Loss of histone H3K27me3 identifies a subset of meningiomas with increased risk of recurrence

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Abstract: Epigenetic patterns on the level of DNA methylation have already been shown to separate clinically relevant subgroups of meningiomas. We here set out to identify potential prognostic implications of epigenetic modification on the level of histones with focus on H3K27 trimethylation (H3K27me3). H3K27me3 was assessed by immunohistochemistry on 232 meningiomas from 232 patients. In 194 cases, trimethylation was detected in tumor cells. In 25 cases, staining was limited to vessels while all tumor cells were negative. Finally, 13 cases yielded equivocal staining patterns. Reduced abundance of H3K27me3 in cases with staining limited to vessels was confirmed by mass spectrometry on a subset of cases. Lack of staining for H3K27me3 in all tumor cells was significantly associated with more rapid progression ($p = 0.009$). In line, H3K27me3-negative cases were associated with a DNA methylation pattern of the more aggressive types among the recently introduced DNA methylation groups. Also, NF2 and SUFU mutations were enriched among cases with complete lack of H3K27me3 staining in tumor cells ($p < 0.0001$ and $p = 0.029$, respectively). H3K27me3 staining pattern added significant prognostic insight into WHO grade II cases and in the compound subset of WHO grade I and II cases ($p = 0.04$ and $p = 0.007$, respectively). However, it did not further stratify within WHO grade III cases. Collectively, these data indicate that epigenetic modifications beyond DNA methylation are involved in the aggressiveness of meningioma. It also suggests that H3K27me3 immunohistochemistry might be a useful adjunct in meningioma diagnostics, particularly for cases with WHO grade II histology or at the borderline between WHO grade I and II.

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Loss of Histone H3K27me3 Identifies a Subset of Meningiomas with Increased Risk of Recurrence

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Abstract

Epigenetic patterns on the level of DNA methylation have already shown to separate clinically relevant subgroups of meningiomas. We here set out to identify potential prognostic implications of epigenetic modification on the level of histones with focus on H3K27 trimethylation (H3K27me3). H3K27me3 was assessed by immunohistochemistry on 232 meningiomas from 232 patients. In 194 cases, trimethylation was detected in tumor cells. In 25 cases, staining was limited to vessels while all tumor cells were negative. Finally, 13 cases yielded equivocal staining patterns. Reduced abundance of H3K27me3 in cases with staining limited to vessels was confirmed by mass spectrometry on a subset of cases. Lack of staining for H3K27me3 in all tumor cells was significantly associated with more rapid progression ($p=0.009$). In line, H3K27me3 negative cases were associated with a DNA methylation pattern of the more aggressive types among the recently introduced DNA methylation groups. Also, *NF2* and *SUFU* mutations were enriched among cases with complete lack of H3K27me3 staining in tumor cells ($p<0.0001$ and $p=0.029$, respectively). H3K27me3 staining pattern added significant prognostic insight in WHO grade II cases and in the compound subset of WHO grade I and II cases ($p=0.04$ and $p=0.007$, respectively). However, it did not further stratify within WHO grade III cases. Collectively, this data indicate that epigenetic modifications beyond DNA methylation are involved in the aggressiveness of meningioma. It also suggests that H3K27me3 immunohistochemistry might be a useful adjunct in meningioma diagnostics, particularly for cases with WHO grade II histology or at the borderline between WHO grade I and II.

Introduction

Meningiomas are the most frequent primary intracranial and spinal tumors. They are classified into WHO grades I to III. Grading is based on histological features [18]. This histological system, as *per se* all classificatory approaches, is imperfect; particularly, WHO grade I meningiomas can recur and infiltrate locally and, conversely, higher grade meningiomas can have an indolent clinical course [10].

Despite its limitations, the WHO grading system is the best currently available algorithm for risk stratification of meningiomas. Thus, it defines clinical management, with clear guidelines for grades I and III, recommending observation and adjuvant radiation, respectively. Grade II meningiomas, however, comprise a histologically heterogeneous group of tumors with behavior that is more challenging to predict, leaving treatment decisions to be determined by institutional multidisciplinary consensus rather than formalized guidelines [10]. Treatment determination hinges on an imprecise balance between likelihood of recurrence and potentially latent treatment-induced morbidity. Thus, further improvement of the classification is needed, potentially employing novel, more reliable biomarkers.

Besides the previously known mutations in *NF2*, genome wide and subsequent targeted sequencing studies of meningiomas have identified recurrent mutations in *AKT1/TRAF7*, *KLF4/TRAF7*, *SMO,* and *PIK3CA* which are strongly associated with histological features and location [2,3,5,26,33,38]. Additionally, mutations in the *TERT* promoter are associated with higher risk of recurrence and shorter progression free survival [11,23,29].

Epigenetic modifications may also indicate the risk of recurrence in meningiomas [5,7,8,12,16,35]. Consequently, recent reports propose risk stratification schemes based on DNA methylation subgroups [21,30].

Besides DNA methylation, a major epigenetic determinant of gene expression and cellular differentiation is the modification of histones, primarily by methylation and acetylation. Particularly modifications of lysine 27 (K27) of histone H3 play a crucial role in tumorigenesis [37]. Methylation of H3K27 is regulated by the EZH2 subunit of the PRC2 complex [4,20,36] and trimethylated H3K27 (H3K27me3) is associated with silencing of genes in the accompanying region [17]. Dysregulation of H3K27 methylation has been identified in several different cancers, including breast, prostate, colon, ovarian cancers, and malignant peripheral nerve sheath tumors [6,15,24,31,37,39,40]. As a result, assessment of H3K27 methylation status, particularly trimethylation (H3K27me3) has entered diagnostic practice as an immunohistochemical tool for several entities [1,22].

