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Teaching Case

Methylation and Molecular Profiling to Aid in Diagnosis and Radiation Treatment for an Intracranial Ewing Sarcoma in a Pediatric Patient: A Case Report

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Introduction

Central nervous system (CNS) tumors have a high mortality rate and often pose a diagnostic challenge because of their heterogeneity and the presence of variable pluripotent or differentiated cell types. Among primary pediatric CNS tumors, undifferentiated small blue round cell tumors are frequently observed, encompassing a variety of tumor types. The availability of molecular characterization, including genomic and epigenomic characterization, helps in the diagnosis of these tumors.[1,2]

Primary intracranial Ewing sarcomas are a group of poorly defined, highly malignant, small round cell neoplasms. They are often associated with a fusion of the EWSR1 gene to other genes, most commonly the FLI1 gene, resulting in a t(11;22) (q24;q12) translocation.[3,4] Ewing sarcomas differ from CNS embryonal tumors, previously known as primitive neuroectodermal tumors (PNETs), in terms of their molecular profiles, treatment approaches, and prognosis. Therefore, an accurate diagnosis is crucial for the management of these patients.

Under the 2020 World Health Organization classification, there is an updated description for round cell sarcomas of soft tissue and bone.[5] This classification defines various distinct subtypes of Ewing sarcoma. These include round cell sarcomas with EWSR1 non-ETS fusions, CIC-rearranged sarcomas, and sarcoma with BCOR genetic alterations. Extracranial undifferentiated small round cell sarcomas were previously referred to as "Ewing-like tumors" and typically lack the characteristic genetic translocations observed in Ewing sarcoma, such as EWSR1, TET/FET, and E26. Instead, they often exhibit distinct mutations identified through next-generation sequencing, such as CIC-FOXO4 fusion or CICDUX4 fusions. These tumors have also been associated with other molecular alterations involving genes such as FOXO4, LEUTX, and NUTM1.[6,7]

Similarly, a subset of intracranial Ewing tumors is referred to as EWSR1-negative tumors. Within these tumors, CIC rearrangements have been identified. Intracranial CIC-rearranged sarcomas are characterized as rare, poorly differentiated mesenchymal tumors of the nonmeningeal epithelial type. They typically exhibit an aggressive clinical course, primarily affecting children but also occurring in adolescents and adults.[8]

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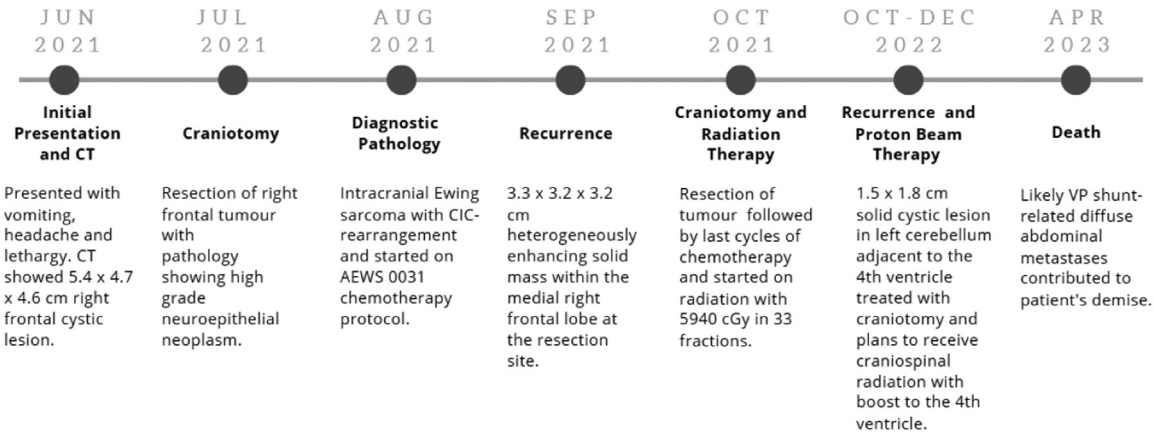

Figure 1 Timeline of the patient's diagnosis and treatments. *Abbreviations:* CT = computed tomography; VP = ventriculoperitoneal.

