DICER1-Associated Central Nervous System Sarcoma with Neural Lineage Differentiation: A Case Report

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Case Report

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Abstract

Background: *DICER1*-associated central nervous system sarcoma (DCS) without evidence of other cancer-related syndromes is rare. Though the morphology of DCS was highly variable, the immunophenotype was predominant myogenic phenotype. Other lineage markers were consistently negative. Herein, our objective was to identify the clinical, pathogenesis, treatment and driver mutation of DCS with neurogenic differentiation through whole-exome sequencing (WES) and RNA sequencing (RNA-seq) of both leukocytes and tumor tissues.

Case presentation: We describe here the case of a 8-year-old female patient presented with a 8-day history of headache, nausea and vomiting. Magnetic resonance imaging (MRI) revealed a heterogeneous mass in left parietal lobe. The patient underwent the craniotomy via left parietal approach. Histologically, the tumor predominately showed fibrosarcoma-like spindle cells with obvious cytoplasmic eosinophilic globules. Immunohistochemically, the tumor stained positively for NF, Syn, MAP-2, Desmin and DICER1. WES of tumor tissues detected the *DICER1* somatic mutation. This case harbored tumor-driving mutations mainly including *AR*, *AXL* and *ETV5* mutations, proved sarcoma-associated genes in other kind of sarcomas growth, in addition to *TP53* and *RAF1* mutations which were common found in DCS. All theses findings indicated the diagnosis of DCS with neurogenic differentiation. This neural lineage differentiation was further confirmed by the result of Gene Ontology (GO) analysis. The patient subsequently received high dose radiotherapy (60Gy) and chemotherapy. The MRI showed no evidence of tumor recurrence at the 12 months' follow-up.

Conclusions: This unusual case of DCS with neuronal differentiation is an important addition to the immuno-phenotypic spectrum of DCS. The prognosis is poor for DCS, and total tumor resection and high dose radiotherapy may assist in prolonging survival. Further research is needed to better understand the behavior and treatment of this rare DCS with neuronal differentiation.

Introduction

Intracranial sarcomas are uncommon tumors with poor prognoses and mainly occurred in adult patients [1]. However, they rarely affect children, and when they do, they are often associated with syndromic disorders [2–4]. DICER1 syndrome is one kind of this rare syndromic disorders with intracranial sarcomas, pinealoblastoma, pituitary blastoma, cystic nephroma, and several other rare tumor entities and always exhibit the combination of germline *DICER1* mutations and somatic *DICER1* mutations [5, 6]. Pediatric intracranial sarcomas involving somatic *DICER1*-mutants without evidence of other cancer-related syndrome are rare [7]. Koelsche C et al reported a group of predominantly pediatric intracranial spindle cell sarcomas to be called "spindle cell sarcoma with rhabdomyosarcoma-like features, *DICER1* mutant" [7]. In this study, a germline *DICER1* mutation was only detected in two of five cases. Then, Kamihara J et al reported another six cases of "DICER1-associated central nervous system sarcoma (DCS) ", In their group, only three cases harbored somatic *DICER1* mutations without germline *DICER1* mutations [8]. Moreover, most of the reported cases showed highly variable morphology, but they

consistent with the positive of myogenic markers. Other lineage markers were consistently negative[5–8]. To the best of our knowledge, there is no report of a DCS with expression of neurogenic markers. Herein, we describe the clinical, Imaging, pathological, molecular profiles and treatment of a rare DCS harboring the somatic *DICER1* mutation with neural lineage differentiation.

Case Presentation

An 8-year-old female presented to the neurosurgery department with intermittent headache, nausea and vomiting for 8 days. Neurological examination revealed no abnormalities. She had no family history of cancer or visceral. Magnetic resonance imaging (MRI) of the brain revealed a heterogeneous mass in left parietal lobe that was predominantly hypointense on T1 (Fig. 1a) and inhomogeneous hyperintense on T2 (Fig. 1b), as well as the fluid attenuation inversion recovery (FLAIR) sequence was iso-intensity (Fig. 1c). After the administration of contrast, the mass displayed heterogeneous enhancement (Fig. 1d, 1e). The patient underwent a craniotomy via left parietal approach, which revealed a well-defined, soft, gray and gelatinous lesion attached to the falx cerebri (5 cm × 5 cm × 5 cm). The lesion was completely resected and postoperative course was uneventful. Postoperative CT scan showed that the intracranial tumor had been completely removed (Fig. 1f).

