Identification of shared genomic aberrations between angiomatous and microcystic meningiomas

Yasuhiro Kuroi, Hiroyuki Akagawa, Makoto Shibuya, Hideaki Onda, Tatsuya Maegawa, and Hidetoshi Kasuya

Department of Neurosurgery, Tokyo Women’s Medical University Medical Center East, Tokyo, Japan (Y.K., H.A., M.S., H.O., T.M., H.K.); Tokyo Women’s Medical University, Institute for Integrated Medical Sciences (TIIMS), Tokyo, Japan (Y.K., H.A., T.M., H.K.); Central Laboratory, Hachioji Medical Center, Tokyo Medical University, Tokyo, Japan (M.S.); Division of Neurosurgery, Kofu Neurosurgical Hospital, Kofu, Yamanashi, Japan (H.O.)

Corresponding Author: Hiroyuki Akagawa, MD, Tokyo Women’s Medical University, Institute for Integrated Medical Sciences (TIIMS), 8-1 Kawada-cho, Shinjuku-ku, Tokyo, 162-8666 Japan (akagawa.hiroyuki@twmu.ac.jp).

Abstract

Background. Angiomatous and microcystic meningiomas are classified as rare subtypes of grade I meningiomas by World Health Organization (WHO). They typically exhibit distinct histopathological features as indicated by their WHO titles; however, these angiomatous and microcystic features are often intermixed. Recently, angiomatous meningiomas were reported to show characteristic chromosomal polysomies unlike the other WHO grade I meningiomas. In the present study, we hypothesize that microcystic meningiomas share similar cytogenetic abnormalities with angiomatous meningioma.

Methods. We performed copy number analysis using single nucleotide polymorphism (SNP) arrays for three angiomatous and eight microcystic meningiomas. Of these, three angiomatous and three microcystic meningiomas were also analyzed by whole exome sequencing and RNA sequencing.

Results. We first analyzed three angiomatous and three microcystic meningiomas for which both frozen tissues and peripheral blood were accessible. Copy number analysis confirmed previously reported multiple polysomies in angiomatous meningiomas, which were entirely replicated in microcystic meningiomas when analyzed on different analytical platforms with five additional samples prepared from formalin-fixed paraffin-embedded tumors. Polysomy of chromosome 5 was found in all cases, along with chromosome 6, 12, 17, 18, and 20 in more than half of the cases including both angiomatous and microcystic meningiomas. Furthermore, next generation sequencing did not reveal any distinctive somatic point mutations or differences in gene expression characterizing either angiomatous or microcystic meningiomas, indicating a common genetic mechanism underlying tumorigenesis.

Conclusions. Angiomatous and microcystic meningiomas have substantially similar genetic profiles represented by the characteristic patterns of multiple polysomies originating from chromosome 5 amplification.

Key Points

- Angiomatous and microcystic meningiomas are hyperdiploid meningiomas.
- Angiomatous and microcystic meningiomas largely share their genetic profiles.
- Chromosome 5 polysomy is a biomarker of angiomatous/microcystic meningiomas.
Meningiomas are the most frequently diagnosed primary brain tumors that account for one-third of all primary brain tumors.\(^1,2\) Most meningiomas (80%) are histologically classified as benign (grade I),\(^3\) which are further subdivided into nine distinct histological variants according to the current World Health Organization (WHO) classifications for meningiomas.\(^4,5\) Among these WHO grade I variants, angiomatous and microcystic meningiomas are distinguished based on the tumor mass consisting either of numerous blood vessels or delicate processes encompassing microcysts, respectively. However, these angiomatous and microcystic features are often intermixed, making it difficult to determine a definitive pathological diagnosis.\(^5\) Such histopathological intermixing also presents a neuroimaging feature resembling high-grade meningiomas based on predisposition to peritumoral brain edema, although angiomatous and microcystic meningiomas do not exhibit aggressive behavior.\(^4,6\)

Recently, angiomatous meningioma was reported to have multiple chromosomal polysomies represented by chromosome 5 amplification, in contrast to most of the other meningiomas having a normal diploid or karyotype with monosomy 22q.\(^7\) In the present study, we hypothesized that microcystic meningioma shares cytogenetic profile similar to angiomatous meningioma. We performed genetic analyses to compare molecular profiles of these closely related but different subtypes of meningiomas. The results confirmed that the characteristic multiple polysomies represented by chromosome 5 amplification in angiomatous meningiomas were entirely replicated in microcystic meningiomas. Furthermore, whole exome and RNA sequencing demonstrated no somatic point mutations or differences in gene expression discriminating angiomatous or microcystic subtypes, indicating a common genetic mechanism underlying tumorigenesis. Chromosome 5 amplification was found in all of the angiomatous/microcystic cases, thereby constituting a potential diagnostic marker and a future therapeutic target.

