validation of the gene expression biomarker was performed using meningiomas and clinical data that
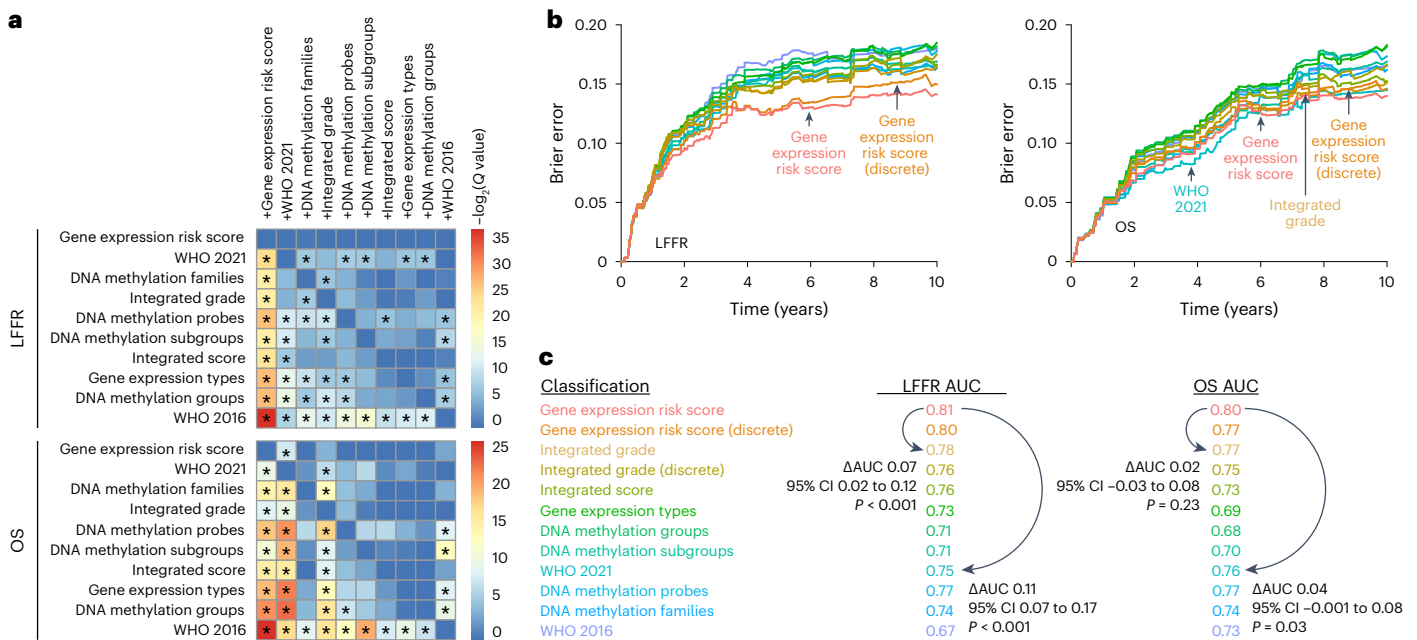
Fig. 3 | Gene expression biomarker comparisons with other meningioma classification systems. a, Heatmap of $-\log_2$-transformed two-sided P values with false discovery rate (FDR) correction (Q values) for pairwise likelihood-ratio tests[32] of improvements in Cox regression models for LFFR or OS. Meningioma classification systems in columns (for example +Gene expression risk score) were combined with meningioma classification systems in rows. The performance of combined models was assessed using 290 consecutive meningiomas from The University of Hong Kong validation cohort with available data to define all ten meningioma classification systems tested. Asterixis denote Benjamini–Hochberg-corrected $Q < 0.01$. Combination with the gene expression risk score improved all other models tested for both LFFR and OS (first column). Conversely, no models improved the gene expression risk score for LFFR (first row, top heatmap), and only WHO 2021 grade provided improvement for OS (first row, bottom heatmap). These findings were additionally validated using multivariate analyses (Supplement Data Tables 10 and 11) and Kaplan–Meier analyses (Extended Data Figs. 5 and 6). **b**, Brier error curves over time for LFFR or OS in the same retrospective validation cohort as in **a**. The gene expression biomarker achieved the lowest Brier error score over time for LFFR across meningioma classification systems and had an error score that was

comparable to WHO 2021 grade and integrated grade over time for OS. **c**, Five-year time-dependent area under the receiver operating characteristic (AUC) for all meningioma classification systems tested. AUC values reflect the performance of each system in all multicenter retrospective clinical validation cohort meningiomas ($N = 866$ meningiomas, 6 institutions) with available data to define each system tested (Supplement Data Tables 4, 10 and 11). Pairwise comparisons were performed for select systems using bootstrap delta-AUC. P values shown are one-sided bootstrap P values. The gene expression biomarker achieved the highest 5-year AUC for LFFR and OS across meningioma classification systems, with a delta-AUC for LFFR of +0.07 (95% CI 0.02 to 0.12, $P < 0.001$) compared with the next best performing system (integrated grade), and a delta-AUC of +0.11 for LFFR (95% CI 0.07 to 0.17, $P < 0.001$) and +0.04 for OS (95% CI −0.001 to 0.08, $P = 0.03$) compared with the current standard of care (WHO 2021 grade). As was the case for AUC calculations, the number of meningiomas included in each delta-AUC comparison varied depending on the number of meningiomas in the multicenter retrospective clinical validation cohort with available data to define the systems tested in each comparison (Supplement Data Tables 4 and 10). Unless specified (as in **a**), P values are not corrected for multiple comparisons.

were prospectively collected from patients enrolled on RTOG 0539 itself ($N = 103$) (Table 1 and Supplementary Table 13). In comparison with clinical risk groups used to allocate patients to postoperative radiotherapy or postoperative surveillance on this study, the gene expression biomarker reclassified 39.8% of meningiomas from RTOG 0539 (Fig. 5d and Supplementary Table 15), including downstaging 30.3% of intermediate clinical risk patients who received postoperative radiotherapy. The gene expression biomarker was prognostic for progression-free survival (PFS) and OS in patients from RTOG 0539 (Fig. 5d,e) and was well calibrated with 5-year PFS of 92.0%, 76.5% and 38.6% for low-, intermediate- and high-risk groups, respectively. Moreover, the gene expression biomarker remained independently prognostic on multivariate analysis incorporating meningioma setting (primary or recurrent), extent of resection, and WHO grade using data from RTOG 0539 (Supplementary Table 14).

Discussion

Here we use targeted gene expression profiling to develop and validate a polygenic biomarker that provides additional information for meningioma outcomes compared with other classification systems, including prediction of postoperative radiotherapy responses. The gene expression biomarker we report is independently prognostic

across all clinical, histological and molecular contexts tested[5,16,17,19,21–24], including WHO 2021 grade[11], the current standard of care. When incorporated into clinical risk groups defined by contemporary trials[7–9] that are consistent with consensus NCCN and EANO guidelines[4,33], the gene expression biomarker potentially refines postoperative management for 29.8% of patients.

DNA methylation profiling[21–24,34], CNV analysis[16–18], DNA sequencing[12–15] and RNA sequencing[18–20,22] have improved understanding of meningioma biology. Unsupervised bioinformatic analyses paired with mechanistic and functional approaches have identified molecular groups and subgroups of meningiomas with distinct biologic drivers, therapeutic vulnerabilities and clinical outcomes[18,19,21,22]. Supervised bioinformatic models incorporating clinical endpoints have refined risk stratification for meningioma local recurrence[16,17,24,34]. The gene expression biomarker reported here provides additional prognostic information for local recurrence and OS when combined with all unsupervised or supervised meningioma molecular classification systems tested. These findings are concordant with pan-cancer analyses examining gene expression, CNV, DNA methylation, protein expression and DNA sequencing data in 10,884 patients, which suggest gene expression encodes the greatest prognostic information across cancer types[28]. Efforts to reduce molecular classification of
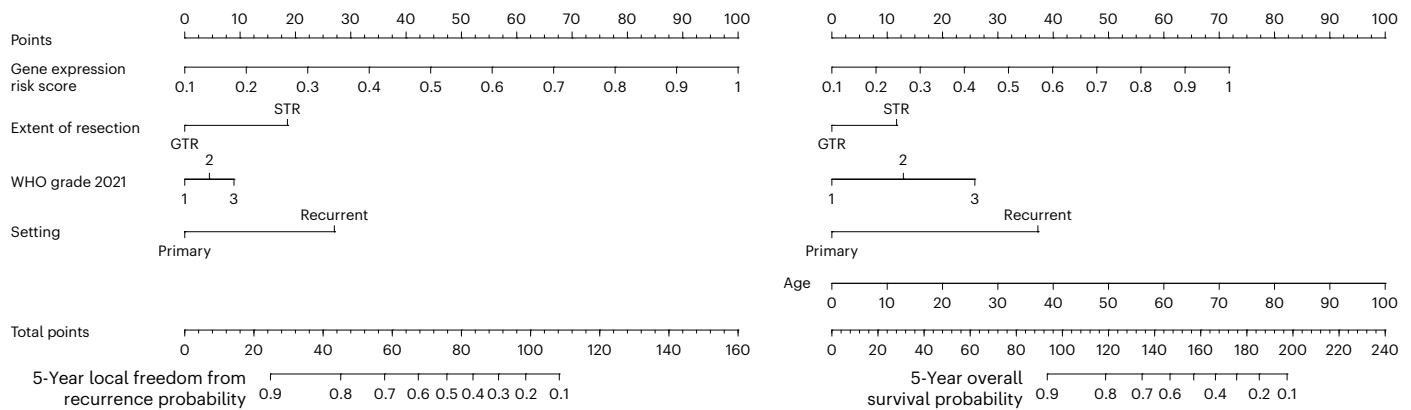
Fig. 4 | Gene expression biomarker nomograms for meningioma outcomes. Nomograms are shown for prediction of 5-year LFFR (left) or OS (right) based on gene expression risk score, setting (primary or recurrent), extent of resection, and WHO 2021 grade using data from the multicenter retrospective clinical validation cohort. Similar nomograms based on WHO 2016 grade are available in Extended Data Fig. 7. To use the nomograms, use a straight edge to draw a vertical line between the variable of interest and the points scale at the top of the nomogram to determine the contribution in points to the total score for each variable. Add up the points from each variable, and then draw a vertical line from the total points scale at the bottom of the nomogram to the 5-year outcome scale to determine the estimated outcome.

meningioma to one or several immunohistochemical stains have thus far not been reproducible[35], and such qualitative or semi-quantitative protein expression biomarkers may not fully capture the quantitative signal of a gene expression-based continuous risk score. Inspection of the genes comprising the biomarker reported here across the cellular architecture of meningiomas from single-cell RNA sequencing[22] reveals that some biomarker genes are enriched in meningioma cells, while other biomarker genes are expressed by the tumor microenvironment (Extended Data Fig. 9). Moreover, the meningioma genes of interest from the literature that we interrogated using targeted gene expression profiling display a range of pairwise correlations in the samples from this study (Supplementary Table 16), and the functional interactions among these genes (which include a long non-coding RNA) or among the cell types in meningiomas, or the relationship(s) of these genes to meningioma histological variants[11], are incompletely understood. Genes included in the biomarker (Supplementary Table 3) comprised genes involved in cell cycle and epigenetic regulation (*CDK6*, *CDKN2A*, *CDC20*, *CKS2*, *CHEK1*, *EZH2* and *MYBL1*) or mitotic stability (*KIF20A*), genes located on recurrent CNVs (*LINC02593* on chromosome 1p, *TMEM30B* on 14q, *USF1* on 1q, and *ARID1B* on 6q), immune-related genes (*CCL21* and *CD3E*), or previously identified prognostic markers in meningioma such as *PGR*, *IGF2* and *COL1A1*.

Current indications for postoperative radiotherapy for patients with meningiomas are controversial, particularly for patients with primary WHO grade 2 meningiomas who are randomized to postoperative surveillance or postoperative radiotherapy on NRG BN-003 and EORTC 1308 after GTR[3,6]. Conflicting retrospective series have variably reported a benefit[9,36–43], or no benefit from radiotherapy in this setting[44–51], which has fueled debate and inspired these international Phase III clinical trials of radiotherapy for patients with meningiomas. The gene expression biomarker reported here improves risk stratification for primary WHO grade 2 meningiomas and may identify favorable WHO grade 2 meningiomas where postoperative radiotherapy could be safely omitted in favor of close surveillance. The gene expression biomarker also identifies primary WHO grade 1 meningiomas with elevated risk of recurrence (Extended Data Fig. 8e). Most meningiomas are WHO grade 1 and are often considered benign, but we found that 6.4% of primary WHO grade 1 meningiomas in the multicenter retrospective clinical validation cohort were classified as gene expression high risk (N = 27), with 5-year LFFR of 43.0%. Of these, only one patient received postoperative radiotherapy (3.7%). The gene expression biomarker also identified 59 primary WHO grade 1 meningiomas (13.9%)

with STR in the multicenter retrospective clinical validation cohort as intermediate risk, and this unfavorable combination was associated with 5-year LFFR of 65.1%. Of these, only three patients (5.1%) received postoperative radiotherapy. In sum, 20.3% of primary WHO grade 1 meningiomas in the multicenter retrospective clinical validation cohort (N = 86 of 423) were reclassified as unfavorable using biomarker/surgical strata, and the overwhelming majority of these patients did not receive radiotherapy (95.3%) although may have benefited from postoperative treatment.

