Global epigenetic profiling identifies methylation subgroups associated with recurrence-free survival in meningioma

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Abstract

Meningioma is the most common primary brain tumor and carries a substantial risk of local recurrence. Methylation profiles of meningioma and their clinical implications are not well understood. We hypothesized that aggressive meningiomas have unique DNA methylation patterns that could be used to better stratify patient management. Samples (n=140) were profiled using the Illumina HumanMethylation450 BeadChip. Unsupervised modeling on a training set (n=89) identified 2 molecular methylation subgroups of meningioma (MM) with significantly different recurrence free survival (RFS) times between the groups: a prognostically unfavorable subgroup (MM-UNFAV) and a prognostically favorable subgroup (MM-FAV). This finding was validated in the remaining 51 samples and led to a baseline meningioma methylation classifier (bMMC) defined by 283 CpG loci (283-bMMC). To further optimize a recurrence predictor, probes

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subsumed within the baseline classifier were subject to additional modeling using a similar training/validation approach, leading to a 64-CpG loci meningioma methylation predictor (64-MMP). After adjustment for relevant clinical variables [WHO grade, mitotic index, Simpson grade, sex, location, and copy number aberrations (CNA)] multivariable analyses for RFS showed that the baseline methylation classifier was not significant (p=0.0793). The methylation predictor however was significantly associated with tumor recurrence (p<0.0001). CNA were extracted from the 450k intensity profiles. Tumor samples in the MM-UNFAV subgroup showed an overall higher proportion of CNAs compared to the MM-FAV subgroup tumors and the CNAs were complex in nature. CNAs in the MM-UNFAV subgroup included recurrent losses of 1p, 6q, 14q and 18q, and gain of 1q, all of which were previously identified as indicators of poor outcome. In conclusion, our analyses demonstrate robust DNA methylation signatures in meningioma that correlate with CNAs and stratify patients by recurrence risk.

Keywords

Meningioma; epigenetics; DNA methylation; recurrence risk; copy number aberrations; molecular subgroups

INTRODUCTION

Meningiomas are thought to arise from the arachnoid (meningothelial) cells in the leptomeninges. They are the most common primary tumors of the central nervous system (CNS) and can cause significant morbidity and poor quality of life for selected patients [59]. Meningiomas have diverse morphological spectra with both epithelial and mesenchymal features. Currently meningioma is classified based on histomorphological criteria of proliferation (i.e. mitotic rate), brain invasion, and morphological aspects into 3 World Health Organization (WHO) grades [41]. The strongest predictors of tumor recurrence to date are the WHO grade and the extent of surgical resection (Simpson grade) [66,24,52]; however there is significant within-grade variation of recurrence-risk for individual patients. While in general the recurrence rate increases with the WHO grade, there are subsets of patients with WHO I meningioma who experience early tumor recurrence and rare patients with WHO grade III who do not experience recurrence at all. However the most challenging group of patients from a clinical management perspective are those with WHO grade II tumors where recurrence is expected in approximately 40% of cases. It is hard to accurately predict tumor recurrence for individual patients with atypical meningioma and therefore is difficult to decide which patients should receive adjuvant radiation therapy versus close monitoring by imaging [72,17,2,43,41,54].

Although numerous molecular alterations have been described in meningioma [62,10,13,65,1,16,9] few of which were linked to prognosis, like 1p36 loss [39,45], 9p21 loss [61], complex karyotypes [17,45], and recently *TERT* promoter mutations [65], these alterations are not yet integrated with the morphological diagnosis in the most recent, revised WHO classification [41] and molecular testing is not routinely performed in the clinic for patients with meningioma. Meningioma recurrence may warrant adjuvant radiation therapy or increased monitoring [54] but these patients are not accurately identified by the

current meningioma classification system. In a similar way those not at risk for meningioma recurrence could be spared the toxicity of radiation therapy if accurately identified. The neuro-oncology community is in need of a better, more robust, perhaps molecular stratification system that could better tailor risk of recurrence and modulate therapy for patients with meningioma.

Methylation profiling of solid tumors has revealed biological subtypes, often with clinical implications [73,57,71,5,48,28,29,68,42,31,27,67,64,70,26,37,25,36,51]. However, little is known about global methylation profiles of meningioma [21,35,74], and their clinical implications are not yet well understood. Moreover, a deeper understanding of methylation in meningioma may increase our understanding regarding meningioma tumorigenesis.

In an attempt to address these issues and improve upon the current methods of risk stratification in meningioma, we hypothesized that aggressive meningiomas have unique DNA methylation profiles, with the potential for clinical relevance that could be used to better stratify patients. To test this hypothesis we have profiled a group of 140 meningioma samples of all histologic grades using the Illumina HumanMethylation450 BeadChip, and have compared these findings with clinical outcome. We identified robust DNA methylation patterns that stratify patients by recurrence risk.