Clinically, they present at extracranial sites with only rare involvement of the bones. These tumors manifest with signs and symptoms of increased intracranial pressure and have a poor prognosis, with a median survival of less than 2 years.[9]

Neuropathologic diagnosis poses a challenge because of the rarity and nonspecific histologic features of these tumors. However, integrated technologies for genetic analysis, such as methylation profiling, have aided in diagnosis and classification. DNA methylation profiling is based on the molecular signatures derived from genome-wide methylation profiles in DNA, and in pediatric CNS tumors, it is superior to standard histopathology. Identification of this CIC rearrangement allows for an accurate diagnosis of intracranial Ewing sarcoma.

Herein, we present a case of an intracranial Ewing CIC-rearranged sarcoma located in the frontal lobe of a 4-year-old male. The patient underwent testing through immunochemical analysis, fluorescence in situ hybridization (FISH), and DNA-methylation-based tumor classification. Additionally, we describe the molecular and radiologic features of this rare tumor, which was treated with surgical resection, chemotherapy, and radiation therapy.

Case Presentation

Patient history

A previously healthy 4-year-old male presented with a 2-week history of headaches and recurrent vomiting. During the physical examination, no focal neurologic deficits, visual changes, seizures, or weakness in the extremities were observed. The boy had no prenatal abnormalities and demonstrated appropriate growth and development for his age. Furthermore, his personal and family medical history was unremarkable. Signs suggestive of constipation were noted at the initial presentation, and he was treated with laxatives. However, despite the treatment, he continued to experience morning vomiting and headaches, and his neurologic symptoms worsened, with increased lethargy. This was the initial presentation for a complicated cancer diagnosis and timeline (Fig. 1).

Neuroimaging findings

A computed tomography (CT) scan of the brain (Fig. 2A) revealed a suspicious lesion in the right frontal lobe measuring 5.4 × 4.7 × 4.6 cm with a cystic appearance and a peripheral enhancing nodule along the anteromedial aspect measuring 1.3 × 1.4 × 2.0 cm. There was minimal surrounding vasogenic edema anterior to the lesion.

Due to the clinical signs of elevated intracranial pressure, the patient was started on 4 mg intravenous dexamethasone and was referred for urgent magnetic resonance imaging (MRI) (Fig. 2B). The MRI of the head redemonstrated the predominantly cystic mass within the right frontal lobe, and the solid anteromedial mass was measured at 2.0 × 1.3 × 1.5 cm. The mass had heterogeneous enhancement and was bright on T2. It resulted in mass effect and shift of the midline by approximately 1.4 cm. The MRI of the spine was unremarkable. Given the intracranial mass and clinical status, surgery was planned.

Therapeutic interventions

With CT and MRI demonstrating a mass, the patient underwent a craniotomy, frontal lobe tumor resection, and duraplasty (Fig. 2C). The surgery was performed without complication.

The specimen obtained during craniotomy was sent for histologic and molecular analysis. Basic pathologic tests

