

Rhabdomyosarcoma arising from retroperitoneal teratoma in an infantile neurofibromatosis type 1 patient

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Key Words:	electron microscope, isochromosome 12p, Neurofibromatosis 1, rhabdomyosarcoma, retroperitoneal, pediatric		
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ABSTRACT

We herein report the case of a 2-year-old girl with neurofibromatosis type 1 (NF1), who presented with a 12-cm mass in the right retroperitoneum and underwent tumor resection. Histologically, the tumor was composed of two distinct components: one was teratoma, showing mature morphology; and the other was embryonal rhabdomyosarcoma. An interphase fluorescence *in situ* hybridization (FISH) analysis of the rhabdomyosarcoma component revealed the absence of isochromosome 12p. Although it is well-known that rhabdomyosarcoma occurs in infantile NF1, and that rhabdomyosarcoma can arise from teratoma as a somatic-type malignancy, to the best of our knowledge, this is the first case of an infantile NF1 patient, who developed rhabdomyosarcoma within a retroperitoneal teratoma. The absence of chromosome 12p alteration suggests a possibility that the rhabdomyosarcoma occurred due to the NF1 background, not as a somatic-type malignancy of germ cell tumor.

Key words:

electron microscope, isochromosome 12p, Neurofibromatosis 1, rhabdomyosarcoma, retroperitoneal, pediatric

INTRODUCTION

Neurofibromatosis type 1 (NF1) is an autosomal-dominant hereditary disorder caused by heterozygous mutation of the tumor suppressor gene, *NF1*. *NF1* codes for neurofibromin which downregulates the rat sarcoma viral oncogene homologue (RAS)/mitogen-activated protein kinase (MAPK) pathway and other pathways associated with cellular proliferation; however, the function is insufficient in patients with NF1. NF1 is characterized by the presence of café-au-lait spots, neurofibroma, and an increased risk of several types of benign and malignant tumor. Rhabdomyosarcomas are the most frequent malignant tumor among young children with NF1.¹

Teratomas commonly occur at the gonads, followed by extragonadal sites, including the mediastinum, retroperitoneum, sacrococcyx and intracranium. Occasionally, non-germ cell-type malignancy arises secondary to teratoma; this is referred to as teratoma with somatic-type malignancy. The somatic-type malignancy can be carcinoma or sarcoma, and one of the most frequent histological types is rhabdomyosarcoma.²

In this report we describe an extremely rare case of an infantile NF1 patient who developed rhabdomyosarcoma in a retroperitoneal teratoma. In the present case, the rhabdomyosarcoma showed characteristics of an NF1-associated malignant tumor, as well as teratoma with somatic-type malignancy. Each of the two phenomena is well known, however, we found no previous reports describing the occurrence of these two situations in a tumor.

CLINICAL SUMMARY

The patient was a 2-year-old girl with a family history of NF1 (her mother, brother and herself). She has no significant growth or development abnormalities with the exception of mildly short height. She was admitted to our hospital with a right upper abdominal mass and appetite loss. She

had multiple café-au-lait spots on her skin, but no neurofibromas. A laboratory test showed mild anemia (Hemoglobin 95 g/L) and a mildly elevated neuron-specific enolase (NSE) level (17.5 ng/mL). Alpha-fetoprotein (AFP) was within the normal limit (2.4 ng/mL).

Abdominal CT showed a right retroperitoneal mass (Fig. 1a). Neither lymph node metastasis nor distant metastasis were detected on FDG-PET/CT. The retroperitoneal mass was almost completely surgically resected, with focal capsular rupture. After surgery, she received VAC chemotherapy (vincristin, actinomyocin D and cyclophosphamide), standard therapy for rhabdomyosarcoma of unfavorable tumor site, and radiation therapy for local control. No recurrence was detected in the 9 months after surgery.

PATHOLOGICAL FINDINGS

The tumor measured $12 \times 10 \times 7.5$ cm in size. Macroscopically, approximately 30% of the tumor (mainly located at the periphery of the tumor) had a fatty yellow color. The remaining 70% of the tumor had a tan-white color with focal hemorrhage and necrosis (Fig. 1b).

