Immunophenotype of Pediatric NTRK fusion mesenchymal tumors

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Research

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Abstract

Background: While ETV6-NTRK3 fusion is common in infantile fibrosarcoma, NTRK1/3 fusion in pediatric tumors is scarce and, consequently, not well known. Herein, we evaluated NTRK1/3 fusion pediatric mesenchymal tumors clinicopathologically and immunophenotypically.

Methods: We reviewed nine NTRK fusion pediatric sarcomas confirmed by fluorescence in situ hybridization and/or next-generation sequencing from Seoul National University Hospital between 2002 and 2020.

Results: One case of TPR-NTRK1 fusion-positive intracranial extra-axial high-grade undifferentiated sarcoma (12-year-old boy), one case of LMNA-NTRK1 fusion-positive low-grade infantile fibrosarcoma of the forehead (3-year-old boy), one case of ETV6-NTRK3 fusion-positive inflammatory myofibroblastic tumor (IMT) (3-month-old girl), and six cases of ETV6-NTRK3 fusion-positive infantile fibrosarcoma (median age: 2.6 months, range: 1.6–5.6 months, M: F = 5:1) were reviewed. The Trk immunopositive pattern was distinctive according to the fusion genes. We notified nuclear positivity in TPR-NTRK1 fusion sarcoma, nuclear membrane positivity in LMNA-NTRK1 fusion sarcoma, and both cytoplasmic and nuclear positivity in ETV6-NTRK3 fusion-positive IMT and infantile fibrosarcomas. Also, the TPR-NTRK1 fusion sarcoma showed robust positivity for CD34/nestin and high mitoses. The LMNA-NTRK1 fusion sarcoma revealed CD34/S100 protein/nestin/CD10 coexpression, and a low mitotic rate. The IMT with ETV6-NTRK3 fusion expressed SMA. Six infantile fibrosarcomas with ETV6-NTRK3 fusion showed variable coexpression of nestin (6/6)/CD10 (4/5)/S100 protein (3/6).

Conclusions: All cases of NTRK1 and NTRK3 fusion-positive pediatric tumors robustly expressed the Trk protein. A Trk immunopositive pattern and CD34/S100/nestin/CD10/SMA immunohistochemical phenotype may suggest NTRK fusion partner genes and diagnoses. LMNA-NTRK1 fusion sarcoma might be a low-grade subtype of infantile fibrosarcoma. Interestingly, more than half of the infantile fibrosarcoma cases were positive for S100 protein and CD10. The follow-up period of TPR-NTRK1 and LMNA-NTRK1 fusion tumors are not enough to predict prognosis. However, ETV6-NTRK3 fusion infantile fibrosarcomas showed an excellent prognosis with no evidence of disease for an average of 11.7 years after gross total resection of the tumor.

Background

Next-generation sequencing (NGS) studies have recently revealed an increasing number of fusion genes in soft tissue sarcomas; these genes have been identified as oncogenic drivers and diagnostic markers of a wide range of adult and pediatric cancers.[1] However, until now, the clinicopathological characteristics of all of these gene fusion tumors have not been clarified.

Among these recent discoveries are neurotrophic receptor kinase (NTRK) gene fusions. NTRK1, NTRK2, and NTRK3 encode the neurotrophin tyrosine kinase receptor family TrkA, TrkB, and TrkC transmembrane proteins.[2] These genes play an essential role in nervous system development and function through activation by neurotrophins.[3] However, NTRK gene fusions transcribe to chimeric Trk proteins either by constitutive activation or overexpression of kinase-confering oncogenic proteins.[2] The ETV6-NTRK3 fusion has been identified in glioblastoma, mammary secretory carcinoma, salivary gland mammary carcinoma, adult lung cancer, papillary thyroid cancer, and mesenchymal tumors including infantile fibrosarcoma, mesoblastic nephroma, inflammatory myofibroblastic tumor (IMT), and gastrointestinal stromal tumors.[1, 2, 4-7] EML4-NTRK3 fusion has been identified in infantile fibrosarcomas and congenital mesoblastic nephroma in addition to ETV6-NTRK3 fusion.[8] The common fusion partners of NTRK1, located on 1q25, are the 5′ exons of various thyroid-expressed genes (tropomyosin 3 [TPM3], translocated promoter region, nuclear basket protein [TPR], and TRK fused gene [TFG]) in the frame on 1q21-23 because NTRK1 is located close to its gene partners.[9] Additional fusion partners of NTRKR1 include RAB GTPase activating protein 1-like (RABGAP1L), chromatin target of PRMT1 (CHTOP), Rho-Rac guanine nucleotide exchange factor 2 (ARHGEF2), neurofascin (NFASC), and brevican (BCAM).[10] TPR-NTRK1 fusion has been identified in pediatric papillary thyroid carcinomas[11] and lipofibromatosis[12, 13], and adults interdigitating dendritic cell sarcoma,[5] fibrosarcoma-like uterine undifferentiated sarcomas,[1] and colorectal adenocarcinomas (Supplementary file: table 2).[14] However, it has never been reported in primary intracranial tumors.[5] Additionally, lamin A/C (LMNA)-NTRK1 fusion has been infrequently reported in congenital infantile fibrosarcoma,[4, 15, 16] cellular mesoblastic nephroma,[17] and lipofibromatosis-like neural tumors.[13]

We have recently encountered pediatric cases of intracranial and forehead sarcomas. Pathologically, they did not fit into any known category of sarcomas or benign mesenchymal tumors. However, RNA sequencing by NGS of our cases revealed TPR-NTRK1, LMNA-NTRK1, and ETV6-NTRK3 fusions. Herein, we report these distinctive cases in detail so that their clinicopathological characteristics can be defined in similar cases.

