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Case report

Germline ATM mutation and somatic PIK3CA and BCOR mutations found in an infant with aggressive orbital embryonal rhabdomyosarcoma

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ABSTRACT

Purpose: To report a case of aggressive infantile orbital embryonal rhabdomyosarcoma harboring germline ATM mutation and 2 somatic mutations as revealed by next-generation sequencing and the potential application for personalized therapy.

Observations: A 7-month-old male developed a rapidly progressive left proptosis over 6 weeks due to a large medial orbital mass. Biopsy revealed embryonal rhabdomyosarcoma. After the first cycle of chemotherapy, re-imaging showed interval tumor enlargement with intracranial extension. Craniotomy, combined with orbital exenteration, was performed. Tumor specimens and blood samples were sent for 596 gene DNA sequencing panels with RNA-sequencing focused on actionable mutations as well as gene fusion detection. Sequencing revealed 3 clinically relevant mutations: a germline ATM loss-of-function (LOF) mutation, a somatic PIK3CA gain-of-function mutation, and a somatic BCOR LOF mutation. No chromosomal translocation was detected. Workup for metastasis was positive for bone marrow involvement. Despite standard high-dose adjuvant chemotherapy in combination with radiation therapy, the patient died 10 months later with metastatic diseases.

Conclusions and importance: This case highlights an aggressive form of embryonal rhabdomyosarcoma in an infantile orbit. The presence of germline mutation may explain the increased chemo-resistance and adverse prognosis, and may be used as the target for genomic-directed therapy.

1. Introduction

Rhabdomyosarcoma (RMS) is a malignant neoplasm consisting of cells with histopathologic features of striated muscle. RMS is divided into two major histologic subtypes, including embryonal (ERMS) and alveolar (ARMS). Orbital RMS remains the most common primary orbital malignancy in children, and its incidence exceeds all other sarcomas combined.[1,2] The majority of orbital RMS is an embryonal subtype, which portends a good prognosis. However, it is rarely found in infants less than 1-year-old. Although the orbital site is proved to have a favorable prognosis for RMS, local invasion and tumor metastasis may occur, resulting in a poor outcome.[1] Despite a significant improvement in failure-free survival for children with RMS over the past decade, the same improvement in outcome has not been seen for infants.[3,4] It is still unclear why RMS biology and response to therapy in infants differ from those of older children or adults. The authors report a case of a rapidly growing embryonal rhabdomyosarcoma of the infantile orbit that failed conventional therapy. In this case, next-generation sequencing revealed a germline ATM mutation, which could play a role as a cancer predisposing mutation conferring cancer susceptibility, chemo-resistance, and unfavorable prognosis. This study was adherent to the tenets of the Declaration of Helsinki.

2. Case report

A 7-month-old male infant, previously healthy, presented with

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progressive left proptosis over six weeks (Fig. 1A). Orbital imaging revealed a large, well-circumscribed mass at the medial aspect of left orbit without intracranial lesion (Fig. 1B). Anterior orbitotomy via a transcaruncular approach for incisional biopsy of the mass was performed. Pathology and immunohistochemistry results showed round cell neoplasm diffusely marked with myogenin, MyoD1, and desmin (Fig. 2), and stained with PAS with diastase lability, consistent with embryonal RMS. According to the Intergroup Rhabdomyosarcoma Study Group (IRSG),[5] he was classified as a low-risk group (Group IIIa, Stage 1). Hence, systemic chemotherapy following ThaiPOG-RMS-13LR protocol, which consists of vincristine, actinomycin D, and cyclophosphamide (VAC regimen), was inducted.[6]

Two weeks after the first cycle of chemotherapy, he developed rapid tumor progression with exposure keratopathy resulting in severe corneal ulcer and impending globe perforation (Fig. 1C). The repeat computed tomography (CT) imaging revealed an interval increase in tumor size with the newly detected intracranial extension via orbital apex (Fig. 1D). He underwent cranio-orbitotomy combined with orbital exenteration for tumor resection. The bony defect at the orbital apex was reconstructed with temporoparietal pericranial flap. Pathology reported a presence of residual tumor at the inferoposterior margin where it invaded into the anterior cavernous sinus. Further workups for distant metastasis, including bone marrow biopsy, CT of chest, and bone scan, were performed. Bone marrow metastasis was detected. According to the IRSG,[5] the patient was reclassified as a high-risk group (Group IV, Stage 4) for more intensive treatment. He received chemotherapy with ifosfamide, etoposide, carboplatin, and VAC regimen as per ThaiPOG-RMS-13HR protocol in combination with radiation therapy (1.8 Gy x 28 fractions; total 50.4 Gy).[6] During chemotherapy course, patient developed febrile neutropenia and had to postpone his treatment a few times.