Light microscopy examination revealed sarcomatous neoplasms predominantly presented with spindle-shaped cells in a fascicular pattern (Fig. 2a). There was a clear boundary between tumor and peripheral brain tissue (Fig. 2b). In some areas, a myxoid stroma matrix was found with a few multinucleated tumor giant cells (Fig. 2c). The nuclei of the tumor cells were with brisk mitotic activity (Fig. 2d). Prominent cytoplasmic eosinophilic globules were easily identified (Fig. 2e, 2f).

Immuno-histochemical studies were performed using an automated Ventana Benchmark Ultra autostainer (Ventana, Tucson, Arizona, USA). Briefly, tissue sections were deparaffinized, antigens retrieved and endogenous peroxidase was blocked with 1% H_2O_2. The primary antibodies were used for target protein detection: Desmin (clone D33, Dako, 1:50), smooth muscle actin (SMA, clone 1A4, Dako, 1:400), MyoD1 (clone 5.8A, Dako, 1:50), myogenin (clone F5D, Dako, 1:50), CD34 (clone QBEnd10, Beckman Coulter, 1:500), S-100 (clone 4c4.9, Zytomed, 1:3000) and Ki67 (clone MIB1, Dako, 1:80). Olig2 (clone H-68, Santa Cruz, 1:200), MAP2 (Abcam Cambridge; 1:1500), Syn (Bio Genex, 1:100), glial fibrillary acidic protein (GFAP, clone 6F2, Dako Cytomation, 1:500), epithelial membrane antigen (EMA, Dako, 1:50), cytokeratin (CK, clone AE¹⁄AE3, Zymed San Francisco, 1:200), NF (clone 2F11, Dako Cytomatio, 1:150), TLE1 (clone sc-9121, Santa Cruz, 1:100), STAT6 (Abcam Cambridge, 1:500), TLE1 (clone sc-9121, Santa Cruz, 1:100), SOX10 (Abcam Cambridge, 1:400), somatostatin receptor 2A (SSTR2A, cloneUMB1, Abcam, 1:200), BRG1 (clone G-7, Santa Cruz, 1:200), INI-1 (clone BAF47, Biosciences, 1:200), DICER1 (clone 4A6, Abcam, 1:100). Revelation was performed using the ultra-VIEW TM DAB systems (Ventana). All tissue sections were counterstained with hematoxylin II/Mayer's hematoxylin (Ventana). Immunohistochemically, the MIB-1-positive cell index was 70%. The tumor cells stained positively for vimentin. The tumor cells had a focal expression of Desmin (Fig. 2g) and did not express myogenin, SMA. DICER1 protein immunostaining was extensively positive in the tumor cells, raising the possibility

of *DICER1* mutation (Fig. 2h). Interesting, the tumor cells stained positively for S-100 (Fig. 2i), Syn (Fig. 2j), MAP-2 (Fig. 2k) and NF (Fig. 2l), and INI-1(Fig. 2m) and BRG1 were retained. But tumor cells were negative for GFAP (Fig. 2n) and Olig-2. The Syn, MAP-2, and NF immunostaining highlighted the neural lineage differentiation of tumor cells. SOX10, CK, EMA, CD34, TLE1, STAT6 and SSTR2A were negative. The tumor cells displayed with a large amount of thin reticulin fibers (Fig. 2o). A diagnosis of primary intracranial spindle cell sarcoma with neurogenic and myogenic differentiation, possibly associated with *DICER1* mutation, was favoured.