Previous meningioma molecular classification studies have largely not reported OS outcomes. A prospective trial of trabectedin in 90 patients with recurrent WHO grade 2 or grade 3 meningiomas examined DNA methylation families in multivariate analysis without including WHO grade as a covariate, and found meningiomas in the malignant DNA methylation family had worse OS compared with nonmalignant families, although all families (including benign and intermediate) experienced poor outcomes[52]. The data we present using meningiomas from RTOG 0539 demonstrate that the gene expression biomarker was prognostic for OS both before and after adjusting for WHO grade on multivariate analysis, and that outcomes remained well calibrated in this prospective, investigator-blinded validation cohort. For patients with meningiomas, prospective trials such as these will be critical to distinguish conventionally higher-risk cases that may safely undergo postoperative surveillance (Extended Data Fig. 8f,g), elucidate which biomarker(s) could be used for stratification (Extended Data Fig. 8h,i) and determine whether the timing of postoperative radiotherapy or other interventions improves OS (Extended Data Fig. 8j). As clinical trials develop, we do not anticipate targeted gene expression profiling will obviate longstanding and robust meningioma classification systems, such as WHO grade[11], or more recent classification systems that are tractable across multiple brain tumor types, such as DNA methylation profiling, which elucidates biological drivers and vulnerabilities to molecular therapy for meningiomas[21,22,53]. We found DNA methylation profiling was only able to predict the gene expression risk score to a moderate degree (Supplementary Methods). Thus, if incorporated alongside other meningioma classification systems and clinical factors such as extent of resection that are already in widespread use, the gene expression biomarker reported here may offer additional benefit to patients with the most common primary intracranial tumor[1], particularly in terms of postoperative radiotherapy response.

This study should be interpreted in the context of its limitations. First, clinical data in the discovery and multicenter validation cohorts
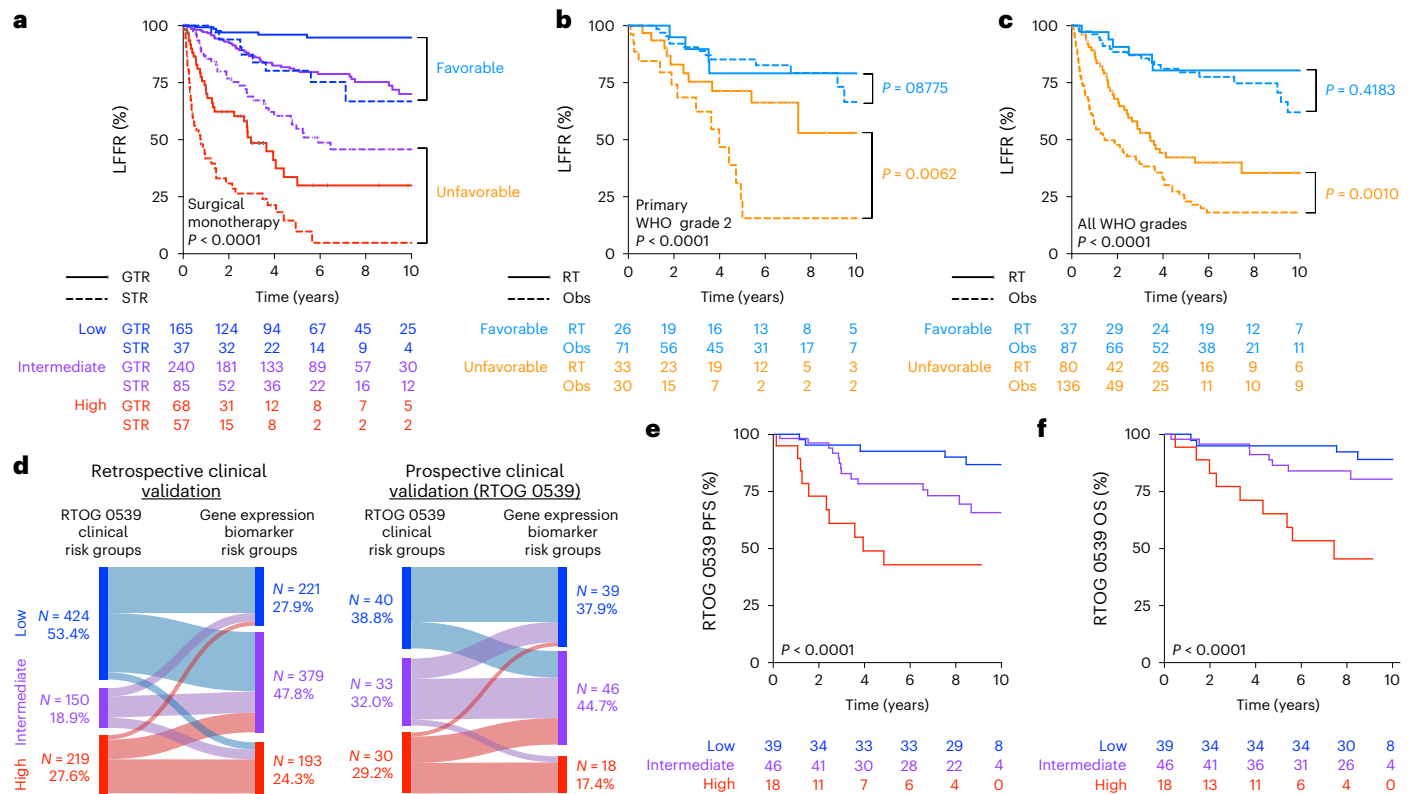
Fig. 5 | Gene expression biomarker prediction of meningioma radiotherapy responses and prognostic validation in samples from a prospective clinical trial. a, Kaplan–Meier curves for LFFR for meningiomas in the multicenter retrospective clinical validation cohort that were treated with surgical monotherapy, stratified by extent of resection and the gene expression risk score. Five-year LFFR was 96.1% for gene expression low-risk meningiomas with GTR, 80.3% for gene expression low risk with STR, 80.5% for gene expression intermediate risk with GTR, 54.9% for gene expression intermediate risk with STR, 30.0% for gene expression high risk with GTR, and 9.8% for gene expression high risk with STR. Meningiomas were grouped as favorable (N = 442) or unfavorable (N = 210) as shown if they had >80% or <80% 5-year LFFR, respectively, for subsequent analyses. P value shown is from a two-sided log-rank test. **b**, Kaplan–Meier curves for LFFR of favorable versus unfavorable primary WHO grade 2 meningiomas in the multicenter retrospective clinical validation cohort that received postoperative radiotherapy (RT) or underwent postoperative observation (Obs). Unfavorable primary WHO grade 2 meningiomas benefited from postoperative radiotherapy (HR 0.33, 95% CI 0.14 to 0.76, two-sided log-rank P = 0.009), while favorable primary WHO grade 2 meningiomas did not (two-sided log-rank P = 0.88). **c**, Kaplan–Meier curves for LFFR of favorable versus unfavorable propensity matched meningiomas in the multicenter retrospective clinical validation cohort that received postoperative radiotherapy or underwent postoperative observation. P values shown are from two-sided

log-rank tests. Cases were first stratified by favorable versus unfavorable criteria, and then matched on the basis of gene expression risk score, extent of resection, and WHO grade (Supplementary Data Table 12). Unfavorable propensity-matched meningiomas benefited from postoperative radiotherapy (HR 0.54, 95% CI 0.37 to 0.78, P = 0.0001), while favorable propensity-matched meningiomas did not (P = 0.42). **d**, Sankey plot of RTOG 0539 clinical risk groups versus gene expression biomarker risk groups in the multicenter retrospective clinical validation cohort (left) or the multicenter prospective clinical validation cohort from RTOG 0539 itself (right). Compared with clinical risk groups used for postoperative radiotherapy stratification in RTOG 0539, the gene expression biomarker reclassified 52.0% (N = 416, Supplementary Data Table 15) of retrospective validation cohort meningiomas, and 39.8% (N = 41, Supplementary Data Table 15) of RTOG 0539 meningiomas. Reclassified meningiomas were better stratified by gene expression risk (Extended Data Fig. 8d). **e**, Kaplan–Meier curves for PFS of patients enrolled on RTOG 0539, stratified by meningioma gene expression risk score. P values are from two-sided log-rank tests. Five-year PFS was 92.0%, 76.5%, and 38.6% for gene expression low-, intermediate- and high-risk groups, respectively (c-index 0.73). **f**, Kaplan–Meier curves for OS of patients enrolled on RTOG 0539, stratified by meningioma gene expression risk score. Five-year OS was 94.7%, 85.7% and 63.0% for gene expression low-, intermediate- and high-risk groups, respectively (c-index 0.73). P value is from a two-sided log-rank test. Unless specified, P values are not corrected for multiple comparisons.

were obtained retrospectively, suggesting our results are susceptible to biases inherent to retrospective research. To address this limitation, we provide additional investigator-blinded, independent validation using meningiomas and clinical data that were prospectively collected from patients enrolled on RTOG 0539. Second, pathology and radiology reviews were performed independently at each institution for meningiomas in the retrospective discovery and validation cohorts. Nevertheless, inter-observer concordance for meningioma WHO grade and imaging characteristics are high[54–56], and any heterogeneity in clinical review across independent cohorts may better represent the heterogeneity intrinsic to routine clinical practice than might be anticipated from central review. To further address this limitation, the meningiomas from RTOG 0539 that were included in this study underwent central pathology and radiology review[7–9,56]. To establish the impact of

the biomarker we report on routine clinical practice, widely available technology with established assay cutoffs and robust, standardized practices for biomarker calculation will be critical. Moreover, further prospective validation is needed to confirm our findings, potentially with benchmarking against other molecular classification systems for meningiomas such as integrated grade, which in our data was the next best performing system. Prospective clinical trials where patients are stratified to postoperative radiotherapy versus observation based on gene expression risk score may be warranted if NRG BN-003 or EORTC 1308 do not show a benefit to postoperative radiotherapy for patients with WHO grade 2 meningiomas after GTR. More broadly, our discovery of a biomarker that identifies meningiomas benefiting from radiotherapy suggests that biopsy-based gene expression risk scores may be useful for guiding observation versus definitive radiotherapy for

incidental meningiomas or other meningiomas that may be managed non-operatively. In contrast to many other tumors, the paradigm of biopsy-based management has not been historically applied to meningiomas, but with identification of meningioma biomarkers that shed light on therapeutic vulnerabilities, longstanding clinical approaches to meningiomas may be revisited.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-023-02586-z.

