BRIEF COMMUNICATION

ARID1A and TERT promoter mutations in dedifferentiated meningioma

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Unlike patients with World Health Organization (WHO) grade I meningiomas, which are considered benign, patients with WHO grade III meningiomas have very high mortality rates. The principles underlying tumor progression in meningioma are largely unknown, yet a detailed understanding of these mechanisms will be required for effective management of patients with these high grade lethal tumors. We present a case of an intraventricular meningioma that at first presentation displayed remarkable morphologic heterogeneity composed of distinct regions independently fulfilling histopathologic criteria for WHO grade I, II, and III designations. The lowest grade regions had classic meningothelial features, while the highest grade regions were markedly dedifferentiated. Whereas progression in meningiomas is generally observed during recurrence following radiation and systemic medical therapies, the current case offers us a snapshot of histologic progression and intratumoral heterogeneity in a native pretreatment context. Using whole exome sequencing and high resolution array-based comparative genomic hybridization, we observed marked genetic heterogeneity between the various areas. Notably, in the higher grade regions we found increased aneuploidy with progressive loss of heterozygosity, the emergence of mutations in the TERT promoter, and compromise of ARID1A. These findings provide new insights into intratumoral heterogeneity in the evolution of malignant phenotypes in anaplastic meningiomas and potential pathways of malignant progression.

Keywords Meningioma, anaplastic, intratumoral heterogeneity, SWI/SNF complex, chromatin remodeling

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Meningiomas are the most common primary tumors of the central nervous system, with 25–30% following an aggressive course with rapid growth, invasion into adjacent structures, and rapid recurrence even after radical resection and adjuvant treatment. These meningiomas are classified as World Health Organization (WHO) grade II or III and are associated with premature mortality (1). Despite an emerging understanding of the genetic changes underlying meningioma formation (2–10), the factors that drive malignant progression in meningioma remain poorly understood (11–13). We encountered a case of a unique untreated intraventricular meningioma, which allowed us to investigate molecular factors associated with tumor progression and dedifferentiation.

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Materials and methods

Pathologic examination

Histopathologic diagnosis and tumor purity of greater than 80% was confirmed by review of the H&E stained sections by two neuropathologists (S.S., M.A.). This study was approved by the human subject institutional review board of the Dana-Farber/Brigham and Women’s Cancer Center.

Immunohistochemistry and RNAscope

Immunohistochemical analysis was performed using commercially available antibodies following standard automated and manual protocols on formalin-fixed, paraffin-embedded (FFPE) tissue. The slides were incubated with antibodies for MIB-1 (Ki-67, Dako, catalogue no. M7240) (Agilent Technologies, Santa Clara, CA), epithelial membrane antigen (EMA, Dako, catalogue no. M0613 clone: E29) (Agilent Technologies), and ARID1A (monoclonal ARID1A/BAF250a, catalogue no. sc-32761X) (Santa Cruz Biotechnology, Santa Cruz, CA) and developed using 3,3′-diaminobenzidine (DAB) as the chromagen (Sigma-Aldrich, St. Louis, MO), before being counterstained with hematoxylin. Appropriate positive and negative controls were used throughout. In situ hybridization of TERT mRNA transcripts was performed using the RNAscope assay with Probe-Hs-TERT (catalogue no. 605511) (Advanced Cell Diagnostics, Hayward, CA), following the manufacturer’s protocols (10,14).

Genomic analysis

Array-based comparative genomic hybridization (aCGH) was performed using a stock 1×1M Agilent SurePrint G3 human CGH microarray chip in a Clinical Laboratory Improvement Amendments (CLIA)—certified laboratory. A minimum of 1.3 μg DNA, corresponding to approximately 6×1-mm punches from FFPE tissue blocks, was obtained for each specimen. Genomic DNA isolated from FFPE blocks was hybridized with a commercial reference DNA sample representing a pool of individuals with normal karyotypes (Promega, Madison, WI). The array platform contains 963,029 probes spaced with a 2.1-kb overall median probe spacing for single nucleotide variants (SNVs) was performed using the MuTect v1.1.4 (18); indel calling was performed using the GATK SomaticIndelDetector tool; and SNVs and indels were annotated using Oncotator. To analyze somatic copy number alterations from whole exome data, we used the ReCapSeg algorithm, which assesses homologue-specific copy ratios from segmental estimates of multipoint allelic copy ratios at heterozygous loci incorporating the statistical phasing software (BEAGLE) and population haplotype panels (HAP-MAP3) (19–21).

Results

A 48-year-old woman presented with 5 days of headache and gait instability, prompting imaging that revealed a 6.9×4.7×5-cm heterogeneously contrast-enhancing mass, centered within the atrium of the right lateral ventricle, causing obstructive hydrocephalus (Figures 1A and 1B). A right parietal craniotomy was performed with gross total resection of the tumor (Figure 1C). The patient’s symptoms improved postoperatively and she remains alive 2 years following the resection.

The histopathologic analysis was notable for the concurrent presence of regions displaying classic features of low grade meningothelial meningioma (WHO grade I), others with features of atypical WHO grade II meningioma, and areas harboring cells with a pronounced increase in the nucleocytoplasmic ratio, frequent mitosis, and loss of discernable features of meningothelial differentiation—hallmarks of dedifferentiation in a WHO grade III anaplastic meningioma (Figure 1D). Consistent with this histologic progression, the MIB-1 proliferation index ranged from less than 1% to more than 90% in corresponding grade I versus grade III regions (Figure 1D). Immunohistochemical results for epithelial membrane antigen (EMA), a marker of meningothelial differentiation, also showed EMA expression in the low grade regions that was entirely lost in the highest grade regions, consistent with the morphologic dedifferentiation (Figure 1D).