After receiving 3 cycles of chemotherapy and completed course of radiotherapy (Fig. 3A), a repeat CT scan revealed progression of the residual tumor at the orbital apex (Fig. 3B) with a new metastatic brain lesion at the right frontal cortex. Eight months after diagnosis, he developed multiple brain, lung, and spine metastases. His treatment plan was changed to palliative care at this stage. The patient died at the age of 17 months, approximately 10 months after the initial diagnosis.

At the time of tumor resection, the tumor specimen and blood sample were sent for comprehensive tumor profiling with 596-gene panel and RNA-sequencing (Tempus xT, Tempus Labs Inc, Chicago, IL, USA) which covers actionable mutations as well as gene fusion. Three clinically relevant mutations identified were a germline ATM LOF (p.Gly696*) mutation, somatic gain-of-function mutation of PIK3CA (p.Asn345Lys) and somatic LOF mutation of BCOR (p.Glu630*), both of somatic mutations have been previously reported in non-fusion related rhabdomyosarcoma. No chromosomal translocation was detected.

3. Discussion

Previous studies have demonstrated that age is an independent prognostic factor in RMS. Although most of RMS present in the first decade of life, it is uncommon in infants. A poorer outcome has been reported for RMS in infants than for older children.[3,4] The study of nonmetastatic RMS patients who received multimodal therapy following the IRSG protocols showed that the estimated 5-year failure free-survival and overall survival rate were 57% and 76% for infants compared with 81% and 87% for children ages 1–9 years, respectively.[4] According to prior studies, prognostic factors related to worse outcomes in infants are alveolar histologic subtype, less aggressive local therapy, and chromosomal translocation.[1,4] Despite the absence of alveolar subtype or chromosomal translocation, our patient had a poor prognosis for survival given the young age of onset, the presence of bone marrow involvement around the time of diagnosis, and eventual development of multiple metastases.

The current treatments of RMS are multimodal, including surgery, radiation therapy, and chemotherapy, depending on tumor staging. Due to recent advances in diagnosis and treatment, significant improvement in survival for children with RMS has been observed over the past decade. On the contrary, a similar improvement in treatment outcome has not been seen for infants.[4] The reason for biological and behavioral differences between infantile RMS and older childhood RMS remains unclear. Comprehensive genomic profiling may elucidate such differences and identify the potential candidate of targeted therapy for treatment selection.

The most remarkable cytogenetic finding of RMS is t (2; 13) or t (1; 13) translocation resulting in PAX3 or PAX7-FOXO1 fusion. It is found in the vast majority of ARMS and is associated with poor prognosis. Our patient did not have chromosomal translocation nor gene fusion. In fusion-negative RMS, a wide range of mutated genes have been identified, including TP53, NRAS, KRAS, PIK3CA, BCOR, CTNNB1, and MyoD1.[7–9] Somatic PIK3CA and BCOR mutations were identified in the tumor of this patient. MyoD1 mutation has been reported in an aggressive form of fusion-negative RMS.[9,10] However, it was not identified during the tumor profiling in this study since the MyoD1 gene was not included in the genome targeted sequencing panel.[11] Interestingly, germline mutations in the ataxia telangiectasia mutated (ATM) gene, which are more commonly reported in lymphoid, breast, and gastric malignancies,[12,13] have rarely been reported in RMS cases.[14]