We here tested for H3K27me3 staining patterns in meningioma in order to detect potentially clinically relevant subgroups identified by this marker.

Materials and Methods

Case cohort

We assessed formalin-fixed paraffin embedded tissue of 232 meningiomas of 232 patients. Since meningiomas at the interface of the common grade I and the less frequent grade II are most challenging to predict in terms of clinical course, the study was intentionally enriched for WHO grade II cases compared to epidemiological distribution (Table 1). Of note, all but two cases of WHO grade II were diagnosed based on mitotic count, the other two based on brain invasion. Of cases with recurrences, only material from the primary lesion was assessed. Based on information from available clinical records, no patient had prior radiotherapy or known neurofibromatosis type 2. Tumor size was estimated by measuring the largest diameter of contrast-enhancing tumor lesions in one plain on available imaging scans (CT or MRI). The cases and clinical data were provided by the Dept. of Neurology Zurich (Switzerland), the Dept. of Neuropathology Frankfurt (Germany), the Dept. of Pathology at the NYU Langone Medical Center (USA), and the Depts. of Neurosurgery, Neurology and Neuropathology Heidelberg (Germany). The tissues from Frankfurt were assessed on tissue micro arrays with two cores of each 2 mm diameter from each case. All other cases were analyzed as whole sections. Research use of tissue and clinical data was in accordance with local ethical regulations. Diagnoses were based on the WHO classification of brain tumors 2016. Cases initially diagnosed based on previous versions of the classification were reviewed (Zurich and Heidelberg samples in Heidelberg, other cases at the respective local institutions).

Immunohistochemistry and molecular analysis

Immunohistochemistry was performed on 4 µm-thick formalin-fixed, paraffin-embedded (FFPE) tissue sections. Tissues were pre-treated for 10 min at 121°C in an autoclave at 210 kPA, subsequently further incubated with Ventana Cell Conditioner 1 immunostainer (Ventana Medical Systems, Tucson, AZ, USA) for 1 h. This pre-treatment was followed by incubation with rabbit monoclonal H3K27me3 antibody C36B11 (1:100, Cell Signaling, Danvers, MA, USA) on a Ventana BenchMark Ultra automated stainer for 2 h. Standard Ventana signal amplification was used including OptiView Amplifier Multimer and incubation with hematoxylin and Blueing reagent for 4 min each. Intratumoral vessels served as positive controls. DNA methylation and panel sequencing data were obtained from previous analyses [30].

Statistics

Fisher's exact test was used to compare categorical factors between H3K27 groups. Mann-Whitney test was used to compare quantitative parameters between H3K27 groups. Time to progression (TTP) was defined as time from initial surgery to 1st recurrence as determined by imaging. Patients without recurrence during follow-up were censored at last follow-up. Kaplan-Meier estimates and log-rank test were used to estimate and compare distribution of TTP between groups. Cox regression was used to assess the impact of factors on TTP. Interaction between WHO grade and H3K27 was tested in Cox regression in order to identify subgroup effects. Cox Regression with Firth correction [13] was used in case of complete separation. For multivariable Cox regression model, multiple imputations of missing values with 100 imputations were performed using the chained equations (mice) algorithm [34]. Associations of staining patterns with mutations were analyzed with Fisher's exact test. P-values below 0.05 were considered statistically significant. Analysis was performed with statistical software R 3.4 (https://www.R-project.org/).

Mass spectrometry for histone modification

Nine frozen meningioma samples (from Zurich and Heidelberg) were available for mass spectrometry analysis. Histones were acid extracted, derivatized via propionylation, digested with trypsin, newly formed N-termini were propionylated as previously described [9] at ActiveMotif (Carlsbad, CA, USA), and then measured 3 separate times using the Thermo Scientific TSQ Quantum Ultra mass spectrometer coupled with an UltiMate 3000 Dionex nano-liquid chromatography system. The data was quantified using Skyline [19].

Results

Of 232 assessed cases, 194 showed positive staining for H3K27me3 in vessels and tumor cells, indicating trimethylation at H3K27 in the majority of meningiomas (Table 1). In 25 cases, however, H3K27me3 staining was limited to vessels while tumor cells were negative for this marker, pointing towards a loss of trimethylation (Fig. 1a, b). Cases with positive staining for H3K27me3 were subsequently tagged "retained" while cases without H3K27me3 in tumor cells were designated as "loss". Of note, 13 cases had retained trimethylation with intermingled areas of negative staining. In these cases with ambiguous staining pattern, the vessels were also faintly stained or negative for H3K27me3 in the areas with negative tumor cells (Fig 1c, d). Thus, this pattern of partial loss is more likely an artifact than due to a sub-clonal event. Consequently, these cases were grouped with the "retained" cases.

