Case study

Pediatric soft tissue tumor of the upper arm with LMNA–NTRK1 fusion

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Summary

A 6-year-old girl was admitted to our hospital because of the presence of a slow-growing tumor in her right elbow. Biopsy specimens showed a round to spindle cell neoplasm with uncertain malignant potential, leading to the decision of surgical resection. Histologically, the resected tumor was encapsulated by fibrous tissue but focally invaded the surrounding skeletal muscles. The tumor was composed of ganglion cell–like short spindle cells with lymphocytic infiltration in the collagenous background. Tumor cells with large bizarre nuclei were occasionally observed, and multinucleated giant cells were scattered at the periphery. Hemangiopericytoma-like patterns and adipose tissue elements were not evident, and mitotic figures were rarely observed (<1 per 10 high-power fields). Immunohistochemically, the tumor cells were positive for S-100 and CD34 and focally positive for epithelial membrane antigen and AE1/AE3. RNA sequencing and subsequent reverse-transcription polymerase chain reaction revealed alternative splicing forms of LMNA–NTRK1 fusion (Ex2-Ex10 and Ex2-Ex15).

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1. Introduction

Recent genome-wide and RNA sequence analyses have constantly identified new fusion genes in soft tissue tumors previously thought to be of unknown origin without characteristic histological features, leading to the establishment of certain tumor entities. Doebele et al [1]...
described that NTRK1 fusions were detected in only 5 of 1272 soft tissue sarcomas. A recent study reported that of 2031 tumors, comprising leukemias, solid tumors, and primary central nervous system tumors and identified in patients under the age of 21 years, 9 harbored NTRK fusions; 5 of these 9 tumors were identified to have NTRK1 fusions [2]. NTRK1 fusions in soft tissue sarcomas are extremely rare; however, they tend to be preferentially observed in pediatric patients [1]. These tumors with NTRK1 fusions, especially LMNA-NTRK1 fusions, have been described to be fibrosarcoma, fibroblastic tumors with hemangiopericytoma- or myopericytoma-like patterns with a relatively higher mitotic rate, or benign to low-grade neuroectodermal tumors [1,2]. Another tumor entity, lipofibromatosis-like neural tumor with a lower mitotic rate, has recently been described [3]. In addition, a recent manuscript described a case of generalized eruptive histiocytosis associated with an LMNA-NTRK1 fusion [4]. We encountered a case of a soft tissue tumor, arising from the right upper arm (elbow) of a 6-year-old girl, detected with LMNA-NTRK1 gene fusion that did not correspond well with the descriptions of either of these abovementioned tumor entities.

2. Materials and methods

2.1. Immunohistochemistry

Immunohistochemical staining was performed using the streptavidin-biotin method [A], with antibodies to the following: epithelial membrane antigen (EMA) (Clone: E29, Dako, Glostrup, Denmark), S-100 protein (Clone: Rabbit Poly, Dako), CD34 (Clone: QBEnd/10, Leica Biosystems, Newcastle, UK), p53 (Clone: DO-7, Dako), and Ki-67 (Clone: MIB-1, Dako). The Ki-67 labeling index was evaluated in representative areas showing the highest immunoreactivity by counting the number of positive cells among 1000 tumor cells.

2.2. RNA-seq

We could not obtain an appropriate histological diagnosis for this tumor with immunohistochemical examination. Because we had been performing extensive RNA-seq analysis to detect recurrent genetic alterations or gene fusions in otherwise histologically undifferentiated sarcomas, we sent this case for RNA-seq. Total RNA was extracted from snap-frozen tissue samples, treated with DNase I (Thermo Fisher Scientific, Waltham, MA), and then subjected to poly(A)-RNA selection that was used for cDNA synthesis. Library preparation for RNA-seq was conducted with NEBNext Ultra Directional RNA Library Prep Kit (New England Bio Labs Inc., Tokyo, Japan) according to the manufacturer’s protocol. Next-generation sequencing was carried out from both ends of each cluster using the HiSeq2500 platform (Illumina, San Diego, CA, USA). The deFuse pipeline (https://bitbucket.org/dranew/defuse) was used to detect gene fusions [5].