**Copy Number Analysis Using SNP Array**

Genomic DNA samples from six frozen meningiomas and their complementary peripheral leucocytes were hybridized to the Affymetrix Genome Wide Human SNP Array 6.0 (Thermo Fisher Scientific, Waltham, MA). The signal intensity data were processed simultaneously with that of the HapMap individuals \((ftp://ftp.ncbi.nlm.nih.gov/hapmap/raw_data/hapmap3_affy6.0/)\) using Affymetrix Power Tools (Thermo Fisher Scientific) and the PennCNV-affy package.\(^8,9\) ASCAT 2 was used to calculate somatic copy number profiles from the processed data of B allele frequency (BAF) and Log R ratio (LRR).\(^10\) Control Affymetrix SNP array data consisting of 45 nonangiomatous/microcystic grade I meningiomas was obtained from the previous study by Tabernero et al.,\(^11\) which was publicly available in the ArrayExpress database \((E-GEOD-42624, https://www.ebi.ac.uk/arrayexpress/)) and analyzed using the same methods.
Table 1  Clinicopathological information of studied cases

| Sample   | Sample type | WHO classification | Sex | Age | Tumor location   | Peritumoral edema | Clinical manifestation |
|----------|-------------|--------------------|-----|-----|------------------|--------------------|------------------------|
| AG517    | FF*         | Angiomatous        | M   | 37  | Parasagittal     | No                 | Headache               |
| AG559    | FF*         | Angiomatous        | M   | 60  | Falx             | Yes                | Headache               |
| AG825    | FF*         | Angiomatous        | M   | 77  | Convexity        | Yes                | Motor paresis          |
| MC409    | FF*, FFPE   | Microcystic        | F   | 67  | Falx             | Yes                | Seizure                |
| MC488    | FF*         | Microcystic        | M   | 64  | Convexity        | Yes                | Seizure                |
| MC505    | FF*         | Microcystic        | F   | 60  | Convexity        | No                 | Motor paresis          |
| MC1      | FFPE        | Microcystic        | M   | 67  | Convexity        | No                 | Asymptomatic           |
| MC2      | FFPE        | Microcystic        | F   | 58  | Convexity        | No                 | Asymptomatic           |
| MC3      | FFPE        | Microcystic        | F   | 66  | Convexity        | Yes                | Seizure                |
| MC4      | FFPE        | Microcystic        | F   | 71  | Convexity        | Unknown            | Seizure                |
| MC5      | FFPE        | Microcystic        | F   | 54  | Convexity        | Unknown            | Unknown                |
| ASA1     | FFPE        | Meningothelial     | F   | 82  | Convexity        | Yes                | Asymptomatic           |
| ASA2     | FFPE        | Fibrous            | F   | 48  | Falx             | No                 | Dizziness, headache    |
| ASA3     | FF          | Meningothelial     | F   | 62  | Convexity        | Yes                | Headache, motor paresis|
| ASA4     | FF          | Meningothelial     | M   | 63  | Convexity        | Yes                | Unknown                |
| ASA5     | FF          | Meningothelial     | F   | 70  | Convexity        | No                 | Unknown                |
| ASA6     | FF          | Meningothelial     | F   | 83  | Posterior petrous| No                 | Trigeminal neuralgia   |
| ASA7     | FF          | Fibrous            | F   | 74  | Posterior petrous| No                 | Asymptomatic           |
| ASA8     | FF          | Meningothelial     | M   | 75  | Convexity        | No                 | Asymptomatic           |
| ASA9     | FF          | Meningothelial     | F   | 65  | Falx             | No                 | Motor paresis          |
| ASA10    | FF          | Meningothelial     | F   | 67  | Parasagittal     | Yes                | Motor paresis          |
| ASA11    | FF          | Meningothelial     | F   | 53  | Clinoideal       | No                 | Asymptomatic           |
| ASA12    | FF          | Meningothelial     | F   | 66  | Convexity        | No                 | Headache               |
| ASA13    | FF          | Meningothelial     | F   | 64  | Tentorial edge   | No                 | Trigeminal neuralgia   |
| ASA14    | FF          | Transitional       | F   | 69  | Sphenoid ridge   | Yes                | Asymptomatic           |

