Pediatric NTRK fusion sarcomas

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Research

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Abstract

Background

While ETV6-NTRK3 fusion is common in infantile fibrosarcoma, NTRK1 fusion in pediatric sarcoma is extremely rare and consequently not well known. Herein, we evaluated NTRK1 fusion pediatric sarcomas to determine their clinicopathological nature and differential diagnosis from classic ETV6-NTRK3 fusion infantile fibrosarcoma.

Methods

We retrieved and reviewed eight NTRK-fusion pediatric sarcomas confirmed by fluorescence in situ hybridization and/or next-generation sequencing from Seoul National University Hospital between 2002 and 2019.

Results

The NTRK1 fusion-positive tumors included one case of TPR-NTRK1 fusion in a 12-year-old boy with an intracranial extra-axial high-grade undifferentiated sarcoma and an LMNA-NTRK1 fusion in a 3-year-old boy with a low-grade infantile fibrosarcoma of the forehead. Six cases of infantile fibrosarcoma with ETV6-NTRK3 fusion were identified (median age: 2.6 months, range: 1.6–5.6 months, M: F = 5:1). The TPR-NTRK1 fusion sarcoma showed nuclear Trk expression, robust CD34/nestin expression (S100 protein-negative), and high mitoses. The LMNA-NTRK1 fusion sarcoma showed nuclear membrane Trk expression, CD34/S100 protein/nestin/CD10 coexpression, and a low mitotic rate. The six cases of infantile fibrosarcoma with ETV6-NTRK3 fusion showed S100 protein (3/6)/nestin(6/6)/CD10 (4/5) coexpression and both cytoplasmic and nuclear Trk expression but were CD34-negative.

Conclusions

All cases of NTRK1 and NTRK3 fusion-positive pediatric sarcomas robustly expressed the Trk protein. A Trk immunopositive pattern and CD34/S100/nestin/CD10 immunohistochemical profile may suggest NTRK fusion partner genes. TPR-NTRK1 and LMNA-NTRK1 fusions might be surrogate markers for pediatric mesenchymal tumors, while LMNA-NTRK1 fusion sarcoma might be a low-grade infantile fibrosarcoma subtype. More than half of the infantile fibrosarcoma cases were S100 protein and CD10-positive. The cases of infantile fibrosarcoma showed good prognoses regardless of tumor cellularity, Ki-67 index, and mitoses.

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. 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 neurotrophic tyrosine kinase receptor family TrkA, TrkB, and TrkC transmembrane proteins. These genes play an essential role in nervous system development and function through activation by neurotrophins. However, NTRK gene fusions transcribe to chimeric Trk proteins either by constitutive activation or overexpression of kinase-conferring oncogenic proteins. 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, low-grade spindle cell sarcoma, and gastrointestinal stromal tumors. EML4-NTRK3 fusion has been identified in infantile fibrosarcomas and congenital mesoblastic nephroma in addition to ETV6-NTRK3 fusion. 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. Additional fusion partners of NTRK1 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].

TPR-NTRK1 fusion has been identified in pediatric papillary thyroid carcinomas and lipofibromatosis and adults interdigitating dendritic cell sarcoma, fibrosarcoma-like uterine undifferentiated sarcomas, and colorectal adenocarcinomas (Supplementary file: table 2). However, it has never been reported in primary intracranial tumors. Additionally, lamina A/C (LMNA)-NTRK1 fusion has been infrequently reported in congenital infantile fibrosarcoma, cellular mesoblastic nephroma, and lipofibromatosis-like neural tumors.

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 sequencing (NGS), such as RNA sequencing or customized gene panel study. They were one case of TPR-NTRK1 fusion-, one case of LMNA-NTRK1 fusion- and six cases of ETV6-NTRK3 fusion-sarcomas. Their clinicopathologic findings are summarized in Table 1.

<table>
<thead>
<tr>
<th>TPR-NTRK1 fusion-positive pediatric sarcoma</th>
<th>LMNA-NTRK1 fusion-positive pediatric sarcoma</th>
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<tr>
<td>Age/Gender</td>
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<td>Site</td>
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<td>Size</td>
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<tr>
<td>Histological grade</td>
<td>High-grade</td>
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<tr>
<td>Nuclear pleomorphism</td>
<td>Uniform cell</td>
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<td>Tumor necrosis</td>
<td>Absent</td>
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<td>Mitotic rate</td>
<td>20/10 HPFs</td>
</tr>
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<td>Ki67 labeling index</td>
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<td>Lipofibromatosis, adult uterine undifferentiated sarcoma</td>
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<td>Known gene fusions of infantile fibrosarcoma</td>
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</table>


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

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, Glostrup, Denmark), S100 protein (1: 3000, DAKO), CD34 (1: 200, Dako), CD10 (RTU, Novocastra, Newcastle, UK), Ki67 (1: 100, MAb MIB-1, Dako), and phosphohistone-H3 (1: 5000, Cell Marque, Rocklin, USA), TLE1 (1: 20, Cell Marque, Rocklin, US), Fli1 (1: 300, Becton and Dickinson, Flasklin Lakes, US), p53 (1: 100, DAKO), ERG (rtu, Ventana, Export, US), CD99 (1: 200, Novocastra (Leica), Munich, 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 inhibitor 1 (NI-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 pH3 immunostain on 4 µm thick 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 targeted gene panel (FIRST brain tumor panel), which was customized and verified by the Department of Pathology of 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. 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, SNV, InDel, 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 LofFreq for SNV/InDel detection, Delly and Manta for Translocation discovery, ThetA2 for purity estimation, and CNVKit for CNV calling, 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 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.

Result

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).