Cases with complete loss of trimethylation showed significantly less favorable outcome and more rapid progression (p=0.009, Fig. 2a). This also held true when limiting the analysis to cases with clearly positive or negative H3K27me3 staining in tumor cells, excluding the cases with ambiguous pattern (Suppl. Fig. 1, p=0.01). While this survival analysis was applied to the entire un-stratified cohort, for potential application in diagnostic routine, however, the added value on top of the current grading is more relevant. Interestingly, when further dissecting this overall discriminatory effect by the WHO grades, it was actually limited to WHO grade I and II (Fig 2b, c). All WHO grade II cases with complete loss were diagnosed as atypical based on mitotic count. In contrast, histologically clearly anaplastic cases could not be further sub-divided for prognostic sub-groups by H3K27me3 staining pattern (Suppl. Fig. 2) and showed in fact a significantly different prognostic impact of H3K27me3 than WHO I/II cases (interaction test p=0.02).
H3K27me3 staining, WHO grade, extent of resection (STR vs. GTR and Simpson grade) were all significantly associated with outcome in a univariable analysis of WHO grade I and II cases (Table 2). H3K27me3 staining pattern remained prognostically relevant when adjusting this subset for WHO grade and extent of resection in a multivariable analysis (Table 3).

Mutational status and DNA methylation subgroups

Mutational data was available for 98 cases. Among the most frequently mutated genes in meningioma, encompassing *AKT1*, *KLF4/TRAF7*, *NF2*, *PIK3CA*, *SMO*, *SUFU* and the *TERT* promoter, only mutations of *NF2* and *SUFU* were significantly more frequent in cases without H3K27 trimethylation ($p<0.001$ and $p=0.029$, respectively, Suppl. Table 1). Other recurrent mutations, including aberrations of genes coding for histones that can also be associated with loss of trimethylation, were not detected.
Also, the DNA methylation status of 87 samples was analyzed in context of the H3K27me3 staining pattern. Case numbers with complete loss of trimethylation were too small to assess association with the previously introduced six individual DNA methylation subgroups [30]. Thus, an analysis for association with the two overarching DNA methylation groups "A" (comprising the three benign subgroups and subgroup intermediate-A) and "B" (comprising subgroups intermediate-B and malignant) was performed. Therein, complete loss of trimethylation was significantly associated with a DNA methylation pattern of the "group B", comprising the subgroups "MC malignant" and "MC intermediate B" (p=0.0046, Fisher's exact test, Suppl. Table 2, Suppl. Fig.3).

Mass spectrometry screen for histone modification

In order to assess the broader landscape of epigenetic regulation by histone modification, we performed a mass spectrometry-based screen for >80 histone modifications. Availability of sufficient frozen tissue and cost per analysis restricted the case selection and sample size. The analysis included two samples with complete loss of H3K27me3, assigned to group B by DNA methylation analysis (subgroups MC malignant and MC intermediate-B) and seven cases with retained trimethylation from group A (each three from MC benign-2 and benign-1 and one from MC intermediate A). Unsupervized clustering of these data yielded a pattern that exactly recapitulated the groups assigned by DNA methylation analysis (Fig. 3). Trimethylation of H3K27me (H3.1 and H3.3) was significantly lower in group B cases ($p=0.003$ and $p=0.04$, respectively).

Discussion

Associations between epigenetic modification and aggressiveness in meningioma have so far mostly been assessed on the level of DNA methylation. Thereby, DNA methylation analysis has evolved as promising candidate to add a molecular layer to upcoming WHO classifications, along with risk-related genetic aberrations like *TERT* promoter or *BAP1* mutations [11,14,23,29,32].

However, the major alternative epigenetic modifier, histone methylation and acetylation, has not been further evaluated for prognostic potential. Yet, associations of H3K27-related regulation of expression and molecular subgroups of meningioma have already been reported. On the basis of chromatin immunoprecipitation sequencing (ChIP Seq) for the regulatory mark H3K27Ac, the chief competing modification to trimethylation, differences between meningioma with *AKT1* vs. *NF2* mutations have been reported [5].

In contrast to ChIP-Seq and DNA methylation analysis, H3K27me3 immunohistochemistry is already implemented in many laboratories and can be readily applied. Its diagnostic potential has already been demonstrated for malignant peripheral nerve sheath tumors (MPNST) and ependymomas [22,27]. In both entities, MPNST and posterior fossa ependymoma, the H3K27me3 staining pattern parallels a distinct DNA methylation. Also in our cohort, the H3K27me3 staining was associated with the previously introduced DNA methylation subgroups. In MPNST, the loss of trimethylation is mechanistically attributable to the perturbed PRC2 complex as result of the EED/SUZ12 alterations [25]. For ependymomas and meningiomas, the functional background is not yet fully deciphered.

Although a merely descriptive finding, our data show that immunohistochemistry for H3K27me3 on meningioma samples can provide a useful tool in neuropathology practice. Complete loss of H3K27me3 staining predicts increased risk of recurrence in meningiomas, for the group of WHO grade I/II cases even independent of histological grade or extent of resection. While complete loss of trimethylation also occurred in WHO grade III cases, the staining pattern did not further stratify for risk-related subgroups among them. This might be due to other factors driving malignancy irrespective of H3K27 status. Also, an effect might be obscured by the study design that did not stratify for treatment. The majority of the high-grade cases might have received adjuvant therapy. However, only limited information on this was available for the present study which prevents definitive assessment of H3K27 trimethylation as a biomarker in WHO grade III meningiomas. Further, the fact that complete loss of trimethylation is associated with worse outcome within the entire cohort but not an obligatory prerequisite for high-grade meningiomas may also explain why a previous study could detect higher H3K27 trimethylation in a subset of WHO grade II cases compared to low grade meningioma [12].