Materials And Methods

Patients

Eight NTRK fusion sarcomas were retrieved from the archives of pediatric sarcomas under 18-year-old from the archives of the Department of Pathology, Seoul National University Children’s Hospital from 2002 to 2019. Among them, authors reviewed clinicopathological and genetic findings of 8 NTRK fusion-positive sarcomas, detected by either fluorescence in situ hybridization (FISH) or next-generation sequencings (NGS), such as RNA sequencing or customized gene panel study. They were one case of ETV6-NTRK3 fusion-positive IMT, one case of TPR-NTRK1 fusion-, one case of LMNA-NTRK1 fusion- and six cases of ETV6-NTRK3 fusion-sarcomas.

Pathology, immunohistochemistry (IHC), and fluorescence in situ hybridization-study (FISH)

All tumors were reviewed by two pathologists (JWK and SHP). Immunohistochemical (IHC) stain performed on an immunostaining system (BenchMark ULTRA system, Ventana-Roche, Mannheim, Germany) using primary antibodies including Trk (1: 50, Cell signaling, Boston, USA), nestin (1: 200, Millipore, Temecula, USA), vimentin (1: 500, DAKO, Grostrup, Denmark), S100 protein (1: 3000, DAKO), CD34 (1: 200, Dako), CD10 (RTU, Novocastra, Newcastle, UK), Ki67 (1: 100,
MAb MIB-1; Dako), and phosphohistoneH3 (1: 5000, Cell Marque, Rocklin, USA), TLE1 (1: 20, Cell Marque, Rocklin, US), Fli1 (1: 300, Becton and Dickinson, Franklin Lakes, US), p53 (1: 100, DAKO), ERG (1: 200, Ventana, Export, US), CD99 (1: 200, Novocastra (Leica), Muchen, Germany), Smooth muscle actin (SMA, 1: 500, DAKO), Desmin (1: 200, DAKO), Myogenin (1: 500, DAKO), cytokeratin (1: 300, DAKO), epithelial membrane antigen (EMA, 1: 300, DAKO), integrase interactor 1 (INI-1, 1: 100, Cell signaling), STAT6 (1: 100, ABCAM, Cambridge, UK) (Supplementary file: table 1). Appropriate positive controls were included, and for the negative control, primary antibodies were omitted. Mitotic activity was assessed with pHH3 immunostain on 4μm thick formalin-fixed, paraffin-embedded (FFPE) slides by counting mitotic figures in 10 high power fields (HPF; area, 2.38 mm2). The immunohistochemical antibodies that were used are summarized in Supplementary file, Table 1.

For ETV6 break-apart FISH study, locus-specific identifier (LSI) Vysis ETV6 fluorescence dual-color break apart DNA probes, ETV6 (CEN) SpectrumGreen and Vysis LSI ETV6 (TEL) SpectrumOrange (Abbott Molecular, Abbott Park, US), was used.

DNA extraction and customized brain tumor gene panel study

On hematoxylin and eosin-stained FFPE sections, representative areas of tumors with at least 90 % tumor cell purity were outlined for microdissection. DNA-extraction from the serial sections of the microdissected tumor tissue using the Maxwell® RSC DNA FFPE Kit (Promega, USA) was carried out according to the manufacturer's instructions.

The customised targeted gene panel (FIRST brain tumor panel and FIRST pan-cancer panel), which was customized and verified by the Department of Pathology of Seoul National University Hospital (SNUH), containing 172 genes and ten fusion genes, 1.7 Mb/run by NextSeq550Dx in Hi-Output. The produced sequencing data was analyzed using the pipeline of SNUH First Brain Tumor Panel Analysis. First, we performed the quality control of the Fastq file and analyzed only the data that passed the criteria. Paired-end alignment to HG19 reference genome was performed using BWA-men and the GATK Best Practice. [18] After finishing the alignment step, an "analysis-ready BAM" was produced, and second quality control was performed to determine if further variant calling is appropriate. In the pipeline, single nucleotide variation (SNV), insertion and deletion (InDel), copy number variation (CNV), and translocation, were analyzed using at least more than two analysis tools, including in-house and open-source software. The open-source tools used were GATK UnifiedGenotyper, SNVer, and LoFreq for SNV/InDel detection[19], Delly and Manta for Translocation discovery[20], THetA2 for purity estimation, and CNVKit for CNV calling[21], respectively. SnpEff annotated detected variants with various databases such as RefSeq, COSMIC, dbSNP, ClinVar, and gnomAD. Then germline variant was filtered using the population frequency of these databases (> 1% population frequency). Finally, the variants were confirmed throughout a comprehensive review of a multidisciplinary molecular tumor board.

RNA extraction, RNA sequencing, and fusion analysis

For RNA sequencing, the tumor RNA was extracted from the paraffin block (tumor fraction: >90%) with Maxwell® RSC RNA FFPE Kit (Promega, USA). The library was generated with SureSelectXT RNA Direct Kit (Agilent, Santa Clara, USA) and sequenced on an Illumina NovaSeq 6000 at Macrogen (Seoul, Republic of Korea). Raw sequencing reads were analyzed with three kinds of algorithms of DIFFUSE, Fusion catcher, and Arriba (https://github.com/suhrig/arriba/) to detect gene fusions, and compared the results.

Briefly, Fastq files were aligned by the STAR aligner on the hg19 reference genome for Arriba analysis. The chimeric alignments file and the read-through alignments file were produced, and fusion candidates were generated with a set of filters that detect artifacts based on various characteristic features.

Results

Clinicopathological findings and follow-up data of the patients

The patient with TPR-NTRK1 fusion sarcoma was a 12-year-old boy presented with headache and diplopia for three months, who did not have any perinatal health problems. A 7.4-cm contrast-enhancing mass was detected in the right temporal lobe on magnetic resonance imaging (MRI) (Fig. 1). Craniotomy revealed a hypervascular, extra-axial tumor with superficial brain invasion. Complete resection of the tumor with adjuvant chemotherapy with Ifosfamide, Carboplatin, and Etoposide (ICE) and radiation therapy (54+7.2 Gy) were administered because the pathology was high-grade undifferentiated sarcoma.