References

1. Ostrom, Q. T. et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2015–2019. *Neuro Oncol.* **24**, v1–v95 (2022).
2. Reihl, S. J. et al. A population study of clinical trial accrual for women and minorities in neuro-oncology following the NIH Revitalization Act. *Neuro Oncol.* **24**, 1341–1349 (2022).
3. Brastianos, P. K. et al. Advances in multidisciplinary therapy for meningiomas. *Neuro Oncol.* **21**, I18–I31 (2019).
4. Goldbrunner, R. et al. EANO guideline on the diagnosis and management of meningiomas. *Neuro Oncol.* **23**, 1821–1834 (2021).
5. Louis, D. N. et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol.* **131**, 803–820 (2016).
6. Chen, W. C. et al. Radiotherapy for meningiomas. *J. Neuro Oncol.* **160**, 505–515 (2022).
7. Rogers, C. L. et al. Low-risk meningioma: initial outcomes from NRG Oncology/RTOG 0539. *Neuro Oncol.* **25**, 137–145 (2023).
8. Rogers, C. L. et al. High-risk meningioma: initial outcomes from NRG Oncology/RTOG 0539. *Int. J. Radiat. Oncol. Biol. Phys.* https://doi.org/10.1016/j.ijrobp.2019.11.028 (2020).
9. Rogers, L. et al. Intermediate-risk meningioma: Initial outcomes from NRG Oncology RTOG 0539. *J. Neurosurg.* **129**, 35–47 (2018).
10. Weber, D. C. et al. Adjuvant postoperative high-dose radiotherapy for atypical and malignant meningioma: a phase-II parallel non-randomized and observation study (EORTC 22042-26042). *Radiother. Oncol.* https://doi.org/10.1016/j.radonc.2018.06.018 (2018).
11. Louis, D. N. et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol.* **23**, 1231–1251 (2021).
12. Youngblood, M. W. et al. Correlations between genomic subgroup and clinical features in a cohort of more than 3000 meningiomas. *J. Neurosurg.* **133**, 1345–1354 (2020).
13. Clark, V. E. et al. Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. *Science* **339**, 1077–1080 (2013).
14. Sahm, F. et al. TERT promoter mutations and risk of recurrence in meningioma. *J. Natl Cancer Inst.* **108**, djv377 (2016).
15. Guyot, A. et al. Analysis of CDKN2A gene alterations in recurrent and non-recurrent meningioma. *J. Neuro Oncol.* https://doi.org/10.1007/s11060-019-03333-6 (2019).
16. Driver, J. et al. A molecularly integrated grade for meningioma. *Neuro Oncol.* **24**, 796–808 (2022).
17. Maas, S. L. N. et al. Integrated molecular-morphologic meningioma classification: a multicenter retrospective analysis, retrospectively and prospectively validated. *J. Clin. Oncol.* **39**, 3839–3852 (2021).
18. Nassiri, F. et al. A clinically applicable integrative molecular classification of meningiomas. *Nature* **597**, 119–125 (2021).
19. Patel, A. J. et al. Molecular profiling predicts meningioma recurrence and reveals loss of DREAM complex repression in aggressive tumors. *Proc. Natl Acad. Sci. USA* **116**, 21715–21726 (2019).
20. Vasudevan, H. N. et al. Comprehensive molecular profiling identifies FOXM1 as a key transcription factor for meningioma proliferation. *Cell Rep.* **22**, 3672–3683 (2018).
21. Choudhury, A. et al. Hypermitotic meningiomas harbor DNA methylation subgroups with distinct biological and clinical features. *Neuro Oncol.* **25**, 520–530 (2023).
22. Choudhury, A. et al. Meningioma DNA methylation groups identify biological drivers and therapeutic vulnerabilities. *Nat. Genet.* **54**, 649–659 (2022).
23. Olar, A. et al. Global epigenetic profiling identifies methylation subgroups associated with recurrence-free survival in meningioma. *Acta Neuropathol.* **133**, 431–444 (2017).
24. Sahm, F. et al. DNA methylation-based classification and grading system for meningioma: a multicentre, retrospective analysis. *Lancet Oncol.* **18**, 682–694 (2017).
25. Sparano, J. A. et al. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. *N. Engl. J. Med.* **379**, 111–121 (2018).
26. Hu, J. C. et al. Clinical utility of gene expression classifiers in men with newly diagnosed prostate cancer. *JCO Precis. Oncol.* https://doi.org/10.1200/po.18.00163 (2018).
27. Spratt, D. E. et al. Development and validation of a novel integrated clinical-genomic risk group classification for localized prostate cancer. *J. Clin. Oncol.* https://doi.org/10.1200/JCO.2017.74.2940 (2018).
28. Smith, J. C. & Sheltzer, J. M. Genome-wide identification and analysis of prognostic features in human cancers. *Cell Rep.* **38**, 110569 (2022).
29. Schaeffer, E. M. et al. *NCCN Clinical Practice Guidelines in Oncology: Prostate Cancer Version 2.2022* (National Comprehensive Cancer Network, 2021).
30. Gradishar, W. J. et al. Breast Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. *J. Natl Compr. Cancer Netw.* **20**, 691–722 (2022).
31. Chen, W. C. et al. A prognostic gene-expression signature and risk score for meningioma recurrence after resection. *Neurosurgery* **88**, 202–210 (2021).
32. Vuong, Q. H. Likelihood ratio tests for model selection and non-nested hypotheses. *Econometrica* **57**, 307 (1989).
33. Nabors, L. B. et al. Central nervous system cancers, version 3.2020. *J. Natl Compr. Cancer Netw.* **18**, 1537–1570 (2020).
34. Nassiri, F. et al. DNA methylation profiling to predict recurrence risk in meningioma: development and validation of a nomogram to optimize clinical management. *Neuro. Oncol.* https://doi.org/10.1093/neuonc/noz061 (2019).
35. Näslund, O, et al. Meningioma classification by immunohistochemistry: a replicability study. *Brain and Spine* https://doi.org/10.1016/j.bas.2022.101711 (2023).
36. Bray, D. P. et al. Adjuvant radiotherapy in Grade II, atypical meningioma of the skull base. *J. Neurol. Surg. B* **83**, 418–422 (2022).
37. Bray, D. P. et al. Adjuvant radiotherapy versus watchful waiting for world health organization grade II atypical meningioma: a single-institution experience. *Neurosurgery* **88**, E435–E442 (2021).
38. Byun, H. K. et al. Adjuvant radiotherapy versus surveillance for Grade 2 intracranial meningiomas: a multi-institutional propensity score-matched study. *Front. Oncol.* **12**, 877244 (2022).
39. Chen, W. C. et al. Histopathological features predictive of local control of atypical meningioma after surgery and adjuvant radiotherapy. *J. Neurosurg.* https://doi.org/10.3171/2017.9.JNS171609 (2019).
40. Dohm, A. et al. Early or late radiotherapy following gross or subtotal resection for atypical meningiomas: clinical outcomes and local control. *J. Clin. Neurosci.* **46**, 90–98 (2017).

41. Kent, C. L. et al. Long-term outcomes for patients with atypical or malignant meningiomas treated with or without radiation therapy: a 25-year retrospective analysis of a single-institution experience. *Adv. Radiat. Oncol.* **7**, 100878 (2022).

42. Pan, P. C. et al. Outcomes following upfront radiation versus monitoring in atypical meningiomas: 16-year experience at a tertiary medical center. *Neuro Oncol. Adv.* **3**, vdab094 (2021).

43. Rydzewski, N. R. et al. Gross total resection and adjuvant radiotherapy most significant predictors of improved survival in patients with atypical meningioma. *Cancer* **124**, 734–742 (2018).

44. Barrett, O. C. et al. Pathologic predictors of local recurrence in atypical meningiomas following gross total resection. *Int. J. Radiat. Oncol. Biol. Phys.* **103**, 453–459 (2019).

45. Graffeo, C. S. et al. Revisiting adjuvant radiotherapy after gross total resection of world health organization Grade II meningioma. *World Neurosurg.* **103**, 655–663 (2017).

46. Keric, N. et al. Impact of postoperative radiotherapy on recurrence of primary intracranial atypical meningiomas. *J. Neuro Oncol.* **146**, 347–355 (2020).

47. Momin, A. A. et al. Adjuvant radiation versus observation with salvage radiation after gross-total resection of WHO grade II meningiomas: a propensity score-adjusted analysis. *J. Neurosurg.* https://doi.org/10.3171/2021.4.JNS21559 (2021).

48. Rebchuk, A. D. et al. Survival and recurrence outcomes following adjuvant radiotherapy for grade 2 intracranial meningiomas: 13-year experience in a tertiary-care center. *World Neurosurg.* **161**, e748–e756 (2022).

49. Sun, S. Q. et al. Management of atypical cranial meningiomas, part 1: predictors of recurrence and the role of adjuvant radiation after gross total resection. *Neurosurgery* **75**, 347–54 (2014). discussion 354-5; quiz 355.

50. Wang, F. et al. Risk factors associated with postoperative recurrence in atypical intracranial meningioma: analysis of 263 cases at a single neurosurgical centre. *Acta Neurochir.* **161**, 2563–2570 (2019).

51. Zeng, Q. et al. Effectiveness of postoperative radiotherapy in patients with atypical meningiomas after gross total resection: analysis of 260 cases. *World Neurosurg.* **162**, e580–e586 (2022).

52. Preusser, M. et al. Trabectedin for recurrent WHO grade 2 or 3 meningioma: a randomized phase II study of the EORTC Brain Tumor Group (EORTC-1320-BTG). *Neuro Oncol.* **24**, 755–767 (2022).

53. Capper, D. et al. DNA methylation-based classification of central nervous system tumours. *Nature* **555**, 469–474 (2018).

54. Morin, O. et al. Integrated models incorporating radiologic and radiomic features predict meningioma grade, local failure, and overall survival. *Neuro Oncol. Adv.* **1**, vdz011 (2019).

55. Magill, S. T. et al. Multiplatform genomic profiling and magnetic resonance imaging identify mechanisms underlying intratumor heterogeneity in meningioma. *Nat. Commun.* **11**, 4803 (2020).

56. Rogers, C. L. et al. Pathology concordance levels for meningioma classification and grading in NRG Oncology RTOG Trial 0539. *Neuro Oncol.* **18**, 565–574 (2016).

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

William C. Chen [1,2,3] ✉, **Abrar Choudhury** [1,2,3,4,32], **Mark W. Youngblood** [5,32], **Mei-Yin C. Polley** [6,32], **Calixto-Hope G. Lucas** [7], **Kanish Mirchia** [1,2,3], **Sybren L. N. Maas** [8,9], **Abigail K. Suwala** [2,10], **Minhee Won** [6], **James C. Bayley** [11], **Akdes S. Harmanci** [12], **Arif O. Harmanci** [13], **Tiemo J. Klisch** [14], **Minh P. Nguyen** [1,2,3], **Harish N. Vasudevan** [1,2], **Kathleen McCortney** [5], **Theresa J. Yu** [15], **Varun Bhave** [16], **Tai-Chung Lam** [17], **Jenny Kan-Suen Pu** [18], **Lai-Fung Li** [18], **Gilberto Ka-Kit Leung** [18], **Jason W. Chan** [1], **Haley K. Perlow** [19], **Joshua D. Palmer** [19], **Christine Haberler** [20], **Anna S. Berghoff** [21], **Matthias Preusser** [21], **Theodore P. Nicolaides** [22], **Christian Mawrin** [23], **Sameer Agnihotri** [24], **Adam Resnick** [25], **Brian R. Rood** [26], **Jessica Chew** [1], **Jacob S. Young** [1,2,3], **Lauren Boreta** [1], **Steve E. Braunstein** [1], **Jessica Schulte** [27], **Nicholas Butowski** [2,28], **Sandro Santagata** [29], **David Spetzler** [22], **Nancy Ann Oberheim Bush** [2], **Javier E. Villanueva-Meyer** [15], **James P. Chandler** [5], **David A. Solomon** [3], **C. Leland Rogers** [6], **Stephanie L. Pugh** [6], **Minesh P. Mehta** [6,30], **Penny K. Sneed** [1], **Mitchel S. Berger** [2], **Craig M. Horbinski** [5,31], **Michael W. McDermott** [30], **Arie Perry** [2,3], **Wenya Linda Bi** [16], **Akash J. Patel** [11], **Felix Sahm** [10], **Stephen T. Magill** [5,33] ✉ & **David R. Raleigh** [1,2,3,33] ✉

[1]Department of Radiation Oncology, University of California San Francisco, San Francisco, CA, USA. [2]Department of Neurological Surgery, University of California San Francisco, San Francisco, CA, USA. [3]Department of Pathology, University of California San Francisco, San Francisco, CA, USA. [4]Medical Scientist Training Program, University of California San Francisco, San Francisco, CA, USA. [5]Department of Neurological Surgery, Northwestern University, Chicago, IL, USA. [6]NRG Statistics and Data Management Center, NRG Oncology, Philadelphia, PA, USA. [7]Department of Pathology, Johns Hopkins University, Baltimore, MD, USA. [8]Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands. [9]Department of Pathology, Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, the Netherlands. [10]Department of Neuropathology, University Hospital Heidelberg and CCU Neuropathology, German Consortium for Translational Cancer Research, German Cancer Research Center, Heidelberg, Germany. [11]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, USA. [12]Department of Neurosurgery, Baylor College of Medicine, Houston, TX, USA. [13]Center for Secure Artificial Intelligence for Healthcare, Center for Precision Health, School of Biomedical Informatics, The University of Texas Health Science Center, Houston, TX, USA. [14]Department of Molecular and Human Genetics, Baylor College of Medicine, and Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, USA. [15]Department of Radiology and Biomedical Imaging, University of California San Francisco, San Francisco, CA, USA. [16]Department of Neurosurgery, Brigham and Women's Hospital, and Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA. [17]Department of Clinical Oncology, The University of Hong Kong, Pokfulam, China. [18]Division of Neurosurgery, Department of Surgery, The University of Hong Kong, Pokfulam, China. [19]Department of Radiation Oncology, Ohio State University, Columbus, OH, USA. [20]Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna, Vienna, Austria.

[21]Division of Oncology, Department of Medicine, Medical University of Vienna, Vienna, Austria. [22]Caris Life Sciences, Phoenix, AZ, USA. [23]Department of Neuropathology, University of Magdeburg, Magdeburg, Germany. [24]Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, PA, USA. [25]Department of Neurological Surgery, Children's Hospital of Philadelphia, Philadelphia, PA, USA. [26]Brain Tumor Institute, Children's National Hospital, Washington, DC, USA. [27]Neurosciences Department, University of California San Diego, La Jolla, CA, USA. [28]Department of Neurology, University of California San Francisco, San Francisco, CA, USA. [29]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. [30]Miami Neuroscience Institute, Baptist Health, Miami, FL, USA. [31]Department of Pathology, Northwestern University, Chicago, IL, USA. [32]These authors contributed equally: Abrar Choudhury, Mark W. Youngblood, Mei-Yin C. Polley. [33]These authors jointly supervised this work: Stephen T. Magill, David R. Raleigh. ✉e-mail: william.chen@ucsf.edu; stephen.magill@northwestern.edu; david.raleigh@ucsf.edu

Methods

Study design

A discovery cohort composed of 173 retrospective meningiomas with well-annotated clinical follow-up data from a single institution was used to identify and optimize a 34-gene expression biomarker and risk score (Fig. 1a, Extended Data Fig. 1 and Supplementary Tables 1–3). The performance of the gene expression biomarker was validated in three cohorts. First, the analytical validity of the gene expression biomarker was tested in a retrospective analytical validation cohort composed of 1,219 meningiomas from 8 international institutions, some of which had sparse or absent clinical follow-up data (Fig. 1a and Supplementary Table 4). Meningiomas from the discovery cohort, which had matched RNA sequencing, were also used for analytical validation of orthogonal approaches for gene expression quantification (Fig. 1a and Supplementary Table 4). Second, the clinical validity and performance of the gene expression biomarker in comparison with other meningioma classification systems were tested in an independent retrospective clinical validation cohort composed of 866 meningiomas with well-annotated clinical follow-up data from 6 international institutions (Fig. 1a, Table 1 and Supplementary Tables 5–9), some of which were also used for analytical validation (Supplementary Table 4). There was no overlap among meningiomas used to identify and optimize the gene expression biomarker in the discovery cohort (Supplementary Table 2), and meningiomas used for clinical validation (Supplementary Tables 4–9). c-Index, log-rank test, Brier error score, time-dependent area under the receiver operant curve (AUC), delta-AUC, the Kaplan–Meier method, multivariate analysis (Supplementary Tables 10 and 11) and propensity matching (Supplementary Table 12) were used to compare gene expression biomarker performance across contemporary molecular and histological classification systems and clinical contexts. Third, a prospectively collected cohort of 103 meningiomas from patients enrolled on RTOG 0539 were used for investigator-blinded, independent clinical validation (Fig. 1a, Table 1 and Supplementary Tables 13 and 14). In total, 4,898 genomic assays were performed and analyzed across 1,856 unique meningiomas to define and compare molecular classification systems (Fig. 1b). Details on data collection, tissue and nucleic acid processing, genomic assays, pathology review, imaging review, statistical analyses, and prognostic and predictive validation (Extended Data Fig. 1) are reported in Supplementary Methods. This study complied with ethical regulations and was approved by the University of California San Francisco (UCSF) institutional review board (13-12587, 17-22324, 17-23196 and 18-24633) and by relevant Institutional Review Boards at all included institutions. As part of routine clinical care, all patients who were included in this study signed a waiver of informed consent to contribute deidentified data to research projects.