ATM functions as a cell cycle checkpoint kinase, controlling the rate

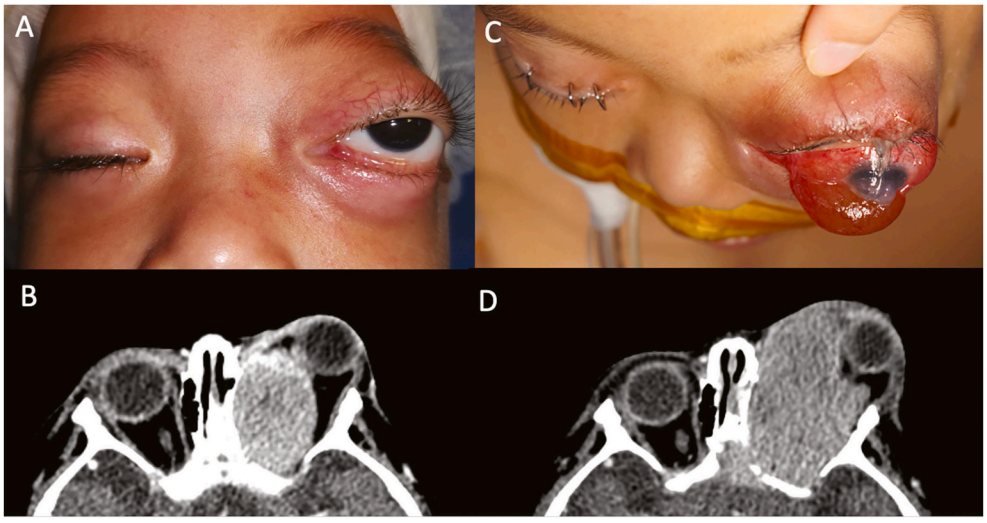

Fig. 1. Preoperative clinical and radiologic images. **A**, Pre-biopsy external photograph showing left proptosis and conjunctival chemosis. **B**, Pre-biopsy axial CT revealing a medial orbital mass. **C**, Post-biopsy external photograph showing interval progression after the first cycle of chemotherapy (1 month after 1A) with severe proptosis, corneal ulcer, and impending globe perforation. **D**, Repeated CT scan revealing progressive enlargement of the medial orbital mass with sinus and intracranial extension via orbital apex.

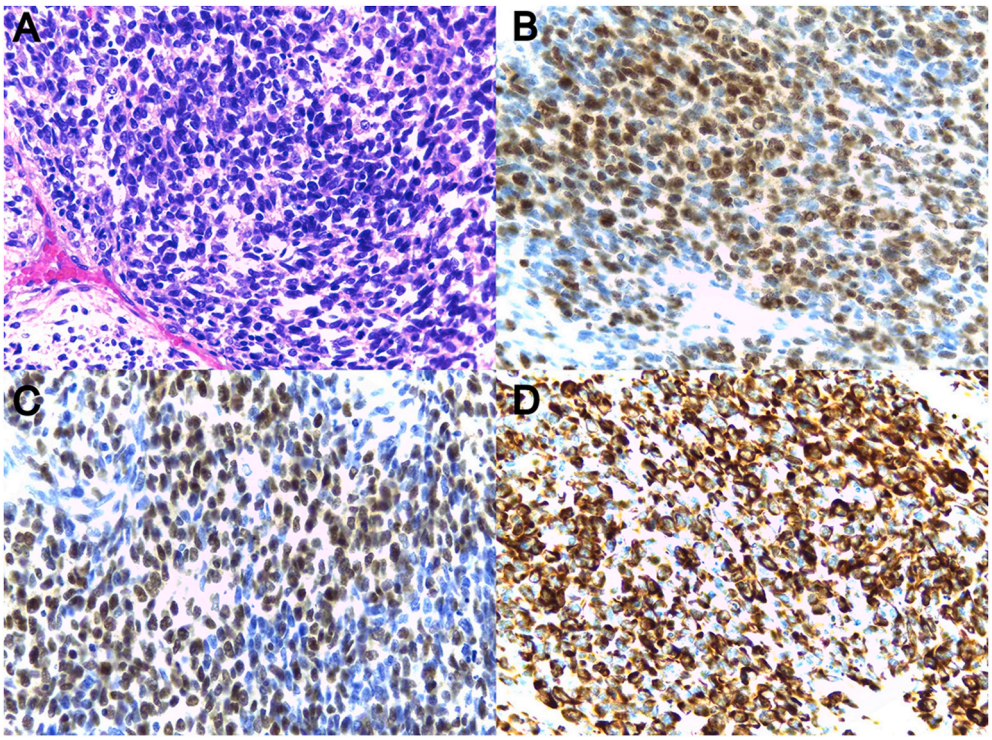

Fig. 2. Pathological examination confirming a diagnosis of embryonal rhabdomyosarcoma. **A**, Hematoxylin and eosin stained section showing small, undifferentiated, hyperchromatic round and spindle cells with scant cytoplasm densely packed in hypercellular area adjacent to a less cellular myxoid area. Immunohistochemical staining revealing diffuse nuclear positivity for myogenin (**B**) and MyoD1 (**C**), and cytoplasmic immunoreactivity for desmin (**D**).