Next, WES analysis was performed in surgical formalin-fixed paraffin-embedded (FFPE) tumor tissues and normal plasm. In brief, WES analysis sequencing libraries were generated using Agilent SureSelect Human All Exon V6 kit (Agilent Technologies, CA, USA). DNA libraries were sequenced on Illumina Hiseq platform and 150 bp paired-end reads were generated and cleaned. Data was mapped to the reference human genome b37 by Burrows-Wheeler Aligner (BWA) software [9]. SAMtools [10] and Picard (http://broadinstitute.github.io/picard/) were used to sort BAM files and do duplicate marking, local realignment, and base quality recalibration. Samtools mpileup and bcftools were used to do variant calling and identify SNP. Variants were selected by applying more filters on germline variants callsets. *DICER1* p.E1813D (c.5439G > T) mutation located within the Ribonuclease III domain was detected in tumor tissues but not in plasm sample suggesting a somatic mutation. A final diagnosis of DCS was established. *TP53* mutation (c. 560-2A > T) was found in this case. It harbored *RAF*1 mutation (p.R191T) which was predicted to cause activation of the MAP kinase signaling pathway. Other tumor-driving mutations including *AR* mutation (p.G473del), *AXL* mutation (p.T45P), *ETV5* mutation (p.F11Y) were detected. Alterations in all genes (including not reported/undetermined significance = 158) are summarized in (Supplementary Table 1). Then we performed GO analysis to identify the potential molecular function of these driver-genes. GO analysis showed that genes involved in chromatin organization (*SATB1, ATAD2* and *CHD4*), neuron development (*TENM4, SECISBP2* and *HS6ST1*), histone deacetylation (*SIN3B, CHD4* and *MTA2*) processes that were mutated in this case.

Additionally, WES indicated that the tumor presented a high level of tumor mutational burden (TMB), but did not show microsatellite instability (MSI). RNA-seq was performed for as previously described [11]. We did not find any gene fusions by RNA-seq.

With DICER1 p.E1813D (c.5439G > T) mutation confirmed, a final diagnosis of DCS was established. The patient received postoperative radiotherapy (60 Gy/30f) with the following cyclophosphamide, doxorubicin and vincristine for the first, third, fifth and seventh chemotherapy cycles. In the second, fourth, sixth and eighth cycles ifosfamide and etoposide were used for chemotherapy. After eight cycles (median duration of 21 days among cycles) of combination chemotherapy, brain MRI revealed no evidence of tumor recurrence at the 12 months' follow-up. The present study was conducted in accordance with the Declaration of Helsinki and under the guidelines of the institutional board on ethics of the Sanbo Brain Hospital. Written informed consent was obtained from the family member (mother) of the patient for the publication of any potentially identifiable images or data included in this article.

Discussion

We describe here a case of DCS haboring somatic DICER1 mutation with neurogenic differentiation. Early reports have described that though the morphology of DCS was highly variable, spindle cell morphology was common with immunopositive myogenic markers (SMA, desmin, and myogenin) [7, 8]. Other lineage markers were consistently negative [7, 8]. In the current case, the tumor predominately showed fibrosarcoma-like spindle cells with brisk mitotic activity, including obvious cytoplasmic eosinophilic globules. These microscopic features are quite similar to previous reports [7, 8]. Unlike previous cases, this case showed the expression of neurogenic markers (NF, Syn, MAP-2) in addition to myogenic marker (Desmin). GO analysis of WES have indicated that genes involved in neuron development were mutated, such as *TENM4, SECISBP2* and *HS6ST1*. We have shown that this case both have neural characteristics and the potential for neural development. The neurogenic differentiation was found in DCS, and this testified the statement of "highly variable heterologous differentiation of DCS" on the other side.

There are additional genetic changes in addition to somatic *DICER1* mutation detected in this patient. The *TP53* mutation and mutations in the MAP-kinase pathway were frequent in SCS-RMSlike-DICER1[7]. *TP53* mutation and *RAF1* gene mutation associated with MAP-kinase pathway had also been observed in the current case. Furthermore, the patient also carried *AR, AXL* and *ETV5* mutations. Recently *Axl* has been proved a candidate for pathogenesis and therapeutic investigations in leiomyosarcoma [12] and osteosarcoma [13]. It was demonstrated that *ETV5* had oncogenic function on Ewing's sarcoma [14]. This may be of interest because *AXL* and *ETV5*, proved sarcoma-associated genes in leiomyosarcoma, osteosarcoma and Ewing's sarcoma growth, can be also detected in DCS cases [12–14]. Similar tumor-driving mutations in this DCS and other kind of sarcoma suggested that there was also an inherent relationship between DCS and some other mesenchymal sarcomas.