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. Six cases of ETV6-NTRK3 fusion sarcomas are all alive without disease for an average of 11.7 years (range: 6.0-17.4 years) except one case who had a huge sacrococcygeal mass and lost follow-up.

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

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. 2). 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, and vimentin (Fig. 2). The robust nuclear positivity of Trk was remarkable (Fig. 3). However, the tumor cells were negative for S-100 protein, SMA, desmin, myogenin, CD99, Fli-1, CD56, STAT6, p53, cytokeratin, and EMA. 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. 2). There was collagen lay down between the tumor cells. Intermixed inflammatory cell infiltration was remarkable, which was pronounced on CD3 IHC (Fig. 2). 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. 2). The nuclear envelope positivity for Trk was remarkable with weak cytoplasmatic staining (Fig. 3).

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 cytoplasmatic stain with some nuclear staining (Fig. 3). Ki-67 labeling indices were 15–60%. ETV6-NTRK3 fusion was verified by fluorescence in situ hybridization in all six cases (Fig. 3) 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. 4).

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 ETV6 and NTRK3 (Breakpoints: 12: 12022903: 15: 88483984, 12: 12022903, 15: 88524591) were 11 and 16, and 25 and 8, respectively (Supplementary Fig. 1–4). Split reads are the reads fragments of the unmatched paired-end alignments. 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.20 Recent reports have described emerging pediatric fusion-positive sarcomas.5,21,22 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 sarcoma. 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, extremely low mitotic activity (near 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 coexpression are generally rare in sarcomas, which can be interpreted as hybridomas or dual differentiation; however, infantile fibrosarcomas often show co-expression of these two antibodies.13,23 Miettinen et al. reported that non-pleomorphic, low-grade spindle cell features characterized LMNA-NTRK1 fusion infantile fibrosarcomas, low mitotic rates (< 5/10 HPFs), and S100 protein-positivity with often CD34-coexpression.23 Our LMNA-NTRK1 fusion sarcoma was consistent with Hung et al.’s and Miettinen et al.’s S100/CD34 coexpressing infantile fibrosarcomas.

**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.24,25 More recently, NTRK1 chromosomal rearrangements have been identified in additional tumor types (Supplementary file, Table 2), 7,17,26,27 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.26 Depending on the direction 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.10 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 12–17.14 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 cytoplasmatic 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 cytoplasmatic staining with the Trk antibody (Mab EPR17341). In contrast, TPM3-NTRK1 fusion tumors showed strong and diffuse cytoplasmatic staining.3 Thus, these differences might be due to various Trk antibody clones.

ETV6-NTRK3 and (rarely) EML4-NTRK1, LMNA-NTRK1, TPM3-NTRK1, and SQSTM1-NTRK1 fusions have been reported in infantile fibrosarcoma (Table 1).4,13 The six cases of classic infantile fibrosarcoma 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.\textsuperscript{4,23} These \textit{NTRK} fusion tumors tend to respond to \textit{NTRK} inhibitors.\textsuperscript{2,8} 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 \textit{TPR-NTRK1} fusion showed good response.\textsuperscript{28} \textit{NTRK} gene fusion could be a novel target of \textit{NTRK} inhibitors for multiple tumor types.\textsuperscript{2}

In conclusion, we report two cases of \textit{NTRK1} fusion and six cases of \textit{NTRK3} fusion pediatric sarcomas that were diagnostically challenging before NGS or FISH; the three types of fusion sarcomas (\textit{TPR-NTRK1}, \textit{LMNA-NTRK1}, and \textit{ETV6-NTRK3}) differed in their H&E morphology, immunoprofile, and Trk immunopositivity patterns. In the case of \textit{LMNA-NTRK1} fusion sarcoma, S100/CD34/CD10-coexpression was a novel finding. While the finding of S100 protein, nestin, and CD10 positivity in infantile fibrosarcoma is new, the \textit{TPR-NTRK1} fusion sarcoma was negative for S100 protein. The most interesting findings were the distinctive Trk immunopositivity patterns according to the fusion subtype and that the CD34/S100/nestin/CD10 immunoprofile could be used to suggest the \textit{NTRK} fusion partner genes. The patients with \textit{NTRK} fusion infantile sarcomas survived for up to 17.3 years without tumor recurrence except for a sacrococcygeal tumor, regardless of the histological grade, cellularity, or ki-67 labeling index. The median survival of our infantile fibrosarcomas was 8.3 years. Furthermore, patients with these fusion tumors may benefit from \textit{NTRK} inhibitor therapy if the tumors cannot be controlled by conventional therapy.\textsuperscript{29}

**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
- LMNA, lamin A/C
- LSI, locus-specific identifier
- Mab, monoclonal antibody
- MRI, magnetic resonance imaging
- NFASC, neurofascin
- NGS, next-generation sequencing
- NTRK, neurotrophic receptor kinase
- RABGAP1L, RAB GTPase activating protein 1-like
- TFG, TRK-fused gene
- TPM3, tropomyosin 3
- TPR, translocated promoter region, nuclear basket protein

**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
Acknowledgments: I express many thanks to our team of the division of Molecular pathology and immunohistochemical laboratories.

Authors’ contributions: S-H Park designed, supervised, and edited this study and manuscript. J Kang wrote the manuscript, J-W Park, J Kang, and S-H Park collected and analyzed the clinicopathological data. 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.

References


Supplementary Figure Captions

**Supplementary Fig. 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 488bp deletion (breakpoints: chromosome 1: 186337018; 1: 156844363).

**Supplementary Fig. 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 Fig. 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 Fig. 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.

Figures

**Figure 1**

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

Figure 3

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 co-expresses CD34, S100, and CD10. (A: nestin, B, F: CD34, C, G: S100 protein, E: CD34D, H: CD10, Bar: 200 micrometers).
Figure 4

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 5

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