MATERIALS AND METHODS

Data collection and tissue samples

This study was approved and carried out in accordance with institutional review board guidelines. One hundred and forty formalin-fixed paraffin embedded (FFPE) meningioma tissue samples were retrospectively collected. The samples were elected based on tissue availability and availability of detailed clinical information. The following samples were not included: radiation induced meningiomas, meningiomas of special morphological subtypes (with grade implication – clear cell, chordoid, rhabdoid, papillary; without grade implication – microcystic, secretory, angiomatous, psammomatous, lymphoplasmacyte-rich, metaplastic), meningioangiomatosis, spinal cord meningiomas, syndromic meningiomas (patients with a clinical diagnosis of neurofibromatosis type 2). Hematoxylin and eosin (H&E) slides were reviewed and the meningioma diagnosis was confirmed by two experienced neuropathologists (AO and KDA). Tumors collected pre-2007 were re-graded according to the 2007 WHO criteria [40]. Tumors with brain invasion in absence of mitotic activity were graded WHO grade II in accordance to our institutional practices and now per 2016 WHO criteria [41]. Therefore all cases were graded per current WHO 2016 criteria. Tissue blocks with >90% tumor were selected for immunohistochemistry and DNA extraction.

Recurrence was defined and recurrence-free survival (RFS) was calculated as previously described [58]. The timepoint of *recurrence* was established by serial imaging review [pre- and postop magnetic resonance imaging (MRI) review or computer tomography studies when MRI was contraindicated and after resolution of post-surgical changes] and documented in the medical charts by the treating physicians (neuroradiologist and neurosurgeon); *recurrence* was defined as either tumor recurrence following gross total

surgical resection or tumor progression (further growth) following subtotal surgical resection. Simpson grade was determined as previously described based on the surgical operative report correlated with the first post-surgical imaging study. For 11 patients Simpson grade was not available or not applicable (i.e. for one intraventricular meningioma) [58].

Immunohistochemistry

Immunohistochemistry with anti-pHH3 (Ser 10) rabbit polyclonal antibody (Cell Signaling Technology, catalog#9701L, dilution 1:100) and anti-Ki-67 mouse monoclonal antibody (DAKO, clone MIB-1, dilution 1:500) was performed as previously described [58]. Mitotic index was recorded as described previously - pHH3-labeled mitoses counted per 1000 meningioma tumor cells that were further split in three categories (2, 3–4, and 5) [58].

Methylation analysis

DNA was extracted using the MasterPure™ Complete DNA and RNA Purification Kit (Epicentre, Madison, WI, USA) per manufacturer's protocol. A total of 500 ng of DNA per sample was processed on Infinium HumanMethylation450 BeadChip methylation platform following quality control and DNA restoration using the Infinium FFPE QC and DNA Restoration Kits (Illumina Inc, San Diego, CA, USA) in the institutional core laboratory. Following methylation profiling, samples were split in 2 groups for analysis: a training dataset (n=89) and a validation dataset (n=51) balanced for the number of recurrences. In order to avoid any potential treatment effect bias, all treated tumor tissues were included in the validation dataset. The training dataset was comprised of 85 newly-diagnosed, non-treated tumors and 4 non-treated recurrent tumors. The validation dataset included 35 newly-diagnosed, non-treated tumors, 7 non-treated recurrent tumors, 7 recurrent treated tumors, 1 newly-diagnosed, treated tumor, and 1 metastatic treated tumor.

Raw data files (*.idat) were imported, processed, and analyzed using R 3.2.2 GUI 1.66 Mavericks build (6996) (The R Foundation for Statistical Computing http://www.R-project.org) and JMP® Genomics 7.0 software (SAS Institute Inc, Cary, NC, USA).

Development of a baseline meningioma methylation classifier (283-bMMC)

Raw data was imported using the ChAMP R package. The following probes were removed from the analysis: probes that have failed to hybridize (with a detection p-value>0.01) in one or more samples, probes with a beadcount <3 in at least 5% of samples, probes that overlapped with single nucleotide polymorphisms and probes that aligned to multiple locations as identified by Nordlund et al [55], as well as probes localized on X and Y chromosomes [53]. In addition, for consideration of future research endeavors we chose to eliminate the probes that were no longer represented in the new Illumina assay, the Infinium MethylationEPIC BeadChip which now replaces the HumanMethylation450 platform (n=31396 probes). Batch effect was present only in the training dataset and the ComBat batch normalization function was applied [53]. The validation dataset was BMIQ normalized [69]. The top 2000 probes based on the median average deviation score from the training dataset (n=89 samples) were introduced in a non-negative matrix factorization (NMF) unsupervised learning method (NMF package) [22]. The NMF algorithm 'brunet' was

selected [12] and run 50 times in order to select the best factorization rank (r) or the number of groups/clusters that best fitted the data. To check for overfitting the same parameters were used on randomized data. The best factorization rank (r=2) was selected based on the highest cophenetic coefficient and the smallest value at which the decrease in the residual sum of squares (RSS) was lower than the decrease of the RSS obtained from random data (Online resource 1). Then, the final NMF model was run 50 times and 2 metagene clusters were defined by 283 probes (NMF class 1 – 98 probes and 34 samples & NMF class 2 – 185 probes and 55 samples) (Online resource 1). Supervised hierarchical clustering (HC) was run on the training dataset with only these 283 probes and the probes respected the NMF classes following clustering. Because 3 samples switched class following HC they were eliminated and the final methylation model (283-bMMC) was defined by 86 samples and 283 probes. The reasons for divergent classification based on analytical methods can be multiple and range from factors pertaining to bioinformatics pipeline analyses to the possibility that these 3 cases may not have fit either group as robustly compared to the remainder of the cases. The clinical information for the 3 eliminated samples is offered in Online Resource 2.