Clinical experience with DCS is very limited. Some DCS cases without germline *DICER1* variants and family history of *DICER1*-related tumors were presumed to represent sporadic disease [8]. It is worth noting that sometimes a few cases without germline *DICER1* variants may present de novo neoplasm in other organs besides intracranial sarcoma as time goes on [7]. That is, generally, even if the DCS patients without *DICER1* germline mutation did not have any other diseases at the time of DCS. It is needed to pay close attention to their general and systemic examination in postoperative follow-up. There was no recurrence and other diseases in our case after 12 months of follow-up.

Sporadic reports of long-term cure indicate that achieving gross total resection, immediate postoperative focal radiotherapy, and prolonged combination chemotherapy may be of importance [8]. Effective regimens include cyclophosphamide, topotecan, ifosfamide, doxorubicin, vincristine and dactinomycin. In addition, while we found that 3 of the DCS patients in the previous study who had been exposed to high dose rate radiotherapy (≥ 59.9 Gy) had relatively good prognosis [8], the treatment of this DCS case included gross total resection, high dose rate radiotherapy (60 Gy) and the following chemotherapy.

In summary, this first report of a patient with DCS with neuronal differentiation further expands the immuno-phenotypic spectrum of DCS. The prognosis is poor for DCS, and total tumor resection and high

dose radiotherapy may assist in prolonging survival. Long-term observation is necessary to better elucidate the biological behavior of DCS with neuronal differentiation .

Abbreviations

DCS
DICER1-associated central nervous system sarcoma

Declarations

Informed Consent

Written informed consent was obtained from the family member (mother) of the patient for the publication of any potentially identifiable images or data included in this article.

Author contributions:

The overall experimental design was conceived and supervised by Qi Xueling. Yao Kun contributed to the analysis of the data and the final draft of the manuscript. Duan Zejun contributed to the analysis of the data and the figure. Feng Jing helped analyze whole-exome sequencing data. All authors and approved the final manuscript.

Data availability statement:

The data that support the findings of this study are available on request from the corresponding author.

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Ethics approval and consent to participate

Our study was carried out according to the ethical guidelines of the ethics committee of Sanbo Brain Hospital. It was approved by the ethics committee of Sanbo Brain Hospital

Informed Consent

Written informed consent was obtained from the family member (mother) of the patient for the publication of any potentially identifiable images or data included in this article.

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Conflicts of interest: All authors declare that they have no conflicts of interest

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Figures

Figure 1

Intracranial mass in patient. a: Axial T1-weighted MRI shows hypointense lesion in the left parietal lobe.b: Axial T1-weighted MRI shows inhomogeneous hyperintensel. c: Axial FLAIR image shows iso-intensity. d, e: Axial and coronal post-contrast T1-weighted MRI shows enhancement of the mass and the adjacent dura. f: Axial CT shows intracranial tumor has been completely removed.