*FF = fresh frozen tumor; FFPE = formalin-fixed paraffin-embedded tumor.
**With peripheral blood sample.
Genomic DNA samples from additional 19 meningiomas without complementary normal tissues were hybridized to the Infinium Asian Screening Array (Illumina, San Diego, CA) to independently verify the results obtained from the Affymetrix array. Processing of the signal intensities and extraction of BAF and LRR data were performed using GenomeStudio 2.0 (Illumina). ASCAT 2 with an option inferring the germline genotypes and GPHMM version 1.3 were used to calculate somatic copy number alterations, without complementary normal tissues were hybridized to the Affymetrix SNP array data from 45 other WHO grade I meningiomas in the ArrayExpress database (E-GEOD-42624, https://www.ebi.ac.uk/arrayexpress/) (Figure 3).11 Half of these 45 control meningiomas showed chromosome 22q deletion, reflecting its highest incidence among grade I meningiomas.2,21 Two out of the six angiomatous/microcystic meningiomas (33.3%) showed chromosome 22q deletion (AG559 and MC488); however, whole exome sequencing (WES) revealed no somatic mutations in NF2, supporting a different genetic etiology from many other grade I meningiomas associated with biallelic inactivation of NF2.22 Direct Sanger sequencing confirmed a total of 61 somatic mutations from the WES data (Supplementary Table 2). The major driver genes for non-NF2 meningioma (TRAFL, KLF4, AKT1, SMO, and POLR2A) were not mutated, except for AG559 harboring the recurrent p.S561N mutation in TRAF7 (COSM1578117).22,23 There were no genes that were commonly mutated in two or more of the angiomatous/microcystic meningiomas. RNA sequencing (RNA-seq) further supported the findings in the SNP array-based copy number analysis and WES, which showed no detectable genomic abnormality discriminating angiomatous and microcystic meningiomas. Density and box plots showing the distribution of RNA-seq read counts (FPKM) equally overlapped one another (Supplementary Figure 1). Hierarchical clustering was inconsistent with the present histopathological diagnosis, forming the closest cluster with the angiomatous AG559 and the microcystic MC488 meningiomas (Supplementary Figure 1). Correlation coefficients of the most distant (MC505 and MC488) and nearest (AG559 and MC488) samples in the hierarchical clusters reached nearly one: \( R^2 = 0.95 \) and 0.98, respectively (Supplementary Figure 2). This line of evidence indicates a substantially similar molecular profile of microcystic and angiomatous meningiomas.

Whole Exome Sequencing
Exome capture was performed using SureSelect Human All Exon V5 kit following manufacturer instructions (Agilent Technologies Inc., Santa Clara, CA). Enriched exome libraries were sequenced using 100 bp paired end reads on a HiSeq 2500 sequencer (Illumina). After quality based read trimming, sequence reads were aligned to the human reference genome GRCh37/hg19 using BWA,14 and duplicate reads marked using the Picard program (http://broadinstitute.github.io/picard/). Base substitutions and indels were detected using MuTect2.16 COSMIC (https://cancer.sanger.ac.uk/cosmic), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), and a panel of normal variants (https://gembdb.ncc.go.jp/omics/docs/others.html) were used as inputs to MuTect2. Detected somatic mutations were annotated using ANNOVAR,16 CADD,17 and FATHMM-Cancer.18 These were subsequently validated using Sanger method, certified by the following criteria: (i) MuTect2 filter qualification; (ii) Splice site, nonsynonymous, and exonic indel mutations; (iii) variant allele frequencies in tumor samples greater than 0.1; (iv) uncommon variants in general populations; and (v) absence in 16 control exomes using the same exon capture kit and analyzed at the same sequencing center. The Sanger validations were performed via standard PCR-based amplification, followed by BigDye Terminator cycle sequencing on a 3130xl Genetic Analyzer (Thermo Fisher Scientific).