Optimization of a meningioma methylation predictor (MMP)

In order to maximize prediction of RFS, we further optimized a meningioma methylation predictor on the training dataset. The 283 probes derived from 283-bMMC were introduced into a support vector machine (SVM) classification method with radial-basis smoothing kernel (RBM) [20,19]. Deviance residuals were calculated and were used further as continuous predictor variables for model selection. The genetic algorithm SAS GENESELECT was used for feature selection. The final model was selected based on the maximum Harrell C Statistic [23] and minimum Akaike and Bayesian information criteria [3] with the lowest possible root mean square error (RMSE). Fifty-fold cross-validation with 30% of the data holdout was run on the final selected model and the RMSE was documented. The RBM predictor probabilities generated were normalized by inverse transformation and a cutoff was calculated by partition analysis using the deviance residual as the dependent variable. The selected model was then independently applied to the validation dataset.

Copy number analysis

Copy number aberration (CNA) information was extracted from the 450k methylation data (intensity values) after a baseline correction for cancer samples using the maximum density peak estimation (MDPE) method (CopyNumber450kCancer R package) [49] All samples were reviewed manually as well as using Integrative Genomics Viewer (IGV) v. 2.3.68 (97) (hg19) for detailed partial/segmental annotation. (For more details and few examples please see Online resource 3).

Survival and other analyses

Summary statistics, Chi-square, Fisher's exact test, two-sample Wilcoxon test (Mann-Whitney), Cox uni- and multivariable analyses, Log-rank and Kaplan Meier survival graphics were generated in R 3.2.2 GUI 1.66 Mavericks build (6996) (The R Foundation for Statistical Computing http://www.R-project.org). Reported median survivals were calculated

via Kaplan-Meier method. Probe (cg loci) annotation was retrieved from the Illumina 450k manifest file and UCSC Genome Browser using the GRCh37/hg19 assembly. Gene ontology and pathway analysis was performed using QIAGEN 's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, CA, USA www.qiagen.com/ingenuity) (v. 27821452). All IPA p-values were calculated using a right-tailed Fisher's exact test. For comparisons of methylation levels a Mann-Whitney test was run after Bonferroni correction for multiple sample comparison. CpG appartenance to promoter region was determined using the Genome Browser tool of Genomatrix Software Suite (v. 3.6). Details on CNAs selection for uni- and multivariable analyses are presented in Online resource 4. P-values   0.05 were considered statistically significant.

RESULTS

Most tumors (98%) were graded based on mitotic activity. No tumor was graded as atypical (WHO grade II) solely based on the presence of atypical morphological features. Overall, 12 tumors had brain invasion of which only 3 were graded as atypical based on brain invasion only. The training dataset consisted of 89 samples and included 25 patients (28%) that experienced tumor recurrence at a median of 1.57 years. Median follow-up time was 7.02 years. The validation dataset (n=51 samples) included 22 patients (43%) that experienced tumor recurrence at a median of 3.6 years and had a median follow-up time of 6.27 years. Detailed clinical characteristics of the datasets are provided in Table 1. Overall, as expected, most patients were females with a F:M ratio of 2.33. A single pediatric patient was included (age<18 years). With respect to location, the non skull base vs. skull base ratio was 1.69.

The training methylation dataset was initially analyzed to identify clinically significant methylation signatures. Using NMF unsupervised classification on top differentially methylated CpG loci, a final baseline meningioma methylation classifier (283-bMMC) was defined by 86 samples and 283 probes (Figure 1). The 283-bMMC separated 2 meningioma methylation subgroups: a clinically favorable prognostic subgroup (MM-FAV) (n=31, hypermethylated CpG loci=98) (median RFS not reached, range: 0.27–16.6 years) and a clinically unfavorable meningioma methylation subgroup (MM-UNFAV) (n=55, hypermethylated CpG loci=185) (log-rank p=0.0014) (median RFS=12.07 years, range: 0.31–17.61 years) (Figure 1). When 283-bMMC was applied to the 51-sample validation dataset it validated the subgroup signatures and the difference in RFS (log-rank p=0.049) with a median RFS of 16.35 years (range: 0.86–16.35) for the MM-FAV group and of 8.27 years (range: 1.10–12.62) for the MM-UNFAV group (Figure 2). Overall, most WHO grade II tumors (~80%) and all anaplastic meningiomas classified as MM-UNFAV, subgroup that included tumors with higher median Ki-67 (MIB-1) proliferation index, significantly shorter RFS and were diagnosed at an increased median age compared to tumors of the MM-FAV subgroup. Also most recurrences (41/46, ~89%, p=0.0001, Fisher's exact test), skull-base meningiomas (~82%) and meningiomas of male patients (~81%) belonged to the MM-UNFAV subgroup (Table 2). Overall, 283-bMMC subgroups were not significant for predicting tumor recurrence after adjustment for WHO grade, mitotic index, Simpson grade, sex, location, and CNAs [p=0.0793, HR=2.97, 95%CI=(0.88–10.05)] (Table 3).