A histological examination revealed two distinct components in the tumor. The gross examination of the yellow fatty lesion predominantly consisted of mature-appearing adipose tissue (Fig. 1c), mixed with foci of various histological types, including epidermal squamous cell nests, salivary glands, chondroid, glial and skeletal muscle tissue (Fig. 1d,e). Neither cytological atypia nor neuroepithelial rosettes was recognized. The gross examination of the tan-white lesion showed a mixture of dense and loose cellularity with myxoid stroma (Fig. 2a), and a dense proliferation of tumor cells with necrotic foci could also be detected (Fig. 2b). On a high-power view, the tumor cells had a short spindle to small round shape with hyperchromatic nuclei and scant cytoplasm (Fig. 2c). The myxoid area consisted of stellate cells with eosinophilic cytoplasms (Fig. 2d). Mitotic cells were frequently observed. Most of the components were composed of undifferentiated mesenchymal cells, but small areas with rhabdomyoblastic differentiation were present, displaying atypical large round cells with abundant densely eosinophilic cytoplasm, obvious nucleoli, focal multinucleation and characteristic cross-striation (Fig. 2e,f). No alveolar growth pattern was detected. We could not find other germ cell tumor components, or organoid mixtures of varied elements.

The boundary of the two tumor components was obscure, and infiltrative growth of dense primitive cells into the intratumoral adipose tissue was recognized (Fig. 1f).

Immunohistochemistry, electron microscopy & FISH

Immunohistochemical staining of Desmin (DE-R-11, Leica Biosystems, Nussloch, Germany), MyoD1 (EP212, Roche, Basel, Switzerland) and Myogenin (F5D, Dako, Glostrup, Denmark) was positive for both eosinophilic cells showing rhabdomyoblastic differentiation and undifferentiated spindle to round cells (Fig. 3a,b,c). Myogenin positivity for undifferentiated mesenchymal cells was 55 % at hot spot. Tumor cells exhibited heterogenous H3K27me3 (C36B11, Cell Signaling Technology, Danvers, MA) expression (Fig 3d). Staining for S100 protein (2A10, Immuno-Biological Laboratories, Gunma, Japan) was almost negative, but tiny foci of positive cells could be found, suggesting the involved background neural elements (Fig. 3e).

The tan-white lesion of the tumor was also observed under a transmission electron microscope. The surgical specimen was fixed in mixture of 1.5% formaldehyde and 1% glutaraldehyde, followed by secondary fixative of 2% osmium tetroxide. Then, the tissue was embedded in EPON 812 Resin, cut into 90-nm sections, and stained with uranyl acetate and lead

citrate. The ultrastructural observation was made with an H-7650 electron microscope (Hitachi High-Technologies, Tokyo, Japan). On electron microscopy, bundles of straight filaments were found at the cytoplasm of the tumor cells (Fig. 4a,b). These filaments might represent primitive Z-bands, but we could not confirm the myoblastic differentiation by ultrastructural findings alone.

A fluorescence *in situ* hybridization (FISH) analysis was performed using 4-µm-thick slides of formalin-fixed, paraffin-embedded tumor tissue with commercially available CEP12 (Vysis CEP 12 SpectrumOrange, Abbott Molecular, Des Plaines, IL, USA) and a 12p telomeric probe (TelVysion 12p SpectrumGreen, Abbott Molecular), according to the manufacturer's instructions on ThermoBrite (Leica Biosystems). A FISH analysis of the undifferentiated cell component revealed the absence of 12p overexpression and isochromosome 12p (i12p) (Fig. 4c).

Consequently, we diagnosed the two components of the tumor as teratoma and embryonal rhabdomyosarcoma, respectively. The macroscopic appearance suggested that the rhabdomyosarcoma component arose from the teratoma which developed previously, rather than from a collision of two tumors.

DISCUSSION

The present report described a retroperitoneal mass occurring in an infantile NF1 patient that was composed of teratoma and an associated undifferentiated tumor component with focal scattered rhabdomyoblasts. We diagnosed the latter component as somatic-type malignancy which arose from the teratoma, but one of the differential diagnosis might be that the component was one of the features of teratomatous mesenchyme with atypical, immature appearance. According to the WHO classification of Tumours of the Urinary System and Male Genital Organs, somatic-type

malignancies in teratoma typically consists of a pure population of atypical mesenchymal of epithelial cells and occupies at least one low-power filed. In contrast, teratoma consists of somewhat organoid admixtures of varied elements and is often mixed with other germ cell tumor components. Because the undifferentiated tumor component in our cases occupied more than 10 cm in diameter and did not contain other germ cell tumor components or organoid elements other than rhabdomyoblastic differentiation, we concluded that the undifferentiated tumor component was somatic-type malignancy in teratoma.

