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BRIEF REPORT

Novel KHDRBS1-NTRK3 rearrangement in a congenital pediatric CD34-positive skin tumor: a case report

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Abstract

Cutaneous spindle-cell neoplasms in adults as well as children represent a frequent dilemma for pathologists. Along this neoplasm spectrum, the differential diagnosis with CD34-positive proliferations can be challenging, particularly concerning neoplasms of fibrohistiocytic and fibroblastic lineages. In children, cutaneous and superficial soft-tissue neoplasms with CD34-positive spindle cells are associated with benign to intermediate malignancy potential and include lipofibromatosis, plaque-like CD34-positive dermal fibroma, fibroblastic connective tissue nevus, and congenital dermatofibrosarcoma protuberans. Molecular biology has been valuable in showing dermatofibrosarcoma protuberans and infantile fibrosarcoma that are characterized by *COL1A1-PDGFB* and *ETV6-NTRK3* rearrangements respectively. We report a case of congenital CD34-positive dermohypodermal spindle-cell neoplasm occurring in a female infant and harboring a novel *KHDRBS1-NTRK3* fusion. This tumor could belong to a new subgroup of pediatric cutaneous spindle-cell neoplasms, be an atypical presentation of a plaque-like CD34-positive dermal fibroma, of a fibroblastic connective tissue nevus, or represent a dermatofibrosarcoma protuberans with an alternative gene rearrangement.

Keywords Cutaneous · Neoplasms · Spindle-cell

Introduction

Cutaneous spindle-cell proliferations form a large spectrum of neoplasms occurring in children and adults. Along this spectrum, infantile fibroblastic, and myofibroblastic tumors constitute a group of lesions with benign to intermediate malignancy potential. They represent the second most common group of soft-tissue tumors occurring in the first year of life. Infantile fibrosarcoma, usually occurring under 2 years of age, is the most common malignant entity before age 1 year and is mainly associated with local aggressiveness. The differential diagnosis between cutaneous spindle-cell neoplasms in children can be challenging, especially regarding the morphologic overlap between tumors of the infantile fibroblastic/myofibroblastic spectrum.

Molecular study is useful in the differential diagnosis of these tumors in that infantile fibrosarcoma and dermatofibrosarcoma protuberans are characterized by quite specific translocations, *ETV6-NTRK3*, and *COL1A1-PDGFB*, respectively. Some cutaneous spindle-cell neoplasms are characterized by CD34-positivity, including fibroblastic connective tissue nevus (FCTN), plaque-like CD34-positive dermal fibroma (PLDF),

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lipofibromatosis, and congenital dermatofibrosarcoma protuberans (CDFSP) in children.

We report a case of pediatric cutaneous congenital skin tumor with CD34-positive spindle cells featuring a novel *KHDRBS1-NTRK3* fusion.

Case report

A 2-month-old female infant was referred to our hospital center for suspected Kasabach-Merritt phenomenon associated with a congenital vascular tumor of the trunk. The child's parents were from Turkey and had no consanguinity and no medical history. She had a 4-year-old brother with no health problems. The infant had shown normal growth for the first 3 months of life and had no medical history. At 1 month of age, the lesion was a 3 × 3-cm infiltrated cutaneous mass of the abdomen that was asymptomatic. The lesion had rapidly increased in size (5.0 × 4.5 cm) (Fig. 1a). A biological test showed anemia (hemoglobin level 7.8 g/dL, normally > 13 g/dL) and thrombocytopenia (58,000/μL, normally > 150,000/μL), without coagulation abnormalities. We suspected the beginning of Kasabach-Merritt phenomenon, but biological values spontaneously normalized within 48 h. Abdominal wall MRI with the infant under sedation showed a cutaneous vascular-like mass infiltrating underlying fat and abdominal muscles (Fig. 1b). The mass was slightly heterogeneous, with hypersignals on short-TI inversion recovery sequence (STIR).