Targeted gene expression profiling and analysis

Targeted gene expression profiling was performed using a hybridization and barcode-based panel (Nanostring nCounter) with internal negative and spike-in positive controls[57] (Supplementary Methods). Positive-control normalized gene counts were standardized by normalization to the geometric mean count of seven meningioma-specific housekeeping genes (Supplementary Table 3). $\log_2$-transformed gene expression values were used for all subsequent analyses. Meningioma-related genes of interest (Supplementary Table 1) were selected on the basis of prognostic or biological relevance in the literature[11–22,31] (Supplementary Methods), and feature selection was performed using a LASSO regularized Cox regression model with the c-index of LFFR in the discovery cohort as the target endpoint (Supplementary Table 2). An optimized set of 34 genes was identified within 1 standard error of the model achieving maximal c-index (Extended Data Fig. 1a and Supplementary Table 3), resulting in a highly discriminatory set of linearly rescaled risk scores between 0 and 1 (Fig. 1c–e and Extended Data Fig. 2). To further reduce overfitting and to facilitate recalibration of the model for data derived from frozen or

FFPE meningiomas, or for data derived from orthogonal approaches for gene expression quantification such as RNA sequencing, bootstrap aggregation was used to train 500 ridge-regression submodels using normalized and $\log_2$-transformed gene counts as input and discovery cohort risk scores as target variables[58].

Gene expression risk score cutoffs were determined using a nested procedure in the discovery cohort and applied without alteration to validation cohorts (Fig. 1d). An initial cutoff was determined in the discovery cohort using the maximally selected rank statistic. The subsets above and below this threshold were again split by maximally selected rank statistic. The lowest risk score group was considered low risk (LFFR cutoff ≤0.3760769, OS cutoff ≤0.4206913), and the highest risk score group was considered high risk (LFFR cutoff >0.5651741, OS cutoff >0.6453035). The intervening risk score groups were combined as intermediate risk (LFFR cutoff (0.3760769, 0.5651741], OS cutoff (0.4206913, 0.6453035]). All model training, calibration and cutoff determination was performed in the discovery cohort ($N = 173$).

Reproduction of molecular classification systems in validation cohort meningiomas

Assignment of validation cohort meningiomas to DNA methylation groups[22] or DNA methylation subgroups[21] (W.C.C., A.C., C.H.G.L., H.N.V., S.T.M. and D.R.R.), DNA methylation families[24] or integrated score[17] (S.L.N.M. and F.S.), or gene expression types[19] (J.C.B., A.S.H., A.H., T.K. and A.J.P.) was performed independently by investigators who developed each of these classification systems. Integrated grade[16] was assigned using CNVs derived from DNA methylation profiles and histological features under supervision of investigators who developed this classification system (S.S. and W.L.B.). DNA methylation probe risk scores were estimated by training a LASSO regularized Cox regression model with LFFR as the endpoint in the discovery cohort using β values of 283 unfavorable CpG loci[23]. The resulting continuous risk score was converted into low-, intermediate- and high-risk groups using the same nested procedure described for the gene expression risk score above. All meningioma classification system assignments were performed by investigators who were blinded to clinical outcomes and other molecular characteristics of the meningiomas included in this study (Fig. 1a).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Raw data from targeted gene expression panels are deposited in the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE222054. Raw DNA methylation data from the UCSF WHO grade 2 or grade 3 validation cohort and the RTOG 0539 validation cohort are available under accession number GSE221029. Raw amplicon and targeted exome sequencing data from discovery and validation cohort meningiomas are deposited in the NCBI Sequencing Reads Archive (https://www.ncbi.nlm.nih.gov/sra) under project numbers PRJNA916225 and PRJNA916253. Matrices containing TPM data from RNA sequencing cohorts used for analytical validation are deposited along with code on GitHub (https://github.com/william-c-chen/Meningioma_GE_Biomarker). Accession numbers and publications containing previously reported data are available in Supplementary Table 4. The publicly available GRCh38 (hg38), CRCh37.p13 (hg19) and Kallisto index v10 datasets were used in this study. Source data are provided with this paper.

Code availability

Raw data from targeted gene expression panels are deposited in the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE222054. Raw DNA methylation data from the UCSF WHO grade 2 or grade 3 validation cohort and the RTOG 0539

validation cohort are available under accession number GSE221029. Raw amplicon and targeted exome sequencing data from discovery and validation cohort meningiomas are deposited in the NCBI Sequencing Reads Archive (https://www.ncbi.nlm.nih.gov/sra) under project numbers PRJNA916225 and PRJNA916253. Matrices containing TPM data from RNA sequencing cohorts used for analytical validation are deposited along with code on github (https://github.com/william-c-chen/Meningioma_GE_Biomarker). Accession numbers and publications containing previously reported data are available in Supplementary Table 4. The publicly available GRCh38 (hg38), CRCh37.p13 (hg19), and Kallisto index v10 datasets were used in this study.

References

57. Geiss, G. K. et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat. Biotechnol.* **26**, 317–25 (2008).
58. Breiman, L. Bagging predictors. *Mach. Learn.* https://doi.org/10.1007/BF00058655 (1996).
59. Baik, B., Yoon, S. & Nam, D. Benchmarking RNA-seq differential expression analysis methods using spike-in and simulation data. *PLoS ONE* **15**, e0232271 (2020).
60. Bi, W. L. et al. Genomic landscape of high-grade meningiomas. *NPJ Genom. Med.* https://doi.org/10.1038/s41525-017-0014-7 (2017).
61. Von Spreckelsen, N. et al. KLF4 K409Q-mutated meningiomas show enhanced hypoxia signaling and respond to mTORC1 inhibitor treatment. *Acta Neuropathol. Commun.* **8**, 41 (2020).
62. Leek, J. T., Johnson, W. E., Parker, H. S., Jaffe, A. E. & Storey, J. D. The SVA package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* **28**, 882–883 (2012).

Acknowledgements

Author contributions

A.C., M.W.Y. and M.Y.C.P. contributed equally as second authors and S.T.M. and D.R.R. contributed equally as last authors. All authors contributed to the acquisition, analysis or interpretation of data. W.C.C., M.Y.C.P., M.W., C.L.R., S.L.P., M.P.M. and D.R.R. performed or supervised statistical analyses. W.C.C., A.C., S.L.N.M., A.K.S., J.C.B., A.S.H., A.H., T.K., H.N.V., V.B., W.L.B., A.J.P., F.S. and D.R.R. performed or supervised bioinformatic analyses. W.C.C., C.H.G.L., K.M. and S.T.M. processed tumor samples and extracted DNA and RNA. W.C.C. and D.R.R. drafted the manuscript. All authors critically edited the manuscript and provided administrative, technical or material support. W.C.C. and D.R.R. conceived and designed the study. S.T.M. and D.R.R. supervised the study and contributed equally.

Competing interests

Additional information

a

b

c

d

Extended Data Fig. 1 | Prognostic and predictive gene expression biomarker development. a, C-index for LFFR models based on meningioma Nanostring targeted gene expression profiling plotted against the natural-log of the lambda parameter during algorithm training. Center and error bars shows the mean C-index +/− estimated SEM. Candidate model performance was estimated using tenfold cross validation in the UCSF discovery cohort (N = 173). An optimal gene set (N = 34 genes, dotted lines, Supplementary Table 3) was selected within 1 standard error of the model achieving maximal c-index to reduce over-fitting. The number of genes in each model is displayed at the top of the graph. In order to further reduce over-fitting, improve calibration and stability, and facilitate risk score calculations using FFPE meningiomas or gene expression quantification from RNA sequencing or microarrays (Extended Data Fig. 4), bootstrap aggregation[58] was used to train 500 ridge-regression sub-models using the normalized and log-transformed gene counts as inputs and discovery cohort risk scores between 0 and 1 as target variables. In brief, this procedure nominates a bootstrap aggregated risk score defined as the arithmetic mean across sub-model risk scores. **b**, Log$_2$ values for Nanostring counts or RNA sequencing data (transcripts per million, TPM) for the 34 genes comprising the gene expression risk score in the frozen meningiomas from the UCSF discovery cohort (N = 173), revealing high concordance with R^2 = 0.81 (two sided F-test P < 2.2 × 10^{-16}). Similarly, a non-regularized Cox model for LFFR using RNA sequencing TPMs for the same 34 genes also achieved excellent performance in the UCSF discovery cohort (N = 173 meningiomas, LFFR c-index 0.89 ± 0.02, OS

