Recurrent BRAF Gene Fusions in a Subset of Pediatric Spindle Cell Sarcomas

Expanding the Genetic Spectrum of Tumors With Overlapping Features With Infantile Fibrosarcoma

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纤维母细胞性/肌纤维母细胞性肿瘤（2013）

- 中间性（局部侵袭性）
  浅表纤维瘤病（掌/跖）
  韧带样型纤维瘤病
  脂肪纤维瘤病

- 中间性（偶见转移型）
  孤立性纤维性肿瘤
  炎症性肌纤维母细胞性肿瘤
  低度恶性肌纤维母细胞肉瘤
  黏液炎症性纤维母细胞肉瘤
  婴儿型纤维肉瘤

- 恶性
  成人型纤维肉瘤
  黏液纤维肉瘤
  低度恶性纤维黏液样肉瘤
  玻璃样变梭形细胞肿瘤
  硬化性上皮样纤维肉瘤
KEY WORDS

- fibrosarcoma
- infantile fibrosarcoma
- BRAF
- NTRK1, NTRK3, fusions
BACKGROUND

先天性/婴儿纤维肉瘤
Congenital/Infantile Fibrosarcoma

• 临床特征：大部分病例发生在1岁以前，1/3出生时即出现
• 主要部位是四肢，躯干和头颈部较少见
• 惰性肿瘤，预后良好，远隔转移率低，5年生存率89%，大部分可通过肿瘤扩大切除治愈
病理特征

- 形态单一、胞质稀少的梭形细胞密集排列成束状，呈鱼骨样外观
- 细胞核多形性不明显，核分裂象活跃
- 可出现较多胶原纤维的区域
- 其他改变：不规则血窦和裂隙状血管形成血管外皮细胞瘤样结构；多少不等的慢性炎性浸润、髓外造血
- 少见：多核巨细胞；圆形细胞区域

部分内容参考王哲主任课件
免疫表型：缺乏特异性

- Vim 阳性
- 局灶SMA、MSA阳性

遗传学改变：

- 大部分先天性病例出现t(12;15)(p13;q25) → ETV6与NTRK3基因融合
ETV6 (ETS variant gene 6)：定位于12p13。编码的蛋白质是ETS转录因子家族中一种序列特异性的转录抑制因子，通过Fli-1结合抑制其活性，在早期造血和血管生成中起着重要作用。

NTRK3 (neurotrophic tyrosine kinase, receptor type 3)：定位于15q25。编码神经营养因子-3 (NT-3) 的跨膜受体，在神经系统的生长发育中起着重要作用。
ETV6-NTRK3信号转导途径

激活RAS-RAF1-MEK-ERK1/2和PI3K-Akt两条酪氨酸途径引起细胞增殖和分化失控，从而引发肿瘤。
ETV6-NTRK3融合基因在肿瘤组织中的表达

- 先天性/婴儿型纤维肉瘤（CFS）：几乎所有患者年龄<两岁，E-N融合基因只发生于CFS，在成人纤维肉瘤、婴儿纤维瘤、肌纤维瘤等组织学相似的梭形细胞肿瘤中不表达。
- 富于细胞型先天性中胚层肾瘤（CMN）：患者发病年龄轻，预后相对较好，可以与CFS共存。
- 急性髓系白血病：较少见或只表达在某些特定亚型。
- 分泌型乳腺癌（SBC）：有学者研究202例乳腺癌标本，5例SBC4例表达E-N融合基因，其他类型乳腺癌皆不表达。
BACKGROUND

Therapy

IFS with ETV6-NTRK3 gene fusions

significant sensitivity to cytotoxic chemotherapy and more recently a promising response to NTRK inhibitors (crizotinib)
病例讨论 (Index Case)

- 女性，16岁
- 腹膜后肿瘤，合并破裂和腹腔内出血
- 术中显示腹膜受累
病例讨论(Index Case)

- H&E：疏松的间质中梭形细胞呈束状排列，细胞染色质细，细胞核不典型，可见淋巴细胞、浆细胞浸润及扩张的血管，核分裂象高达6/10HPF

- IHC：SMA (+)、caldesmon (灶+);

On the basis of these findings, a diagnosis of IFS was assigned.

- FISH：未检测到ALK、SS18、ROS1及RET基因重排
FIGURE 2. A SEPT7-BRAF gene fusion was identified in the index case by targeted RNA sequencing. A pericentric inversion of chromosome 7 resulted in the fusion of SEPT7 (7p14.2) and BRAF (7q34) (A). RNA sequencing fusion junction reads and subsequent confirmatory RT-PCR showed SEPT7 exon 11 fused in-frame to BRAF exon 11 (B). FISH further confirmed BRAF gene rearrangement with break-away centromeric (red) and telomeric (green) signals (C, left, arrows) as well as SEPT7-BRAF fusion with come-together SEPT7 (red) and BRAF (green) signals (C, right, arrows).
# Patient Selection

## TABLE 1. Clinicopathologic and Genetic Findings of Pediatric Spindle Cell Sarcomas With Features Reminiscent of Infantile Fibrosarcoma