To better understand the molecular underpinnings of the histologic heterogeneity within this tumor, we analyzed DNA extracted from core punches from each of these three regions, using high resolution aCGH, Sanger sequencing of the TERT promoter, and whole exome sequencing (Figure 2).

The aCGH data showed partial single-copy loss of chromosomes 1p, 3q, 6q, 7p, 11q, 18q (monosomy 18), and 22q (monosomy 22) in all three areas (Figure 2A). The atypical and anaplastic regions both harbored an expanded loss of chromosome 11q; additional unique losses of 4 and 11p; and regions of 5, 15q, and 17q that were not detected in the grade I areas. Expansion of 5p and 9q losses was distinct to the grade III area, as were gains on chromosome 8 and 10q. Copy number analysis of the whole exome sequencing data showed a similar pattern of losses and further revealed progressive loss of heterozygosity across the three regions and evidence for subclonal chromosomal losses in area 2 that become clonal in area 3 (Figure 2B).

Because whole exome sequencing does not cover the promoter region of the TERT gene, which has been reported to be mutated in meningiomas with histologic progression (2), we performed Sanger sequencing of this region. We found wild-type sequences in the grade I area but the c.228 C>T mutation in both the grade II and III areas. Using RNAscope in situ hybridization (14), we found that the expression of TERT mRNA is low in grade I areas and sharply increased in higher-grade areas (Figure 2C). The whole exome sequencing data revealed a low frequency of other mutations in the three samples, with the notable
The presence of an ARID1A frameshift deletion (p.LHH2017fs) in the grade II and III areas (Supplementary Table 1). Coupled with single-copy loss of chromosome 1p, the frameshift would lead to a sharp decrease in ARID1A protein levels. Indeed, ARID1A levels are markedly reduced in the high grade areas on IHC (Figure 2D).

**Discussion**

The mutation and copy number data reveal a concentric, rather than partially overlapping, pattern of alterations; nearly all mutations and copy number alterations in the areas with
atypical histopathology were also observed in the sample with higher grade anaplastic features. This finding suggests a common clonal origin to the distinct areas. Notably, this tumor was newly diagnosed, without prior surgical or chemoradiation treatment. It is interesting to speculate that the de novo molecular heterogeneity observed in our case reflects a set of clonal sweeps where each ensuing clone consists of a subclone of the previous dominant clone (Figure 2E). One might imagine that therapeutic interventions such as chemotherapy and radiation may precipitate a different pattern in which multiple surviving subclones compete for dominance.

The shared pattern of single-copy loss, involving chromosomes 1p, 3q, 6q, 18q, and 22q, is consistent with previously reported cytogenetic changes in high grade meningiomas (22). We found these alterations even in the areas with grade I histologic features, which had a low proliferative index and intact EMA expression, suggesting that additional alterations may be needed for histologic progression. Such cytogenetic aberrations are uncommon in meningioma with grade I histology, and the presence of these aberrations in our case suggests that the mechanisms of chromosomal instability that drive accumulation of the recurrent genomic aberrations in meningioma were present in these well-differentiated areas as well. Noncontiguous losses on 15q and 17q and the unique whole arm loss of 9q observed only in the anaplastic region contrast with previously observed gains of 9q, 15q, and 17q in some high grade meningiomas and reflect the current incomplete understanding of the specific oncogenic drivers within these broad regions (13,22). No CDKN2A (9p21.3) deletions, a frequent finding among aggressive meningiomas (13,23), were observed.

The differences in mutational profiles between the low grade (grade I) and high grade (grades II and III) areas are intriguing. The NF2 gene was intact in all three samples, with more than 100X coverage at each site of all exons after manual review. Although it was reported in a prior study that NF2-mutated meningiomas may also exhibit greater chromosomal instability than NF2 wild type counterparts, our

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Figure 2 Genetic heterogeneity within a high grade meningioma. (A) High resolution aCGH was performed on DNA extracted from core punches taken from area 1 (WHO grade I), area 2 (WHO grade II), and area 3 (WHO grade III), using 1×1M Agilent SurePrint G3 human CGH microarray chip (3,31). In the results shown here, red indicates gain and blue indicates loss. (B) Results of copy number analysis from exome sequencing data of the three areas are shown as allelic copy ratios. Red indicates gain and blue indicates loss. (C) Results of in situ hybridization of TERT mRNA transcripts using a TERT-specific RNAscope probe in FFPE sections of the meningioma samples (10,14). (D) Immunohistochemical results for ARID1A. (E) A schematic representation of clonal evolution leading to dedifferentiation and intratumoral heterogeneity. Scale bar, 10 μm.
case showed genomic progression with apparently intact NF2 (24). Mutations in the TERT promoter and in ARID1A were most notable. Telomerase activation is well recognized as a feature of malignant meningiomas (25), with a recent report that TERT promoter mutations are specific to meningiomas that recur displaying histologic progression (2). The observation of differential TERT expression within regions of the same tumor that had not been treated further supports the role of increased TERT expression in the malignant progression of meningioma. Moreover, sequencing studies have revealed that the genes encoding subunits of mammalian SWI/SNF complexes are mutated in over 20% of all human cancers (26–28). ARID1A is important in ensuring correct lineage progression and in limiting self-renewal (29). It is interesting to propose that the concomitant activation of TERT and the compromise of the SWI/SNF complex or other chromatin remodeling complexes through a range of genetic or epigenetic events (30) may synergize in allowing meningioma cells to develop high grade malignant phenotypes (12) and dedifferentiate.

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Supplementary data

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