Primary Intracranial Spindle Cell Sarcoma, **DICER1-Mutant, with MDM2 Amplification Diagnosed on the Basis of Extensive Molecular Profiling**

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**ABSTRACT:** Primary intracranial spindle cell sarcoma is an extremely rare mesenchymal tumor, the molecular pathogenesis of which is poorly understood. Because of the lack of specific markers, diagnosis sometimes relies on ruling out all possible differential diagnoses, often making it difficult to reach a definitive diagnosis. In this case study, we report a 69-year-old female patient for whom the integration of multi-layered molecular analyses contributed to making the diagnosis. The disease exhibited aggressive clinical behavior, requiring two sequential surgeries because of rapid regrowth within a short period. Primary and recurrent tumors exhibited similar histological features, in which spindle-shaped cells arranged in interlacing fascicles without any specific architectures, implicating sarcomatous tumors. In immunohistochemistry testing, tumor cells were immunopositive for vimentin but lacked any specific findings that contribute to narrowing down the differential diagnoses. Seeking further diagnostic clues, we performed DNA methylation-based analysis. The copy number analysis revealed MDM2 gene amplification and loss of heterozygosity of 22q. Moreover, dimension reduction clustering analysis implicated a methylation pattern comparable to aggressive types of sarcomas. In addition, an in-house next-generation sequencing panel (“Todai-OncoPanel”) analysis identified somatic mutations in DICER1, NF2, and ATRX genes. Taken together, we finally made the diagnosis of primary intracranial spindle cell sarcoma, DICER1-mutant, with MDM2 gene amplification. This case report suggests that even for the tumors with insufficient morphological and immuno-histological diagnostic clues, integration of multi-layered molecular analyses can contribute to making the diagnoses as well as to understanding the rare tumors by elucidating unexpected genetic and epigenetic features.

**KEYWORDS:** Whole exome sequencing, DNA methylation, brain neoplasms, sarcoma

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**Introduction**

Primary intracranial spindle cell sarcoma (IC-SCS) is an extremely rare mesenchymal tumor; only a limited number of cases have been reported in the literature to date.1-4 The tumor is considered to originate from fibroblastic elements within the brain parenchyma or its meningeal coverings.3 Because of its rarity, its molecular pathogenesis remains poorly understood. Patients with IC-SCS are treated on a case-by-case basis.5 Therefore, the prognosis of patients with the disease is generally poor.3

Recently, next-generation sequencing techniques have been incorporated in clinical practice.6,7 At our institution, a platform for DNA and RNA targeted panel sequencing, named as “Todai-OncoPanel” (TOP), has also been launched and prospectively tested to improve our understanding of the molecular background of diseases and identify therapeutic targets.8,9 In addition, the utility of recent genome-wide DNA methylation-based classification methods has become increasingly recognized.10,11 Indeed, such high-throughput approaches can sometimes identity pivotal information leading to a revision of the diagnosis.

Here, we describe a patient with a primary IC-SCS for whom extensive molecular profiling attempts contributed to making the diagnosis and revealed several genetic features such as DICER1, NF2, and ATRX gene mutations and MDM2 gene amplification. This report not only improves our understanding of this rare brain tumor case but also underscores the diagnostic impact of molecular profiling.
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Case Presentation

A 69-year-old woman with conscious disturbance was referred to a previous hospital. Computed tomography scanning and subsequent magnetic resonance imaging (MRI) T2-weighted images (T2WI) showed intraparenchymal hematoma at the right frontal lobe with surrounding edema (Figure 1A). MRI gadolinium (Gd) enhanced T1-weighted images (T1WI) revealed an enhancing mass lesion at the medial margin of hematoma (Figure 1B), indicating a tumorous lesion as the bleeding source. The patient had a prior medical history of uterine myoma and hypothyroidism, and no previous history of severe head injury or cranial irradiation. No abnormalities were noted regarding tumor markers including CEA, AFP, CA19-9, and CA125. Suspecting high-grade meningioma or solitary fibrous tumor/hemangiopericytoma (SFT/HPC), tumor resection was performed using an interhemispheric approach. The intraoperative findings revealed that the tumor mass was fibrous and hemorrhagic; discontinuity between the tumor and falx was confirmed by direct observation (Figure 1C). Because the tumor residue was very small (Figure 1D), a wait-and-see strategy was applied first. However, only 2.5 months later, she was urgently transferred because of sudden conscious disturbance. MRI T1WI revealed a well-demarcated enhancing lesion, suggesting tumor regrowth (Figure 1E). A second surgery was carried out; near-total resection was achieved again. She was subsequently referred to our institution to undergo detailed pathological examination and postsurgical treatments. Based on the tentative diagnosis of sarcomatous tumor, she underwent stereotactic radiotherapy (50Gy in 10 fractions) and 5 cycles of chemotherapy with ifosfamide, carboplatin, and etoposide (ICE). The patient is doing well and her disease remains stable at 14 months after initiating postoperative treatment (Figure 1F).