Of note, a challenge in application of H3K27me3 staining remains that different specificities have been reported for the various available antibodies [28]. More advanced proteomic methods including mass spectrometry will potentially elucidate which specific methylation status is detected at which specific sites and by which clones. Importantly, these studies may identify whether there is actually a functional background and relevance of these discrepant antibody specificities. By now, incorporation of this immunohistochemical biomarker as outlined here has the potential to predict which meningiomas are more likely to recur, helping to identify those patients that may benefit from adjuvant radiation or a more

stringent clinical and radiological follow-up. Future larger and prospective studies stratifying patients based on H3K27me3 status are warranted to further validate its use in diagnostic routine and its correlation with mutations and DNA methylation.

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References

1 Bender S, Tang Y, Lindroth AM et al. (2013) Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. Cancer cell 24: 660-672

2 Bi WL, Greenwald NF, Abedalthagafi M et al. (2017) Genomic landscape of high-grade meningiomas. NPJ Genom Med 2:

3 Brastianos PK, Horowitz PM, Santagata S et al. (2013) Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. Nat Genet 45: 285-289

4 Cao R, Wang L, Wang H et al. (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298: 1039-1043

5 Clark VE, Erson-Omay EZ, Serin A et al. (2013) Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. Science 339: 1077-1080

6 Cleven AH, Al Sannaa GA, Briaire-de Bruijn I et al. (2016) Loss of H3K27 tri-methylation is a diagnostic marker for malignant peripheral nerve sheath tumors and an indicator for an inferior survival. Modern Pathology:

7 Di Vinci A, Brigati C, Casciano I et al. (2012) HOXA7, 9, and 10 are methylation targets associated with aggressive behavior in meningiomas. Transl Res 160: 355-362

8 Gao F, Shi L, Russin J et al. (2013) DNA methylation in the malignant transformation of meningiomas. PloS one 8: e54114

9 Garcia BA, Mollah S, Ueberheide BM et al. (2007) Chemical derivatization of histones for facilitated analysis by mass spectrometry. Nat Protoc 2: 933-938

10 Goldbrunner R, Minniti G, Preusser M et al. (2016) EANO guidelines for the diagnosis and treatment of meningiomas. Lancet Oncol 17: e383-391

11 Goutagny S, Nault JC, Mallet M, Henin D, Rossi JZ, Kalamarides M (2014) High incidence of activating TERT promoter mutations in meningiomas undergoing malignant progression. Brain Pathol 24: 184-189

12 Harmanci AS, Youngblood MW, Clark VE et al. (2017) Integrated genomic analyses of de novo pathways underlying atypical meningiomas. Nat Commun 8: 14433

13 Heinze G, Schemper M (2001) A solution to the problem of monotone likelihood in Cox regression. Biometrics 57: 114-119

14 Juratli TA, Thiede C, Koerner MVA et al. (2017) Intratumoral heterogeneity and TERT promoter mutations in progressive/higher-grade meningiomas. Oncotarget 8: 109228-109237

15 Karczmarski J, Rubel T, Paziewska A et al. (2014) Histone H3 lysine 27 acetylation is altered in colon cancer. Clinical proteomics 11: 24

16 Kishida Y, Natsume A, Kondo Y et al. (2012) Epigenetic subclassification of meningiomas based on genome-wide DNA methylation analyses. Carcinogenesis 33: 436-441

17 Kondo Y, Shen L, Cheng AS et al. (2008) Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nature genetics 40: 741-750

18 Louis DN, Perry A, Reifenberger G et al. (2016) The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta neuropathologica 131: 803-820
19 MacLean B, Tomazela DM, Shulman N et al. (2010) Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. Bioinformatics 26: 966-968
20 Nekrasov M, Klymenko T, Fraterman S et al. (2007) Pcl-PRC2 is needed to generate high levels of H3-K27 trimethylation at Polycomb target genes. The EMBO journal 26: 4078-4088
21 Olar A, Wani KM, Wilson CD et al. (2017) Global epigenetic profiling identifies methylation subgroups associated with recurrence-free survival in meningioma. Acta neuropathologica 133: 431-444
22 Panwalkar P, Clark J, Ramaswamy V et al. (2017) Immunohistochemical analysis of H3K27me3 demonstrates global reduction in group-A childhood posterior fossa ependymoma and is a powerful predictor of outcome. Acta neuropathologica 134: 705-714
23 Peyre M, Gauchotte G, Giry M et al. (2017) De novo and secondary anaplastic meningiomas: a study of clinical and histomolecular prognostic factors. Neuro Oncol:
24 Puppe J, Drost R, Liu X et al. (2009) BRCA1-deficient mammary tumor cells are dependent on EZH2 expression and sensitive to Polycomb Repressive Complex 2-inhibitor 3-deazaneplanocin A. Breast Cancer Research 11: R63
25 Reuss DE, Habel A, Hagenlocher C et al. (2014) Neurofibromin specific antibody differentiates malignant peripheral nerve sheath tumors (MPNST) from other spindle cell neoplasms. Acta neuropathologica 127: 565-572
26 Reuss DE, Piro RM, Jones DT et al. (2013) Secretory meningiomas are defined by combined KLF4 K409Q and TRAF7 mutations. Acta neuropathologica 125: 351-358
27 Rohrich M, Koelsche C, Schrimpf D et al. (2016) Methylation-based classification of benign and malignant peripheral nerve sheath tumors. Acta neuropathologica 131: 877-887
28 Rothbart SB, Dickson BM, Raab JR et al. (2015) An Interactive Database for the Assessment of Histone Antibody Specificity. Mol Cell 59: 502-511
29 Sahm F, Schrimpf D, Olar A et al. (2016) TERT Promoter Mutations and Risk of Recurrence in Meningioma. J Natl Cancer Inst 108:
30 Sahm F, Schrimpf D, Stichel D et al. (2017) DNA methylation-based classification and grading system for meningioma: a multicentre, retrospective analysis. Lancet Oncol 18: 682-694
31 Schlesinger Y, Straussman R, Keshet I et al. (2007) Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. Nature genetics 39: 232-236
32 Shankar GM, Abedalthagafi M, Vaubel RA et al. (2017) Germline and somatic BAP1 mutations in high-grade rhabdoid meningiomas. Neuro Oncol 19: 535-545
33 Strickland MR, Gill CM, Nayyar N et al. (2017) Targeted sequencing of SMO and AKT1 in anterior skull base meningiomas. J Neurosurg 127: 438-444
34 Van Buuren S, Groothuis-Oudshoorn K (2011) mice: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software 45(3): 1-67