One patient with LMNA-NTRK1 fusion sarcoma was a 3-year-old boy who presented with a growing mass on his left forehead, which had been present since his neonatal period as pea size, and it has recently grown rapidly to 4.0×3.5×3.0 cm. It protruded from the forehead and was covered with eroded skin. The patient underwent complete surgical excision, and the cut surface of the tumor exhibited a homogenous tan-colored solid appearance (Fig. 1).

ETV6-NTRK3 fusion-positive IMT was developed in a 3-months-old girl who presented with sudden onset dyspnea and systemic cyanosis. Chest CT showed a mass on the left lower thorax, looked like the mass of the lower lobe of the left lung (Fig. 2). The mass was emobilized under the impression of arteriovenous malformation at the local hospital. However, the symptom and signs were not relieved, and the mass had grown continuously to 5.6×5.2×3.3 cm. The lobectomy of the left lower lobe was conducted to remove the tumor. Grossly, the mass was well encapsulated and well separated from the left lower lobe of the lung (Fig. 2). The tumor arose in the extrapulmonary sequestration, which was diagnosed as IMT by full pathological examination and next generation sequencing with customized First pan-cancer gene panel.

The patients' median ages of six ETV6-NTRK3 fusion-positive infantile fibrosarcomas at the time of surgery were 2.6 months (range: 1.6-5.6 months of age). The male to female ratio was 5: 1. The patients had presented with a mass on the tongue, buttock, right shoulder, left foot, right abdominal cavity, and sacrococcygeal area, respectively. Five tumors were completely resected, and adjuvant chemotherapies were given, which are summarized in Table 1. The remaining massive sacrococcygeal tumor, involving the spinal cord, was initially subtotally resected and underwent three times of operation with one cycle of chemotherapy, but the follow-up of the patient was lost.
These follow-up data were also summarized in table 1. The patients with TPR-NTRK1 fusion and LMNA-NTRK1 sarcomas fared relatively well for 18 months and 11.6 months follow-up period, without tumor recurrence or neurological defects. Five patients with ETV6-NTRK3 fusion-positive infantile sarcomas are all alive without disease for an average of 11.7 years (range: 6.0-17.4 years), but one case who had a huge sacrococcygeal mass lost follow-up.

**Result of pathology, immunohistochemistry (IHC), and fluorescence in situ hybridization (FISH)**

Histopathology of TPR-NTRK1 fusion sarcoma showed a sheet of small oval-to-spindle cells with dilated blood vessels. Scanning power microscopy revealed a tiger-striped pattern due to vague layers of cellular and less-cellular areas with keloid type collagen deposits (Fig 3). The tumor cells exhibited relatively uniform oval nuclei with fine chromatin and clear-to-eosinophilic cytoplasm. A high mitotic rate (25/10 per high-power fields) and a high Ki-67 labeling index (36.0%) were present; however, necrosis was not observed. The tumor cells were also robustly positive for Trk (1: 50, Cell Signaling, Boston, US), CD34, nestin, p53, and vimentin (Fig. 4). The robust nuclear positivity of Trk was remarkable (Fig. 5). However, the tumor cells were negative for S-100 protein, SMA, desmin, myogenin, CD99, Fli-1, CD56, STAT6, cytokeratin, andEMA. TLE1 was weakly positive for the tumor cell nuclei. INI1 was retained.

LMNA-NTRK1 fusion tumor was composed of vaguely fascicular spindle cells with bland-looking elongated nuclei and inconspicuous nucleoli (Fig. 3). There was collagen lay down between the tumor cells. Intermixed inflammatory cell infiltration was remarkable, which was pronounced on CD3 IHC (Fig. 4). The Ki-67 index was moderately high (18.2%), but many of them might be infiltrated inflammatory cells. Mitosis was absent on pH3 IHC. There was neither necrosis nor hemorrhage. Therefore, this tumor was much less cellular and much more bland-looking than TPR-NTRK1 or ETV6-NTRK3 fusion sarcoma. The tumor cells were robustly and diffusely positive for Trk, S100-protein, CD34, and nestin (Fig. 4 and 5), but negative for CD56, SMA, desmin, myogenin, STAT6, EMA, CK, CD1a, CD21, CD35, CD43, WT-1(c-terminal), MelanA, HMB45, BRAF and ALK. The nuclear envelope-positivity for Trk was remarkable with weak cytoplasmic staining (Fig. 3).

Histopathology of the ETV6-NTRK3 fusion-positive IMT was composed of vaguely fascicular bland-looking spindle cells intermixed with lymphoplasma cells (Fig. 2 and 3). Intratumoral hemorrhage and the congestion of large blood vessels were present, most likely due to the previous embolization. The tumor cells were positive for Trk and SMA, but negative for S100, CD34, ALK, CD10, desmin, myogenin, CD99, CD56, CK, EMA and STAT6. There was no necrosis. Mitosis was very low (1/10HPF) but Ki-67 index was 36%, possibly due to positivity in the inflammatory cells. ETV6 break FISH was positive and pan-cancer panel resulted in ETV6-NTRK3 fusion (split read: 339, spanning read: 40).

