Diverse outcomes in SMARCB1-deficient rhabdoid tumors: A single institute experience

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Abbreviations:

RT	rhabdoid tumor
ERT	extra-cranial rhabdoid tumor
RTK	rhabdoid tumor of the kidney
AT/RT	atypical teratoid/rhabdoid tumor
MRI	magnetic resonance imaging

Abstract: Rhabdoid tumors (RTs) are a rare and aggressive pediatric cancer that commonly show alterations in the tumor suppressor gene *SMARCB1*. However, RT prognosis is still poor, with no standard treatment, predictive biomarkers for its aggressiveness, or chemo- and radio-sensitivity. Herein, four cases of extra-cranial RTs are described, two of which were in long-term survivors. These two surviving cases were positive for p16, whereas the other two were p16-negative. These findings suggest that p16 expression may represent a potential positive prognostic biomarker in RTs; nevertheless, further studies are required.

Introduction

Rhabdoid tumors (RT) are exceedingly rare and aggressive pediatric tumors that can arise from different tissues. More than 95% of RTs display biallelic alteration of *SMARCB1*.[1] Lack of SMARCB1 expression and either deletion or mutation in its coding gene are used to diagnose RTs.[2,3] Prognosis of RT is generally poor; however, there are examples of long-term survival.[4-6] To date, no standard protocols are available for RT diagnosis and treatment. An exclusively clinical approach is often not appropriate for RT investigation, with additional genetic and molecular analyses being required. In this study, we describe four extra-cranial RT

(ERT) cases, including two long-term survivors, using clinical features, and genetic and immunohistochemical analyses. This information can be used to develop improved combined strategies for diagnosing RT.

Results

Clinical histories of the four ERT cases are summarized in Table 1.

Case 1

A 2-month-old female presenting abdominal distension and gross hematuria, was found to have an intra-abdominal tumor in the left kidney (Supplemental Figure S1A).[7] The left kidney was completely resected and evidence of metastasis to the perinephric lymph-nodes was found. Wilms tumor was initially suspected; however, the tumor pathology revealed round or oval nuclei with prominent nucleoli and eosinophilic inclusions in the cytoplasm, which are characteristic of RT. The patient was initially treated with dactinomycin/vincristine.[8] The diagnosis was changed to RT of the kidney (RTK) after consultation with experts. The patient was treated with dactinomycin/vincristine/doxorubicin and cobalt radiation, followed by vincristine/doxorubicin/cyclophosphamide/etoposide/cisplatin.[9] Despite the treatment, intraperitoneal dissemination occurred, and the patient died 11 months

after the initial diagnosis. Additional analysis of RT tissues revealed no *SMARCB1* expression (Supplemental Figure S2A).

Case 2

A 5-month-old male presented with a mass on his chest wall, which was suspected as cancer suspicious given its rapid growth and structural heterogeneity as determined by magnetic resonance imaging (MRI) (Supplemental Figure S1B).[3,10] The tumor was completely resected and pathologically diagnosed as Ewing sarcoma. Four cycles of alternative chemotherapy with vincristine/doxorubicin/cyclophosphamide and ifosfamide/etoposide were used. The tumor showed aggregated, small, round, or polygonal cells with irregularly shaped hyperchromatic nuclei, prominent nucleoli, and no typical eosinophilic cytoplasmic inclusions. Karyotype and immunohistochemical analyses further revealed that the tumor harbored the t(1;22)(p36;q11.2) translocation, which can cause RT, and was SMARCB1-negative (Supplemental Figure S2B). Hence, the tumor was diagnosed as RT after remission. The tumor locally relapsed on the chest wall 18 months post-treatment, and was completely resected again. Multimodal chemotherapy with vincristine/etoposide, tetrahydropyranyl-doxorubicin, cyclophosphamide, and cisplatin, commonly used for pediatric

patients with advanced neuroblastoma at the time, was started and local radiation was administered. The patient has survived for over 15 years.

Case 3

A 2-month-old male with abdominal distension showed a large tumor in the left kidney detectable by computerized tomography imaging (Supplemental Figure S1C). RTK was suspected because gross hematuria and hypercalcemia were observed. Total left kidney resection was performed. Rhabdoid cells showing enlarged nuclei, prominent nucleoli, and eosinophilic inclusion bodies in the cytoplasm were found in the tumor. No expression of SMARCB1 was detected (Supplemental Figure S2C). After two treatment cycles with carboplatin/etoposide,[11] a metastatic lesion occurred in the right lung (Supplemental Figure S1D). The metastatic tumor was resected and postoperative chemotherapy with vincristine/tetrahydropyranyl-doxorubicin/cyclophosphamide and ifosfamide/etoposide was administered unsuccessfully. The patient died 9 months after diagnosis.

Case 4

A 10-month-old female with fever and recurrent vomiting was found to have a tumor surrounding the ureter and iliac artery and vein by MRI (Supplemental Figure

S1E). After partial resection, the patient was initially diagnosed with Ewing sarcoma. Subsequent RT diagnosis was determined based on pathological findings of prominent nucleoli, oval nuclei with mitotic figures, and SMARCB1-negative expression (Supplemental Figure S2D). Five cycles of alternative therapy with vincristine/doxorubicin/cyclophosphamide and ifosfamide/carboplatin/etoposide, concurrent with local radiotherapy were administered. Complete remission was eventually achieved. The patient has survived for over 11 years without recurrence.