35 Vengoechea J, Sloan AE, Chen Y et al. (2013) Methylation markers of malignant potential in meningiomas. J Neurosurg 119: 899-906
36 Viré E, Brenner C, Deplus R et al. (2006) The Polycomb group protein EZH2 directly controls DNA methylation. Nature 439: 871-874
37 Wei Y, Xia W, Zhang Z et al. (2008) Loss of trimethylation at lysine 27 of histone H3 is a predictor of poor outcome in breast, ovarian, and pancreatic cancers. Molecular carcinogenesis 47: 701-706
38 Yesiloz U, Kirches E, Hartmann C et al. (2017) Frequent AKT1E17K mutations in skull base meningiomas are associated with mTOR and ERK1/2 activation and reduced time to tumor recurrence. Neuro Oncol 19: 1088-1096
39 Yoo KH, Hennighausen L (2012) EZH2 methyltransferase and H3K27 methylation in breast cancer. Int J Biol Sci 8: 59-65
40 Yu J, Yu J, Rhodes DR et al. (2007) A polycomb repression signature in metastatic prostate cancer predicts cancer outcome. Cancer research 67: 10657-10663

Figure legends

Figure 1: Examples of meningiomas positive (a) and negative (b) for H3K27me3 staining, and examples with indeterminate staining pattern (c, d). Scale bar: 50 µm

Figure 2: Kaplan-Meier curves showing time-to-recurrence for all analyzed meningiomas (a) and restricted to cases of WHO grade I/II (b), stratified for H3K27me3 staining, with number of patients/events given in parenthesis. Hazard ratio for H3K27me (c): The first five lines (I, II, I/II, III, all) are based on univariable Cox regression models for H3K27me in the respective WHO grade subgroup. Line 6 (I/II adjusted) is based on the multivariable Cox regression model in the subgroup of WHO grade I/II patients (table 3). Wald test p-values are given.

Figure 3: Unsupervised clustering of mass spectrometry for histone modifications. Two samples are derived from meningioma methylation Group (MG) B, seven from meningioma methylation Group A. Highlighted are H3.1K27me3 and H3.3K27me3.

Table 1 Characteristics of the Cohort

Parameter	Subcategory	retained		loss		p
n / [%]		207	89%	25	11%	
WHO Grade	I	48	23%	1	4%	
	II	137	66%	18	72%	0.02
	III	22	11%	6	24%	
Sex	F	123	59%	14	56%	0.67
	M	80	39%	11	44%	
Simpson Grade	1	61	29%	10	40%	
	2	63	30%	6	24%	
	3	13	6%	5	20%	0.24
	4	33	16%	3	12%	
	5	2	1%	0	0%	
EOR	GTR	155	78%	22	88%	0.31
	STR	43	22%	3	12%	
Subtype	Secretory	8	4%	0	0%	
	Meningiothelial	7	3%	0	0%	
	Transitional	8	4%	1	4%	
	Fibrous	8	4%	0	0%	
	Psammomatous	5	2%	0	0%	
	Angiomatous	5	2%	0	0%	
	Microcystic	5	2%	0	0%	
	Metaplastic	1	0%	0	0%	0.86
	Lymphoplasmacyte-rich	1	0%	0	0%	
	Atypical	126	61%	17	68%	
	Chordoid	10	5%	1	4%	
	Clear cell	1	0%	0	0%	
	Anaplastic	20	10%	6	24%	
	Papillary	1	0%	0	0%	
	Rhabdoid	1	0%	0	0%	
Age (median [IQR])		56.00	[46.00, 66.00]	56.00	[47.00, 70.00]	0.75
Diameter (median [IQR])		42.00	[34.00, 50.00]	47.00	[45.00, 66.00]	0.19

Legend to Table 1:

Characteristics of the case cohort in absolute numbers and percentages. f – female, m – male, retained – tumor cells were positive for H3K27me3 staining, loss – complete loss of H3K27me3 staining in tumor cells. Extent of resection (EOR) was available for 198 cases in the categories “gross total resection” (GTR) and “subtotal resection” (STR) and for 172 cases additionally as Simpson grade. Age and diameter in millimeters are given in median values with inter-quartile ranges (IQR). p – p-values. For categorical parameters, p-value of Fisher’s exact test is given. For continuous parameters, p-value of Mann-Whitney test is given.