After a multidisciplinary discussion, a deep skin biopsy was performed. Gross examination revealed a whitish and fibrous aspect. The lesion corresponded to a dermohypodermal proliferation of monotonous spindle cells arranged in a fascicular and storiform pattern. This proliferation included the cutaneous appendages, without destroying them, and dissected the hypodermic adipose tissue with a honeycomb pattern. No vascular inflection, no necrotic areas, and no epidermal ulceration were seen in the tumor. Some mitotic activity was observed (five mitosis per 2 mm^2). On immunohistochemical studies, tumor cells showed strong and diffuse immunostaining for CD34 but were negative for desmin, smooth muscle actin, caldesmon, cytokeratin AE1-AE3, epithelial membrane antigen, and protein S100 as well as CD31 and E26 oncogene homolog, excluding a vascular tumor (Fig. 2). The proliferative index (Ki67) was 10%. Quantitative RT-PCR analysis and fluorescent in situ hybridization results of formalin-fixed paraffin-embedded material were negative for *COL1A1-PDGFB* rearrangements characterizing dermatofibrosarcoma protuberans. RNA-sequencing of frozen material was used to investigate the presence of specific *ETV6-NTRK3* translocation of congenital infantile fibrosarcoma. The tumor featured an undescribed rearrangement involving exon 7 of KH RNA binding domain containing, signal transduction-associated 1 (*KHDRBS1*) and exon 12 of neurotrophic tyrosine receptor kinase 3 (*NTRK3*) (Fig. 3). Fusion was initially detected on RNAseq data by two different algorithms (Defuse and FusionMap). Observed alignments allowed to predict and to model the chimeric RNA transcript. Specific primers were designed and tested by specific PCR on independent cDNA obtained from the tumoral RNA. Sanger Sequencing of the obtained fragment was then performed and analyzed and revealed a specific frame fusion of *KHDRBS1-NTRK3*.

A second-opinion pathologic review was requested concomitantly, arguing in favor of plaque-like CD34-positive dermal fibroma (PLDF), without excluding a fusion-negative CDFSP.

Because the tumor was not easily extractable, with multidisciplinary and multicenter consultation, the decision was to follow up every 3 months and to discuss targeted therapy with tropomyosin-receptor kinase (TRK) inhibitors if the tumor increased and/or presented local aggressiveness. The tumor grew slowly from 5 cm (at 2 months of age) to 7 cm (at 8 months of age). At age 10 months, clinical and imaging evaluation revealed a stable lesion.

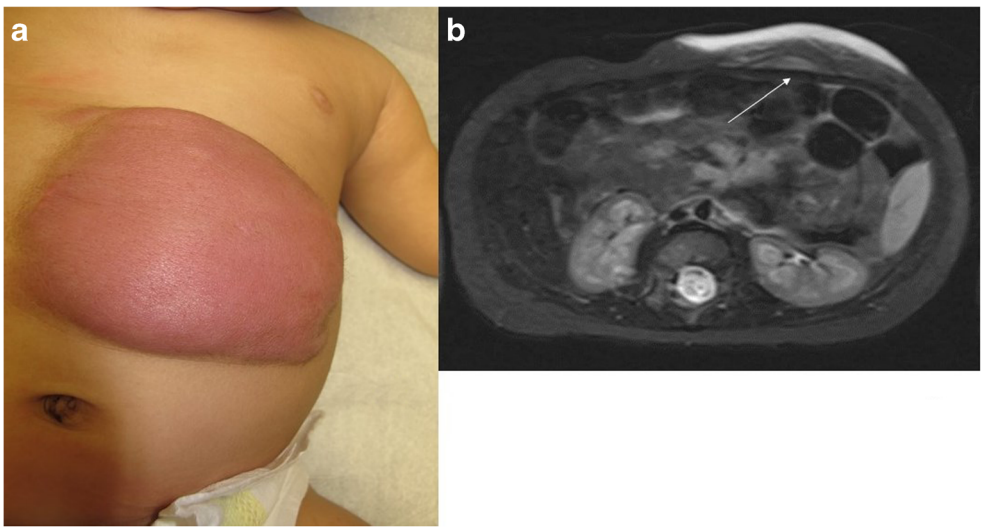

Fig. 1 **a** Voluminous infiltrate congenital vascular cutaneous mass of the abdomen in a female infant at age 2 months. **b** MRI, T2 STIR sequence, and axial section at age 2 months. The tumor showed hypersignals on STIR sequence