Tumors in the MM-UNFAV group had complex CNA pattern including enrichment of −1p, +1q, −2p, −3p/+3, −4,+5, −6q,+9, −10,+12,+13q, −14q,+15q, −16, −18,+20,+21q,+22q compared to tumors in the MM-FAV group which consisted mainly of +1p and −22q (Figures 1 and 2). Overall, among recurrent CNAs in the samples overall, −1p, +1q, −6q, −14q, and −18q were significantly linked to meningioma recurrence (Online resource 4).

In order to maximize prediction of aggressive meningioma and improve classification for the poor prognostic subgroup (Online resource 5), the 283 probes defining 283-bMMC were introduced in a RBM prediction algorithm. Unsupervised variable selection followed by RBM resulted in an optimized 64-CpG locus meningioma methylation predictor (64-MMP) with a negligible 50-fold cross-validation RMSE of 0.15 (Figure 3a). Partition analysis of the inverse transformed predictor probabilities returned the cutoff of 0.3813. Predictor probabilities > 0.3813 (64-MMP-UNFAV) separated all recurrences (24/24) and patients classified in this poor prognosis group had significantly decreased median RFS (1.48 years, range: 0.31–12.07) compared to those with predictor probabilities $\leq$ 0.3813 (64-MMP-FAV) (median RFS=not reached, range: 0.27–17.61) (log-rank p<0.0001) (Figure 3a)

When applied to the validation dataset, the 64-MMP significantly improved stratification (log-rank p =0.0004) with a median RFS of 3.60 years (range: 0.45–10.84) for the 64-MMP-UNFAV group compared to 16.35 years (range: 0.10–16.35) for the 64-MMP-FAV group (Figure 3b).

Overall, the 64-MMP was predictive of tumor recurrence after adjustment for WHO grade, mitotic index, Simpson grade, sex, location, and CNAs [p<0.0001, HR=12.16, 95%CI=(5.16–28.67)] (Table 3).

The 283-bMMC probes were located on 157 gene regions (52 genes for the MM-FAV group and 105 for the MM-UNFAV group). The subset of 64-MMC probes were located on 44 gene regions (identified with details in Online Resource 6). An overview of the gene function associated with the 283-bMMC probes and their subcellular location is provided in Online resource 7. The signaling and metabolic canonical pathways mapped were G-protein coupled receptor signaling, axonal guidance signaling, cAMP-mediated signaling, WNT/β-catenin signaling, and AMPK signaling. Molecules were also involved in thrombin signaling, glucocorticoid receptor signaling, protein kinase A signaling, ERK/MAPK, PI3K/AKT and PTEN signaling, mTOR, p53, NF-kB signaling, but also in IL-6, IL-8 and IL-12 signaling. For direct and indirect relationships among these molecules as well as relationships with other molecules that have been described to be involved in meningioma pathogenesis see Online resource 7.

In order to evaluate the biologic relevance of the genes involved in the predictors, we performed gene ontology analyses of the bMMC gene regions containing at least 3 hypermethylated CpG in the gene regulatory sites. These hypothetically involved genes (identified in yellow in Online resource 6) were *DUSP6, GJB6, SLC38A4, STRA6* of the MM-FAV group and *REC8* and *IER* of the MM-UNFAV group. Molecule activity predictor analysis showed that molecules activated in the MM-UNFAV group support are usually inactivated in the MM-FAV group and vice-versa (Online resource 7) and from the

biological standpoint are involved in pathways that ultimately lead to cell proliferation and tumorigenesis; whereas this effect is less pronounced in the MM-FAV group. Interestingly, molecule predictor activity predicted NF2 inactivation in both groups of tumors. Comparison analysis showed a significant activation of the WNT/β-catenin pathway in the MM-UNFAV group compared to the MM-FAV group (Online resource 8).

DISCUSSION

In this study we demonstrate robust DNA methylation signatures in meningioma able to stratify patients by recurrence risk. By using a training/validation approach and unsupervised analyses we defined a robust basic methylation classifier (bMMC) able to separate 2 groups of meningioma patients with significant differences in RFS (MM-FAV vs. MM-UNFAV) (Figures 1–2). In order to improve the prognostic effect and separation of the groups, we further optimized a meningioma methylation predictor (64-MMP) that resulted in a significantly improved effect on RFS that remained statistically significant in a multivariable model, after adjustment for relevant clinical, morphological, and molecular variables (Figure 3, Table 3). These signatures provide proof-of-concept that methylation signatures could be used in addition to the current clinical (Simpson grade) and histological (WHO grade) predictors [66,24,52,41] to more accurately identify patients prone to tumor recurrence. With such information, recurrence risk might be more accurately predicted for individual patients to tailor decisions for follow-up with serial imaging and/or neurological status assessment versus the option of adjuvant radiation therapy.