Histopathology of ETV6-NTRK3 fusion sarcomas showed highly cellular and relatively uniform small spindle cells with a high mitotic rate (10/40-10 HPFs). There were neither necrosis nor prominent inflammatory cell infiltration in all cases. These infantile fibrosarcomas were diffusely and robustly positive for Trk (100%), S100 protein (50%, 3/6 cases), nestin, CD10 (80%, 4/5 cases), and vimentin (100%), but negative for CD34, SMA, desmin, myogenin, and CD56. The Trk IHC showed a diffuse cytoplasmic stain with some nuclear staining (Fig. 5). Ki-67 labeling indices were 15-60%. ETV6-NTRK3 fusion was verified by FISH in all six cases (Fig. 5) and additionally by RNA sequencing in two cases.

The targeted gene panel revealed a TPR-NTRK1 fusion of TPR on chromosome 1q25 (position 186337018) and NTRK1 on chromosome 1q21-q22 (position 156844363) with amplification of NTRK1 and H3F3A on chromosome 1 in case 1 (Fig. 6).

RNA sequencing of an intracranial sarcoma (12-year-old boy) confirmed TPR-NTRK1 fusion (Breakpoint: 1: 186337018, 1: 156844363), and a forehead tumor (3-year-old boy) resulted in LMNA-NTRK1 fusion (Breakpoint: 1: 156104766, 1: 156844698). The number of split reads in TPR and NTRK1 was 35 and 31, respectively, and there were two discordant mates, and 37 split reads in LMNA and 53 in NTRK1, with seven discordant mates. Two cases of infantile fibrosarcomas performed RNA sequencing showed ETV6-NTRK3 fusion (Case 4: Breakpoints: and the split reads of alignment is an alignment where both mates align uniquely, but that does not satisfy the paired-end constraints. A discordant alignment is an alignment where both mates align uniquely, but that does not satisfy the paired-end constraints.

**Discussion**

Primitive small round cell sarcomas and infantile fibrosarcomas are rare childhood sarcomas that pose diagnostic and therapeutic challenges. Recently, confirmative diagnosis of neoplasms has been made possible at the genomic level by identification of driver mutation or marker gene alterations.[22] Recent reports have described emerging pediatric fusion-positive sarcomas.[5, 23, 24] Our pediatric sarcomas had distinct immunohistochemical profiles. The TPR-NTRK1 fusion-positive tumor was a CD34-positive, dural-based, high-grade undifferentiated sarcoma with features that did not fit the classifications of existing types of sarcomas. In contrast, our LMNA-NTRK1 fusion-positive tumor was a low-grade spindle cell mesenchymal tumor of the forehead that was first noticed early in the neonatal period. The LMNA-NTRK1 fusion-positive tumor was difficult to diagnose before RNA sequencing by NGS because of its unusual pathology and immunohistochemical profile, namely, a combination of prominent inflammatory cells, no mitotic activity (0/10 HPF), and S100/CD34 coexpression. However, Hung et al. ’s case of infantile fibrosarcoma also showed prominent inflammatory cells.[4] S100-protein and CD34 co-positivity are generally rare in sarcomas, which can be interpreted as hybridomas or dual differentiation; however, infantile fibrosarcomas often show coexpression of these two antibodies.[15, 25] Miettinen et al. and Wong et al. reported that non-pleomorphic, low-grade spindle cell neoplasm with LMNA-NTRK1 fusion, which was infantile fibrosarcoma.[16, 25] Miettinen et al. ’s case showed low mitotic rates (<5/10 HPFs), and S100 protein/CD34 coexpression.[25] Wong et al. ’s case was CD34/vimentin-positive. Our LMNA-NTRK1 fusion sarcoma was consistent with Hung et al. ’s and Miettinen et al. ’s S100 protein/CD34 coexpressing infantile fibrosarcomas. The main differential diagnosis of this LMNA-NTRK1 fusion tumor was IMT, but it can be ruled out by immunoprofile (SMA-negative, but S100/CD34 coexpression).

NTRK1 encodes TRKA receptor tyrosine kinase, which has a high affinity for nerve growth factor.[3] Genetic alterations of NTRK1 by translocations, amplifications, deletions, and point mutations have been reported in various tumor types, suggesting the potential role of Trk in oncogenesis.[26, 27] More
recently, NTRK1 chromosomal rearrangements have been identified in additional tumor types (Supplementary file, Table 2), [9, 19, 28, 29] suggesting that, while oncogenic activation through NTRK1 fusion is not frequent, it can occur in various cancers. Interestingly, a significant number of NTRK1-associated gene fusions have developed as a result of intrachromosomal gene fusion.[10] Depending on the directions of transcription of NTRK1 and its fusion partner, intrachromosomal fusions can occur either through simple interstitial deletion (e.g., LMNA-NTRK1) or through a more complex break/inversion mechanism (e.g., TPM3-NTRK1 or TPR-NTRK1) if the two genes are transcribed in opposite directions.[12] A 737-kbp deletion yielded the 5’ end of LMNA (localized to 1q22), including exons 1–10 fused to the 3’ end of NTRK1 (also localized to 1q22) and exons 12–17. [16]

Pan-Trk IHC can be used to detect NTRK fusion tumors; however, the expression site within the tumor cell differs according to the fusion partner genes.[4] Our LMNA-NTRK1 fusion tumor cells showed strong nuclear envelop and cytoplasmic staining. In contrast, our TPR-NTRK1 fusion sarcoma revealed strong nuclear staining for Trk (clone A7H6R), consistent with Hechtman et al.’s report using the same clone (monoclonal antibody [Mab] EPR17341). However, Rudzinski et al. reported that the LMNA-NTRK1 fusion sarcoma in their study showed diffuse and strong cytoplasmic staining with the Trk antibody (Mab EPR17341). In contrast, TPM3-NTRK1 fusion tumors showed strong and diffuse cytoplasmic staining.[1] Thus, these differences might be due to various Trk antibody clones.

