

Case Report

A novel case of an infantile fibrosarcoma-like tumor with *KIAA1549-BRAF* translocation and an oncogenic NF2p.Q459* SNV with potential clinical significance

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Abstract

We report a case of a right gluteal mass from the sacroiliac joint to the knee of an infant girl. Biopsy showed histopathological features similar to infantile fibrosarcoma (IFS). However, unlike most IFS, no *ETV6-NTRK3* fusion gene abnormality was detected. Molecular analysis with TruSight RNA Pan-Cancer Panel detected the presence of *KIAA1549-BRAF* translocation and an oncogenic NF2p.Q459* SNV with potential clinical significance. A review revealed that the combination of this patient's tumor site with the presence of a *KIAA1549-BRAF* translocation abnormality and an accompanying single nucleotide variant has not been previously described. The detection of this translocation abnormality raises the possibility that the spindle cell tumors in infants with an absence of the *ETV6-NTRK3* fusion gene abnormality might have a distinct pathogenetic mechanism different from the previously known IFS and congenital mesoblastic nephroma. Furthermore, the discovery of *BRAF* translocation and its aberrant signaling of the mitogen-activated protein kinase (MAPK) pathway in this tumor contributes to the promise of clinical benefit of using the MEKi trametinib for the treatment of progressive disease that is refractory to conventional chemotherapy.

Introduction

Infantile fibrosarcoma (IFS) is a rapidly growing and locally aggressive painless mass most commonly occurring in the extremities of young children under the age of two years. Most commonly *ETV6-NTRK3* fusion is detected in IFS which it is therefore used as a diagnostic marker. Though number of new gene alterations have been described in recent years [1] and increases the genetic spectrum of IFS-like tumours IFS-like tumors represent a subset of childhood sarcomas with similar morphological features as IFS but without harboring the characteristic *ETV6-NTRK3* fusion. These alternative rearrangements involve other kinase genes, such as *NTRK3*,

NTRK2, *NTRK1*, *BRAF* and *MET* with having various fusion partners [1,2]. Currently, these tumors are included with IFS category in 2020 WHO classification of soft tissue and bone tumours [2]. We report a case with IFS-like morphology with *KIAA1549-BRAF* fusion with an oncogenic NF2p.Q459* single nucleotide variant (SNV), which to our knowledge, is the first case of this kind reported in the limb.

Results

Clinical history

A three-month old appropriately grown and developed female was prematurely born at 28 weeks' gestation via

emergency Caesarean section due to full length cord prolapse. Antenatal history included three episodes of antepartum hemorrhage and one-week history of premature rupture of the membranes prior to delivery.

At one month of age, the patient was noted to have developed an asymmetric swelling on the right buttock. On imaging (US, MRI) a large heterogeneous soft tissue mass was demonstrated, measuring 8.8. x 3.3. x 4.9 cm, with a focal cystic component up to 2.4 cm. The mass mainly involved the gluteus maximus and extended within the pelvis to the obturator internus and piriformis muscles and down to the posterior aspect of the right thigh to the knee level. The lesion was in close contact with the right-sided iliac vessels and their branches, the rectum and the urinary bladder. The right femur showed focal mild periosteal reaction only. There were no lung metastases identified on CT. The child was started on VAC chemotherapy, comprising vincristine, dactinomycin and cyclophosphamide as per protocol, following initial histological diagnosis of IFS. Upon reviewing the family history, it was revealed that the patient's father, aunt and grandmother had "fatty tissue deposits" in their extremities, the nature of these lesions, however, had been unknown.

Histopathology findings

The biopsy demonstrated a moderately cellular tumour comprising monomorphic spindled cells arranged into intersecting fascicles (Figure 1a). The cells had uniform round to oval nuclei with fine nuclear chromatin and occasional nucleoli. Mitotic figures were easy to see (Figure 1b) and proliferation index with Ki67 was increased (Figure 1c). Very rare lesional cells expressed desmin (Figure 1d). Pan-cytokeratin (AE1/AE3), smooth muscle actin, myogenin, CD34, S100 and SOX10 were all negative (not shown). Nuclear expression of INI1/BAF47 was retained (not shown). Occasional nuclei were positive with p53, and there was no staining with BRAF and pan-TRK (not shown).

Molecular findings

As initial genetic analysis for t(12;15) was negative, TruSight RNA Pan-Cancer Panel (1385 gene panel) was initiated. RNA was extracted from formalin-fixed paraffin-embedded tissue using RNAsort FFPE RNA Isolation kit (Cell Data Sciences, Fremont, CA). Fragment length was assessed with an RNA 6000 chip on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA-sequencing library was prepared using 100 ng total RNA with the Illumina TruSight RNA Pan-Cancer Panel Kit (Illumina, Inc., San Diego, CA). The sample was subjected to targeted RNA sequencing on an Illumina MiSeq Dx platform.