In the past, several studies focused on investigating promoter methylation mainly using a targeted approach for a limited number of genes [4,6–8,11,30,14,15,34,38,47]. With time, molecular advances allowed interrogation at a larger number of CpG loci [35] culminating with the methylation assays from Illumina (27k and 450k) interrogating approximately 27.000 and 450.000 CpG loci [74,21]. To the best of our knowledge only three studies addressed global methylation in meningiomas [35,74,21]. Kishida et al. in the first global methylation meningioma study [35] interrogated 30 WHO grade I and II meningiomas using methylated CpG island amplification microarrays for 6157 genes and identified 198 significantly different genes among 3 clusters obtained by unsupervised hierarchical clustering and consensus clustering analysis. Cluster 1 or the low methylator phenotype contained less aggressive tumors and Clusters 2 and 3 were enriched with recurrent tumors. They concluded that 5 of 198 genes which were selected based on pathway analysis (*HOXA6, HOXA9, PENK, UPK3A* and *IGF2BP1*) could successfully identify recurrent tumors and could be used as epigenetic biomarkers to effectively predict a pattern of global DNA methylation in meningioma. They validated their findings in a new set of 32 WHO grade I and II meningioma tumors. Interestingly several of their hypermethylated genes of the more aggressive Cluster 3 (*ADARB1, IER3, REC8*) were also hypermethylated in our MM-UNFAV subgroup. Moreover 2 loci of *REC8* and 1 locus of *IER3* were part of our 64-MMP. To note is that *IER3* and *REC8* had an increased number of hypermethylated CpG islands (at least 3) in the promoter region and were selected as candidates for gene downregulation and upstream/downstream molecule activity prediction (Online resource 7). In contradiction with Kishida's findings we noted that some genes in our cohort were hypermethylated in the MM-FAV group (*GATA2* and *RAP1GAP*) whereas they reported

these genes as hypermethylated in the more aggressive tumors of Cluster 3. Also C13orf33 was hypermethylated in our MM-UNFAV group whereas this gene was hypomethylated in Cluster 3 tumors. Although only 6 genes were in common between our and Kishida's study, several members of the same gene family were present in both. These included genes encoding ephrins, solute carrier proteins, ankyrin repead domain and Rab family of proteins, zinc finger proteins, and calcium-voltage gated channel subunits. On the other hand none of the 5 proposed epigenetic biomarkers were selected by our meningioma predictive model [35].

Gao et al. were the first group to introduce the Illumina 450k platform to meningioma profiling [21]. They profiled 19 WHO grade I, II and III meningiomas and similar with Vengoechea et al [74], Gao's group looked for differences in methylation between benign and malignant meningioma groups. They concluded that WHO grade II and III meningiomas were globally hypomethylated compared to WHO grade I tumors but they did not offer much details and probe identification except for 15 hypomethylated genes that were further investigated for expression analysis. They also mentioned that 26 genes were hypermethylated in malignant meningiomas of which 9 were *BARHL2, TLX3, FOXR1, HOXA11, HOXA6, HOXA9, OTX2, MAL2,* and *PAX3*. None of these genes were selected by our meningioma predictor model although in some instances members of the same gene family were present in our gene list (solute carrier proteins, paired box, and forkhead box transcription factors). It is interesting to notice that hypermethylation of *HOXA11, HOXA6,* and *HOXA9* were reported in aggressive meningiomas by several studies [35,21,15] but our analysis did not identify these genes as important predictors of outcome. However, on manual inspection of all unfiltered CpG loci located on *HOXA11, HOXA6,* and *HOXA9,* there were significant differences with increased levels of methylation in the MM-UNFAV group compared to MM-FAV group (findings limited to the validation dataset) (Online resource 9) confirming the findings in the literature.

Vengoechea et al. [74] profiled 33 WHO grade I and II meningiomas using the 27k Illumina methylation array and validated their finding on additional 7 tumor replicates and 12 additional independent WHO grade I and II meningioma samples using the 450k Illumina methylation array. They performed hierarchical unsupervised clustering analysis with the top differentially methylated probes between WHO grade I and atypical (WHO grade II) meningiomas (95 in the discovery set and 87 in the validation set) but they didn't offer details on which of these probes were predominantly hypermethylated in the more aggressive (WHO grade II) meningiomas. They selected 9 probes based on differential methylation between the groups; however based on their presented results their association was weak [74].

A generally recognized epigenetic meningioma biomarker of interest is *TIMP3* hypermethylation, which along with TIMP3 underexpression has been described as common in anaplastic meningiomas [7,8]. This gene did not include predictive hypermethylated loci in our analysis but on manual inspection *TIMP3* had significant differential methylation in 3 loci with hypermethylation in 2 loci of the MM-FAV group and one locus of the MM-UNFAV group (validation dataset only) (Online resource 9). This is likely because our model was developed mainly on WHO grade I and II tumors (there was only 1 WHO grade

III tumor in the training dataset). Further global methylation studies should be designed to include more anaplastic tumors.

Meningiomas are complex from a genomic perspective, and a number of molecular alterations have been described to date. The most common cytogenetic alteration, 22q loss, is observed across WHO grades, whereas losses of chromosomes 1p, 6q, 10, 14q, 18q and gains of chromosomes 1q, 9q, 12q, 15q, 17q, 20q enrich the WHO grade II and III tumors [76,45,39,16,60,75]. Chromosome 9p loss is frequently associated with anaplastic astrocytomas [61,75]. These findings were generally supported by our analyses with more complex karyotypes clustering in the MM-UNFAV subgroup [17]. Interestingly in our cohort several tumors showed gains of 22q and in support of the published literature when present, they were more likely categorized as of unfavorable clinical course (MM-UNFAV) [44,63,46,75] (Figures 1 and 2). However, on survival analysis this was not significant (Online resource 4). Chromosome 22q gain is an interesting phenomenon that should be further investigated.