2.3. Reverse-transcription polymerase chain reaction

To confirm the finding obtained by RNA-seq, we also performed-reverse transcription polymerase chain reaction (RT-PCR). Using extracted total RNA, cDNA was synthesized with SuperScript First-Strand Synthesis System (Thermo Fisher Scientific, Waltham, MA) for RT-PCR [6]. RT-PCR was performed according to the manufacturer’s protocol as follows: initial denaturation at 94°C for 2 minutes and 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C

Fig. 1  Magnetic resonance imaging shows an intramuscular mass with slightly high intensity in the T1-weighted image (A) and with high intensity in the T2-weighted image (B).
for 30 seconds, followed by 72°C for 2 minutes. The PCR product was electrophoresed on a 2% agarose gel. PCR products with the appropriate and expected sizes were excised from the agarose. Purified PCR products were sequenced with dideoxynucleotides (BigDye Terminator v3.1; Applied Biosystems, Foster City, CA) and specific primers, purified using a BigDye

**Fig. 2** Histology from biopsy specimens. The tumor is composed of proliferating spindle-shaped cells in the collagenous background. A, The ganglion cell–like cells are scattered throughout the lesion along with lymphocytic infiltration. Immunohistochemically, the tumor cells are diffusely positive for CD34 (B), and scattered cells are positive for S-100 protein (C). D, The tumor cells are focally positive for EMA. A-D, original magnification ×200.

**Fig. 3** A, Low-power view of the resected specimen shows a monotonous tumor with collagenous stroma. B, The tumor is composed of proliferating plasmacytoid tumor cells with lymphocytic infiltration. Some tumor cells have enlarged hyperchromatic nuclei (C), and a few mitotic figures are also observed (D). A, ×100; B and C, ×200; D, ×400.
X Terminator Purification Kit (Applied Biosystems), and analyzed with a capillary sequencing machine (3730xl Genetic Analyzer; Applied Biosystems). The primer sequences used are as follows: \( \text{LMNA} \) exon 2, 5'-ACCAAGAAGGGTGACCT-3'; \( \text{NTRK1} \) exon 10, 5'-CAAGGAGCAGCGTAGAAAGG-3'; exon 13, 5'-CGTGCCGCATATACTCAAAG-3'; and exon 15, 5'-ATGATGCGGTGACACTCTGG-3'. Three independent PCRs were performed using a combination of the \( \text{LMNA} \) primer and either of the \( \text{NTRK1} \) primers to detect \( \text{LMNA-NTRK1} \) fusion.

3. Case report

A 6-year-old girl noticed a mass on her right elbow in January 2016 and was admitted to our hospital for a slow-growing tumor. The tumor was approximately 5 cm in diameter. Tenderness was observed, but Tinel’s sign was not evident. Magnetic resonance imaging revealed a mass with slightly high intensity on T1-weighted imaging (Fig. 1A) and high intensity on T2-weighted imaging (Fig. 1B). A biopsy was performed, and on histological examination, the specimen...
showed proliferation of round- to spindle-shaped cells (Fig. 2A). Immunohistochemistry revealed that the tumor cells were diffusely positive for CD34 (Fig. 2B), partially positive for S-100 protein (Fig. 2C), and focally positive for EMA (Fig. 2D), leading to a tentative diagnosis of a hybrid nerve sheath tumor. However, the possibility of a round to spindle cell neoplasm with uncertain malignant potential could not be ruled out. Therefore, surgical resection of the tumor was performed.

Grossly, the cut surface of the solid tumor was white to yellowish in color, without apparent bleeding and necrosis. Histologically, the resected tumor was encapsulated by fibrous tissue but focally invaded the surrounding skeletal muscles. The tumor was uniform throughout the lesion (Fig. 3A). The tumor was composed of ganglion cell–like short spindle cells with lymphocytic infiltration in the collagenous background (Fig. 3B). Tumor cells with large bizarre nuclei were occasionally observed (Fig. 3C), and multinucleated giant cells were scattered at the periphery. Hemangiopericytoma-like patterns were not evident, and an admixture with adipose elements was also not observed. Mitotic figures were rarely observed (<1 per 10 high-power fields) (Fig. 3D). Immunohisto-
chemically, the tumor cells were positive for CD34 (Fig. 4A) and S-100 protein (Fig. 4B), focally positive for EMA (Fig. 4C) and AE1/AE3, but negative for other markers including myogenic markers. Tumor cells were immunohistochemically also positive for p53 (40% of tumor cells), and the MIB-1 labeling index was approximately 25% (Fig. 4D). However, we could not obtain an appropriate histological diagnosis for this tumor.

Frozen tissue samples were available for this case, and RNA sequencing revealed the presence of an LMNA-NTRK1 fusion transcript. Subsequent RT-PCR showed alternative splicing forms of LMNA-NTRK1 (Ex2-Ex10 and Ex2-Ex15) (Fig. 5A-C).

The patient is presently alive and well, 10 months after surgery, without recurrence or metastasis.