RNA Sequencing
Libraries were prepared using the TruSeq RNA Sample Preparation Kit v2 (Illumina) according to manufacturer instructions. Each library was paired end sequenced (2 x 75 bp) by using the TruSeq SBS Kit v4-HS, on a HiSeq2500 sequencer (Illumina). RNA-seq transcript data were analyzed using the combination of TopHat2 and Cufflinks.19 We annotated the assembled transcripts to the UCSC annotation (hg19) obtained from the Illumina iGenomes website (http://jp.support.illuminacom/sequencing/sequencing_software/igencode.html?langsel=ja) and used Cufflinks to estimate fragments per kilobase of exon model per million mapped fragments (FPKM). Cufflinks outputs were thoroughly explored and visualized using CummeRbund (http://compbio.mit.edu/cummeRbund/index.html).

Results
We performed comprehensive genetic analyses of three angiomatous and three microcystic meningiomas with access to both frozen tissues and peripheral blood samples (Table 1). Although partial histopathological intermixing was observed in two angiomatous (AG559 and AG825) and one microcystic (MC409) meningiomas (Figure 1), our histopathological diagnosis was clearly supported by low-density appearance of microcystic meningiomas on computed tomography similar to reported characteristics in the literature (Figure 2).20,21

Copy number analysis using SNP array (Affymetrix Genome-Wide Human SNP Array 6.0) confirmed previously reported multiple polysomies in angiomatous meningiomas,7 which was also replicated in microcystic meningiomas (Figure 3). Similar to the previous report,7 polysomies of chromosome 5 and 6 were found in all six cases, along with chromosome 12, 17, 18, and 20 in more than half of the cases including both angiomatous and microcystic meningiomas (Supplementary Table 1). Angiomatous or microcystic specific copy number alteration was not observed in both chromosomal arm-level and focal gene-level analyses using GISTIC 2.0.13 These characteristic chromosomal polysomies were not observed in the control Affymetrix SNP array data from 45 other WHO grade I meningiomas in the ArrayExpress database (E-GEOD-42624, https://www.ebi.ac.uk/arrayexpress/) (Figure 3).11 Half of these 45 control meningiomas showed chromosome 22q deletion, reflecting its highest incidence among grade I meningiomas.2,21 Two out of the six angiomatous/microcystic meningiomas (33.3%) showed chromosome 22q deletion (AG559 and MC488); however, whole exome sequencing (WES) revealed no somatic mutations in NF2, supporting a different genetic etiology from many other grade I meningiomas associated with biallelic inactivation of NF2.22 Direct Sanger sequencing confirmed a total of 61 somatic mutations from the WES data (Supplementary Table 2). The major driver genes for non-NF2 meningioma (TRAFL, KLF4, AKT1, SMO, and POLR2A) were not mutated, except for AG559 harboring the recurrent p.S561N mutation in TRAF7 (COSM1578117).22,23 There were no genes that were commonly mutated in two or more of the angiomatous/microcystic meningiomas. RNA sequencing (RNA-seq) further supported the findings in the SNP array-based copy number analysis and WES, which showed no detectable genomic abnormality discriminating angiomatous and microcystic meningiomas. Density and box plots showing the distribution of RNA-seq read counts (FPKM) equally overlapped one another (Supplementary Figure 1). Hierarchical clustering was inconsistent with the present histopathological diagnosis, forming the closest cluster with the angiomatous AG559 and the microcystic MC488 meningiomas (Supplementary Figure 1). Correlation coefficients of the most distant (MC505 and MC488) and nearest (AG559 and MC488) samples in the hierarchical clusters reached nearly one: \( R^2 = 0.95 \) and 0.98, respectively (Supplementary Figure 2). This line of evidence indicates a substantially similar molecular profile of microcystic and angiomatous meningiomas.
To further confirm the characteristic multiple polysomies identified in microcystic meningiomas, we added the SNP array based copy number analysis using different types of analytical platforms (Methods). Six microcystic meningiomas whose DNA samples were prepared from FFPE tissues were analyzed using an Illumina BeadChip array (Infinium Asian Screening Array), including MC409 as a positive control (Supplementary Figure 3). Fourteen nonangiomatous/microcystic control samples were from two FFPE tissues and 12 frozen tissues (Table 1). Copy-number calculations for these additional meningiomas were performed without data from matched normal tissues, which would allow for less cumbersome and cost-effective molecular diagnostics. Although lower resolution due to DNA damage common in FFPE samples, the result of MC409 was entirely replicated as that in the former analysis, and the other microcystic meningiomas also exhibited identical patterns of multiple polysomies.