The most commonly activated signaling and metabolic canonical pathways described in meningiomas are the RB/p53 pathway, MAPK and PI3K/AKT, PLCγ/PKC-calcium signaling pathway, the cyclooxygenase-2 signaling pathway, mTOR, WNT/β-catenin, Notch, and Hedgehog signaling pathways [16,33,32,50]. This is overall in keeping with our findings (Online resource 7), noting that comparative pathway analysis identified a stronger activation of the WNT/β-catenin signaling pathway in the MA subgroup opposing the activation of retinoate biosynthesis and leukocyte extravasation signaling in the MB subgroup (Online resource 8)

There are several potential limitations of our study. First, our study is limited in that the training/discovery dataset is somewhat unbalanced for WHO grades and included only one newly diagnosed anaplastic meningioma. Although the validation dataset separated all anaplastic meningiomas accurately (all WHO grade III meningiomas in the MM-UNFAV arm) (Figures 1 and 2), this model should be interpreted with care when applied to WHO grade III meningiomas. Second, although retrieval of copy number information from the 450k array intensity values has been validated in the literature by multiple molecular platforms and the 450k platform has been identified as having the sensitivity of SNP arrays for copy number alteration detection [49,18,56] the results should be interpreted with care. Third, our prediction analyses based on hypothetical downregulation of the 6 genes presented in Online resource 7, should be interpreted with care having in mind that epigenetic regulation is more complex and involves other regulatory molecules that were not taken into consideration here, such as microRNA expression and histone/chromatin modifications.

Our study is novel in that it utilizes global methylation assays and mathematical modeling on a substantive cohort of clinically annotated, non-treated meningiomas to date (n=89) to offer robust methylation meningioma signatures (283-bMMC model) that characterize clinical-biological subgroups (MM-FAV and MM-UNFAV). Importantly a subset of these 283 markers (64-MMP) proved as stronger predictors indicating that this predictor probability could potentially be used for prognostication in the clinical setting (64-MMP).

Moreover a significant strength of our study is the correlation with clinical prognostic factors and extensive available patient follow-up time. Our findings are important because our basic methylation model and methylation predictor could potentially be applied to newly diagnosed meningioma patients in order to better predict tumor recurrence. An improved, more accurate recurrence prediction is likely to facilitate patient management decisions in the clinic. While validated in our cohort, further work is needed, and we hope this work will stimulate further investigation to evaluate methylation signatures that can inform therapeutic decisions for patients with meningioma.