c-index 0.84 ± 0.02), and outperformed 10,000 randomly sampled sets of 34 genes (one-sided bootstrap P < 0.0001). **c**, Limitations to using RNA sequencing for targeted gene expression biomarker discovery, in comparison to using RNA sequencing for targeted gene expression biomarker validation or implementation (as described for **b** and as shown in Extended Data Fig. 4). The distribution of univariate LFFR Cox model two-sided unadjusted Wald test P-values for all RNA sequencing genes in the UCSF discovery cohort are shown (N = 58,830 genes, N = 173 meningiomas). A background uniform distribution is evident, with a peak towards lower P-values. Between P = 0.0 and 0.2, at least 6904 of 17437 P-values (40%) could be expected to be false positives rather than related to true biological function. Spike-in experiments and simulations report false discovery rates across bioinformatic methods and experimental conditions for observational whole transcriptomic approaches between 10-75%, depending on the ground-truth prevalence[59]. **d**, Log$_{10}$ β coefficient magnitudes (red for positive coefficients, blue for negative coefficients) versus log$_2$ transformed P-values from individual Cox models from the discovery cohort RNA sequencing are shown, demonstrating challenges with feature selection using sparse observations in high dimensional space. Despite the limitations in using RNA sequencing to discover clinical biomarkers, we show RNA sequencing can be used to validate and implement the 34-gene expression biomarker that was developed using Nanostring targeted gene expression profiling (Extended Data Fig. 4).
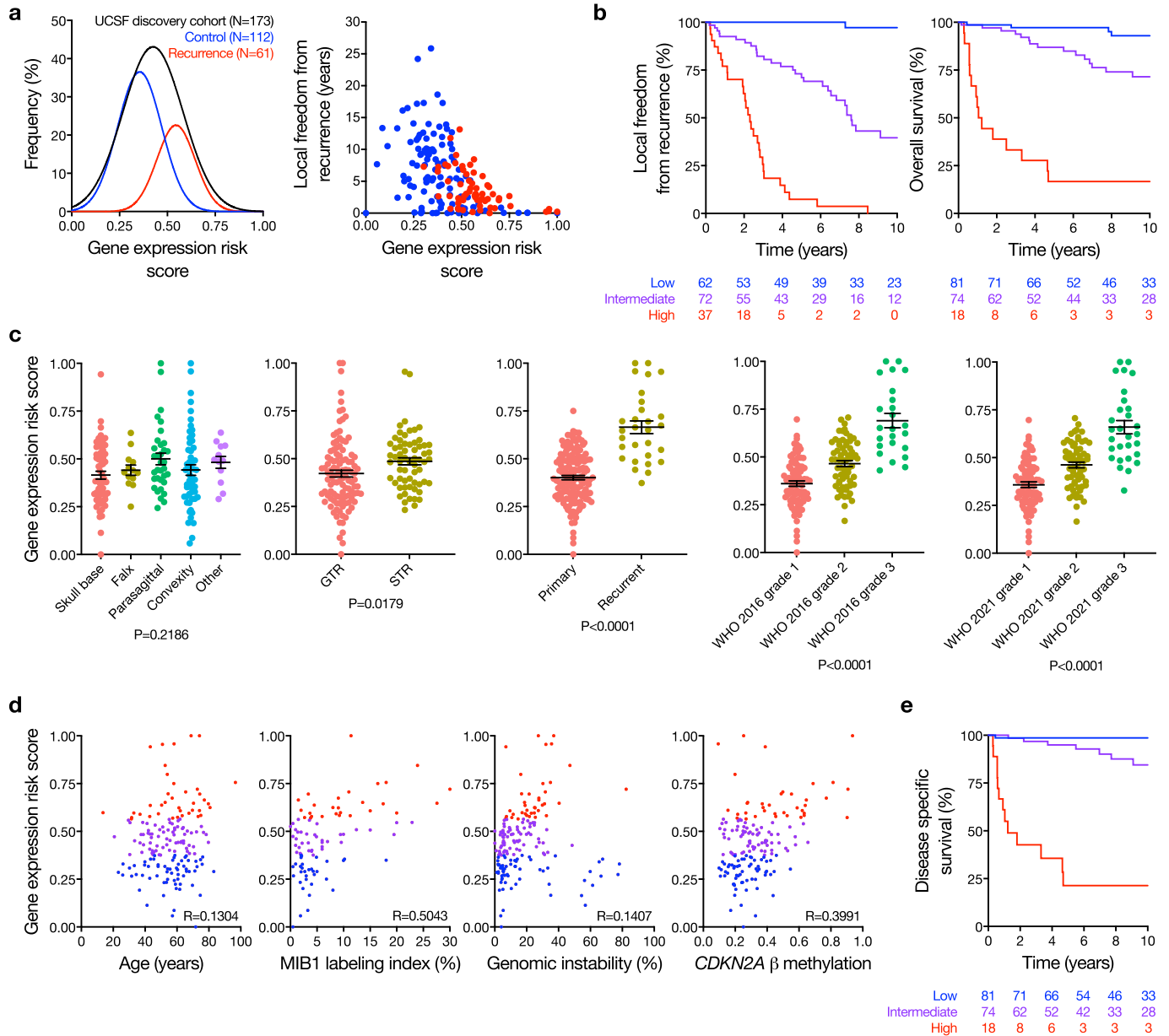
Extended Data Fig. 2 | Gene expression biomarker characteristics in the discovery cohort. a, Gaussian fits (left) to the distribution of gene expression risk scores in the UCSF discovery cohort, stratified by cases with local recurrence (red) or without local recurrence (blue) on clinical follow up. The gene expression risk score was also plotted against the time to censorship or local recurrence (right), and higher risk score correlated with greater risk of local recurrence and shorter time to recurrence. **b**, Kaplan Meier curves for LFFR or OS in the UCSF discovery cohort stratified by the gene expression risk score. **c**, Gene expression risk score distributions stratified by clinical characteristics in the UCSF discovery cohort. Mean +/- standard error measurements are shown for gene expression risk scores stratified by tumor location (skull base, N = 60; falx, N = 13; parasagittal, N = 34; convexity, N = 54; other, N = 10), extent of resection (GTR, N = 110; STR, N = 63), setting (primary, N = 144; recurrent, N = 29), WHO 2016 grade (grade 1, N = 83; grade 2, N = 66; grade 3, N = 24), and WHO 2021 grade (grade 1, N = 81; grade 2, N = 61; grade 3, N = 29). There was no significant difference across meningioma locations (ANOVA, two-sided unadjusted P = 0.22), but gene expression risk scores were higher among subtotally resected meningiomas (Student's *t*-test, two-sided P = 0.02), recurrent meningiomas

(Student's *t*-test, two-sided P < 0.0001), and were stratified by WHO 2016 or 2021 grade (P < 0.0001). Convexity meningiomas arise adjacent to the cerebral convexity underlying the calvarium, while parasagittal meningiomas abut or involve the parasagittal sinus along the calvarial midline, falx meningiomas involve the falx without extending superiorly to the parasagittal sinus, and skull base meningiomas arise adjacent to the bones of the skull base. **d**, UCSF discovery cohort gene expression risk score scatter plots across clinical or molecular variables associated with meningioma biology or outcomes (blue, low risk; purple, intermediate risk; red, high risk). There was no clear association between patient age and gene expression risk score, but risk score was loosely correlated with MIB1 labeling index[39], genomic instability as defined by the proportion of non-centromeric, non-acrocentric chromosomes affected by copy number gain or loss[60], and DNA methylation of the *CDKN2A* locus[21]. P values shown are from a two-sided, unadjusted F-test. Thus, the gene expression biomarker correlated with surrogate markers of aggressive meningiomas. **e**, Disease specific survival among patients in the UCSF discovery cohort stratified by gene expression risk score.
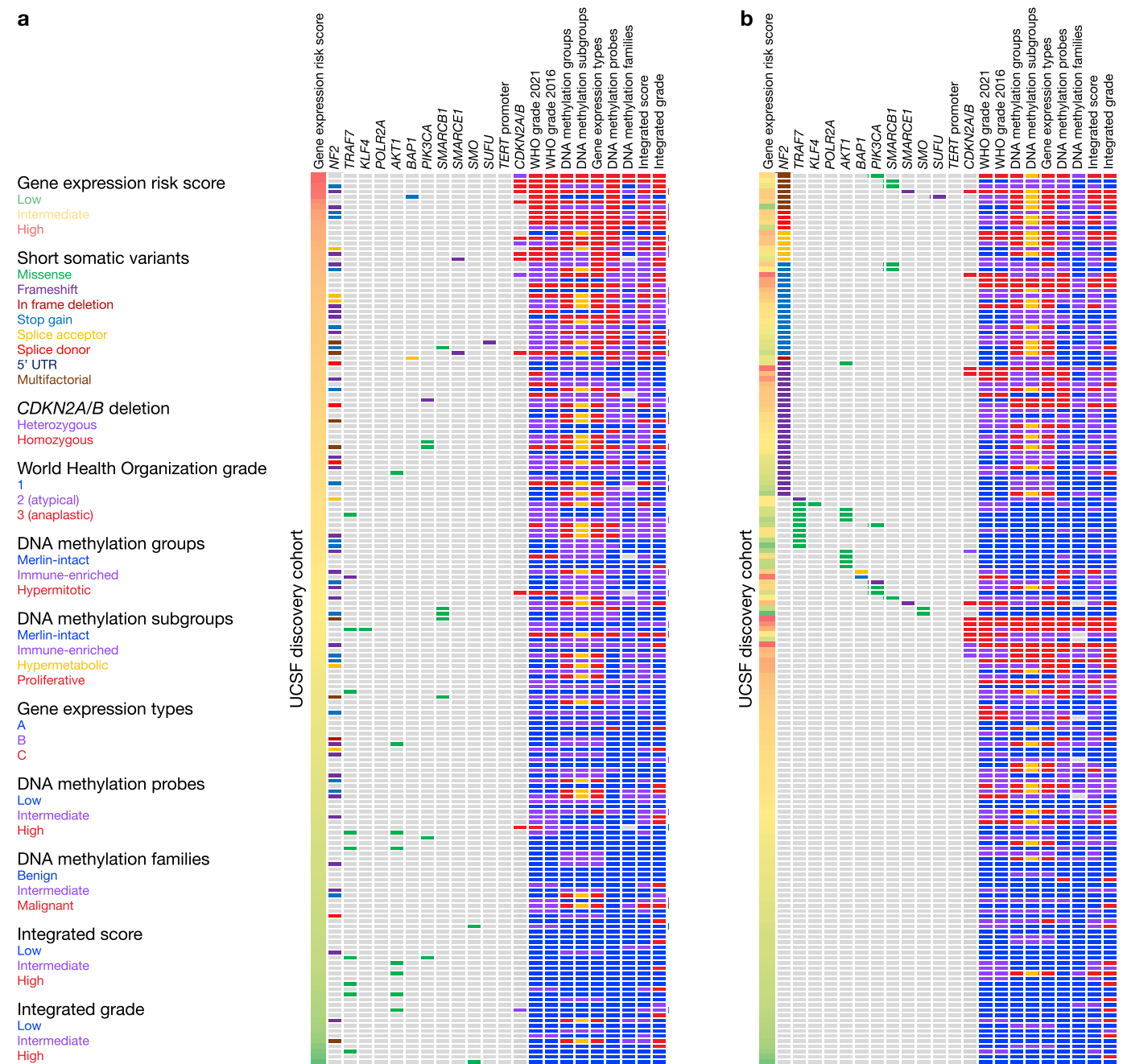
Extended Data Fig. 3 | Gene expression biomarker across somatic short variants in the discovery cohort. Targeted DNA sequencing of recurrent somatic short variants was performed on 171 meningiomas from the UCSF discovery cohort (98.8%). **a**, Oncoplot distribution of identified pathogenic short somatic variants with variant allele frequency (VAF) of at least 5.0% (N = 98 variants, median VAF 38.0%, interquartile range [IQR] 29-43%, median sequencing depth 551.5, IQR 354-856). Consistent with prior reports, variants in *NF2* were most common (N = 67, 39.2%), followed by *TRAF7* (N = 10, 5.8%) and *AKT1* (N = 8, 4.7%). A minority of meningiomas (N = 16, 9.4%) were identified without alteration of *NF2* or loss of chromosome 22q, but with a characteristic pathogenic variant in one of the following genes: *TRAF7, AKT1, PIK3CA, SMARCB1, SMARCE1, SMO, SUFU, KLF4,* or *POLR2A*. The majority of these were WHO 2021 grade 1 meningiomas (N = 9, 60.0%), and were associated with favorable histologic

characteristics and outcomes (median MIB1 labeling index 2.0%, range 0.5-4.0, 5-year LFFR 90.9%). *TERT* promoter C228T and C250T hotspot mutations were not identified in the discovery cohort. *BAP1* mutations were rare (N = 5, 2.9%) and correlated with high histological grade and poor outcomes (N = 3 [60.0%] WHO 2016 grade 2 or 3, 5-year LFFR 40.0%). Homozygous *CDKN2A/B* loss, derived from meningioma DNA CNVs (Supplementary Methods), was identified in 10 meningiomas from the UCSF discovery cohort (5.8%, 80.0% WHO 2016 grade 3, 20.0% WHO 2016 grade 2) and was associated with poor outcomes (5-year LFFR 14.2%). These findings were supported by targeted DNA sequencing of recurrent somatic short variants in 35 consecutive clinical validation cohort meningiomas from The University of Hong Kong using the same approach. **b**, Same oncoplot from the UCSF discovery cohort as in **a**, but ordered by VAF instead of gene expression risk score.
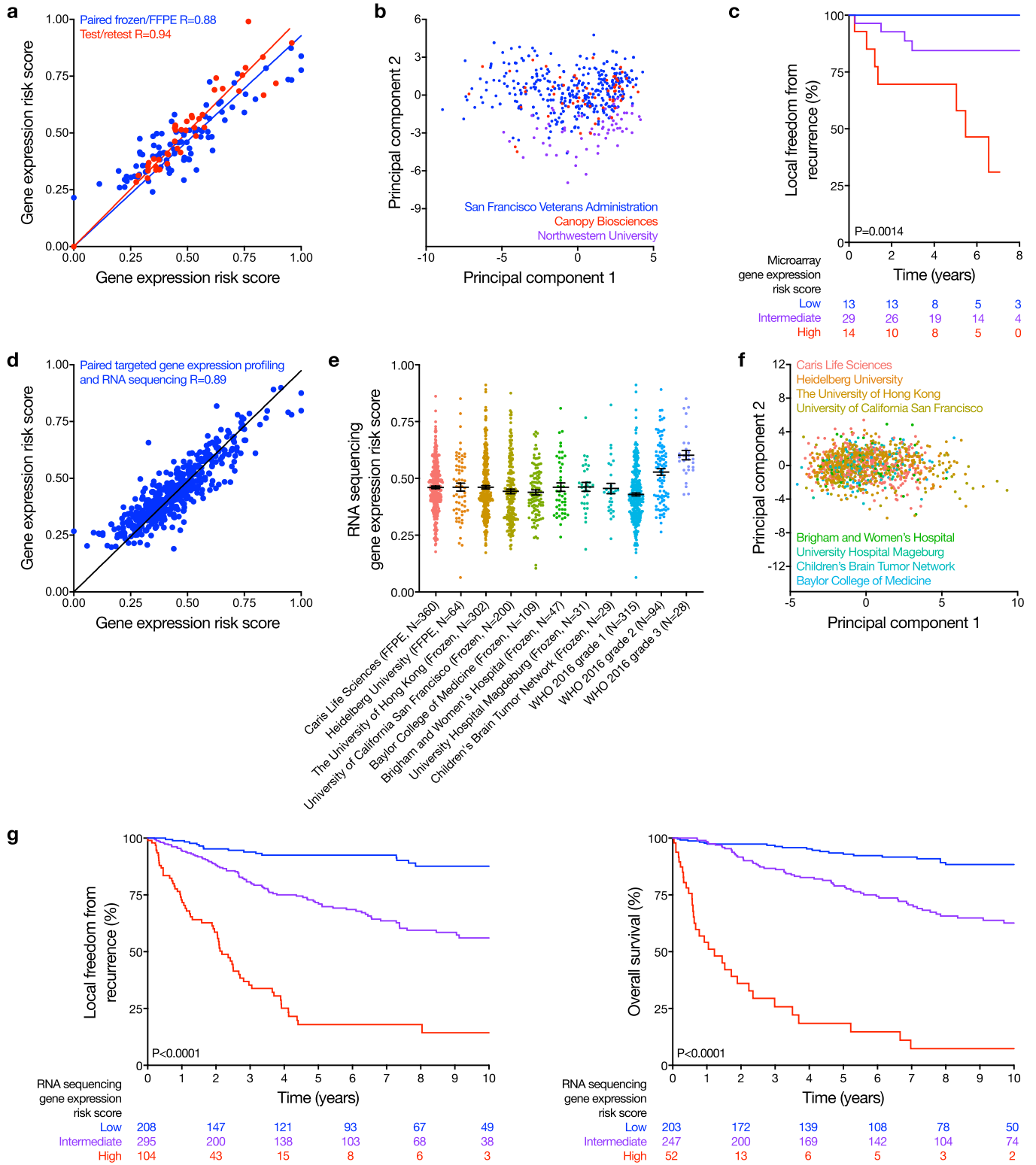
Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | Gene expression biomarker characteristics in the analytical validation cohort. Gene expression risk score concordance across multiple conditions and replicates. Test-retest conditions (combined N = 44, R = 0.94, P < 0.0001) were comprised of varying probe batches (N = 10, R = 0.98, P < 0.0001), within probe batch testing of technical replicates (N = 12, R = 0.98, P < 0.0001), and test-retest conditions for meningiomas with serial RNA extraction on the same FFPE block or frozen tumor chunk at least 4 weeks apart (N = 22, R = 0.94, P < 0.001). Gene expression risk scores on paired frozen/FFPE meningiomas also demonstrated high concordance (N = 90, R = 0.88, P < 0.001), and FFPE gene expression risk scores provided excellent discrimination of outcomes across FFPE clinical validation datasets, including a prospective clinical trial (Figs. 2, 3, 5 and Extended Data Figs. 5, 6, 8). **b**, Principal component analysis on FFPE gene expression risk scores from meningiomas processed at multiple laboratories spanning academic institutions (Northwestern University, San Francisco Veterans Administration) or Clinical Laboratory Improvement Amendments (CLIA) certified private industry (Canopy Biosciences), demonstrating no laboratory batch effects. **c**, Publicly available microarray and clinical data were used to test the performance of the gene expression risk score on a non-Nanostring platform (N = 33 of 34 genes available). No paired microarray/Nanostring data was available to train a calibration model, which precluded direct comparison. Thus, the RNA sequencing calibration model described below (and described in further detail in the Supplementary Methods)