SMARCB1 **and p16 immunohistochemical analyses**

Homozygous deletion of *SMARCB1* was observed in cases 2 and 4, whereas partial deletion of exon 6 occurred in case 1. C157T in codon 53, exon 2, of *SMARCB1* was homozygously present in the primary tumor of case 3, suggesting a copy number-neutral loss of heterozygosity.

p16 expression varied among the four tumors. The tumors of the two deceased cases were p16-negative, whereas strong or partial p16 expression was observed in the two long-term survival cases (Fig. 1).

Discussion

RTs are extremely rare and difficult to diagnose. Negative SMARCB1 immunostaining is generally used to diagnose RTs; however, other SMARCB1-negative tumors have been reported.[12,13] Another RT hallmark is the presence of rhabdoid cells; however, some RTs have very few or none of these cells.[12] Therefore, lack of classic rhabdoid cells within an undifferentiated tumor does not exclude the possibility of RT. Cases 1, 2, and 4 were initially diagnosed as other pediatric malignancies and later RT diagnosis was supported by *SMARCB1* alteration and central pathological review. RTs are currently meticulously diagnosed according to patient age, clinical history, tumor site, SMARCB1 loss, and pathological observations, including presence or absence of rhabdoid cells.

Classification of RTs is controversial. Atypical teratoid/rhabdoid tumors (AT/RT) can be classified as three distinct molecular subtypes: SHH, TYR, or MYC.[14,15] Chun et al.[16] recently reported that the AT/RT-MYC-like subgroup is molecularly similar to ERTs. Additionally, AT/RT-MYCs occur in older children compared to other AT/RT subgroups.[17] Another study identified age at diagnosis as the most important RT prognostic factor.[18] Based on these observations, MYC-expressing ERTs may be relatively curable with multimodal treatments. Noteworthy, Frühwald et al.[15] reported that AT/RT-TYR patients aged ≥1 year have the best prognosis

among the three subgroups. All cases described herein showed cMYc weak-positive/negative staining (Supplemental Figure S3). Further studies are necessary to clarify the distinct clinical, biological, genetic, and molecular features of the different RT types.

RTs commonly show reduced *p16* expression due to alterations in SMARCB1, a direct regulator of *p16*-promoter.[19] Interestingly, both long-term survival cases had p16-positive tumors, whereas the other two cases had p16-negative tumors. Furthermore, absence of genomic alterations, other than in *SMARCB1*, is observed in RTs.[20,21] Venneti et al.[22] reported 64% of ERTs as p16-positive, compared with 32% of AT/RTs, and discussed the discrepancies in downstream regulators of *p16*. The clinical outcome of these cases was not shown, however, the RTs were diverse. This result is consistent with our observations for p16 status and suggests the complexity of the pathways that regulate not only the cell cycle but also the chemo- and radio-sensitivity of the tumors.

In our cases, the two RTK patients (cases 1 and 3) were younger at diagnosis and their tumors were extremely aggressive. The other two extra-renal ERT patients (cases 2 and 4) were older at diagnosis, underwent complete tumor resection and local radiation, and survived long-term. Our cases indicate that p16

expression in RT is useful for predicting chemo- and radio-sensitivity, although further immunohistochemical data from a larger sample are needed to confirm it. To improve the clinical, biological, and molecular understanding of RTs, international collaboration is required to comprehensively collect these rare tumors, treat patients with comparable therapies, and analyze the tumors molecularly and pathologically. The European Rhabdoid Registry has recently provided uniform treatment regimens for all AT/RT, RTKs, and soft-tissue RTs, which will help in this endeavor.[23]

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical

restrictions.

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Legends

Figure 1 Immunohistochemical staining for p16 in cases of rhabdoid tumor. Cases 1 (A) and 3 (C) are p-16 negative, whereas cases 2 (B) and 4 (D) are p-16 positive. All sections were stained simultaneously. Magnification: ×40, Scale bar: 50 μm.

Table 1 Clinical characteristics of four rhabdoid tumor cases

Supplemental Figure S1 Images of rhabdoid tumors in each case. (A) Tumor in the left kidney visualized by computerized tomography imaging (CT) (case 1). (B) Large protruding tumor on the chest wall visualized by magnetic resonance imaging (MRI) (Arrow, case 2). (C) Large tumor in the left kidney, CT (case 3). (D) Lung metastatic lesion, CT (Arrow, case 3). (E) Heterogeneous mass in the pelvis, MRI (case 4).

Supplemental Figure S2 BAF47/SMARCB1-negative tissues in rhabdoid tumors following immunohistochemical staining. (A) Case 1, (B) Case 2, (C) Case 3, (D) Case 4. All sections were stained simultaneously. Magnification: ×40, Scale bar: 50 μm.

Supplemental Figure S3 Immunohistochemical staining for cMyc in rhabdoid tumors. Weakly positive in the tumors of case 1 (A), case 2 (B), and case 4 (D), and negative in case 3 (C). All sections were stained simultaneously. Magnification: ×40, Scale bar: 50 μm.