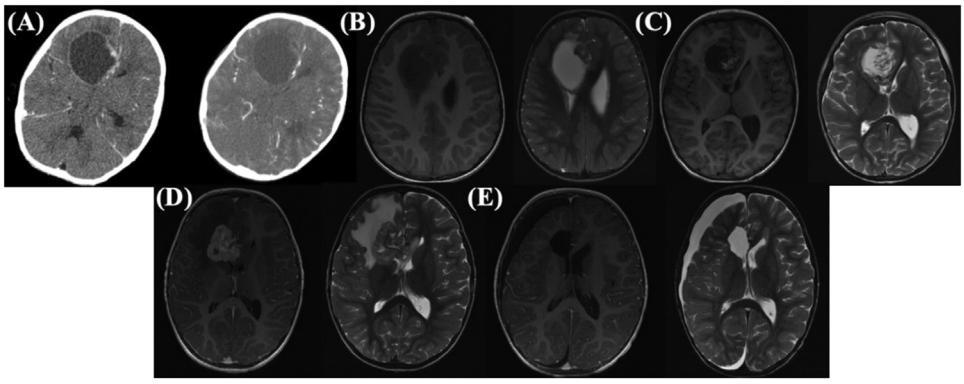

Figure 2 (A) Preoperative axial computed tomography at initial diagnosis showing a large cystic lesion in the right frontal lobe measuring approximately 5.4 × 4.7 × 4.6 cm. (B) Preoperative brain MRI T1-weighted (left) and T2-weighted (right) showing a 2.0 × 1.3 × 1.5−cm cystic mass with peripheral solid enhancement within the right frontal lobe. (C) Postoperative axial MRI showing a right frontal craniotomy with slightly decreased mass effect and persisting findings of early/mild trapping of the left lateral ventricle with transependymal cerebrospinal fluid regression. (D) MRI showing the interval development of a 3.3 × 3.2 × 3.2−cm heterogeneously enhancing solid mass within the medial right frontal lobe at the resection site, indicative of local recurrence. (E) Postoperative MRI of the right frontal lobe after excision. *Abbreviations:* MRI = magnetic resonance imaging.

demonstrated CD99 and GFAP expression as well as a Ki-67 index of 5% to 10%, and p53 was strongly expressed. These pathologic findings were suggestive of a high-grade neuroepithelial neoplasm. The differential included a high-grade pediatric glioma, neuroepithelial tumor (with or without BCOR alterations), and CNS embryonal tumors. Given the uncertainty in diagnosis and the poorly differentiated nature of the tumor, further comprehensive genomic profiling and methylation studies were performed for molecular reclassification. Next-generation sequencing did not find any variations in BRAF, IDH1, or IDH2. This led to a pathology review at an outside center. As part of this review, methylation testing was performed under research categorization—thus, not clinically approved—which identified a CIC-DUX4 fusion. Fluorescence in situ hybridization was performed following a standardized approach from Yoshimoto et al,[10,11] using a dual-color breakapart probe for the CIC gene locus (19q.13.2) This testing was consistent with a CIC gene rearrangement in 182 of 200 nuclei (91%). The final molecular pathology confirmed a diagnosis of CNS Ewing sarcoma family tumor with CIC alteration and PTENp. F341V SNV, without a P53 mutation.

Unfortunately, between the patient's original resection and the first cycles of chemotherapy, an MRI scan (Fig. 2D) showed significant regrowth of his tumor within the prior resection cavity. Interval development of a 3.3 × 3.2 × 3.2−cm heterogeneously enhancing solid mass within the medial right frontal lobe at the resection site was noted. Considering the histologic, molecular, and clinical findings, a multimodal approach to therapy, including chemotherapy followed by image re-evaluation, consideration of second-look surgery, and then adjuvant focal radiation, was proposed. Chemotherapy was started according to the Children's Oncology Group AEWS1031 protocol[12] with compressed cycles of vincristine, doxorubicin, and cyclophosphamide alternating with ifosfamide and etoposide followed by focal irradiation. After 3 cycles of treatment, improvement was noted on imaging with a decrease in the size of the recurrent mass seen on MRI. The patient proceeded with a second resection followed by radiation therapy (Fig. 2E) starting at week 9 of chemotherapy.

Radiation was delivered using an intensity modulated radiation therapy technique with a total dose of 5940 cGy in 33 fractions to the resection cavity (Fig. 3). An initial dose of 54 Gy in 30 fractions was delivered to the resection cavity with a 10-mm clinical target volume (CTV) margin. This was followed by a cone down to the resection cavity with a 5-mm CTV margin for the final 3 fractions. It is worth noting that there was consideration for proton therapy to potentially limit the dose to healthy tissue. However, a decision was made to treat with photons to minimize delays, given the added complexity of receiving treatment out of the country, which would have been necessary for proton therapy.