was adapted to microarray data as an exploratory analysis, yielding prognostic risk groups as shown in the Kaplan Meier plot (P = 0.0014, Log-rank test). **d**, Concordance of gene expression risk scores derived from RNA sequencing or Nanostring targeted gene expression profiling on the same meningiomas (N = 469 meningiomas, R = 0.89, F-test two-sided unadjusted P < 0.0001). **e**, Distribution of gene expression risk scores (mean +/- SEM is shown) derived from RNA sequencing of cohorts overlapping (UCSF, The University of Hong Kong, N = 502) or non-overlapping (Caris Life Sciences, Heidelberg University, Brigham and Women's Hospital, University Hospital Magdeburg, Children's Brain Tumor Network, Baylor College of Medicine, N = 640) with the discovery or clinical validation cohorts, comprising 1142 unique meningiomas. Gene expression risk scores remained well distributed across all datasets, including RNA sequencing of pediatric meningiomas (Children's Brain Tumor Network, N = 29), meningiomas with *KLF4 or AKT1* somatic short variants[61] (University Hospital Magdeburg, N = 31), or FFPE (N = 428) or frozen (N = 718) meningiomas, and demonstrated similar stratification by 2016 WHO histological grade as with Nanostring targeted gene expression profiling analyses (Extended Data Fig. 2c). **f**, Principal component analysis of gene expression risk scores across RNA sequencing cohorts after correction for batch effects using the COMBAT[62] pipeline in the sva package in R. **g**, LFFR or OS stratified by gene expression risk scores from RNA sequencing of cohorts with available clinical data (UCSF discovery, The University of Hong Kong, and Baylor College of Medicine).
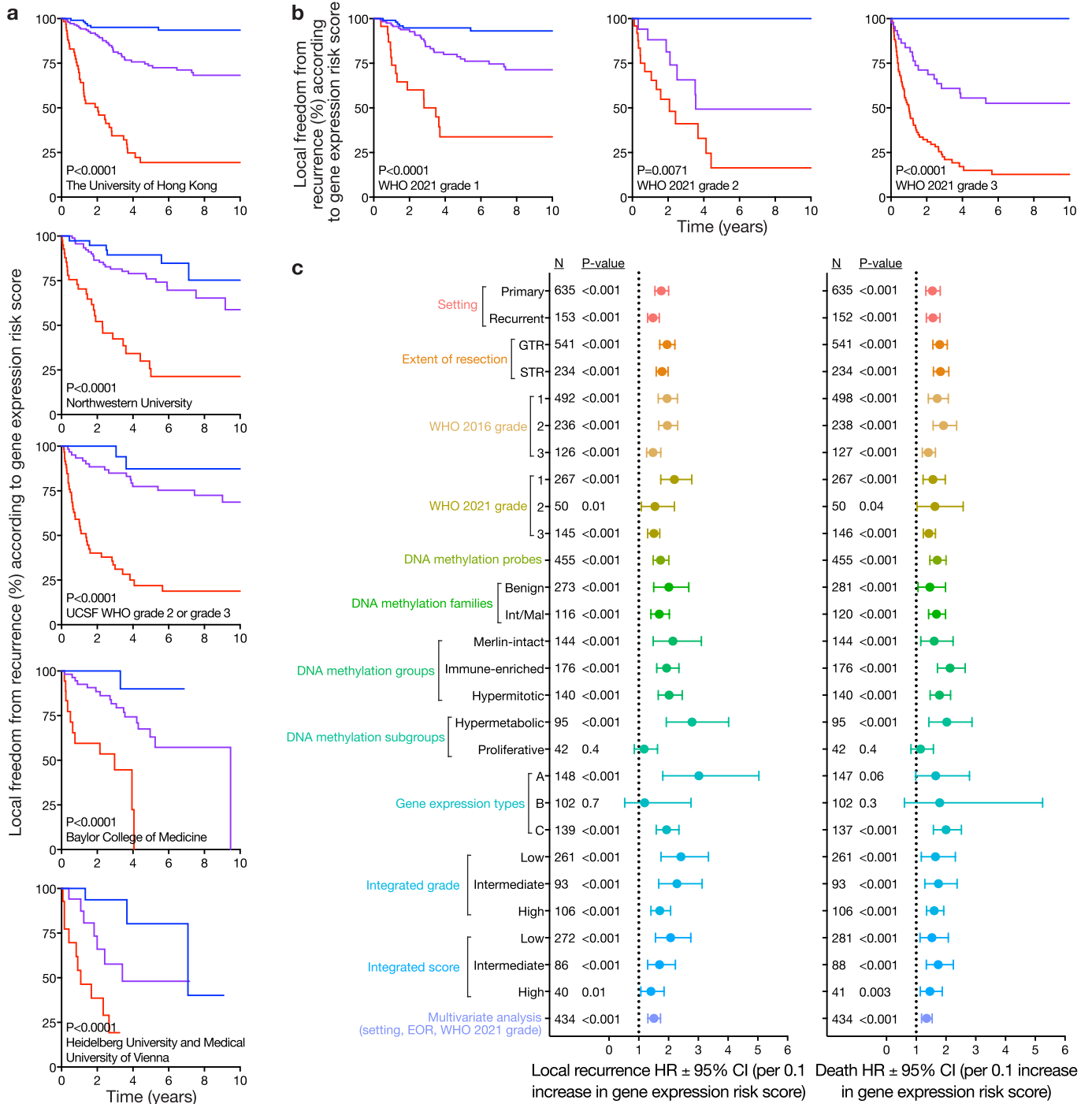
Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5 | Gene expression biomarker characteristics in the clinical validation cohort. A, Kaplan Meier curves for LFFR stratified by gene expression risk score (blue lines, low risk; purple lines, intermediate risk; red lines, high risk) for individual clinical validation cohorts, including The University of Hong Kong (Frozen N = 339; c-index=0.80; low risk N = 122, 5-year LFFR 95.1%; intermediate risk N = 151, 5-year LFFR 73.6%; high risk N = 66, 5-year LFFR 19.5%), Northwestern University (Frozen and FFPE N = 180; c-index=0.74; low risk N = 42, 5-year LFFR 90.0%; intermediate risk N = 98, 5-year LFFR 76.0%; high risk N = 42, 5-year LFFR 21.4%), UCSF WHO grade 2 or grade 3 (FFPE N = 158; c-index=0.78; low risk N = 24, 5-year LFFR 87.4%; intermediate risk N = 69, 5-year LFFR 77.5%; high risk N = 65, 5-year LFFR 22.0%), Baylor College of Medicine (Frozen N = 116; c-index=0.77; low risk N = 35, 5-year LFFR 90.0%; intermediate risk N = 61, 5-year LFFR 63.0%; high risk N = 20, 5-year LFFR 0.0%), and Heidelberg University plus the Medical University of Vienna (FFPE N = 61 with LFFR data; c-index=0.76; low risk N = 24, 5-year LFFR 80.4%; intermediate risk N = 23, 5-year LFFR 48.1%; high risk N = 14, 5-year LFFR 19.3%). The gene expression risk score remained well calibrated across multiple independent clinical validation cohorts comprising both frozen and FFPE meningiomas. When assessed separately within each independent retrospective cohort site, the gene expression risk score remained independently prognostic in multivariate analysis combining the risk score with WHO 2016 grade (P < 0.001 in all cases, two-sided unadjusted Wald test P-value). **B**, Kaplan Meier curves for LFFR in clinical validation cohort meningiomas stratified by gene expression risk score within WHO 2021 grades, demonstrating that the gene expression biomarker remained discriminatory across WHO 2021 grade 1 (low risk N = 114, intermediate risk N = 127, high risk