Another differential diagnosis of the undifferentiated tumor component was malignant peripheral nerve sheath tumor (MPNST) with rhabdomyoblastic differentiation, a so-called "malignant triton tumor". Although rhabdomyoblasts in malignant triton tumor show positive immunostainings for Desmin, MyoD1 and Myogenin, MPNST component other than rhabdomyoblasts typically does not show immunoreactivity for these myogenic markers. In contrast, tumor in our case showed constant positivity for Desmin, MyoD1 and Myogenin to the undifferentiated mesenchymal cells, as well as to the rhabdomyoblasts. Apparent immunoreactivity for markers of skeletal-muscle differentiation indicated the diagnosis of rhabdomyosarcoma, though ultrastructural findings were not sufficient to prove myogenic differentiation. One might also doubt the diagnosis of rhabdomyosarcoma because nearly half of the tumor cells in immunostained slides was negative for myogenic markers. However, Morotti RA reported that 84% cases of embryonal rhabdomyosarcoma displayed immunostaining with MyoD1 in less than 50% tumor cells.³ The distribution of Myogenin was also patchy in the majority of embryonal type rhabdomyosarcoma.³ Therefore, the immunopositivity of myogenic markers in our case was not unusual for that of embryonal rhabdomyosarcoma.

Although several types of tumor can occur in NF1 patients, the disease onset is associated

with age to some degree. In NF1 patients, rhabdomyosarcoma mostly occurs during childhood (mean age: 2.5 years),⁴ while MPNST generally occurs after the twenties.⁵ The diagnosis of rhabdomyosarcoma in our case corresponded with these age-related predilections. Crucis et al. reported that rhabdomyosarcoma occurring in patients with NF1 was an invariable embryonal histology, and that it was associated with a rather good outcome with a 5-year overall survival rate of 87%.⁴

Germ cell tumors are well known to have chromosome 12p overexpression or i12p, and teratoma with somatic-type malignancy also shows frequent 12p alteration.⁶ However, descriptions of teratoma with somatic-type malignancy are mostly separated in individual organs, and there are few reports on cases involving pediatric patients. We summarized the previous case reports of teratoma with somatic-type malignancy in children published between 2008 and 2017 (Table). According to these studies, 14 of 17 cases were female, and none of the cases arose in the testis. Three cases showed rhabdomyosarcoma as a somatic-type malignancy, at least two of them developed distant metastases, and all the three died of their disease. The results suggested that rhabdomyosarcoma arising from teratoma is associated with an adverse prognosis in pediatric patients. Unlike our case, none of the cases were affected by NF1, and none of the studies investigated chromosome 12p alteration.

Regarding the relation of NF1 and teratoma, only 2 cases of teratoma arising from NF1 were reported on the literature before. One case was congenital retroperitoneal teratoma,¹⁷ and another case was testicular teratoma of a 23-year-old man with inguinal herniorrhaphy history.¹⁸ These rare occurrences seemed to be not enough to suggest the association of NF1 and teratoma.

Although the mechanism of developing rhabdomyosarcoma in teratoma of NF1 patient was not precisely elucidated, embryonal rhabdomyosarcoma in general population frequently acquire mutations along Ras pathway, including NRAS, HRAS, KRAS, NF1.¹⁹ On the other hand, genomic analysis of germ cell tumors revealed frequent mutations and amplifications of KRAS, which located on chromosome 12p, as well as almost universal chromosome arm 12p gain.²⁰ Therefore, we expected the involvement of both germline NF1 mutation and 12p alteration for developing rhabdomyosarcoma in present case, but it did not show 12p alteration. The lack of 12p alteration in our case possibly indicate that the secondary tumor arose due to the NF1 background, not as a somatic-type malignancy of a 12p-related germ cell tumor. If so, our case might be expected to have a better prognosis, similar to that of rhabdomyosarcoma in the NF1 population.

In this report, we presented an extremely rare case of rhabdomyosarcoma arising from a retroperitoneal teratoma in an infantile NF1 patient. Our case showed the possibility that teratoma in an NF1 patient might have the potential to develop rhabdomyosarcoma unrelated to 12p alteration; however, the accumulation of other cases is needed.

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DISCLOSURE STATEMENT: None declared.