ETV6-NTRK3 and (rarely) EML4-NTRK3, LMNA-NTRK1, TPM3-NTRK1, and SQSTM1-NTRK1 fusions have been reported in infantile fibrosarcoma (Table 1).[4, 15] The six cases of classic infantile fibrosarcoma and one IMT in our study had an ETV6-NTRK3 fusion verified by ETV6 break-apart FISH and/or RNA sequencing. The diffuse cytoplasmic Trk positivity in our cases is consistent with that in previous reports of Trk immunopositivity patterns.[4, 25]

Although ETV6-NTRK3 is a genetic hallmark of infantile fibrosarcoma, it has been reported in the ALK-negative IMTs. So far, six cases of ETV6-NTRK3 fusion-positive IMTs have been published.[5-7] Chang et al. reported that ALK-altered thoracic IMTs was 73% (24/33) and the remaining ALK-negative IMTs had ROS1 fusion (15%, 5 cases) or ETV6-NTRK3 fusion (9%, 3 cases) or RET fusion (3%, 1 case). Our IMT case is unique because it occurred in the extrapulmonary sequestered lung, had ETV6-NTRK3 fusion, and the patient was the youngest of reported ETV6-NTRK3 fusion-positive IMTs. The infantile IMTs have been notified [30] and the previously reported youngest patient with ETV6-NTRK3 fusion-positive IMT was 2-year-old.[6]

These NTRK fusion tumors tend to respond to NTRK inhibitors.[2, 10] LOXO-101 is an orally bioavailable tyrosine kinase inhibitor that inhibits Trk catalytic activity with a low nanomolar potency. A phase 1 study with LOXO-101 in non-small cell lung cancer harboring TPR-NTRK1 fusion showed a good response. [31] NTRK gene fusion could be a novel target of NTRK inhibitors for multiple tumor types.[2]

In conclusion, we report two cases of NTRK1 fusion and seven cases of NTRK3 fusion pediatric sarcomas and IMT that were diagnostically challenging without molecular features; the three types of fusion-positive mesenchymal tumors (TPR-NTRK1, LMNA-NTRK1, and ETV6-NTRK3) differed in their H&E morphology, immunoprofile, and Trk immunopositivity patterns. In case of LMNA-NTRK1 fusion sarcoma, S100/CD34/CD10-coexpression was a novel finding. While the immunophenotype of S100 protein, nestin, and CD10 positivity in infantile fibrosarcoma is a new finding, the TPR-NTRK1 fusion sarcoma was positive for CD34 and nestin but negative for S100 protein. The Trk immunopattern and the CD34/S100/nestin/CD10 immunophenotype could be used for differential diagnosis and can suggest the NTRK fusion partner gene. The sacrococcygeal infantile fibrosarcoma was unable to achieve complete resection, but the exact outcome is unknown due to the loss of follow-up. However, the remaining patients with ETV6-NTRK3 fusion-positive infantile fibrosarcomas survived for up to 17.3 years, without tumor recurrence after complete resection of the tumor. The median survival of our infantile fibrosarcomas was 8.3 years. The patients with these fusion-positive tumors may benefit from NTRK inhibitor therapy if the tumors cannot be controlled by conventional treatment.[32]

**List Of Abbreviations**

ARHGEF2, Rho-Rac guanine nucleotide exchange factor 2
BCAN, brevican
CHTOP, chromatin target of PRMT1
FFPE, formalin-fixed paraffin-embedded
FISH, fluorescence in situ hybridization
HPF, high-power field
ICE, ifosfamide, carboplatin, and etoposide
IHC, immunohistochemical
IMT: inflammatory myofibroblastic tumor
LMNA, lamin A/C
LSI, locus-specific identifier
Mab, monoclonal antibody
MRI, magnetic resonance imaging
Declarations

Ethics approval and consent to participate: The institutional review board of Seoul National University Hospital approved this study (IRB No: 1905-001-1029 and 1905-108-1035). The authors complied with the Declaration of Helsinki and the Human Research Protection Program.

Consent for publication: All materials had been obtained for medical care of the patients, which were anonymized and retrospectively reviewed. No extra-human materials were obtained for this study. Under the Korean Bioethics and Safety Act, additional consent to publish was waived.

Availability of data and materials: All the genetic data can be found in our SNUH's big data server managing by the center for precision medicine.

Competing interests: The authors declared that they have no competing interests

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Authors’ contributions: S-H Park designed, supervised, and edited this study and manuscript. J Kang wrote the manuscript, J-W Park, J Kang, JM Lim, and S-H Park collected and analyzed the clinicopathological data. Jaemoon Koh, JM Bae and S-H Park performed the histological examination of sarcomas. JK Won, H Yoon, and JH Shin analyzed the genetic data of tumors. S-K Kim did surgical resection of the tumor, and JY Choi and HJ Kang have treated and made follow-up the patients by chemotherapy. All authors read and approved the final manuscript.