Histopathological analyses

Clinical specimens were obtained and analyzed according to the institutional guidelines for the study of human tissues. This study was approved by the research ethics committees of the University of Tokyo (No. G10028 and G10114). Written informed consent was obtained from the patient.

For histopathological diagnosis, 4-µm-thick sections from formalin-fixed, paraffin-embedded tissue blocks were subjected to hematoxylin and eosin staining and immunohistochemistry (IHC) staining using a Ventana BenchMark XT automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA) according to the department’s standard protocol. The antibodies used are summarized in Supplemental Table S1. Histological diagnoses were made by expert pathologists (A.S-U., and T.U.). Additionally, SYT-SSX1 or 2 chimeric genes were examined by polymerase chain reaction (PCR).
Resected specimens of the primary and recurrent tumors shared similar histological and IHC staining features. Both tumor tissues comprised dense, spindle-shaped neoplastic cells with hyperchromatic, irregular nuclei, arranged in an interlacing fascicle-like pattern (Figure 2A and B). The numbers of mitotic cells were 7 and 10 to 40 per 10 high-power fields, and Ki-67 labeling indices were approximately 50% and 70% in the primary and recurrent tumors, respectively, suggesting highly proliferative tumors.

The findings from IHC staining are summarized in Table 1. The cells were immuno-negative for epithelial membrane antigen (EMA), progesterone receptor (PgR), and signal transducer and activator of transcription 6 (STAT6), which indicated inconsistency with meningioma and SFT/HPC (Figure 1C and D). The possibility of glioma, schwannoma, melanoma, and myosarcoma also appeared unlikely because of the negative-staining for glial fibrillary acidic protein (GFAP), S-100 protein, human melanoma black-45 (HMB-45), desmin, and alpha-smooth muscle antigen (αSMA) (Figure 1E). The possibility of spindle cell rhabdomyosarcoma was also thought to be low as rhabdomyoblasts or rhabdomyoblast-like cells were not observed morphologically, however, the possibility was not eliminated since we could not assess MyoD1 staining with this case. Although the tumor cells were immune-positive for vimentin, which is consistent with mesenchymal tumors, the marker is non-specific and less helpful for excluding other possible diagnoses (Figure 1F). Besides, FLI-1, and CD99, and partially positive for CD34 and p53, but those findings did not suggest any specific cellular differentiation. The absence of SYT-SSX1 or 2 fusion gene, examined by PCR, indicated synovial sarcoma unlikely. In sum, as the tumors did not fit neatly into any specific diagnostic categories, we tentatively made the diagnosis of primary IC-SCS. Seeking additional diagnostic clues and potential therapeutic targets, we performed TOP and DNA methylation array analyses.

**DNA methylation array data analysis**

Genomic DNA was extracted from formalin-fixed paraffin-embedded tumor tissue collected from the second surgery and from peripheral blood lymphocytes and subjected to enrichment of target fragments using a SureSelectXT Custom kit (Agilent Technologies). Genome-wide methylation profile was evaluated using the Infinium MethylationEpic BeadChip (EPIC) (Illumina) following the manufacturer’s instructions. The beta-value was calculated for each CpG site using the following equation as previously reported.15

The EPIC methylation array data of the recurrent tumor were then analyzed using the Brain Tumor Methylation Classifier (v11b2) (www.molecularneuropathology.org).10 However, the tumor did not match with any of the 91 established methylation classes with sufficient calibrated scores, indicating that the likelihood of any well-established central nervous system (CNS) tumor classes was very low. On the other hand, the copy number (CN) variation profile elucidated aberrant chromosomal instability, including murine double minute 2 (MDM2) (12q15) amplification (Figure 3A). The finding was later confirmed in TOP CN analysis (CN = 5.870, log R ratio = 1.553) as well as heterogeneously positive staining for MDM2 on IHC (Figure 2H). In addition, the CN analysis also revealed the loss of heterozygosity (LOH) of chromosome 22q which contains the NF2 gene locus. Along with the somatic mutation of NF2 detected by the TOP analysis, a 2-hit inactivation of the gene was indicated.18