AUTHER CONTRIBUTIONS:

A.A., K.M., S.Y. participated in conception and design of the study; A.A. drafted the manuscript; A.A., K.M., C.F., M.K., S.N., N.K., T.N. performed the pathological and immunohistochemical interpretation of the tissues; T.T. performed FISH analysis.

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Case [Ref. No]	Age	Gender	Primary location	Histology of germ cell tumor	Histology of secondary somatic tumor	Metastases	Outcome
1 [7]	0	М	Sacrococcygeal	Mature teratoma	Neuroblastoma	NA	NED
2 [7]	0.3	F	Sacrococcygeal	Mature teratoma	Papillary adenocarcinoma	NA	DOD
3 [7]	1.5	М	Retroperitoneum	Immature teratoma	Rhabdomyosarcoma, Liposarcoma, Chondrosarcoma, Neurogenic sarcoma	NA	DOD
4 [8]	2	F	Mandible	Mature cystic teratoma	Squamous cell carcinoma	None	NED
5 [9]	2	М	Posterior fossa	Immature teratoma	Medulloblastoma	None	DOD
6 [10]	3	F	Sacrum	Mature teratoma	Primitive neuroectodermal tumor	None	NED
7 [7]	3	F	Ovary	Mature teratoma	Adenocarcinoma	NA	NED
8 [11]	3	F	Retroperitoneum	Teratoma	Nephroblastoma	None	NED
9 [7]	4	F	Sacrococcygeal	Immature teratoma	Adenocarcinoma	Lung, Liver	DOD
10 [12]	5	F	Sacrococcygeal	Teratoma	Sarcoma	None	NED
11 [13]	5	F	Sacrococcygeal	Mature teratoma	Malignant steroidogenic tumor	None	NED
12 [14]	6	F	Sacrococcygeal	Mature cystic teratoma	Neuroblastoma	Bone	DOD
13 [7]	10	F	Retroperitoneum	Immature teratoma	Neuroblastoma	NA	DOD
14 [15]	12	F	Ovary	Mature cystic teratoma	Primitive neuroectodermal tumor	None	NED
15 [16]	12	F	Mediastinum	Mature cystic teratoma	Embryonal rhabdomyosarcoma	Bone, Kidney, Liver	DOD
16 [7]	13	F	Ovary	Yolk sac tumor + Mature teratoma	Adenocarcinoma	NA	DOD
17 [7]	13	F	Mediastinum	Mature teratoma	Embryonal rhabdomyosarcoma	Bone	DOD
Present Case	2	F	Retroperitoneum	Teratoma	Embryonal rhabdomyosarcoma	None	NED

Abbreviations: Ref, Reference; M, male; F, female; NA, not available; NED, no evidence of disease; DOD, died of disease.

Table / Figure legends

Figure 1:

(a) Abdominal computed tomography demonstrated a right retroperitoneal mass (arrows). (b) The post-fixation cut surface of the tumor. The tumor was composed of two components: the tanwhite region represents the rhabdomyosarcoma component (R); and the fatty yellow region with cartilaginous foci represents the teratoma component (T). The teratoma component showed mature-appearing adipose tissue (c), cartilage, glands (d) and skeletal muscle (e). (f) A lowpower-view of the boundary region of the two distinct components.

Figure 2:

The rhabdomyosarcoma component showed alternating cellular and myxoid pattern (a) with several necrotic foci (b). (c) Mitotically active undifferentiated cell proliferation. (d) Stellate cells with eosinophilic cytoplasms in myxoid areas. (e) Focal rhabdomyoblastic differentiation with a elie4 few cross-striations (f).

Figure 3:

Immunohistochemical analyses of the rhabdomyosarcoma component of the tumor. The tumor cells expressed Desmin (a), MyoD1 (b) and Myogenin (c). They also showed partial nuclear staining for H3K27me3 (d). Limited areas of S100 protein-positive cells was could be recognized, suggesting the involved background neural elements (e).

Figure 4:

Electron microscopic and fluorescence in situ hybridization (FISH) analyses of the

rhabdomyosarcoma component of the tumor. (**a**) Electron microscopy showed a bundle of filaments in the tumor cells, but indefinite for rhabdomyoblastic differentiation (N, nucleus). (**b**) A higher magnification view of the boxed area in Fig. 2**a**. (**c**) FISH showed 2 red signals (chromosome 12 centromere) and 2 green signals (telomeric regions of 12p), indicating the absence of 12p overexpression and isochromosome 12p.









