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Loss of CDKN1C in a Recurrent Atypical Teratoid/Rhabdoid Tumor

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Abstract

Atypical teratoid/rhabdoid tumor (AT/RT) is a malignant tumor that is commonly associated with biallelic alterations of *SMARCB1*. Recurrent or refractory AT/RT has not been molecularly characterized as well. We present the case of a child with recurrent AT/RT who underwent clinically integrated molecular profiling (germline DNA and tumor DNA/RNA sequencing). This demonstrated a somatic lesion in *CDKN1C* alongside hallmark loss of *SMARCB1*. This data allowed us to explore potential personalized therapies for this patient and expose a molecular driver that may be involved in similar cases.

Keywords

Atypical Teratoid/Rhabdoid Tumor (AT/RT); Pediatric brain tumor; CDKN1C; Precision Medicine; SMARCB1

INTRODUCTION

Atypical teratoid/rhabdoid tumor (AT/RT) is a malignant embryonal tumor that occurs primarily in infants and young children.¹ It is the most commonly occurring embryonic CNS tumor in infants less than one year of age.² Histologically, AT/RT is an embryonal tumor with poorly differentiated elements along neuroectodermal, epithelial and mesenchymal lineages (teratoid), with areas of rhabdoid cells.³ AT/RT shares common histological features with other embryonal tumors such as medulloblastoma (MB), but is distinguished by inactivation of *SMARCB1* (INI-1) or, rarely, *SMARCA4* (BRG1), subunits of the SWI/SNF complex.^{4,5}

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Since the association of AT/RT and loss of *SMARCB1* was made, AT/RT is now widely diagnosed through immunohistochemistry analysis of the absence of INI-1.¹ Current treatment options for AT/RT include surgery, radiotherapy, chemotherapy, or a combination of all three.⁶ Unfortunately, AT/RT is difficult to cure, and historical overall median survival is 8.5 months.⁷ Recent use of intensive multi-agent systemic and intrathecal chemotherapy along with radiation therapy and maximal surgical resection has improved 2-year overall survival to 70%.⁸ Nevertheless, AT/RT remains a devastating tumor and half of all patients will develop progression or recurrence by two years.⁸

INI-1 is a subunit of the SWI/SNF chromatin-remodeling complex.⁹ Biallelic loss of *SMARCB1* can lead to genetic instability, overexpression of cell cycle activators, and tumorigenesis.¹⁰ Widespread genomic characterization of AT/RT has so far documented only DNA alterations in *SMARCB1*, (or the additional SWI/SNF subunit *SMARCA4*), with distinct molecular subgroups at the RNA and epigenetic level.¹¹ Molecular characterization of recurrent or refractory AT/RT is much scarcer in the current literature.

We report a case of recurrent AT/RT in a 12-month old child, for whom we performed molecular characterization, which showed mutation in *CDKN1C* in addition to the hallmark biallelic loss of *SMARCB1*. *CDKN1C* alterations were previously found at the RNA and protein expression level, but not at the genomic level.¹² This finding further stresses the role of the *CDKN1C* pathway in AT/RT. This data allowed us to explore potential personalized therapies for this patient and expose a molecular driver that may be involved in similar cases.

HISTORY

At 6 weeks of age, an infant girl presented with respiratory distress, irritability, lethargy, and a bulging fontanelle. Her past medical history was notable for pneumonia at birth. MR imaging revealed a $4.6 \times 3.0 \times 3.6$ cm right posterior fossa mass with obstructive hydrocephalus (Fig. 1A). She underwent a sub-occipital craniotomy and gross total resection of the tumor.