Figure 1 Histopathological images of six cases of angiomatous and microcystic meningiomas. Hematoxylin and eosin stained sections of angiomatous (a–c) and microcystic (d–f) meningiomas. Representative images providing bases for histopathological diagnosis are shown. Partial histopathological intermixture is observed in (b) AG559, (c) AG825, and (d) MC409 (lower panels).
represented by chromosome 5 amplification, which were not observed in the 14 nonangiomatic/microcystic control meningiomas (Figure 4).

**Discussion**

Microcystic meningioma is a rare histopathological subtype accounting for only 1.6% of all intracranial meningiomas. A previous study indicated distinct patterns of hyperdiploidy in this type of meningiomas; Ketter et al. identified 16 hyperdiploid meningiomas from 677 consecutive surgical cases using conventional and fluorescence in situ hybridization-based karyotyping techniques, six of which were WHO grade I meningiomas having microcystic features. However, until the present study, there was no report of genetic analyses using DNA microarrays and next-generation sequencing that focused on microcystic meningiomas. Copy-number analysis confirmed nonrandom patterns of chromosomal polysomies, which were totally identical to that previously reported in angiomatic meningiomas. Chromosome 5, 6, 12, 17, 18, and 20 were frequently amplified, among which amplification of chromosome 5 was found in all of the angiomatic/microcystic cases. In contrast, chromosomal deletions frequently observed in meningiomas at high risk of recurrence, such as 1p, 4p, 6q, 10q, 18q, and 14 losses, were not observed in the present angiomatic/microcystic cases, except for MC505 showing monosity 14 (Figure 3). Careful follow-up is required for cases with these high-risk deletions, such as ASA12 (Figure 4). Next generation sequencing (WES and RNA-seq) further demonstrated no somatic point mutations or differences in gene expression that characterize angiomatic or microcystic meningiomas, indicating a common genetic mechanism in tumorigenesis.

Chromosome instability (CIN), including the multiple polysomies observed in angiomatic/microcystic meningiomas, is one of the major hallmarks of tumor cells; however, the exact mechanisms inducing CIN are not fully understood. Several explanations have been postulated, such as telomere dysfunction and epigenetic alterations. CIN due to telomere fusion is triggered when telomere attrition reaches critical shortening and induces cell apoptosis. However, in many cancer cells, the telomerase reverse transcriptase (TERT) gene on chromosome 5p15.33 is upregulated through the promoter hotspot mutations (C228T and C250T) or gene amplification, which lead to both sustained cell proliferation and clonal chromosomal alterations. In recent studies, TERT promoter mutations were also detected in meningiomas, particularly in higher-grade meningiomas, and were reported to be poor prognostic factors. Therefore, it was initially hypothesized that TERT activation due to chromosome 5 amplification was potentially responsible for the cytogenetic character of angiomatic/microcystic meningiomas. However, RNA-seq showed no detectable expression of TERT (FPKM = 0 in all six tumors), reflecting that the
present angiomatous/microcystic cases did not demonstrate aggressive behaviors.