N = 26), WHO 2021 grade 2 (low risk N = 7, intermediate risk N = 17, high risk N = 26), and WHO 2021 grade 3 meningiomas (low risk N = 2, intermediate risk N = 46, high risk N = 98). Shown are two-sided unadjusted Log-rank P-values. **C**, Forest plots of hazard ratios (HR) with 95% confidence intervals (CI) for local recurrence (left) or death (right) for each 0.1 increase in gene expression risk score are shown (center and error bars denote the hazard ratio and 95% confidence interval). The gene expression biomarker was prognostic across all molecular classification systems tested for both recurrence and survival. HRs according to gene expression risk score across meningioma settings, extent of resection (EOR), and WHO grades from Fig. 2b are re-presented for ease of comparison to HRs in molecular classification systems. P values shown are from two-sided unadjusted Wald's tests. Overall, in the retrospective clinical validation cohort, LFFR Harrel's c-index/Uno's c-index for the gene expression biomarker was 0.78/0.77 (N = 854), while LFFR c-index was 0.68/0.66 for WHO 2016 grade (N = 854), 0.72/0.71 for WHO 2021 grade (N = 462), 0.72/0.73 for integrated score (N = 398), 0.73/0.73 for integrated grade (N = 460), 0.68/0.69 for DNA methylation groups (N = 460), 0.69/0.70 for DNA methylation subgroups (N = 460), 0.74/0.73 for DNA methylation probes (N = 455), and 0.70/0.71 for gene expression type (N = 389). OS Harrel's c-index/Uno's c-index for the gene expression biomarker was 0.78/0.78 (N = 863), while OS c-index was 0.72/0.72 for WHO 2016 grade (N = 863), 0.74/0.73 for WHO 2021 grade (N = 463), 0.73/0.72 for integrated score (N = 410), 0.75/0.75 for integrated grade (N = 460), 0.66/0.66 for DNA methylation groups (N = 460), 0.68/0.68 for DNA methylation subgroups (N = 460), 0.73/0.74 for DNA methylation probes (N = 455), and 0.70/0.67 for gene expression type (N = 386).
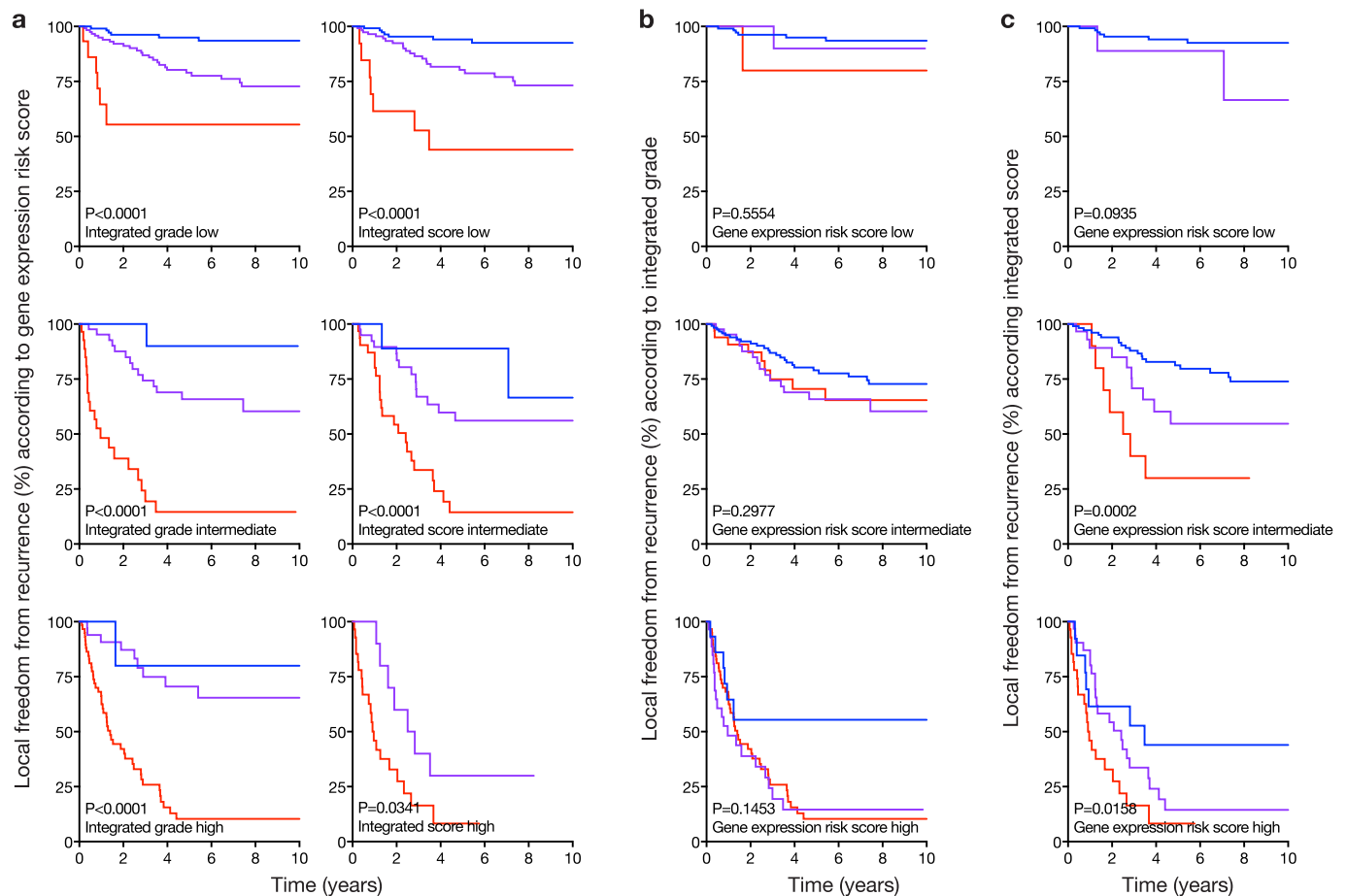
Extended Data Fig. 6 | Molecular classification comparisons in the clinical validation cohort. Kaplan Meier curves are shown for LFFR (shown are two-sided unadjusted Log-rank P-values) in clinical validation cohort meningiomas stratified by molecular risk groups (blue lines, low risk; purple lines, intermediate risk; red lines, high risk) using the gene expression biomarker in **a**, or 2 contemporary supervised meningioma classification systems based on combined molecular and clinical features: integrated grade[16] based on CNVs and mitoses in **b**, or integrated score[17] based on CNVs, DNA methylation families[24], and WHO 2016 grade in **c**. In **a**, the gene expression biomarker remained robustly discriminatory across integrated grade or integrated score risk groups, concordant with the independent prognostic value of the gene expression risk score on multivariate analyses (Supplementary Tables 10, 11) and within groups from the 6 other molecular and/or histological classification systems tested (Fig. 3a). The converse was examined in **b** and **c**, where integrated grade was unable to discriminate outcomes across gene expression risk score groups, and integrated score had limited discriminatory power for intermediate and high gene expression risk score groups and was not discriminatory for low gene expression low risk groups.
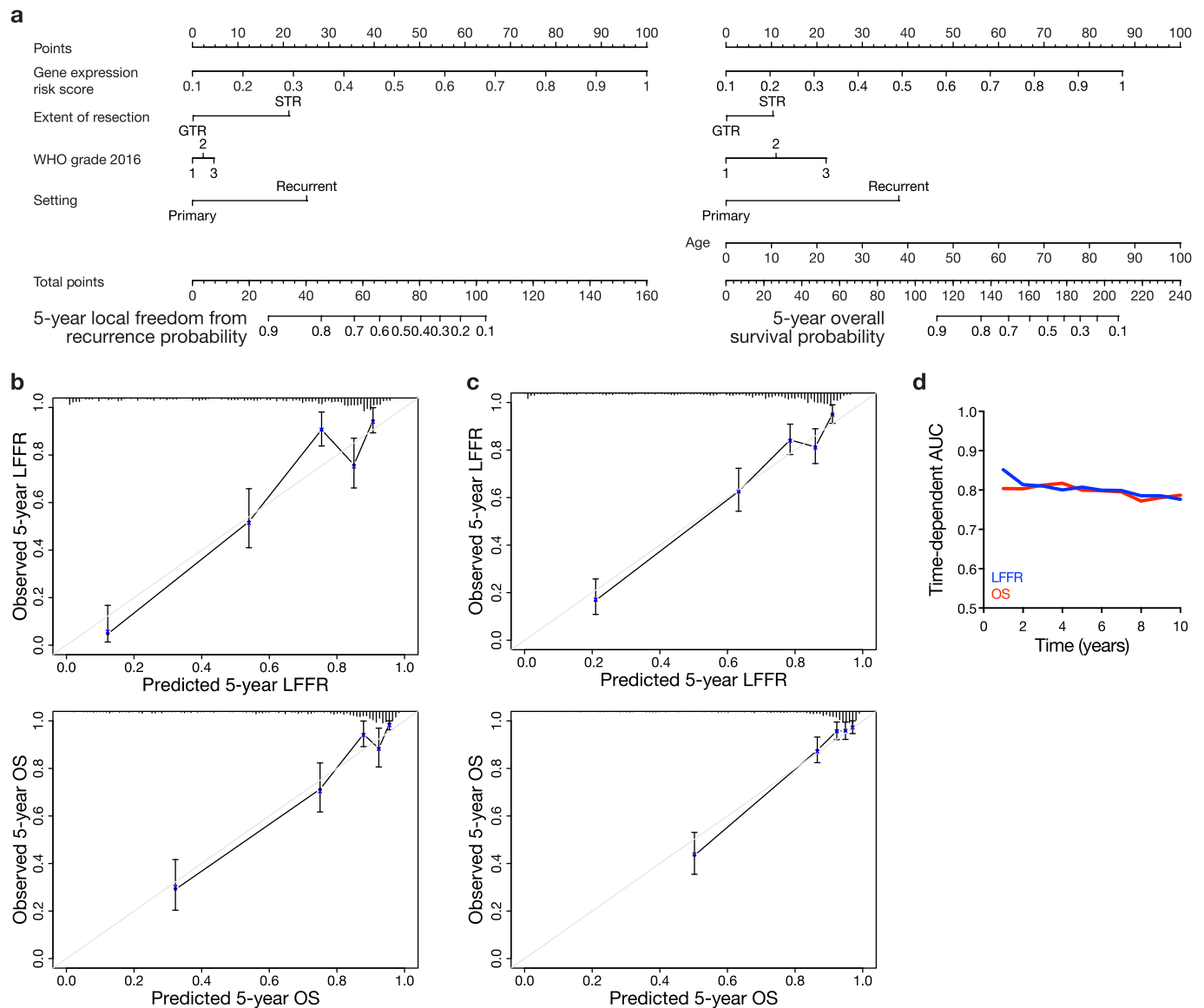
Extended Data Fig. 7 | Gene expression biomarker nomograms for meningioma outcomes. a, Nomograms are shown for prediction of 5-year LFFR or OS based on gene expression risk score, extent of resection, setting, and WHO 2016 histologic grade. To use the nomograms, use a straight-edge to draw a vertical line between the variable of interest and the points scale at the top of the nomogram to determine the contribution in points to the total score for each variable. Add up the points from each variable, and then draw a vertical line from the total points scale at the bottom of the nomogram to the 5-year outcome scale to determine the estimated outcome. **b**, Calibration curves are shown for the models corresponding to the nomograms in Fig. 4a for LFFR (top) and OS (bottom) using the gene expression risk score, extent of resection, primary vs recurrent status, and WHO 2021 grade (and the addition of age for OS). The *calibrate* function from the *rms* package in R was used, with B = 1000 iterations and N = 75 samples per group. Center and error bars denote the predicted 5-year LFFR versus the observed 5-year LFFR calculated via the Kaplan Meier method, with a 95% confidence interval. **c**, Calibration curves corresponding to the nomograms in **a**, for LFFR (top) and OS (bottom) using 150 samples per group. Center and error bars denote the predicted 5-year LFFR versus the observed 5-year LFFR calculated via the Kaplan Meier method, with a 95% confidence interval. **d**, Time dependent AUC is shown for LFFR and OS for the retrospective clinical validation cohort (N = 866) as a function of time.
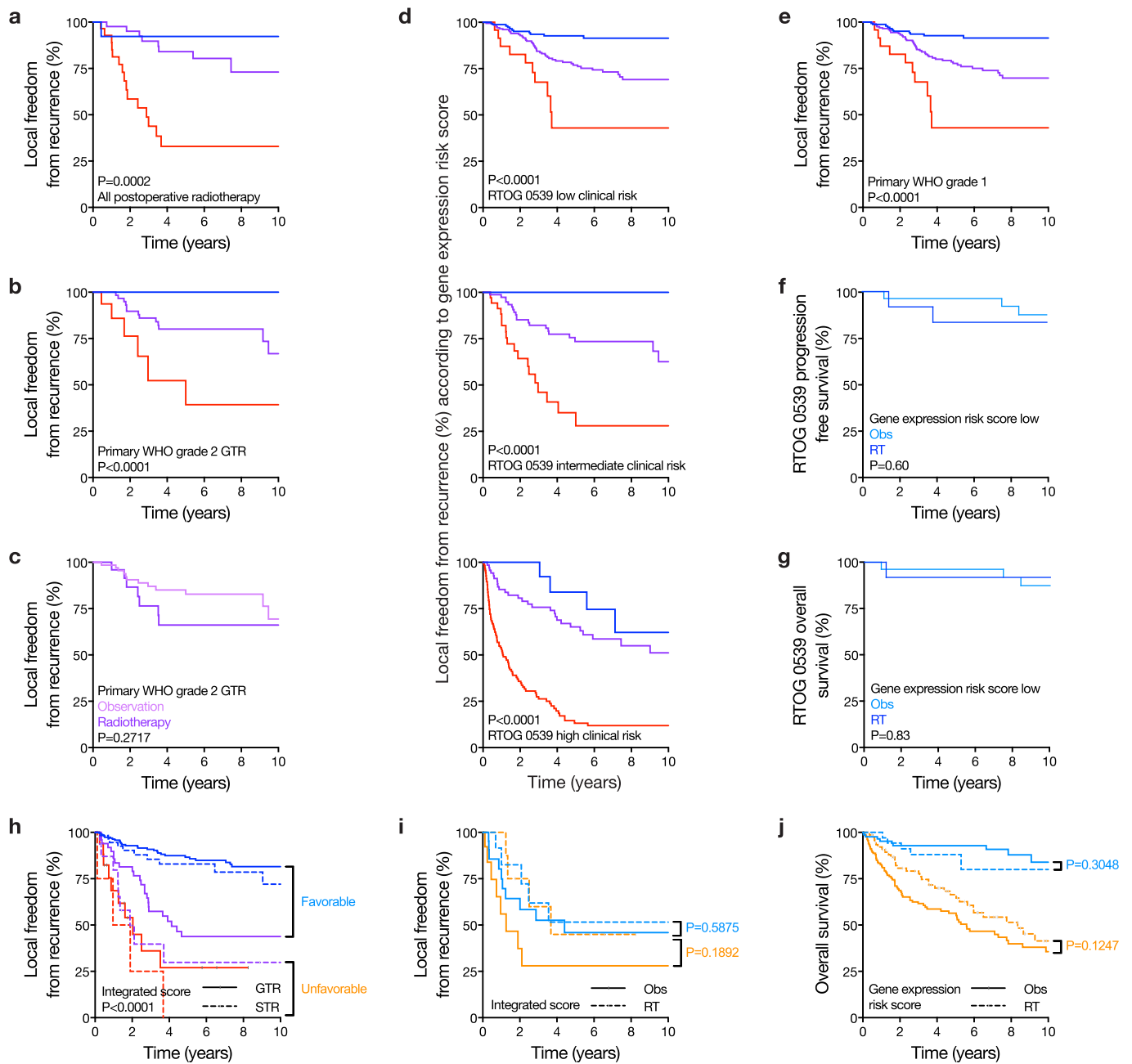
Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | Molecular classification systems and response to treatment. Kaplan Meier curves are shown for LFFR or OS (shown are two-sided unadjusted Log-rank P-values) in retrospective clinical validation cohort meningiomas or prospective RTOG 0539 meningiomas, stratified by gene expression risk score (blue lines, low risk; purple, intermediate risk lines; red lines, high risk), extent of resection, postoperative observation (Obs), or postoperative radiotherapy (RT). **a**, Primary retrospective clinical validation cohort meningiomas receiving postoperative external beam radiotherapy (N = 89) stratified by gene expression risk score (low risk N = 14, intermediate risk N = 45, high risk N = 30), revealing the gene expression biomarker remained prognostic among patients receiving postoperative radiotherapy. **b**, Primary WHO 2016 grade 2 meningiomas with GTR from the retrospective clinical validation cohort stratified by gene expression risk score (N = 21 low risk, N = 63 intermediate risk, N = 18 high risk). The gene expression risk score remained prognostic among gross totally resected primary WHO grade 2 meningiomas (N = 102, HR for local recurrence of 1.75 per 0.1 increase, 95% CI 1.18-2.59, P = 0.0057). **c**, Primary WHO grade 2 meningiomas with GTR from the retrospective clinical validation cohort stratified by postoperative radiotherapy (N = 28) or observation (N = 74). Patients with meningiomas meeting these criteria were eligible for 2 Phase III randomized multi-institutional trials (NRG BN003 and ROAM-EORTC 1308) examining clinical outcomes with postoperative radiotherapy versus observation. Postoperative radiotherapy did not offer a benefit to patients with meningiomas meeting these criteria in the retrospective clinical validation cohort. **d**, Retrospective clinical validation cohort meningiomas stratified by gene expression risk score across RTOG 0539 clinical risk groups (low clinical risk, primary WHO grade 1 meningiomas; intermediate clinical risk, recurrent WHO grade 1 meningiomas or primary WHO grade 2 meningiomas status post GTR; high clinical risk, recurrent or STR WHO grade 2 meningiomas or WHO grade 3 meningiomas after any resection). The gene expression biomarker remained prognostic across RTOG 0539 low clinical risk (gene expression risk score low risk N = 173, intermediate risk N = 224, high