Table 2 Univariable Cox regression models on WHO grade I/II cases

Parameter		**N**	**Events**	**HR**	**95%-CI**	**p-value**
H3K27me3	loss vs. retained	204	61	2.64	1.28-5.46	0.0088
WHO grade	II vs. I	204	61	2.91	1.55-5.47	0.0009
Extent of resection	STR vs. GTR	195	54	2.03	1.10-3.73	0.02
Simpson grade	per degree increase	171	47	1.34	1.04-1.72	0.02
Age	per 10 year increase	204	61	0.92	0.75-1.13	0.43
Sex	male vs. female	200	59	1.25	0.74-2.10	0.41
Diameter	per 10 mm increase	60	20	1.04	0.80-1.34	0.77

Legend to Table 2:

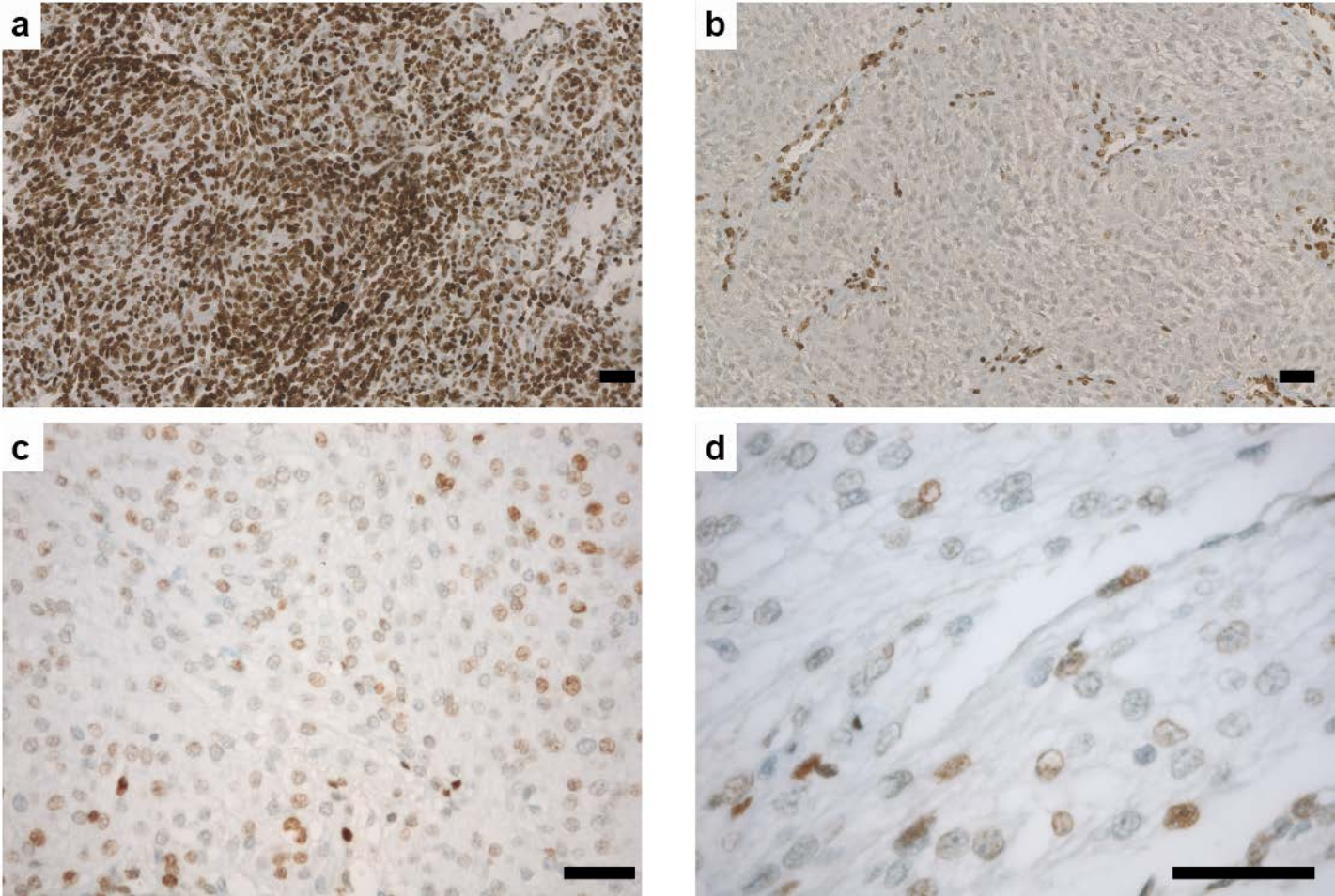
N – number of cases included in analysis, HR – hazard ratio, CI – confidence interval, GTR – gross total resection, STR – subtotal resection, mm – millimeter.