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References

1. Chiang S, Cotzia P, Hyman DM, Drilon A, Tap WD, Zhang L, Hechtman JF, Frosina D, Jungbluth AA, Murali R et al: NTRK Fusions Define a Novel Uterine Sarcoma Subtype With Features of Fibrosarcoma. Am J Surg Pathol 2018, 42(6):791-798.
2. Amatu A, Sartore-Bianchi A, Siena S: NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. ESMO Open 2016, 1(2):e00023.
3. Barbacid M: Structural and functional properties of the TRK family of neurotrophin receptors. Ann N Y Acad Sci 1995, 766:442-458.
4. Hung YP, Fletcher CDM, Hornick JL: Evaluation of pan-TRK immunohistochemistry in infantile fibrosarcoma, lipofibromatosis-like neural tumour and histological mimics. Histopathology 2018, 73(4):634-644.
5. Pavlick D, Schrock AB, Malicki D, Stephens PJ, Kuo DJ, Ahn H, Turpin B, Allen JM, Rosenzweig M, Badizadegan K et al: Identification of NTRK fusions in pediatric mesenchymal tumors. Pediatr Blood Cancer 2017, 64(8).
6. Chang JC, Zhang L, Drilon AE, Chi P, Aalooj R, Borsu L, Benayed R, Travis WD, Ladanyi M, Antonescu CR: Expanding the Molecular Characterization of Thoracic Inflammatory Myofibroblastic Tumors beyond ALK Gene Rearrangements. J Thorac Oncol 2019, 14(5):825-834.
7. Allassiri AH, Ali RH, Shen Y, Lum A, Strahlendorf C, Deyell R, Rassekh R, Sorensen PH, Laskin J, Marra M et al: ETV6-NTRK3 Is Expressed in a Subset of ALK-Negative Inflammatory Myofibroblastic Tumors. Am J Surg Pathol 2016, 40(8):1051-1061.
8. Church AJ, Calicchio ML, Nardi V, Skalova A, Pinto A, Dillon DA, Gomez-Fernandez CR, Manoj N, Haimes JD, Stahl JA et al: Recurrent EML4-NTRK3 fusions in infantile fibrosarcoma and congenital mesoblastic nephroma suggest a revised testing strategy. Mod Pathol 2018, 31(3):463-473.
9. Kim J, Lee Y, Cho HJ, Lee YE, An J, Cho GH, Ko YH, Joo KM, Nam DH: NTRK1 fusion in glioblastoma multiforme. PLoS One 2014, 9(3):e91940.
10. Vaishnavi A, Le AT, Doebel RC: TRKing down an old oncogene in a new era of targeted therapy. Cancer Discov 2015, 5(1):25-34.
11. Prasad ML, Vyas M, Horne MJ, Virk RK, Morotti R, Liu Z, Tallini G, Nikiforova MN, Christison-Lagay ER, Udelsman R et al: NTRK fusion oncogenes in pediatric papillary thyroid carcinoma in northeast United States. Cancer 2016, 122(7):1097-1107.
12. Agaram NP, Zhang L, Sung YS, Chen CL, Chung CT, Antonescu CR, Fletcher CD: Recurrent NTRK1 Gene Fusions Define a Novel Subset of Locally Aggressive Lipofibromatosis-like Neural Tumors. Am J Surg Pathol 2016, 40(10):1407-1416.
13. Bartenstein DW, Coe TM, Gordon SC, Friedmann AM, Senna MM, Kelleher CM, Antonescu CR, Nazarian RM, Hawryluk EB: Lipofibromatosis-like neural tumor: Case report of a unique infantile presentation. JAAD Case Rep 2018, 4(2):185-188.
14. Creancier L, Vandenberghie I, Gomes B, Dejean C, Blanchet JC, Meilleroux J, Guimbaud R, Selves J, Kruczynski A: Chromosomal rearrangements involving the NTRK1 gene in colorectal carcinoma. Cancer Lett 2015, 365(1):107-111.

15. Davis JL, Lockwood CM, Albert CM, Tsuchiya K, Hawkins DS, Rudzinski ER: Infantile NTRK-associated Mesenchymal Tumors. Pediatr Dev Pathol 2018, 21(1):68-78.

16. Wong V, Pavlick D, Brennan T, Yelensky R, Crawford J, Ross JS, Miller VA, Malicki D, Stephens PJ, Ali SM et al. Evaluation of a Congenital Infantile Fibrosarcoma by Comprehensive Genomic Profiling Reveals an LMNA-NTRK1 Gene Fusion Responsive to Crizotinib. J Natl Cancer Inst 2016, 108(1).

17. Wegert J, Vokuhl C, Collidor G, Del Castillo Velasco-Herrera M, Farnдон SJ, Guzzo C, Jorgensen M, Anderson J, Slater O, Duncan C et al. Recurrent intragenic rearrangements of EGFR and BRAF in soft tissue tumors of infants. Nat Commun 2018, 9(1):2378.

18. Van der Auwera GA, Carneiro MO, Hilt C, Poplin R, Del Angel G, Levy-Moonshine A, Jordan T, Shaker K, Roazen D, Thibault J et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinformatics 2013, 43:11 10 11-11 10 33.

19. Wu G, Diaz AK, Paugh BS, Rankin SL, Ju B, Li Y, Zhu X, Qu C, Chen X, Zhang J et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. Nat Genet 2014, 46(5):444-450.

20. Rausch T, Zichner T, Schlattl A, Stutz AM, Benes V, Korbel JO: DELLY: structural variant discovery by integrated paired-end and split-read analysis. Bioinformatics 2012, 28(18):i333-i339.

21. Tavleish E, Shain AH, Botton T, Bastian BC: CNVkit: Genome-Wide Copy Number Detection and Visualization from Targeted DNA Sequencing. PLoS Comput Biol 2016, 12(4):e1004873.

22. Yim J, Lee WS, Kim SK, Kang HJ, Bae J, Park SH: Intracranial Ewing sarcoma with whole genome study. Childs Nerv Syst 2019, 35(3):547-552.

23. Schaefer IM, Hornick JL: Diagnostic Immunohistochemistry for Soft Tissue and Bone Tumors: An Update. Adv Anat Pathol 2018, 25(6):400-412.

24. Le Loarer F, Pissaloux D, Coindre JM, Tirode F, Vince DR: Update on Families of Round Cell Sarcomas Other than Classical Ewing Sarcomas. Surg Pathol Clin 2017, 10(3):587-620.

25. Mittetin M, Felisiak-Golabek A, Luina Contreras A, Gdog J, Kaplan RN, Kiliin JK, Lasota J: New fusion sarcomas: histopathology and clinical significance of selected entities. Hum Pathol 2019, 86:57-65.