After his last course of chemotherapy and radiation, the patient remained clinically well. Unfortunately, surveillance imaging 1 year postradiation demonstrated a solid cystic-enhancing lesion measuring 1.5 × 1.8 cm (Fig. 4) in the deep left cerebellum adjacent to the fourth ventricle, suggesting tumor recurrence. The patient underwent a suboccipital craniectomy and gross removal of the posterior fossa lesion, which was confirmed to be metastatic Ewing sarcoma. Salvage craniospinal radiation therapy with a fourth ventricular boost was recommended, and the patient was referred for out-of-country proton beam treatment. Unfortunately, the patient's

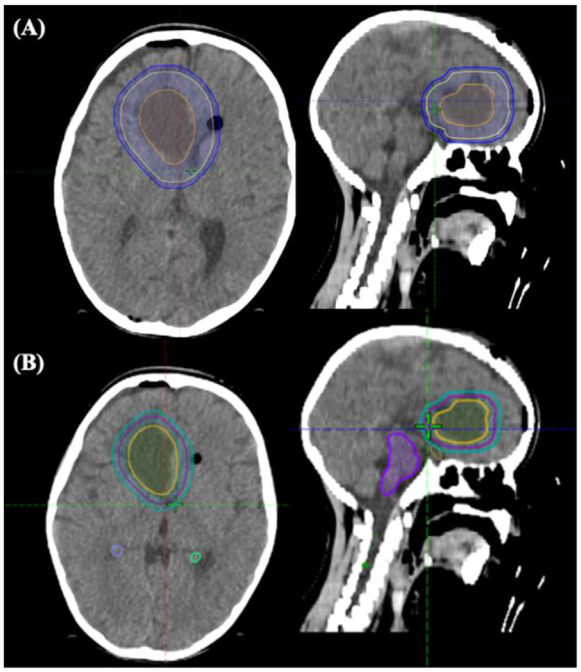

Figure 3 (A) Computed tomography and magnetic resonance fused images showing a radiation plan of 5400 cGy in 30 fractions to the resection cavity, with a cone down to a total dose of 5940 cGy in 33 fractions. The GTV is shown in gold, the CTV (GTV + 10 mm) in yellow, and the PTV (CTV + 3 mm) in blue. (B) Boost plan with the GTV in yellow, CTV (GTV + 5 mm) in purple, and PTV (CTV + 3 mm) in teal. *Abbreviations:* CTV = clinical tumor volume; GTV = gross tumor volume; PTV= planning target volume.

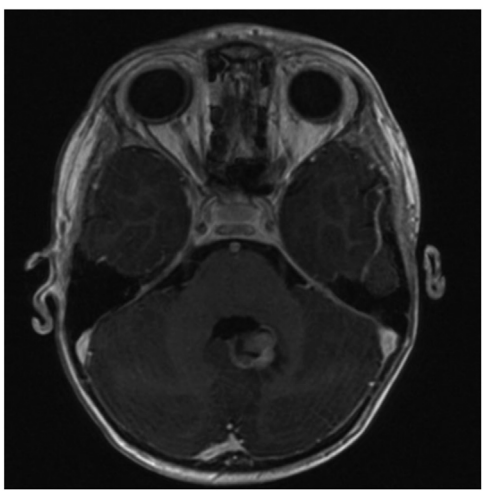

Figure 4 Magnetic resonance imaging showing a solid cystic enhancing lesion in the deep left cerebellum adjacent to the posterior margin of the fourth ventricle. The lesion measured 1.5 × 1.8 cm, causing mild edema in the adjacent brain with significant mass effect.

condition rapidly declined 2 months after salvage radiation therapy due to diffuse abdominal progression of metastatic disease, most likely a result of peritoneal dissemination through the ventriculoperitoneal shunt.

Discussion

CIC-rearranged sarcomas account for approximately 70% of the molecular subtypes in Ewing-like undifferentiated small blue round cell tumors that lack EWSR rearrangements.[13] The features of CIC-rearranged sarcomas overlap with other small blue round cell tumors and are often misdiagnosed as central nervous system embryonal tumors, medulloblastoma, neuroepithelial tumors, malignant meningioma, or even melanoma. Standard Ewing sarcomas can be detected using a FISH assay. However, the FISH assay for EWSR1 is not specific and has been reported to have a false-negative rate of 14% for CIC-rearranged sarcomas.[14] Therefore, DNA methylation profiling has emerged as a robust approach for classifying these tumors.[15]