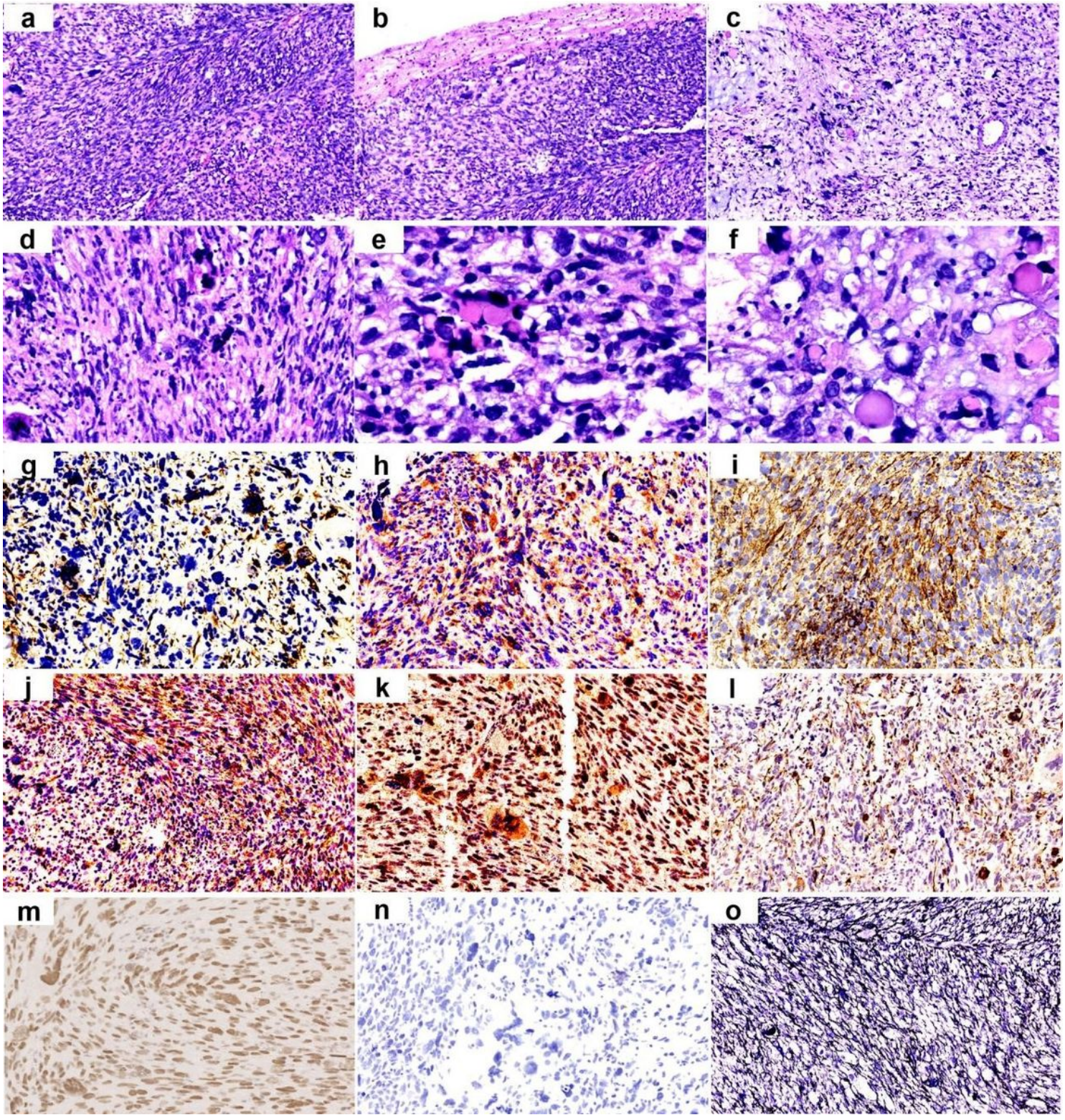

Figure 2

Histopathological features of the DICER1-associated central nervous system sarcoma. a: The tumor presents with spindle-shaped cells with a fascicular pattern of growth.b:The interface of the tumor and the normal brain is well demarcated. c: Focal myxoid stroma matrix and a few multinucleated giant cell; d: Mitoses were frequent. e f: Cytoplasmic eosinophilic globules. g: Desmin highlights myogenic differentiation. h: DICER1 protein expression in the majority of tumor cells. i: S-100

immunohistochemistry in case. j: Strong Syn immunostaining in a signifcant number of tumor cells. k: MAP-2 expression in majority of neoplastic cells. l: NF immunostaining. m: INI-1 immunostaining. n: Immunohistochemistry staining showed that tumor cells are negative for GFAP (n). o: A large of reticulin fibres. (a-c ×100d, g-o×200; e, f ×400).

Supplementary Files

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- SupplementaryTable1.doc
- CAREchecklistEnglish.jpg