risk N = 27), RTOG 0539 intermediate clinical risk (gene expression risk score low risk N = 32, intermediate risk N = 80, high risk N = 38), and RTOG 0539 high clinical risk groups (gene expression risk score low risk N = 16, intermediate risk N = 75, high risk N = 128). **e**, Primary WHO grade 1 meningiomas from the retrospective clinical validation cohort (equivalent to RTOG 0539 low clinical risk meningiomas) stratified by gene expression risk score (N = 173 low risk, N = 224 intermediate risk, N = 27 high risk, 5-year LFFR 92.7%, 77.3%, and 43.0% for low, intermediate, or high risk meningiomas, respectively). **f** and **g**, Prospective validation cohort meningiomas from RTOG 0539 identified as low risk by the gene expression biomarker stratified by postoperative radiotherapy (N = 12 WHO 2016 grade 2 or 3 or recurrent WHO 2016 grade 1 meningiomas) or observation (N = 27 primary WHO 2016 grade 1 meningiomas). These analyses showed favorable outcomes for prospectively collected meningiomas with low gene expression risk scores across clinical risk strata, consistent with findings from retrospective clinical validation cohort meningiomas. More broadly, these data support the hypothesis that the gene expression biomarker may be useful for identifying meningiomas where postoperative radiotherapy could be safely omitted, even in the setting of conventionally high risk clinical features. **h**, Meningiomas treated with surgical monotherapy from the retrospective clinical validation cohort stratified by integrated score[17] (the only contemporary molecular classification system potentially providing additional prognostic information for LFFR within gene expression biomarker strata, Extended Data Fig. 6c) and extent of resection. Favorable (light blue) and unfavorable (yellow) groups were identified using the same criteria for identification of biomarker/surgical strata (Fig. 5a). **I**, Favorable and unfavorable strata based on integrated score were unable to identify meningiomas benefitting from postoperative radiotherapy even after propensity matching on integrated score, extent of resection, and WHO 2016 grade. **j**, OS in the same meningiomas as Fig. 5c (that is propensity matched favorable and unfavorable retrospective clinical validation cohort meningiomas based on biomarker/surgical strata), demonstrating a trend towards benefit with postoperative radiotherapy for unfavorable meningiomas.
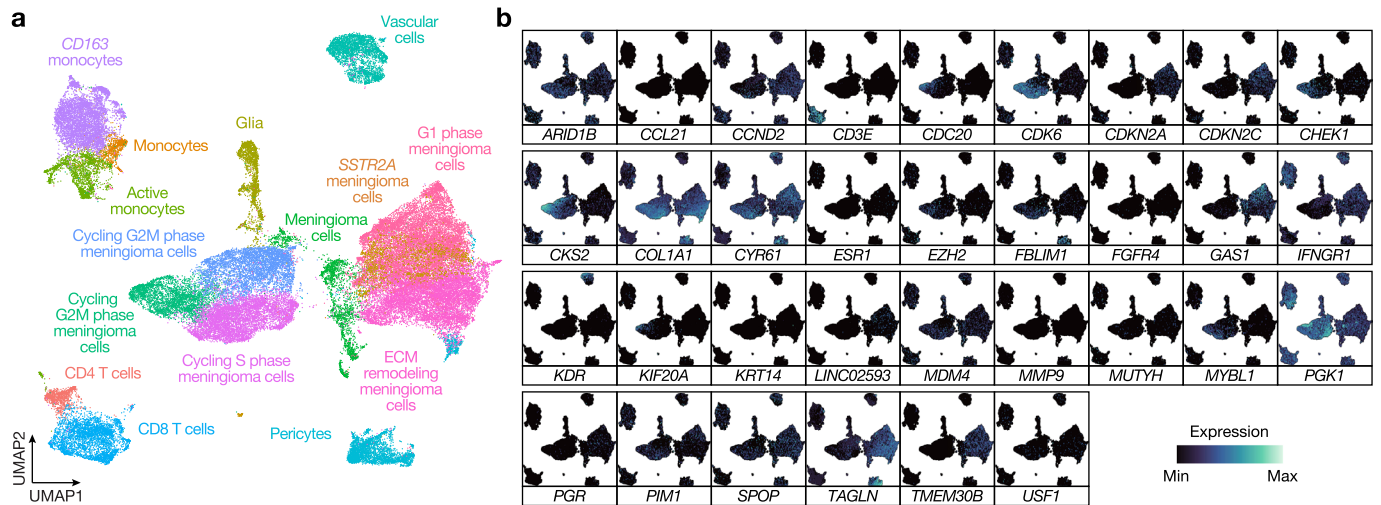
Extended Data Fig. 9 | Biomarker risk score gene distribution in tumor versus microenvironment cell types from meningioma single-cell RNA sequencing. a, Single-cell RNA sequencing uniform manifold approximation and projection (UMAP) of 57,114 transcriptomes from 8 human meningioma samples and 2 human dura samples shaded by cell clusters that were defined using cell signature gene sets, cell cycle analysis, and differentially expressed cluster marker genes, as previously reported[22]. Image is reproduced with permission.

b, Feature plots showing normalized biomarker risk score gene expression across reduced dimensionality clusters of meningioma and tumor microenvironment cells. 33 of 34 biomarker genes were available for analysis in meningioma single-cell RNA sequencing data from **a**, although several were sparsely captured in single-cell RNA data (a known limitation that can be overcome using bulk RNA sequencing or Nanostring hybridization targeted gene expression profiling).

nature portfolio

Corresponding author(s):	William Chen, Stephen Magill, David Raleigh
Last updated by author(s):	Sep 5, 2023

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software or code was used to collect data. Raw data was provided in native format or standard data formats such as .cvs.
Data analysis	Custom code is provided at: https://github.com/william-c-chen/Meningioma_GE_Biomarker. The publicly available GRCh38 (hg38), CRCh37.p13 (hg19), and Kallisto index v10 datasets were used in this study. Source data are provided with this paper. RNA sequencing analysis was performed using a pipeline comprised of FastQC for quality control and Kallisto (v0.46.2) for pseudo alignment and transcript abundance quantification using default settings. DNA methylation Illumina EPIC beadchip data was processed with pre-processing and β-value calculations were performed using the SeSAMe (v1.12.9) pipeline (BioConductor 3.13) with default settings. Amplicon reads were aligned to the GRCh38/hg19 reference genome using hisat2 (v2.2.1) with default settings. Variant calling was performed using samtools (v1.10) with mpileup and call commands in the bcftools program using default settings. Statistical analyses were performed in R (v3.6.2) or Graphpad Prism (v9.3.1). The performance of the targeted gene expression risk score was assessed using c-index, log-rank test, univariate and multivariate Cox regression via the survival package (v3.2-13) in R, time-dependent area under the receiver operant curve (AUC) using the survivalROC package (v1.0.3) and the Kaplan Meier method, and Brier error score using the pec package (2021.10.11)77–84. Unless specifically indicated otherwise, c-indexes reported in the text are Harrel's c-index as implemented in the survival package in R. Uno's c-index85 was also calculated in some instances as a second check, using the Est.Cval function from the survC1 package by Hajime Uno, with default settings and tau=5 years (v1.0-3). Likelihood-ratio tests for model comparison were conducted using the lrtest function in the lmtest package in R (v0.9-39). Nomograms were generated using Cox regression models using the nomogram function in the rms package in R (v6.2-0). Imputation of clinical variables was not used for any analyses. Propensity matching was performed using the MatchIt package (v4.3.3) in R. Nanostring data was processed using the nSolver software (v4.0).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our <u>policy</u>

Raw data from targeted gene expression panels are deposited in the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE222054. Raw DNA methylation data from the UCSF WHO grade 2 or grade 3 validation cohort and the RTOG 0539 validation cohort are available under accession number GSE221029. Raw amplicon and targeted exome sequencing data from discovery and validation cohort meningiomas are deposited in the NCBI Sequencing Reads Archive (https://www.ncbi.nlm.nih.gov/sra) under project numbers PRJNA916225 and PRJNA916253. Matrices containing TPM data from RNA sequencing cohorts used for analytical validation are deposited along with code on github (https://github.com/william-c-chen/Meningioma_GE_Biomarker). Accession numbers and publications containing previously reported data are available in Supplementary Table 4. The publicly available GRCh38 (hg38), CRCh37.p13 (hg19), and Kallisto index v10 datasets were used in this study. Source data are provided with this paper.

Human research participants

Reporting on sex and gender	Sex data was collected on patients and reported in aggregate. 112 of 166 patients in the discovery cohort, 543 of 801 patients in the retrospective clinical validation cohort, and 68 of 103 patients in the prospective clinical validation cohort, were female sex.
Population characteristics	The median age of patients was 57.0, 58.9, and 57.0 years, in the discovery, retrospective validation, and prospective validation cohorts, respectively. Ethnicity data was not collected for the majority of patients, however the retrospective validation cohort included centers from Hong Kong, Europe, and North America.
Recruitment	Patients in the discovery and retrospective cohorts were collected in institutional retrospective databases and reflect characteristics of patients treated at largely tertiary academic centers. At least one validation cohort reflected consecutive patients treated at a center serving as the primary neurosurgical center for a large metropolitan area (University of Hong Kong), thus better reflecting a general population of meningioma patients. Patients from RTOG 0539, a prospective clinical trial, were enrolled from both community based and academic centers and thus better reflected a broad meningioma patient population. Nevertheless, clinical data in the discovery and multicenter validation cohorts were obtained retrospectively, suggesting our results are susceptible to biases inherent to retrospective research, as well as to selection bias related to the largely academic and tertiary center setting of the discovery and validation cohorts. Thus, generalization to broader community based patient care will require future validation.
Ethics oversight	Institutional review board (IRB) at UCSF and all participating centers.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Cohorts included in the study represented available retrospective datasets at participating centers. No pre-specified sample size calculation was used. The prospective validation cohort represented all patients from RTOG 0539 with available tissue. The sample size and event rate invluded in this study represents one of the largest reported for meningioma.
Data exclusions	All patients with available tissue and successful extraction of RNA and performance of the biomarker assay were included.
Replication	Gene expression risk score concordance across multiple conditions and replicates. Test-retest conditions (combined N=44, R=0.94, P<0.0001) were comprised of varying probe batches (N=10, R=0.98, P<0.0001), within probe batch testing of technical replicates (N=12, R=0.98, P<0.0001), and test-retest conditions for meningiomas with serial RNA extraction on the same FFPE block or frozen tumor chunk at least 4 weeks apart (N=22, R=0.94, P<0.001). Gene expression risk scores on paired frozen/FFPE meningiomas (also representing test-retest given re-extraction of RNA from different areas of tumor) also demonstrated high concordance (N=90, R=0.88, P<0.001), and FFPE gene expression risk scores provided excellent discrimination of outcomes across FFPE clinical validation datasets. Concordance of gene expression risk scores

derived from RNA sequencing or Nanostring targeted gene expression profiling on the same meningiomas (N=469 meningiomas, R=0.89, F-test two-sided unadjusted P<0.0001) was also shown. All performed attempts at replication are reported.

Randomization | Not applicable. Covariates were controlled for using standard univariate and multivariate statistical analysis, and propensity matching.

Blinding | For the prospective validation cohort, investigators were blinded to clinical data, and all analyses were performed completely independently by statisticians at the NRG. This represents blinded independent validation.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration | NCT00895622. This trial was previously completed, and we utilized tissue samples from this completed trial.

Study protocol | N/A. We used data from a previously completed trial which has been reported multiple times.

Data collection | N/A. We used data from a previously completed trial.

Outcomes | N/A. We used data from a previously completed trial, outcomes were previously defined in the trial.