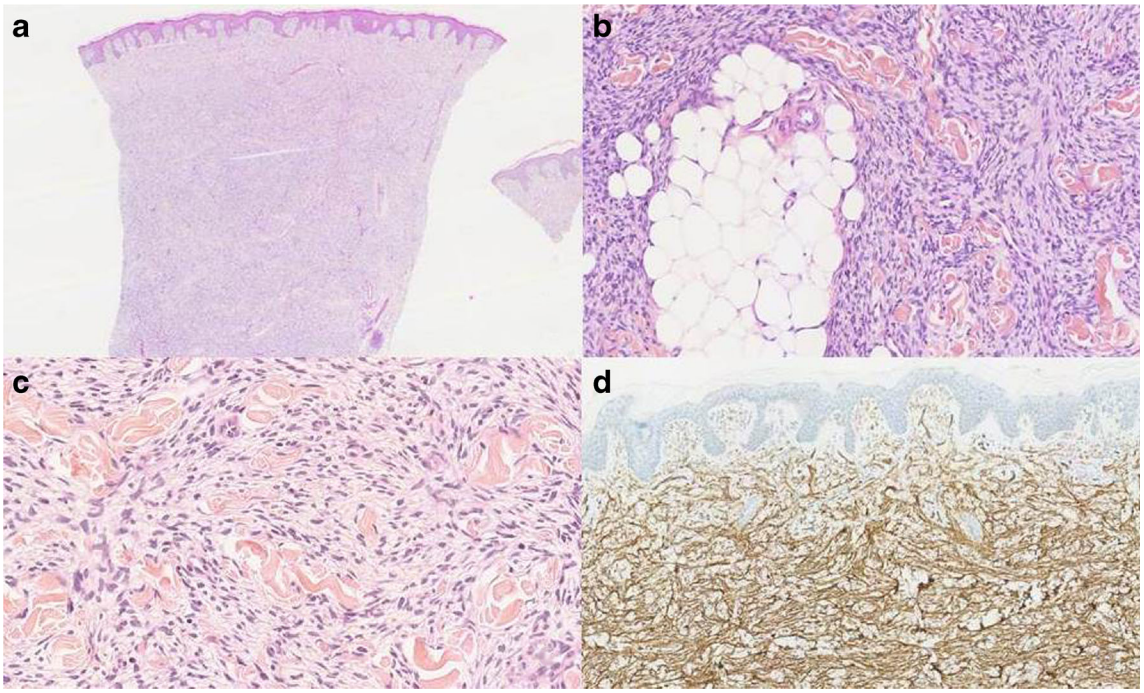

Fig. 2 **a** Hematoxylin, eosin, and saffron (HES) staining showing dermal and hypodermal infiltration with the proliferation (HES × 25). **b** Fascicular pattern associated with honeycomb pattern of fat infiltration (HES × 100). **c** HES staining showing the monotonous aspect of the proliferation associated with scattered collagen bundles (HES × 400). **d** CD34 immunostaining showing diffuse positivity of the proliferation

Discussion

This case of congenital CD34-positive dermohypodermal spindle-cell neoplasm occurred in a female infant and harbored a novel *KHDRBS1-NTRK3* fusion. This tumor could belong to a new subgroup of pediatric cutaneous spindle-cell neoplasms, be a FCTN, a PLDF, or a CDFSP with an alternative gene rearrangement.

Infantile CD34-positive spindle-cell tumors of the skin represent a poorly defined group that includes lipofibromatosis, CDFSP, and the more recently described FCTN and PLDF.

PLDF is a frequently congenital neoplasm or occurring in the early months of life, mostly in girls. The tumor, usually located on the trunk or the extremities, presents as an atrophic and erythematous/pigmented plaque that involves the superficial dermis, or deep dermis and hypodermis when congenital. The proliferation is composed of monotonous spindle cells, with a fascicular or storiform pattern, with variable cellularity and very low mitotic activity. This diagnosis could not be applied to our case, as we did not found any altered and fragmented elastic fibers, which is a key element for the diagnosis of PLDF, and there was no specific orientation of tumor cells, as observed in PLDF [1–3]. Also, no molecular anomalies have been identified in PLDF to our knowledge.

FCTN mainly involves the chest of children and young adults and presents a plaque-like appearance. This tumor is composed of dermohypodermal fascicles with haphazardly distributed spindle cells, entrapping adnexa, and fat without destroying them. The presence of epidermal papillomatous hyperplasia and adipose tissue in the reticular dermis, not observed in our case, is common in FCTN (70 and 61% respectively). Spindle cells show a variable smooth muscle actin positivity and a patchy and weak staining for CD34 (87% of cases), which was strong and diffuse in our case [3, 4]. Conversely, mitotic activity is uncommon in FCTN whereas significant in our case.

Lipofibromatosis is a rare and benign skin or soft-tissue tumor (20% congenital), mainly affecting males, presenting as a subcutaneous nodule, mostly located on extremities which is different for clinical presentation of our case. This lesion is composed of mature adipose tissue and a fibroblastic component, featuring a fascicular pattern. Fibroblastic spindle cells show a variable staining for CD34 and smooth muscle actin [2, 3]. More recently, a subset of locally aggressive neoplasms, called lipofibromatosis-like neural tumors, has been highlighted and is associated with a S100 protein staining (not observed in lipofibromatosis and in our case) and *NTRK1* rearrangements [5].