The tumor histopathologic diagnosis was an atypical teratoid/rhabdoid tumor (WHO grade IV, see Pathology section below). Due to her age and diagnosis, she received adjuvant chemotherapy according to Dana-Farber Cancer Institute pediatric AT/RT protocol, IRSIII, which includes 10 cycles of multi-agent chemotherapy with various combinations of vincristine, cisplatin, etoposide, cyclophosphamide, doxorubicin, temozolomide, actinomycin, and intrathecal cytarabine and hydrocortisone.⁸

During consideration for proton beam therapy, surveillance scans showed evidence of progressive disease, a deep 3 mm lesion not amenable to surgical resection. Chemotherapy was changed to methotrexate, ifosfamide, carboplatin, etoposide; this was followed by triple tandem autologous stem cell transplant with carboplatin/thiotepa conditioning. She was free of new disease for six months, at which time new lesions appeared in the suprasellar region and basal ganglia. The patient began a metronomic therapy regimen with bevacizumab, cyclophosphamide, etoposide, celecoxib, fenofibrate and thalidomide; progressive disease was noted within two months. The patient underwent palliative subtotal resection of a $41 \times$

43 mm right frontal lobe lesion, followed by palliative conformal radiation therapy (20 fractions of 2 Gy for total dose of 40 Gy) localized to the primary residual right frontal lobe lesion. Following completion of radiation, she began single agent therapy with alisertib, an aurora kinase A (AURKA) inhibitor, obtained through compassionate use, and she has remained with stable disease for 12 months.

PATHOLOGY

Histology shows a malignant small round blue cell tumor with focal neuronal rosettes and glandular structures. Immunophenotype was consistent with the diagnosis of AT/RT: INI-1 loss, focal immunoreactivity for epithelial markers (EMA, AM5.2, AE1/AE3), mesenchymal markers (smooth muscle actin, desmin) and neuroglial markers (S100, GFAP, synaptophysin) (Fig. 2A–D).

INTEGRATIVE CLINICAL SEQUENCING

The patient's recurrent tumor (fresh frozen) was entered into the University of Michigan MI-ONCOSEQ study for whole exome (tumor and germline DNA) and transcriptome (tumor RNA) sequencing. Sequencing revealed germline *SMARCB1* exon 7 deletion, plus one copy loss in tumor, consistent with biallelic inactivation. Additionally, the tumor demonstrated a frameshift insertion (p.A17fs) at a variant frequency of 46% in *CDKN1C* (Table 1). Additional research immunohistochemistry performed on recurrent tissue revealed an increase in AURKA expression and reduced CDKN1C expression (Fig. 2E–F), when compared to multiple other pediatric brain tumor specimens (data not shown). Results of her tumor sequencing were discussed in a CNS precision medicine tumor board teleconferenced with clinicians at multiple children's hospitals, members of which made recommendation to consider adjuvant therapy with an aurora kinase A inhibitor, based on recent reports of efficacy in children with recurrent AT/RT.¹³

DISCUSSION

AT/RT is an aggressive infant and childhood brain tumor and despite current optimal multimodal treatment, up to 50% of patients will develop progression or recurrence by two years.⁸ Current therapy for AT/RT combining maximal surgery, cytotoxic chemotherapy, and radiotherapy, while sometimes successful, is not directed at the molecular mechanisms driving AT/RT oncogenesis. Primary AT/RT has been characterized to harbor solitary DNA alterations in *SMARCB1*.¹¹ *SMARCB1/INI1* is an established tumor suppressor and key component of the SWI/SNF chromatin remodeling complex.¹⁴ However, few efforts have been made to molecularly characterize recurrent AT/RT.

We performed clinically integrated sequencing of a recurrent AT/RT, which demonstrated a mutation in *CDKN1C* in addition to the hallmark biallelic loss of *SMARCB1*. Robust loss of CDKN1C was confirmed by immunohistochemistry in the recurrent tumor (Fig. 2F). To our knowledge, this is the first reported mutation in *CDKN1C* in an AT/RT. CDKN1C is a cell cycle inhibitor via inhibition of the Cyclin D/CDK4 or CDK6 complexes involved in the early G₁ cell cycle progression.¹⁵ A loss-of-function mutation in *CDKN1C* is the cause of

the cellular overgrowth in Beckwith-Wiedemann syndrome.¹⁶ Cyclin D appears to play a role in *SMARCB1*-driven AT/RT oncogenesis, since its overexpression has been demonstrated in AT/RT, and genetic ablation of Cyclin D blocks formation of rhabdoid tumors *in vivo*.¹⁷ Interestingly, recent work has shown that *SMARCB1*/*INI1* promotes expression of *CDKN1C* via epigenetic promotor deacetylation. The loss of *SMARCB1*/*INI1* in AT/RT likely results in loss of normal *CDKN1C* tumor suppressive activity and promotion of AT/RT growth.¹⁸ The loss-of-function mutation in *CDKN1C* in our patient's recurrent AT/RT further strengthens this association.