4. Discussion

Recent genome-wide and RNA sequence analyses have identified new fusion genes in tumors, including soft tissue tumors, previously thought to be of unknown origin without characteristic histological features. NTRK1 fusions in soft tissue sarcomas are extremely rare. Doebele et al [1] described that NTRK1 fusions were detected in only 5 of 1272 soft tissue sarcomas; 3 of these patients were less than 5 years old. Furthermore, only 1 among the 5 cases was an LMNA-NTRK1 fusion-positive fibrosarcoma [1]. In addition, a recent study demonstrated that of 2031 tumors, comprising leukemias, solid tumors, and primary central nervous system tumors and identified in patients under the age of 21 years, 5 tumors harbored NTRK1 fusions and that 2 of these 5 tumors were identified to have LMNA-NTRK1 fusions [2]. However, the frequency of NTRK1 fusion sarcomas in Japan is still not clear. We could not obtain an appropriate histological diagnosis in this case and were unaware of the fact that this tumor could harbor NTRK1 fusion. We incidentally identified this case to be having LMNA-NTRK1 fusion by RNA-seq, and we also confirmed this finding by RT-PCR.

Regarding the soft tissue tumors with NTRK1 fusion, a recent study has reported 2 pediatric cases of soft tissue sarcomas with NTRK1 fusion, one of which had an LMNA-NTRK1 fusion and another had TPM3-NTRK1 fusion [7]. Another recent study described a case of infantile fibrosarcoma with LMNA-NTRK1 fusion in a 1-month-old infant who showed remarkable response to the tyrosine kinase inhibitor (TKI) crizotinib [8]. Two soft tissue tumors with LMNA-NTRK1 fusion have been described in a recent report [2]. One of them was a fibrosarcoma arising in a 1-year-old boy, and the other was a benign to low-grade neuroectodermal tumor in a 14-year-old adolescent girl [2]. Two of these three abovementioned pediatric fibroblastic tumors with LMNA-NTRK1 fusion showed high cellularity of spindle cells, suggesting that these tumors were low-grade sarcomas. The remaining case with LMNA-NTRK1 fusion was described to be a metastatic fibrosarcoma without information on the primary tumor [2]. Two pediatric cases with NTRK1 fusion showed histological hemangiopericytoma and myopericytoma patterns and high mitotic rates [7]. However, in our case, hemangiopericytoma and myopericytoma patterns were not evident, and mitosis was rarely observed. The two aforementioned cases were described to be immunohistochemically positive for myogenic markers, but our case was negative for myogenic markers. However, the current case was positive for CD34 and S-100 protein. It would be interesting to note that CD34 positivity is reminiscent of infantile fibrosarcoma with NTRK3 fusion [9] and that the LMNA-NTRK1 fusion, detected in the current study, has been reported in spitzoid melanomas with neurogenic differentiation [10].

In view of the proliferation of spindle-shaped cells with lymphocytic infiltration, we considered the possibility of inflammatory myofibroblastic tumor, of which a subset has been shown to have an ETV6-NTRK3 fusion [11,12]. However, in our case, this possibility was ruled out based on the absence of immunohistochemical positivity for myogenic markers.

A recent study demonstrated a subset of soft tissue tumors with distinct lipofibromatosis (LPF)–like morphology with NTRK1 fusions, with predominance of LMNA-NTRK1 fusions [3]. These tumors were described to occur in children and adults ranging from 4 to 36 years old and were immunohistochemically positive for S-100 and CD34. Furthermore, these tumors showed lower mitotic rates [3]. Our case did not fit well with these previously described tumors having LMNA-NTRK1 fusion, although our case did seem to be closely related to LPF-like neural tumors except for the absence of the adipose tissue element. In addition, a recent study demonstrated that an infantile spindle cell sarcoma with neural features harbored TFG-MET fusion [13].

Two forms of LMNA-NTRK1 fusion transcripts were detected in our case. The LMNA (exon 2)–NTRK1 (exon 10) variant could be the active form of tyrosine kinase because the other one is expected to not contain the protein kinase domain (Fig. 5D), although we did not confirm this on functional studies. However, the real target activity of TKI could also depend on other genetic abnormalities; detailed investigations for the presence of other genetic alterations were not carried out in this present case.

In addition to the low frequency in sarcomas, NTRK1 fusions have also been detected in various tumors including papillary thyroid carcinoma, lung adenoscarcoma, intrahepatic cholangiocarcinoma, colorectal carcinoma, and glioblastoma [14-18]. Currently, several tyrosine receptor kinase (TRK)–targeting compounds are under clinical development [19]. Recent studies demonstrated a positive response to the TRK inhibitor LOXO-101 in 2 tumors with LMNA-NTRK1 fusion [1,20]. Furthermore, 1 of those 2 tumors was an undifferentiated soft tissue sarcoma [1]. However, the presence of an NTRK1 fusion does not necessarily indicate that a tumor would respond to a TRK inhibitor as much as expected. We need to keep in mind that the status of other genetic alterations besides NTRK1 fusion might affect the therapeutic efficacy of TKI [21]. Although the patient in this case is well and alive...
with no evidence of recurrence or metastasis, treatment with these TRK inhibitors would be applicable for such patients.

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References