Telomere dysfunction is also implicated in defective DNA double-strand break repair. For example, the MRE11 gene encoding a double-strand break repair nuclease, which was mutated in the microcystic MC488 meningioma (Supplementary Table 1), plays a central role in double-strand break repair and maintenance of telomere integrity via the MRE11/RAD50/NBS1 complex. Not only somatic mutations, but also germline mutations for cancer predisposition were reported in this gene. In fact, the p.L473S mutation of MRE11 (rs771843497) detected in MC488 was reported as a germline mutation in a patient with hereditary breast cancer according to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/). Further investigation is needed because the biological and clinical significance of the p.L473S mutation has not been determined. However, given the nonmalignant nature and the absence of recurrently mutated cancer driver genes among angiomatous/microcystic meningiomas, it was suggested that dosage-balanced genes associated with the frequently amplified chromosomes might confer the tumorigenesis of these hyperdiploid meningiomas rather than somatic driver mutations. In this theory, pathogenic hyperdiploidy disrupts stoichiometric balance of gene products forming active multiprotein complexes and pathways associated with cell proliferation signals.
Hyperdiploid meningiomas represented by angiomatous meningiomas were reported to have characteristic changes in DNA methylation,\textsuperscript{30} which were also known to contribute to CIN.\textsuperscript{41} Sahm et al.\textsuperscript{40} reported that meningiomas with good postoperative prognosis were clearly subdivided into three classes according to their DNA methylation profiles: the first class denoted as MC-ben-1 was represented by meningiomas with \(\text{NF2}\) loss; the second MC-ben-2 by non-\(\text{NF2}\) meningiomas harboring \(\text{TRAF7}, \text{KLF4}, \text{AKT1},\) or \(\text{SMO}\) mutations; and the third MC-ben-3 by multiple chromosomal gains, most frequently affecting chromosome 5, which include microcystic as well as angiomatous meningiomas. Their analysis further demonstrated that the assignment to these MC-ben-1 to -3 classes had higher power for predicting good postoperative prognosis than the WHO grading.\textsuperscript{40} Although a cause-effect relationship between such DNA methylation changes and hyperdiploidy needs to be clarified, integrated diagnosis using genetic and epigenetic examinations will become increasingly important for clinical decision making in surgical strategies and additional therapeutic options.\textsuperscript{25,26,40}

The present study has a few limitations. The number of the samples was limited because of the rarity of angiomatous/microcystic subtypes. Further replication studies with larger sample sizes are required, especially in transcriptome or methylome analysis. Single-cell RNA sequencing, for example, might uncover angiomatous or microcystic specific genetic characteristics that could not be detected by the present bulk tumor analysis. In addition to the comparison between

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**Figure 4** Copy number profiles calculated using Illumina SNP array data without matched normal samples. GISTIC 2.0\textsuperscript{12} visualizes copy number profiles across the dataset of 20 meningiomas, including six microcystic meningiomas from our archival FFPE samples. FFPE = formalin-fixed paraffin-embedded.
Angiomatous and microcystic subtypes, comparing them with nonhyperdiploid meningiomas is left for future work.

In conclusion, the present study indicated that angiomatous and microcystic meningiomas have substantially similar genetic profiles represented by the characteristic patterns of multiple polysomies originating from chromosome 5 amplification. Although the mechanism underlying such chromosomal aberrations and divergent morphological features needs to be further investigated, polysomy of chromosome 5 can be a promising biomarker providing a novel genetic classification for this angiomatous/microcystic type of meningioma. Dosage-imbalanced genes associated with frequently amplified chromosomes may be potential therapeutic targets for the angiomatous/microcystic meningioma in the future.

Supplementary Material

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

Keywords

Angiomatous meningioma | microcystic meningioma | copy-number analysis | whole-exome sequencing | RNA sequencing.

Funding

This work was supported by JSPS KAKENHI (Grant Number 18K16573 [to Y.K.]).

Acknowledgments

We thank the donors and the supporting medical staff for making this study possible. We also thank Mitsuhiro Amemiya and Akira Saito (StaGen Co. Ltd., Tokyo, Japan) for data processing of next generation sequencing.

Authorship Statement

M.S., H.O., and H.K. designed the study and H.K. coordinated it. H.K. performed the surgical procedures and provided clinical data and inputs to the project. M.S. performed the histopathological evaluations. Y.K., H.A., M.S., H.O., T.M., and H.K. were involved in the acquisition, analysis, or interpretation of data for the work. Y.K. drafted the manuscript and all other co-authors revised it critically for important intellectual content. All authors have read and approved the final version.

Conflict of interest statement. None declared.

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