While *CDKN1C* mutations were not seen in a large dataset of sequenced AT/RT, samples in this dataset were from primary, not recurrent tumors.¹¹ Our case raises the possibility that this additional alteration may be seen in other recurrent AT/RT. Loss of *SMARCB1*/*INI1* also promotes rhabdoid tumor cell growth by loss of Aurora Kinase A (AURKA) regulation.¹⁹ AURKA activation has been associated with genetic instability and promotion of tumor cell proliferation.¹⁹ AURKA inhibitors such as alisertib have been used to target *INI1*-deficient tumors, such as AT/RT.¹³ A recent case series documented disease stabilization in four patients with recurrent AT/RT using alisertib as mono-therapy.¹³ It has been demonstrated that *SMARCB1* regulates *CDKN1C* through *LIN28B*, a protein involved in early embryogenesis.¹⁴ Interestingly, *LIN28B* has also been shown to upregulate AURKA, thus possibly tying these pathways together (see schematic, Fig. 3).²⁰ Looking at this pathway, it is not clear that alisertib will impact *CDKN1C* loss. However, due to the previously published efficacy of alisertib in patients with recurrent *INI1*, this therapy was chosen for our patient.

The need for improved therapies targeting the unique molecular features of AT/RT is paramount. We report the case of a child who was found to have a novel loss-of-function mutation in *CDKN1C* in her recurrent AT/RT, strengthening the evidence for its role in AT/RT tumorigenesis. Further clarification of this oncogenic pathway in rhabdoid tumors should enable the development of improved targeted therapies in this patient population.

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FIGURE 1.

(A) MRI of the patient's initial AT/RT at 6 weeks of age, which reveals a large enhancing right posterior fossa mass, primarily extra axial in location, crossing the midline to the left. (B) MRI of the patient's recurrent AT/RT at 1 year of age, demonstrating an enhancing lesion in the deep left frontal lobe inferior to the frontal horn of the left lateral ventricle (one of three metastatic lesions at that time).

FIGURE 2.

(A) Hematoxylin and eosin staining shows a densely cellular tumor with round to oval nuclei with scant eosinophilic cytoplasm. There is mitotic activity, focal gland-like architecture and neuronal rosettes. (B) INI-1 staining is diffusely lost in tumor cells with normal staining in endothelial cells as normal internal control. (C) Smooth muscle actin, focal expression (mesenchymal differentiation). (D) Cam5.2, focal expression (epithelial differentiation). (E) Aurora Kinase A staining is grossly overexpressed in tumor cells. (F) CDKN1C staining is diffusely lost in tumor cells with hematoxylin counter-staining in endothelial cells as normal internal control.

FIGURE 3.

Proposed mechanism of impact of loss of CDKN1C on AT/RT tumorigenesis displaying LIN28B regulation tumor-promoting pathways AURKA and CDKN1C.

TABLE 1

Tumor sequence data obtained from sequencing analysis through the MI-ONCOSEQ program at the University of Michigan.

Event/Gene	Alteration	Comments
Human pathogens	Not detected	
Gene fusions	No driving fusion detected	
chr6q and chr22	Copy loss	
<i>SMARCB1 (INI1)</i>	Germline exon 7 deletion, plus one copy loss in tumor	Loss of SMARCB1 (cyclin D inhibitors, EZH2 inhibitors)
<i>CDKN1C</i>	Frameshift insertion, p.A17fs	Not previously reported in AT/RT but pathway reported to be repressed by loss of SMARCB1