With the ever-increasing expansion of genomic data, future correlational studies should integrate multi platform analyses (i.e. methylation, copy number, mutational, expression) with clinical information in order to stratify risk and impact patient management in meningioma with the ultimate goal to foster precision therapeutic approaches and improve patient outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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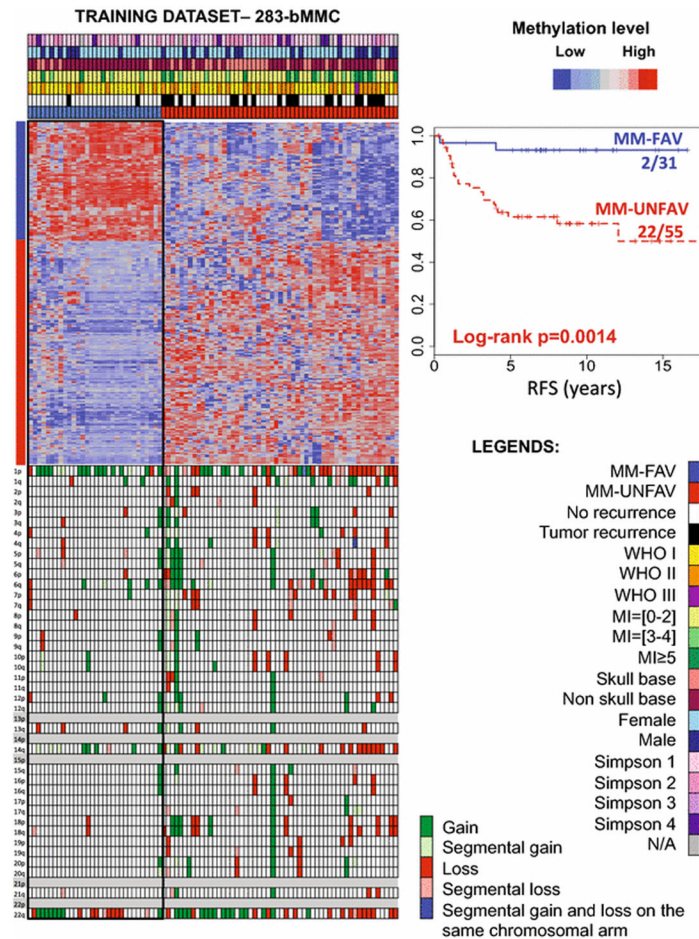
Figure 1.
Supervised hierarchical clustering with the 283 probes in the training dataset (n=86) separates 2 differentially methylated groups of tumors. The copy number information is represented at the bottom. Each row represents a probe and each column represents a sample. Methylation values are shown after batch normalization. Kaplan-Meier survival analysis illustrates significantly decreased tumor recurrence time for patients with meningioma from the MM-UNFAV group.
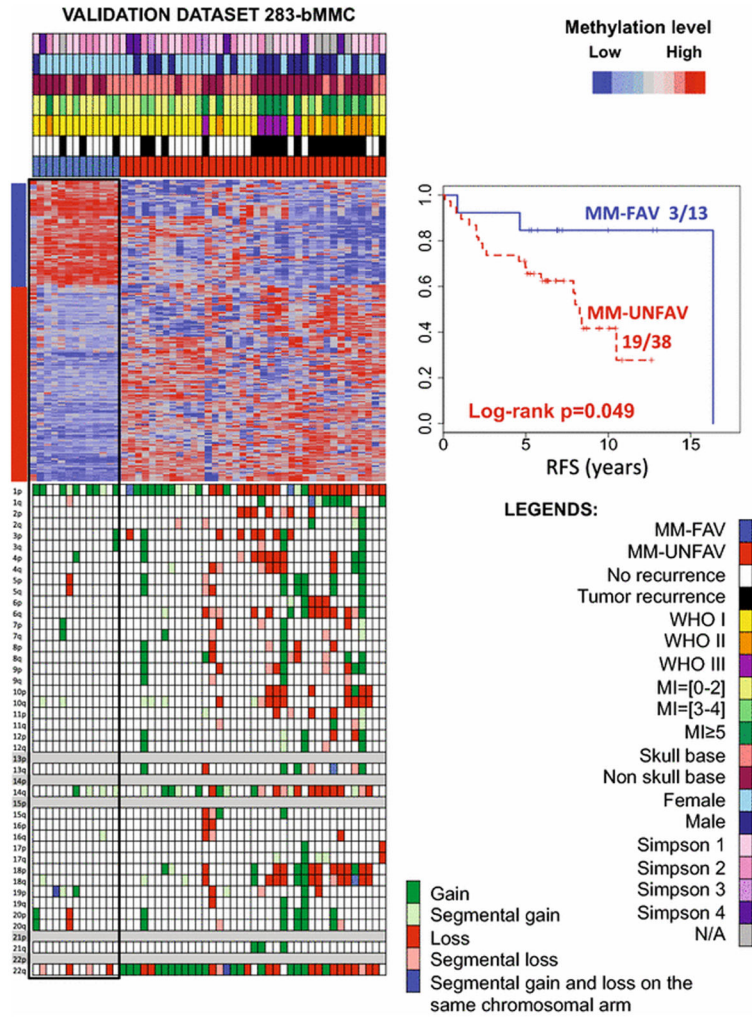
Figure 2.
Supervised hierarchical clustering with the 283 probes in the validation dataset (n=51) separates 2 differentially methylated groups of tumors. The copy number information is represented at the bottom. Each row represents a probe and each column represents a sample. Methylation values are shown after BMIQ normalization. Kaplan-Meier survival analysis illustrates significantly decreased tumor recurrence time for patients with meningioma from the MM-UNFAV group.
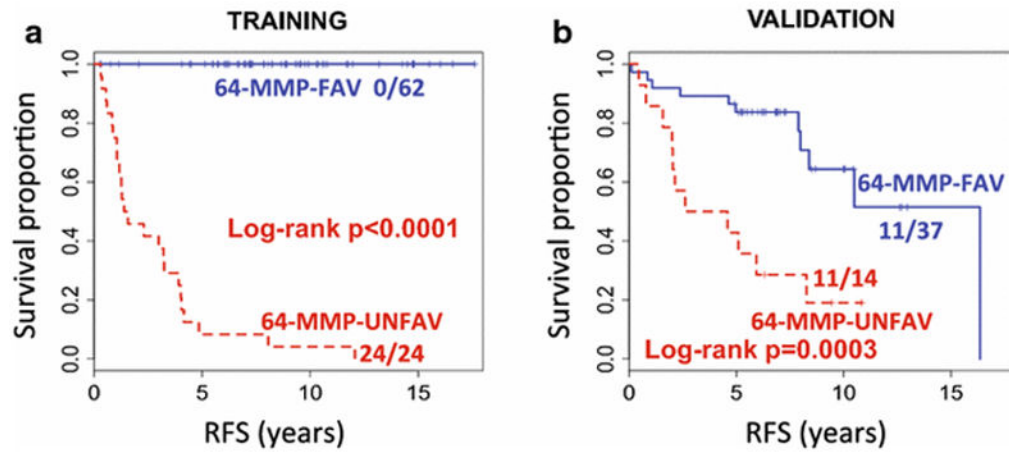
Figure 3.
The 64-MMP model developed on the training dataset (a) and then applied to the validation dataset (b) significantly separates risk groups for meningioma recurrence.

Table 1

Clinical characteristics of the test (n=89) and validation (n=51) datasets (n=140).