26. Greco A, Miranda C, Pagliardini S, Fusetti L, Bongarzone I, Pierotti MA: Chromosome 1 rearrangements involving the genes TPR and NTRK1 produce structurally different thyroid-specific TRK oncogenes. Genes Chromosomes Cancer 1997, 19(2):112-123.

27. Greco A, Pierotti MA, Bongarzone I, Pagliardini S, Lanzi C, Della Porta G: TRK-T1 is a novel oncogene formed by the fusion of TRP and TRK genes in human papillary thyroid carcinomas. Oncogene 1992, 7(2):237-242.

28. Wiesner T, He J, Yelensky R, Esteve-Puig R, Botton T, Yeh I, Lipson D, Otto G, Brennan K, Murali R et al. Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. Nat Commun 2014, 5:3116.

29. Ross JS, Wang K, Gay L, Al-Rohil R, Rand JV, Jones DM, Lee HJ, Sheehan CE, Otto GA, Palmer G et al. New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing. Oncologist 2014, 19(3):235-242.

30. Tanaka M, Kohashi K, Kusuhara S, Konoshita Y, Uda Y, Souzaki R, Kinosita Y, Oda Y et al. Inflammatory myofibroblastic tumors of the lung carrying a chimeric A2M-ALK gene: report of 2 infantile cases and review of the differential diagnosis of infantile pulmonary lesions. Hum Pathol 2017, 66:177-182.

31. Khotskaya YB, Holla VR, Farago AF, Mills Shaw KR, Meric-Bernstam F, Hong DS: Targeting TRK family proteins in cancer. Pharmacol Ther 2017, 173:58-66.

32. Okimoto RA, Bivona TG: Tracking Down Response and Resistance to TRK Inhibitors. Cancer Discov 2016, 6(1):14-16.

33. Sartore-Bianchi A, Ardini E, Bosotti R, et al. Sensitivity to Entrectinib Associated With a Novel LMNA-NTRK1 Gene Fusion in Metastatic Colorectal Cancer. J Natl Cancer Inst 2016;108.

34. Doebele RC, Davis LE, Vaishnavi A, et al. An Oncogenic NTRK Fusion in a Patient with Soft-Tissue Sarcoma with Response to the Tropomyosin-Related Kinase Inhibitor LOXO-101. Cancer Discov. 2015;5:1049-1057.

35. Bender J, Anderson B, Bloom DA, et al. Refractory and metastatic infantile fibrosarcoma harboring LMNA-NTRK1 fusion shows complete and durable response to crizotinib. Cold Spring Harb Mol Case Stud. 2019;5.

36. Zhoa N, Schafner R, Li T, et al. A primary undifferentiated pleomorphic sarcoma of the lumbosacral region harboring a LMNA-NTRK1 gene fusion with durable clinical response to crizotinib: a case report. BMC Cancer. 2018;18:842.

37. Wiesner T, He J, Yelensky R, et al. Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. Nat Commun. 2014;5:3116.

38. Farago AF, Taylor MS, Doebele RC, et al. Clinicopathologic Features of Non-Small-Cell Lung Cancer Harboring an NTRK Gene Fusion. JCO Precis Oncol. 2018;2018.

39. Wong V, Pavlick D, Brennan T, et al. Evaluation of a Congenital Infantile Fibrosarcoma by Comprehensive Genomic Profiling Reveals an LMNA-NTRK1 Gene Fusion Responsive to Crizotinib. J Natl Cancer Inst. 2016;108.

**Supplementary Figure Captions**

**Supplementary Figure 1A.** RNA sequencing confirmed TPR-NTRK1 fusion using the Arriba fusion gene calling method. 1) Circular plot. 2) The fusion gene retained the protein tyrosine kinase domain. 3) The schematic view showed TPR-NTRK1 fusion by 488 bp deletion (breakpoints: chromosome 1: 186337018; 1: 156844363).
**Supplementary Figure 1B.** RNA sequencing confirmed *LMNA-NTRK1* fusion using the Arriba fusion gene calling method (breakpoints: chromosome 1: 156104766; 1: 156844698). 1) The schematic view showed *LMNA-NTRK1* fusion. 2) Circular plot. 3) The fusion gene retained the protein tyrosine kinase domain.

**Supplementary Figure 1C.** RNA sequencing confirms *ETV6-NTRK3* fusion in the fifth case of infantile fibrosarcoma using the Arriba fusion gene calling method. 1) The schematic view showed *ETV6-NTRK3* fusion (breakpoints: chromosome 12: 12022903; 15: 88483984). 2) Circular plot. 3) The fusion gene retained the protein tyrosine kinase domain.

**Supplementary Figure 1D.** RNA sequencing confirmed *ETV6-NTRK3* fusion in the 6th case of infantile fibrosarcoma using the Arriba fusion gene calling method. 1) The schematic view showed *ETV6-NTRK3* fusion (breakpoints: chromosome 12: 12022903; 15: 88524591). 2) Circular plot. 3) The fusion gene retained the protein tyrosine kinase domain.