CDFSP is a cutaneous fibroblastic tumor of intermediate malignancy associated with local aggressiveness and a high rate of recurrence but rare metastases; it represents an important differential diagnosis with PLDF. The tumor is usually on the trunk or the proximal extremities and begins as a red-blue plaque that slowly grows before becoming nodular, resembling a vascular lesion in the early stage. It can be congenital, as was the present case. The proliferation infiltrates the dermis and hypodermis with a typically storiform pattern and often

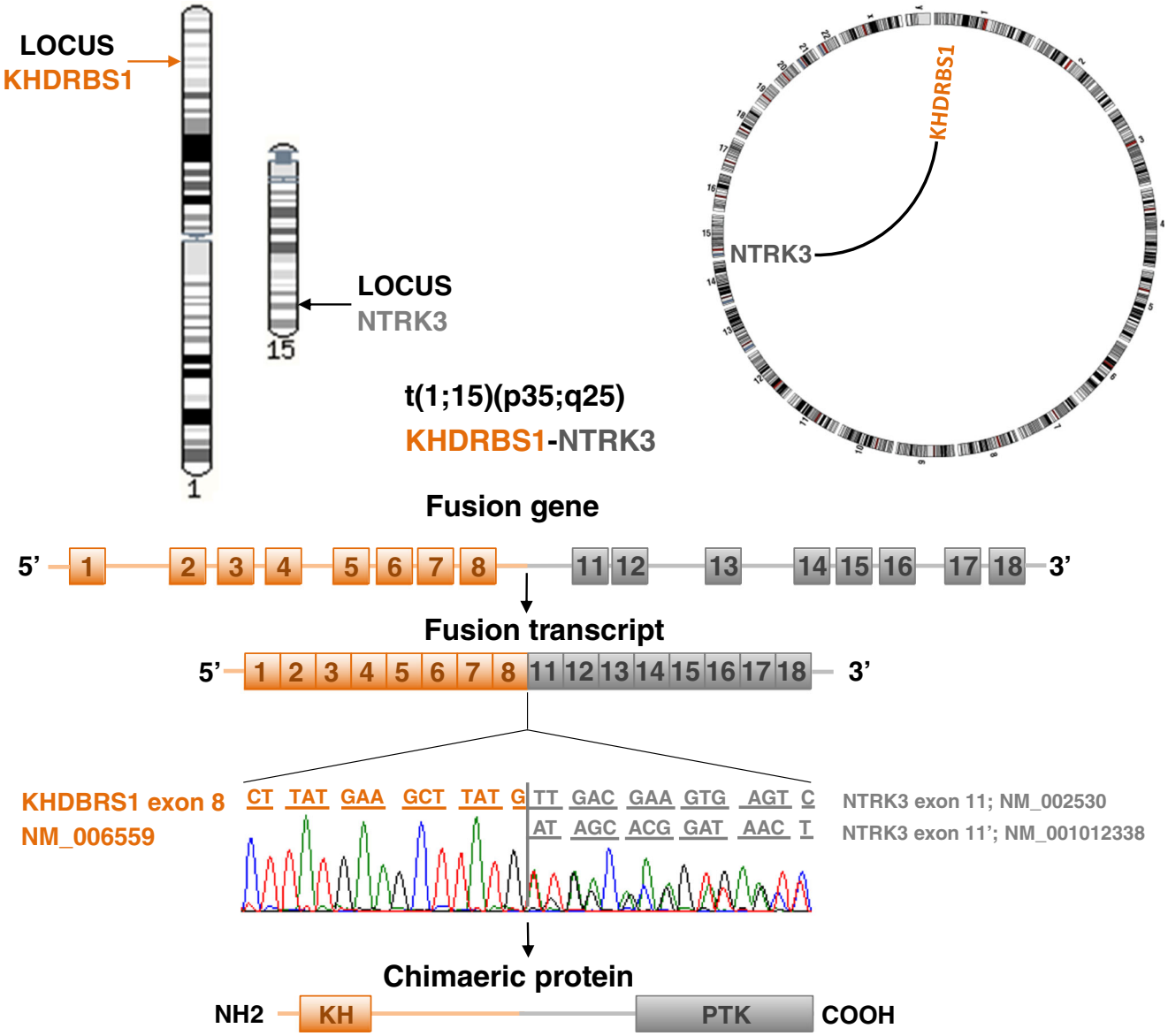

Fig. 3 Circular genome view. *KHDRBS1-NTRK3* fusion involving exon 7 of *KHDRBS1* (1p35.2) and exon 12 of *NTRK3* (15q25.3)

permeative adipose tissue infiltration (honeycomb pattern), a low cellular atypia and low mitotic activity, like in our case. However CDFSP is characterized by *COL1A1-PDGFB* translocation in 90% of cases, and even when negative, no *NTRK* fusions are observed [2, 3, 6].