	Test	Validation	Overall
WHO grade			
# (%) [recurrences]			
I	54 (60.67) [10]	36 (70.59) [10]	90 (64.29) [20]
II	34 (38.20) [14]	9 (17.65) [6]	43 (30.71) [20]
III	1 (1.12) [1]	6 (11.76) [6]	7 (5) [7]
Median F/U (range) (years)	7.02 (0.27–17.61)	6.27 (0.10–16.35)	6.75 (0.10–17.61)
Median age (range) (years)	57.26 (20.31–86.48)	56.62 (5.95–79.58)	57.02 (5.95–86.48)
Simpson grade	45 (50.56)	24 (47.06)	69 (49.29)
# (%)	24 (26.97)	12 (23.53)	36 (25.71)
1	6 (6.74)	3 (5.88)	9 (6.43)
2	9 (10.11)	6 (11.76)	15 (10.71)
3	5 (5.62)	6 (11.76)	11 (7.86)
4			
N/A			
Mitotic index			
2	59 (66.29)	27 (52.94)	86 (61.43)
3–4	16 (17.98)	12 (23.53)	28 (20)
5	14 (15.73)	12 (23.53)	26 (18.57)
Median MIB-1 index (range)	3.4 (0.2–36.4)	3.4 (0.3–54.1)	3.4 (0.2–54.1)
Sex			
F	65 (73.03)	33 (64.71)	98 (70)
M	24 (26.97)	18 (35.29)	42 (30)
Location			
Skull-base	29 (32.58)	23 (45.10)	52 (37.14)
Non skull-base	60 (67.42)	28 (54.90)	88 (62.86)

Abbreviations: F-female, F/U-follow-up, M-male, WHO-World Health Organization.

Table 2

Comparison of clinical and histopathological characteristics between the 283-bMMC subgroups.

	MM-FAV	MM-UNFAV	p-value
WHO grade			
# (%) [recurrences]			
I	35 (39.77) [4]	53 (60.23) [16]	0.0189 [*]
II	9 (21.43) [1]	33 (78.57) [18]	
III	0 (0) [0]	7 (100) [7]	
Median F/U (range) (years)	7.18 (0.27–16.60)	5.93 (0.10–17.61)	<0.0001 [***]
Median age (range) (years)	53 (24.82–82.15)	58.26 (5.95–86.48)	<0.0001 [***]
Simpson grade	23 (33.82)	45 (66.18)	
# (%)	14 (41.18)	20 (58.82)	
1	1 (11.11)	8 (88.89)	
2	4 (26.67)	11 (73.33)	
3	2 (18.18)	9 (81.82)	0.3990 [*]
4			
N/A			
Mitotic index			
2	33 (38.82)	52 (61.18)	
3–4	7 (26.92)	19 (73.08)	0.0658 [*]
5	4 (15.38)	22 (84.62)	
Median mitotic index (range)	1 (0–7)	2 (0–24)	0.1439 [***]
Median MIB-1 index (range)	2.3 (0.2–22.6)	5 (0.3–54.1)	<0.0001 [***]
Sex			
F	36 (37.89)	59 (62.11)	0.0294 [**]
M	8 (19.05)	34 (80.95)	
Location			
Skull-base	9 (17.65)	42 (82.35)	0.0052 [**]
Non skull-base	35 (40.70)	51 (59.30)	

Abbreviations: F-female, F/U-follow-up, M-male, WHO-World Health Organization.

[*] Fisher's exact test,

[**] Chi-square test,

[***] Two-sample Wilcoxon test (Mann-Whitney)

Table 3

Cox univariable and multivariable analyses (n=137, events=46).

| Variable | Univariable | | | Multivariable[**] | | | | | |
| | | | | (with 283-bMMC) | | | (with 64-MMP) | | |
	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
283-bMMC									
UNFAV vs. FAV	5.01	1.97–12.73	0.0007	2.97	0.88–10.05	0.0793			
64-MMP									
UNFAV vs. FAV	19.47	9.48–40	<0.0001				12.16	5.16–28.67	<0.0001
CNA									
1–2 vs. 0	2.91	1.39–6.11	0.0048	2.63	0.99–6.96	0.0512	2.42	0.95–6.18	0.0645
3 vs. 0	6.48	3.19–13.17	<0.0001	3.93	1.34–11.55	0.0129	3.41	1.10–10.54	0.0330
WHO grade									
II vs. I	2.90	1.53–5.50	0.0011	1.52	0.63–3.63	0.3480	0.98	0.37–2.58	0.9633
III vs. I	11.86	4.91–28.65	<0.0001	2.86	0.61–13.31	0.1806	1.39	0.25–7.66	0.7023
Mitotic index									
3–4 vs. 2	3.03	1.41–6.50	0.0044	2.14	0.83–5.56	0.1162	1.45	0.56–3.75	0.4468
5 vs. 2	7.05	3.55–14.00	<0.0001	5.18	1.72–15.63	0.0035	2.98	1.01–8.76	0.0476
Simpson grade [*]									
2 vs. 1	1.21	0.55–2.65	0.6291	2.53	0.98–6.49	0.0542	2.38	0.91–6.20	0.0757
3 vs. 1	3.52	1.29–9.60	0.0138	3.94	1.16–13.44	0.0285	4.63	1.22–17.57	0.0243
4 vs. 1	1.62	0.64–4.12	0.3095	2.57	0.84–7.88	0.0999	1.76	0.58–5.36	0.3172
Sex									
M vs. F	1.86	1.04–3.33	0.0376	0.96	0.44–2.09	0.9179	0.97	0.43–2.16	0.9367
Location									
Skull-base vs. Non skull-base	1.26	0.69–2.29	0.446	1.87	0.75–4.70	0.1815	2.15	0.90–5.15	0.0865

Abbreviations: CNA-copy number aberrations, F-female, M-male, WHO-World Health Organization.

*
126 cases (38 events) with complete data were introduced in the univariable model

**
126 cases (38 events) with complete data across all variables were introduced in the model