Finally, considering a probable diagnosis of mesenchymal histology, we performed DNA methylation-based clustering...
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Analysis, employing the following 2 public datasets. First, the Methylation450K BeadChip array (450K) data of 198 brain tumors with mesenchymal histology (meningioma, 90; hemangioblastoma, 25; schwannoma, 23; SFT/HPC, 16; Ewing sarcoma, 14; Ewing sarcoma with CIC mutation, 13; chordoma, 9; melanotic schwannoma, 8) were extracted from 2801 CNS tumor dataset (GSE90496, “DKFZ-CNS-MES cohort”).10 Second, 450K data of 206 sarcomas in The Cancer Genome Atlas (TCGA)-SARC project were obtained (phs000178. v1.p1, “TCGA-SARC cohort”).16 The processed data derived from these 2 cohorts and EPIC data from the present case were merged; only the CpG probes shared among the cohorts were retained; all CpG probes with one or more blank values were removed; finally 339,041 CpG probes were retained and used for further analysis. Unsupervised clustering analysis with t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction was performed on the 10,000 most variably methylated probes using Rtsne package v.0.13. According to the previous study, the following non-default parameters were applied: theta = 0, pca = FALSE, max_iter = 2500.10

As shown in the tSNE plot, the present case was plotted distantly from the DKFZ-CNS-MES clusters but closely to those of the TCGA-SARC cohort, particularly with aggressive types of sarcomas (Figure 3B).

CLINICAL PANEL SEQUENCING ANALYSIS (TOP ANALYSIS)

Custom-made probes for TOP analysis were designed to hybridize and capture the exons of 464 cancer-related genes, as previously reported.9 Massive parallel sequencing of the isolated fragments was performed with the HiSeq 2500 or NextSeq platform (Illumina). We detected somatic single-nucleotide mutations, insertions/deletions, and copy number variations using Karkinos (https://github.comgenome-rcast/karkinos).19 The raw sequencing data has been deposited in the Japanese Genotype-Phenotype Archive (http://trace.ddbj.nig.ac.jp/jga) under the accession number JGAS00000000164.

Findings of the TOP analysis are summarized in Table 2. We obtained several diagnostic clues, such as somatic single-nucleotide alterations (missense mutations) in DICER1, NF2, and ATRX, for the latter 2 of which with the annotation “likely oncogenic,” based on an automated database search.20 DICER1 mutation has been well documented in recent literature4,21 and is currently regarded as diagnostically critical for intracranial sarcomas with rhabdomyosarcoma-like features in the latest fifth edition of WHO classifications of central nervous system tumors,22 although this present case did not show typical histological and IHC features. ATRX mutation was validated by IHC, showing loss of α-thalassemia/mental retardation syndrome X-linked (ATRX) expression (Figure 2G). In particular, the presence of ATRX mutation further minimized the possibility of meningioma, since the mutation has been reported to be extremely rare in the disease.23,24 Any other mutations diagnostically critical or typical for meningiomas and SFT/HPC were not detected. The total mutation rate was estimated as 4.48/Mb. Taken all together, we concluded that the diagnosis of primary IC-SCS, DICER1-mutant, with MDM2 amplification would be most plausible.

DISCUSSION

In this study, we described a case of primary IC-SCS, DICER1-mutant, with MDM2 amplification, to which extensive molecular profiling was carried out. In this present case, despite the lack of any specific diagnostic clues in the radiological, morphological, and immuno-histological features, integration of multi-layered molecular analyses contributed to narrowing down the differential diagnoses.

Of note, MDM2 gene amplification was highlighted by CN analyses on two separate analysis platforms. MDM2 gene amplification is known as a hallmark of some subtypes of sarcomas, such as dedifferentiated liposarcoma (DDLPS), well-differentiated liposarcoma, and intimal sarcoma.25 In this case, however, the tumors did not show any evidence of adipose tissue differentiation which is mandatory for the diagnosis of DDLPS. This CN alteration might be indicative of the aggressiveness of the tumor.