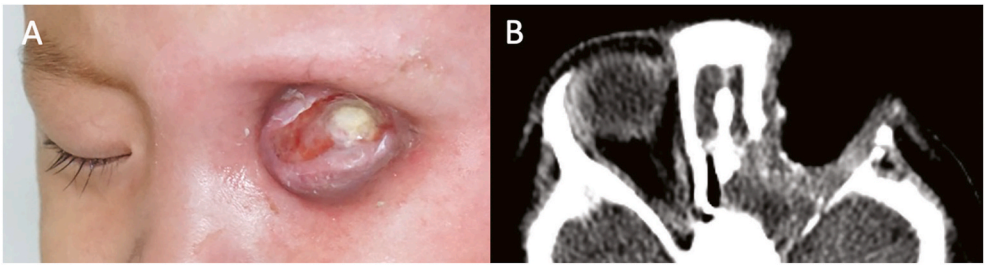

Fig. 3. Postoperative clinical and radiologic images 4 months after the tumor resection. **A**, External photograph showing relatively well-healed left orbital socket. **B**, Postoperative CT revealed the growth of a residual tumor at the left orbital apex and posterior ethmoid. A new metastatic lesion at the frontal cortex is not shown here.

of cell growth and division. Additionally, it plays a significant role in the repair of DNA double-strand breaks by homologous recombination (HR), which is essential for the maintenance of DNA integrity during the replication phase. Loss of function mutation of the ATM gene results in increased cancer susceptibility due to impaired DNA damage response (DDR) and genomic instability. Therefore, the presence of germline ATM LOF mutation in this patient may explain the increased chemoresistance and adverse prognosis. In cancer cells with inadequate HR function, other DNA repair mechanisms such as nucleotide (NER) or base excision repair (BER), all dependent on poly (ADP-ribose) polymerase (PARP), are used to repair damaged DNA. If PARP is inhibited, HR-deficient cells will lack enough DDR function, leading to cancer cell death through synthetic lethality.[12] The concept of synthetic lethality is that cells have evolved a complex DDR, which is in charge of repairing DNA damage and promoting the maintenance of genome integrity. Defects in DDR are associated with increased mutational load and genome instability and are a well-recognized cause of neoplastic transformation and proliferation. Cells harboring DDR defects can become reliant on other repair pathways for survival, which makes DDR targeting an attractive therapeutic strategy. In our case, when the ATM gene is mutated, the cancer cell can still rely on PARP for DNA damage response. Thus, inhibiting PARP will likely lead to cancer cell death. This makes PARP inhibitor, such as olaparib, a potential targeted therapy for cancer patients with ATM mutation. There is an ongoing clinical trial for other pediatric cancer using olaparib, but its use in infantile RMS has not been reported.[15] Additionally, PIK3CA inhibitors could be another group of therapeutic candidates, although their use in RMS is still investigational.[16,17] Unfortunately, our patient was unable to receive these new treatments or enroll in these clinical trials due to geographical and financial constraints.

4. Conclusions

Both germline DNA damage response gene mutation and somatic mutations were found in this unfortunate case of metastatic infantile orbital RMS. In our opinion, for the RMS cases that fail conventional therapy, next-generation sequencing may be offered to detect the precise genetic mutations, which may predict clinical outcomes and identify potential targeted therapy. This technology will likely play a significant role in personalized cancer management in the near future.

Patient consent

Photographs were collected with the parent's written informed consent for publication.

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Authorship

All authors attest that they meet the current ICMJE criteria for authorship.

Intellectual property

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

Research ethics

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

IRB approval was obtained (required for studies and series of 3 or more cases).

Written consent to publish potentially identifying information, such as details or the case and photographs, was obtained from the patient(s) or their legal guardian(s).

Authorship

The International Committee of Medical Journal Editors (ICMJE) recommends that authorship be based on the following four criteria:

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2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Declaration of competing interest

The authors report no conflicts of interest.

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