Studies on molecular profiling have suggested that these CIC-DUX4 tumors are characterized by either t(4;19)(q35;q13) or t(10;19)(q26;q13) translocations.[10,11] These tumors involve the fusion of CIC, a transcriptional repressor on 19q13.1, and DUX, a double homeobox transcriptional factor located on either 4q35 or 10q26.3. These fusions have been implicated in oncogenesis, tumor development, and metastatic involvement. This case report demonstrated the difficulty in accurately characterizing intracranial small round blue cell tumors. Initial pathology and next-generation sequencing can classically identify the majority of pediatric soft-tissue sarcomas, but almost 5% of these tumors cannot be characterized in this way.[10] Therefore, when initial testing is inconclusive, methylation testing can be used to identify specific rearrangements such as a CIC-DUX4 fusion, as seen in this case.

Currently, there is no unified treatment protocol for CNS Ewing sarcoma family tumors with CIC alterations. To date, complete tumor resection is the main cornerstone of therapy.[16,17] A study by Chen et al showed that gross total resection led to a survival of 38 months compared with 20 months for patients who had partial resections.[17] Additionally, adjuvant chemotherapy is often used, which consists of multiagent anthracycline-based therapy with regimens such as compressed cycles of vincristine, doxorubicin, and cyclophosphamide alternating with ifosfamide and etoposide, as was the case with our patient.[12] Studies investigating neoadjuvant chemotherapy have found a high frequency of relapse in localized disease, rapid onset of drug resistance, disease progression, and inferior outcomes.[9,18,19] Antonescu et al suggested that CIC-rearranged sarcomas are genetically specific tumors with distinct biologic behavior,[19] and further studies have found that CIC-rearranged sarcomas are

less sensitive to standard Ewing sarcoma chemotherapy regimens.[20,21]

These molecular differences raise the question of how to optimally manage CIC-rearranged sarcomas. Due to the rare occurrence of these tumors, there is sparse evidence on the optimal radiation therapy approach. A study of 14 patients with intracranial Ewing sarcoma and peripheral primitive neuroectodermal tumors treated surgically demonstrated that adjuvant radiation therapy was a statistically significant prognostic factor for increased median survival. Additionally, they found that adjuvant radiation therapy with or without adjuvant chemotherapy yielded a 100% 2-year survival rate.[17] Moreover, Jiang et al performed an extensive review of intracranial Ewing sarcoma and peripheral primitive neuroectodermal tumors, suggesting that gross total resection combined with adjuvant chemotherapy and radiation therapy might be the optimal treatment approach for improved patient survival.[22] When considering possible radiation therapy techniques, previous studies have suggested that Ewing-like sarcomas can be treated with focal radiation unless metastases are found at initial presentation. This differs from CNS embryonal tumors, which require craniospinal irradiation along with a boost to the primary tumor site.[3,4]

This case study illustrates the occurrence of a rare intracranial CIC-rearranged sarcoma. Methylation analysis of CIC fusions serves as a valuable tool in the diagnosis of EWSR1-negative sarcomas and was used in our case to lead to a diagnosis of an intracranial Ewing sarcoma. The initial diagnosis of a high-grade neuroepithelial neoplasm would have led to adjuvant craniospinal radiation. This treatment carries significant toxicity and long-term implications for pediatric patients. However, due to the revised diagnosis of a CNS Ewing sarcoma tumor with CIC alteration, adjuvant focal radiation therapy was a reasonable approach. Unfortunately, the patient did have a cerebellar recurrence. There are still questions regarding the optimal use of radiation therapy for intracranial Ewing sarcomas and the use of focal versus craniospinal radiation therapy treatment. Further studies and retrospective analysis are needed to optimize the treatment pathway for these rare tumors.

Conclusions

Intracranial Ewing sarcoma is a rare diagnosis that requires molecular characterization for accurate diagnosis. The optimal treatment paradigm for these patients is not clear at this time.

Disclosures

Glenn Bauman has received grants or contracts from the Ontario Institute for Cancer Research, Siemens Healthineers, and Invicro, is the clinical lead of the Ontario Institute for Cancer Research and the Director of the Western Centre for Translational Cancer Research, and has been involved with Advanced Accelerator Applications. No other disclosures were reported.

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