Table 3 Multivariable Cox regression model on WHO grade I/II cases

Parameter		**HR**	**95%-CI**	**p-value**
H3K27me3	loss vs. retained	2.30	1.09-4.81	0.028
WHO grade	II vs. I	2.71	1.40-5.24	0.003
Extent of resection	STR vs. GTR	1.59	0.86-2.96	0.14
Age	per 10 year increase	0.90	0.74-1.09	0.26
Sex	male vs. female	0.99	0.57-1.71	0.96

Legend to Table 3:

Multivariable Cox regression model with missing value imputation on 204 cases with 61 events. HR – hazard ratio, CI – confidence interval, GTR – gross total resection, STR – subtotal resection. Since extent of resection in the categories STR vs. GTR was known for more samples than exact Simpson grade and both parameters are closely related, only STR vs. GTR was included in the analysis. The number of cases with information on tumor diameter was too low to be included in multivariable analysis.


a
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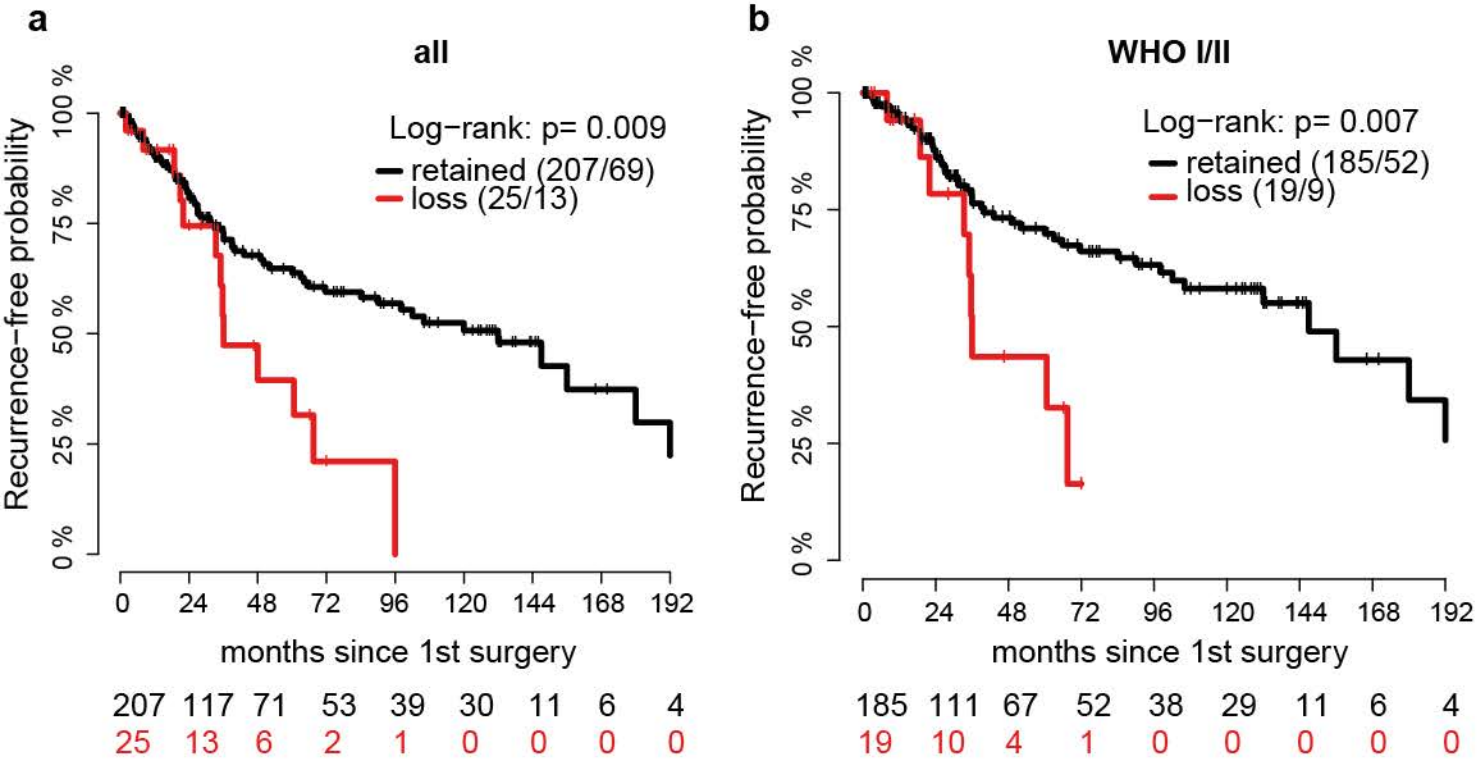
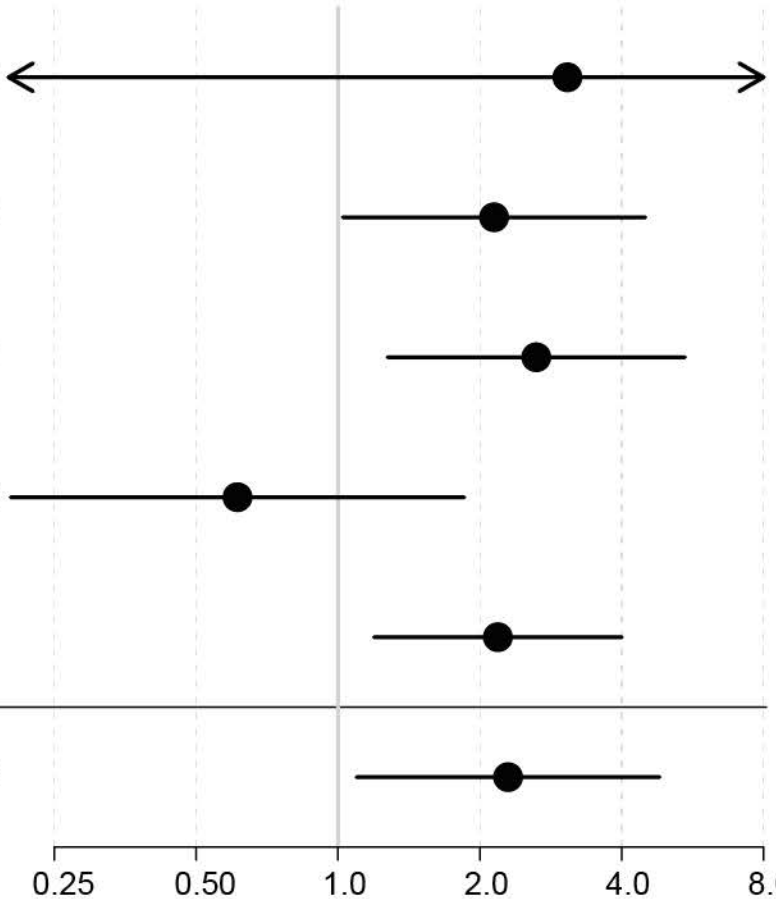
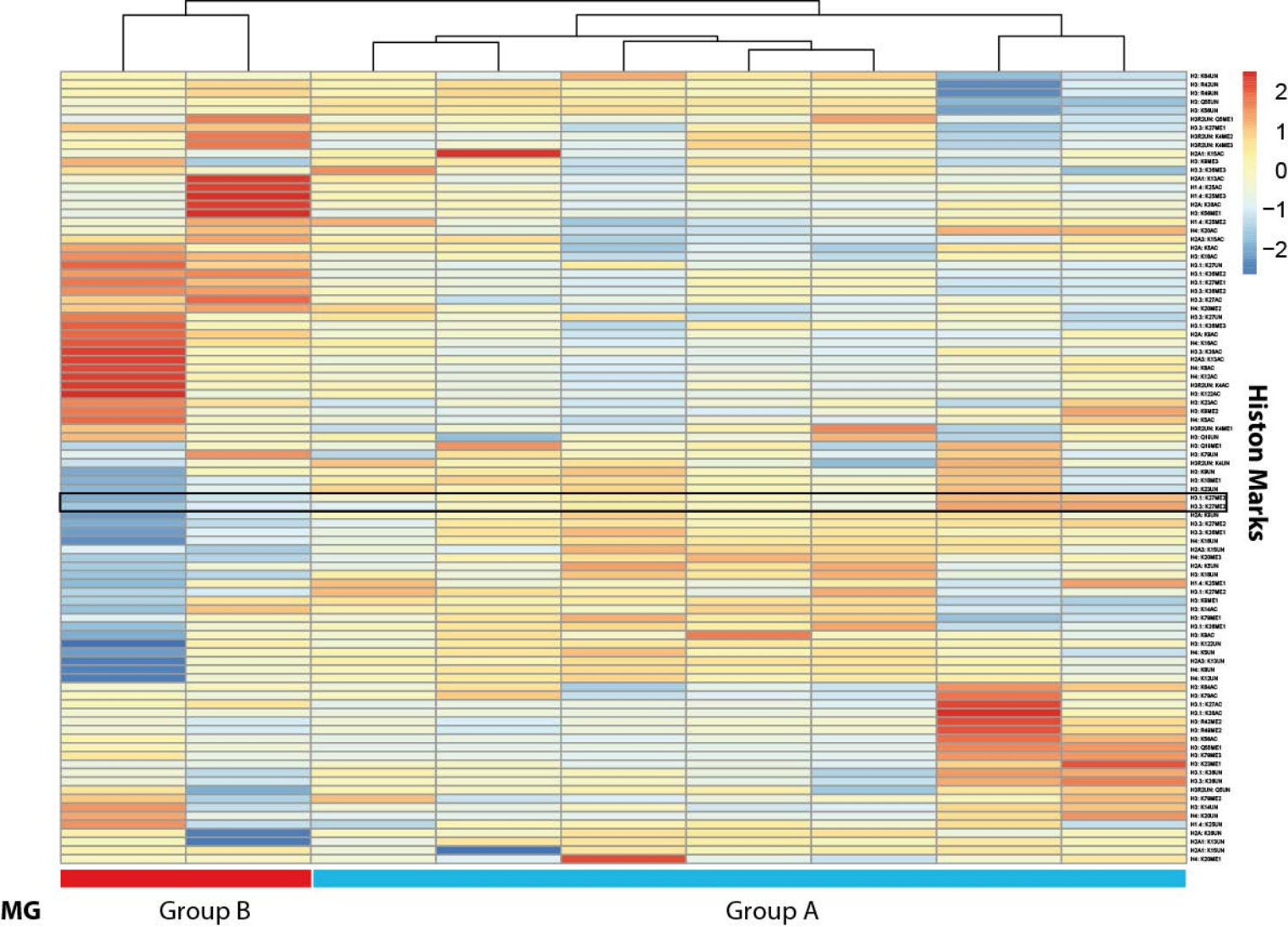


c

WHO	N	HR [95%CI]	p−value
I	49	3.07 [0.02;26.00]	0.512
II	155	2.14 [1.02; 4.49]	0.043
I/II	204	2.64 [1.27; 5.45]	0.009
III	28	0.61 [0.20; 1.85]	0.385
all	232	2.18 [1.19; 4.00]	0.011
I/II adjusted	204	2.30 [1.09; 4.81]	0.028




2
1
0
−1
−2
Histon Marks
MG
Group B
Group A