On the other hand, NGS-based TOP analysis elucidated several unanticipated findings, such as somatic mutations in

| ANTIBODY | PRIMARY TUMOR | RECURRENT TUMOR |
|----------|---------------|-----------------|
| EMA      | Negative      | Negative        |
| PgR      | Negative      | Negative        |
| CD34     | Negative      | Partly positive |
| STAT6    | Negative      | Negative        |
| GFAP     | Negative      | Negative        |
| S-100    | Negative      | Negative        |
| p53      | n/a           | Partly positive |
| HMB-45   | Negative      | Negative        |
| CD99     | Positive      | Weakly positive |
| FLI-1    | n/a           | Positive        |
| αSMA     | Negative      | Negative        |
| h-Caldesmon | n/a     | Negative        |
| Desmin   | n/a           | Negative        |
| MDM2     | n/a           | Positive        |
| CDK4     | n/a           | Negative        |
| ATRX     | n/a           | Loss of expression |
| Vimentin | n/a           | Positive        |
| Ki-67    | ~50%          | ~70%            |

Table 1. Summary of immunohistochemical staining tests.
DICER1, NF2, and ATRX genes. Of note, as mentioned earlier, primary intracranial sarcoma, DICER1-mutant is newly described as an independent diagnosis entity in the recently released fifth edition of WHO classifications of central nervous system tumors. The disease arises most typically in children (the median age at diagnosis is 6 years), and is characterized...
by myogenic rhabdomyosarcoma-like features, such as positivity for desmin (14/17 cases; 82%) and SMA (17/17). ATRX mutation is also observed in some cases. Although the present case with DICER1 mutation would fulfill the current diagnostic criteria, several atypical aspects exist, such as the lack of evidence of myogenic differentiation. The immunostaining assessment of MyoD1, lacking in the present case, might have provided further diagnostic clues. Moreover, MDM2 amplification has never been reported in this new disease entity and its significance remains unknown.

Recently, the use of clinical sequencing has gradually widespread. This method enables the detection of a range of diagnostic and actionable genomic events, including mutational load and fusion-gene detection. While we applied this modality for diagnostic purposes, neither critical genomic alterations nor any actionable targets were identified in this case, except for the aforementioned some implicative findings. According to the literature, the successful rates for clinical sequencing for detecting any “actionable” events that can be targeted therapeutically are reported to be 37% to 45%. For TOP analysis, we previously reported that the detection rate of some actionable alterations was 32.2% among all the tested patients. Therefore, the intrinsic limitation should be recognized.

Simultaneously, the diagnostic utility of DNA methylation array data has been also acknowledged in cancers including brain tumors and sarcomas. The DNA methylation status is considered as highly robust and reliable, as it is a consequence of features reflecting both the cellular origin and somatically acquired changes. Indeed, novel diagnostic classification has been emerging based on such analyses. In the present case, DNA methylation-based unsupervised clustering with external cohort data revealed that the tumor was not consistent with any common CNS mesenchymal tumor classes but rather with aggressive types of sarcomas. We expect that a combination of clinical sequencing and DNA methylation analysis can yield diagnostic accuracy.

With respect to treatment, there is no established standard of care for this disease because of its rarity and limited understanding of the molecular pathogenesis. In this case, we treated the patient with maximum safe surgical resection followed by irradiation and concurrent chemotherapy with an ICE regimen referring to a retrospective study of pediatric intracranial sarcoma in which the treatment led to a favorable outcome. While the clinical course of the present case remains stable, careful long-term follow-up is warranted.

In conclusion, this case report suggests that even for the tumors with insufficient morphological and immuno-histological diagnostic clues, integration of multi-layered molecular analyses can contribute to making the diagnoses as well as to understanding the rare tumors by elucidating unanticipated genetic and epigenetic features.

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Author Contributions

T.N., S.Takayanagi, and S.Tanaka conceptualized and contributed to the design of the study. T.N., S.Takayanagi, S.Tanaka, A.S-U., S.K., T.U., A.M. H.A., and H.M. performed data analysis and interpretation. K.N., M.Y., and S.S. provided samples and clinical data. T.N., S.Takayanagi, and S.Tanaka wrote the manuscript with support from all authors. S.Takayanagi, S.Tanaka, and N.S. supervised the study. S.Takayanagi approved the final version of the manuscript on behalf of all authors.

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Supplemental Material

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