Fibrosarcoma can also show CD34 positivity, especially when developed from a dermatofibrosarcoma protuberans. Infantile fibrosarcoma is a malignant tumor in childhood, mainly occurring before age 1 year (36–80% congenital). The tumor most often involves the extremities (two thirds of cases) and presents as a quickly growing and ulcerated mass. The proliferation, densely cellular, is composed of monotonous spindle cells showing a herringbone or diffuse pattern and high mitotic activity. Hemorrhagic and necrotic areas are common. Infantile fibrosarcoma has a relatively favorable prognosis with low mortality rate (5%) but frequent recurrence (5–50%) and is characterized by *ETV6-NTRK3* or an alternative rearrangement (70% of cases) [2, 3, 7].

The increase in the next-generation sequencing has led to the discovery of various recurrent rearrangements in solid tumors, with some rearrangements being oncogenic factors and other passenger events. The main molecular mechanisms underlying the ability of gene rearrangement to induce tumorigenesis include abnormal expression of one of the genes involved in the fusion, aberrant functional activity of the chimeric protein, or inactivation of a truncated tumor suppressor gene.

The functional role of the *KHDRBS1-NTRK3* chimeric protein remains to be determined, but when upregulated, both partners have shown a role in tumor cell proliferation. *KHDRBS1* or Src associated in mitosis, 68 kDa (SAM68), is

a KH domain RNA-binding protein and part of the signal transduction and activation of RNA metabolism (STAR) family of RNA binding proteins. The physiological function of these proteins in RNA processing is not completely elucidated, but SAM68 has a role in transcriptional and posttranscriptional regulation of gene expression in cancer cells. Its regulatory networks contribute to important processes involved in cancer initiation and progression [8].

Rearrangements of NTRK genes are found at a low level in various tumors [9, 10]. Conversely, high rates of NTRK fusions are frequently found in some rare entities, namely, secretory breast cancer and mammary analogue secretory cancer in adults as well as congenital infantile fibrosarcoma and congenital mesoblastic nephroma in children [11–13].

TRKA, TRKB, and TRKC belong to the TRK family proteins, encoded by *NTRK1*, *NTRK2*, and *NTRK3* genes, respectively. These tropomyosin-tyrosine-kinases are high-affinity receptors for nerve growth factors, which play a role in the development of central and peripheral nervous system [10]. *NTRK* fusions involve the 3′ portion of the gene, encoding the catalytic tyrosine kinase domain, and the 5′ portion of a variable partner gene, which drives the expression of the chimeric gene. Activation of a TRK receptor activates the downstream Ras/MAPK/ERK, PI3K/AKT/mTOR or phospholipase C-gamma signaling transduction pathways.

Identification of these translocations has increased in interest because of the recent availability of TRK inhibitors (entrectinib, larotrectinib) that have demonstrated dramatic efficiency in patients with NTRK rearranged tumors [10, 14, 15]. Otherwise, pan-TRK immunohistochemical staining is now available and seems to be a reliable tool for detecting NTRK fusions in tumors [16].

To conclude, we report an undescribed *KHDRBS1-NTRK3* rearrangement in a CD34-positive spindle-cell skin tumor in an infant. This case may represent a new subgroup of pediatric fibroblastic tumor, an atypical presentation of a plaque-like CD34-positive dermal fibroma or of a fibroblastic connective tissue nevus or a CDFSP with an alternative gene fusion. Further investigations are needed to determine whether these last three entities could harbor *NTRK* rearrangements.

Contributions The first author and last author (M. Tallegas and A. Maruani) have written the first draft of the manuscript. A. Binet performed the skin samples which were analyzed by MC. Machet and S. Fraitag (histological findings). Molecular findings were performed by G. Pierron and S. Reynaud. Management of the child was organized by A. Jourdain and D. Orbach.

Compliance with ethical standards

The study followed the ethical declaration of Helsinki.

Conflict of interest The authors declare that they have no conflict of interest.

Disclaimer Each author listed on the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript.

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