**Table**

Table 1. Clinicopathological comparison of our two cases of *TPR-NTRK1* and *LAMA-NTRK1* fusion-positive sarcomas and 6 cases of our infantile fibrosarcoma.
|                  | TPR-NTRK1 fusion-positive | LMNA-NTRK1 fusion-positive | ETV6-NTRK3 fusion-positive |
|------------------|---------------------------|-----------------------------|---------------------------|
| **Pediatric sarcoma** |                           |                             |                           |
| **Age/Gender**    | 12 y/ male                | 3 y/ male                    | 6 mo/female                |
| **Site**          | Dura, parieto-occipital   | Forehead dermis and subcutaneous tissue | Left lower lobe of |
| **Size**          | 6.0x5.0x3.0cm             | 4.0x3.5x3.0cm                | 5.6x3.5x3.0cm              |
| **Histology**     | Mixed oval to spindle cells | Spindle cells intermixed with prominent lymphoplasma cells | Spindle myofirol prominent lymphoplasma cells |
| **Histological grade** | High-grade                | Low-grade                    | Low-grade                  |
| **Nuclear pleomorphism** | Relatively uniform cells without pleomorphism | Uniform cells without pleomorphism | Uniform cells withl |
| **Tumor necrosis** | Absent                    | Absent                       | Absent                     |
| **Mitotic rate**  | 25/10 HPFs                | 0/10 HPFs                    | 1/10 HPFs                  |
| **Ki67 labeling index** | 36.0%                     | 18.2%                        | 37%                        |
| **Immunopositive markers** | Trk/CD34/Nestin/vimentin/p53 | Trk/S100/CD34/Nestin/vimentin/ | Trk/SMA/vimentin |
| **Trk positive pattern** | Nucleus                   | Nuclear membrane             | Cytoplasmic                |
| **Immunonegative or retained expression (RE) markers** | S100/CD56/SMA/desmin/myogenin/STAT6/EMA/CK/ERG/CD99/CD21/CD35/GFAP/Olig2/p16 | SMA/desmin/myogenin/STAT6/CD56/EMA/CK/CD1a/CD21/CD35/CD43/WT-1 (c-terminal)/MelanA/HMB45/BRAF/ALK | S100/desmin/myc |
| **Genetic abnormalities other than NTRK1 fusion** | NTRK1 amplification (copy number: 11), H3F3A amplification (copy number: 12) | absent | absent |
| **Final diagnosis** | Undifferentiated sarcoma  | Infantile fibrosarcoma, low grade | Inflammatory myc |
| **Treatment**     | Surgery+ vincristine, doxorubicin, cyclophosphamide (6 cycles)/ifosfamide, carboplatin, etoposide (6 cycles), alternative, total 12 cycles | Surgery only | Surgery only |
| **Outcomes and follow-up** | NED (1.4 years) | NED (0.8 years) | NED (0.3 years) |
| **Known mesenchymal tumors with this type of fusion** | Lipofibromatosis, adult uterine undifferentiated sarcoma | Infantile fibrosarcoma, lipofibromatosis-like neural tumor, Undifferentiated sarcoma, Cellular mesoblastic nephroma | Infantile fibrosarcoma |
NED: no evidence of disease

Figures

A-E) Case 1 with TPR-NTRK1 fusion.: MRI reveals A) T1-low, B-D) T2-high dura-based mass with enhancement. E) The tumor was located in the right temporal convexity and right cerebellar tent. The inlet is the cut surface of the tumor, yellowish, and solid without hemorrhage or necrosis. F, G) Case 2 with LMNA-NTRK1 fusion tumor: T2 weighted MRI revealed low-density mass on the left forehead. The cut surface of the tumor shows a gray-white solid appearance without hemorrhage or necrosis.
Figure 2

A) Chest CT of the ETV6-NTRK3 fusion-positive inflammatory myofibroblastic tumor (IMT) reveals a heterogeneously enhancing tumor in the left lower part of the thorax. B) The mass arises from extrapulmonary sequestration, supplied by the left phrenic artery, which is separated from the lower lobe of the lung. C) The cut surface of the tumor is hemorrhagic and has congested large vessels. D) This is the microscopic picture of the squared part of figure C. It is a well-encapsulated, but partly adhered to the lower lobe of the left lung. The tumor arose from the extrapulmonary sequestration and pushed the left lower lobe of the lung. Hemorrhage was developed by previous embolization of large vessels of the sequestrated lung, under the impression of arteriovenous malformation.
Figure 3
A) Histology of the intracranial undifferentiated sarcoma with TPR-NTRK1 fusion shows alternating cellular areas with collagen bands show a tiger-like appearance. The tumor cells are oval to short spindle cells. B) The forehead mesenchymal tumor with LMNA-NTRK1 fusion shows relatively low cellular spindle cell mesenchymal tumor with keloid type collagen laydown. C) The pulmonary inflammatory myofibroblastic tumor with ETV6-NTRK3 fusion shows bland-looking spindle cells with intermixed lymphoplasma cells. D) A sacrococcygeal Infantile fibrosarcoma with ETV6-NTRK3 fusion shows fascicular spindle cells with high cellularity (A-D: H&E, bar: A, B, C-D: 500, 300, 200 μm).

Figure 4
A, B) Sarcoma with TPR-NTRK1 fusion shows co-positive for nestin and CD34. C, D) ETV6-NTRK3 fusion-positive infantile fibrosarcoma is co-positive for S100 protein and CD34. E-H) The LMNA-NTRK1 fusion sarcoma has lots of CD3-positive T-cell infiltration and robustly coexpressed CD34, S100, and CD10. (A: nestin, B, F: CD34, C, G: S100 protein, E: CD34D, H: CD10, Bar: 200 μm).
Trk immunohistochemistry shows A) Nuclear positivity in TPR-NTRK1 fusion sarcoma (Case 1), B) mostly nuclear membrane, and some cytoplasmic stain in LMNA-NTRK1 fusion sarcoma (Case 2), and C) mainly cytoplasmic stain in ETV6-NTRK3 fusion sarcoma. In figure B, the Trk-negative cells are infiltrating inflammatory cells (A, B, C: Trk IHC, bar: A, B: 200 micrometers, C: lower bar: 100 micrometers). D. locus-specific identifier (LSI) FISH study using ETV6 fluorescence dual-color break apart DNA probes show one fused and one widely separated SpectrumGreen and SpectrumOrange signals in an infantile fibrosarcoma with ETV6-NTRK3 fusion-positive.
Figure 6
The custom NGS panel revealed TPR-NTRK1 fusion (IGV capture).

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalsupplementaryTable12.pdf
- Additionalfiguresupplementaryfigure1.pdf