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Site</th>
<th>SMA</th>
<th>CD34</th>
<th>S100</th>
<th>Desmin</th>
<th>Gene Fusions</th>
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<tbody>
<tr>
<td>1</td>
<td>16 y</td>
<td>F</td>
<td>RP</td>
<td>Patchy+</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>SEPT7-BRAF*,†</td>
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<tr>
<td>2</td>
<td>3 y</td>
<td>F</td>
<td>Pelvis</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>BRAF rearrangement†</td>
</tr>
<tr>
<td>3</td>
<td>6 mo</td>
<td>F</td>
<td>Pelvis</td>
<td>Focal+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>BRAF rearrangement†</td>
</tr>
<tr>
<td>4</td>
<td>1.5 y</td>
<td>M</td>
<td>T6-12 spine (extradural)</td>
<td>NA</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>CUX1-BRAF†‡,†</td>
</tr>
<tr>
<td>5</td>
<td>2 d</td>
<td>M</td>
<td>Thigh</td>
<td>Focal+</td>
<td>-</td>
<td>-</td>
<td>Rare cells</td>
<td>BRAF rearrangement†</td>
</tr>
<tr>
<td>6</td>
<td>1 y</td>
<td>M</td>
<td>Foot</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>EML4-NTRK3*,†</td>
</tr>
<tr>
<td>7</td>
<td>7 wk</td>
<td>M</td>
<td>RP</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>8</td>
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<td>F</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Unknown</td>
</tr>
<tr>
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<td>Lumbar</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>10</td>
<td>3 y</td>
<td>F</td>
<td>RP</td>
<td>-</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*By targeted RNA sequencing.
†By FISH.
‡By FoundationOne, Foundation Medicine.
§By whole transcriptome sequencing.

F indicates female; IHC, immunohistochemistry; M, male; NA, not available; RP, retroperitoneum.
MATERIALS AND METHODS

• Targeted RNA Sequencing (Index Case/#1 & case#6)
• Whole Transcriptome Sequencing (case#7)
• FISH:
  ✓ index case/#1: BRAF break-apart and SEPT7-BRAF fusion
  ✓ case#6: EML4 and NTRK3 break-apart
  ✓ case#7: TPM3-NTRK1 fusion
• Reverse Transcription-Polymerase Chain Reaction Sequencing (Index Case/#1 & case#7)
RESULTS
RESULTS

FIGURE 4. EML4-NTRK3 fusion in an IFS involving the foot of a 21-month-old boy (case #6). Targeted RNA sequencing showed fusion of EML4 exon 2 to NTRK3 exon 14, resulting from a t(2;15) translocation (A, B). The chimeric protein contains tyrosine kinase domain from NTRK3 but no known functional domain from EML4 (B). The downstream exons after the breakpoint of NTRK3 (exon 14) showed upregulated mRNA level in contrast to the 5’ NTRK3 exons and other samples in the same data set (B, orange dots). FISH confirmed rearrangement of both EML4 and NTRK3 genes, with break-apart of centromeric (red) and telomeric (green) signals (C).
FIGURE 5. **TPM3-NTRK1** fusion in a 7-week-old infant retroperitoneal IFS (case #7). Whole transcriptome RNA sequencing identified a 2.7 Mb inversion-fusion at 1q21.3 locus, leading to a **TPM3-NTRK1** candidate gene fusion (A). The RT-PCR confirmed an in-frame fusion of **TPM3** exon 6 to **NTRK1** exon 9 (B). FISH also confirmed **NTRK1** rearrangement (split of green and yellow signals) and fusion of **TPM3** (red) with **NTRK1** (green, telomeric/3’ of **NTRK1**) (arrows) (C). **NTRK1** mRNA expression was upregulated compared with other samples in the same platform (D, left). Exonic expression levels showed high expression of **NTRK1** exons downstream to the break in exon 9 (orange dots); the fusion retaining the entire tyrosine kinase domain in the chimeric protein (D, right).
BRAF mutations

✓ melanoma
✓ papillary thyroid carcinoma
✓ non-small cell lung cancer
✓ colorectal cancer
✓ hairy cell leukemia
✓ Langerhans cell histiocytosis
✓ ovarian tumors
**BRAF related fusions**

- Gliomas, carcinomas, and melanocytic neoplasms through either intrachromosomal or interchromosomal translocations
- Soft tissue tumors
  - Only in a subset of myxoinflammatory fibroblastic sarcomas: characterized by superficial acral location and composed of alternating myxoid and solid areas, with a histiocytoid phenotype and Reed-Sternberg-like tumor cells
  - 1 case of chest wall malignant spindle cell neoplasm: KIAA1549-BRAF fusion
EML4-NTRK3 fusion

2 cases (including our case)

- located in the extremity
- A boy (9 month): Composed of elongated spindle cells and CD34 (-); lung metastasis which responded to chemotherapy and radiation therapy
- Our case: a more primitive appearance comprised of round to short spindle cells and CD34 (+)
- Both cases showed a brisk mitotic activity (>10/10HPF)
NTRK1-related fusion

- **SQSTM1-NTRK1** (1 case): IFS
- **LMNA-NTRK1** (1 case):
  - male; infant
  - developed local recurrence and metastasis to bilateral lungs and S5 vertebral body.
  - refractory to chemotherapy but responded to crizotinib
- **TPM3-NTRK1**: our case
- Other soft tissue tumor types: lipofibromatosis-like neural tumors & a group of spindle cell sarcomas with prominent myopericytic/hemangiopericytic pattern
SEPTIN gene family

- **SEPT7**: has GTPase activity and is associated with cytoskeleton structure and organelle transport.
- **SEPT9**: previously reported in a case of T-cell prolymphocytic leukemia with SEPT9-ABL1 fusion, which was resistant to both imatinib and dasatinib therapy.
- **Others (SEPT2, SEPT5, SEPT6, SEPT9, SEPT11)**: involved as 3’ partners in hematological malignancies with MLL-SEPTIN genes fusions.
CONCLUSION

- Our findings of recurrent BRAF gene fusions in a group of tumors with features reminiscent of IFS expand the spectrum of fusion-positive spindle cell sarcomas to also include older children and adolescents and predilection for intra-abdominal sites.

- Pediatric tumors with IFS-like phenotype, the molecular work-up should ideally include testing for abnormalities in other kinases, such as BRAF, NTRK1, MET, if the tumor lacks the canonical ETV6-NTRK3 fusion.
感谢聆听