Fusion calling

Reads were aligned to the hg19 human genomic scaffold using the Illumina STAR aligner (v2.6.1) or the DRAGEN

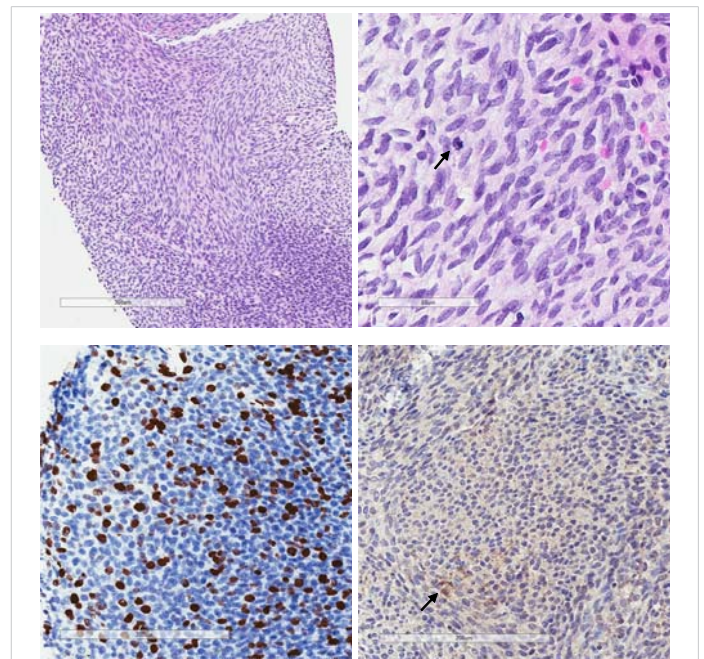


Figure 1: Moderately cellular tumour comprises spindle cells, arranged into intersecting fascicles (A, H&E, x20). The cells had uniform round to oval nuclei with fine nuclear chromatin and mitotic figures are easy to see (arrow) (B, H&E, x40). Immunostaining with Ki-67 showed increased proliferation activity (C, x20). Only a few lesional cells (arrow) were immunoreactive with desmin (D, x20).

aligner (v3.7.5). Fusion transcripts were identified using the Manta structural variant caller (v1.5.0) or the DRAGEN fusion caller (v3.7.5), respectively. Genes identified by both fusion callers were manually checked using Integrative Genomics Viewer (IGV) (v2.9.4) [3]. RNA fusion transcript was visualized using the St. Jude Cloud website [4]. Of note, a minimum of one gene in a putative gene fusion was needed to be present in the TruSight Pan-Cancer gene panel in order to be detected.

SNV calling

The Sequence was aligned to the hg19 human genomic scaffold using the Illumina STAR aligner (v2.6.1a) and single nucleotide variants were identified using the Strelka SNV / indel variant caller (v2.9.9). Genes identified as of potential diagnostic relevance were manually checked using IGV. Only "stop", "truncation", "frameshift", "splice", "deletion", "insertion" and "missense" variant types were considered for analysis. Subsequently, only established oncogenic hotspot mutations found in COSMIC and/or ClinVar databases were reported. Variants of unknown significance were not reported. This RNA-based test could not distinguish between somatic and germline variants, and all variants were assumed to be somatic.

Using the above criteria, we detected *KIAA1549 - BRAF* fusion with the presence of an in-frame fusion between exon 15 of the *KIAA1549* gene (NM_020910) and exon 11 of the *BRAF* gene (NM_004333). The kinase domain of *BRAF*, encoded by exons 11 to 18 was retained in the predicted fusion protein (Table1, Figure 2, Figure 3). An oncogenic

NF2p.Q459 SNV was also detected. The sequence change created a premature translational stop signal (p.Q459*) in the NF2 gene. It was predicted to result in an abnormal disruption/ termination of translation into protein product. We concluded this mutation to be a variant with potential clinical significance (Table 2). The missense variant of *PMS2* was thought to be a variant of unknown clinical significance in this case (Table 2).

Discussion

The most frequently detected gene fusion in IFS is *ETV6-*

NTRK3, t(12:15)(p13;q25). Infantile spindle cell sarcomas with similar histopathological features that lack this fusion abnormality, developed through oncogenic activation of various other genes involved in kinase signalling pathway, have recently been reported (e.g.; *NTRK3*, *NTRK2*, *NTRK1*, *BRAF*, *MET*). Moreover, there is significant variation in gene fusion partners (e.g.; *LMNA*, *TPR*) [1,2], and breakpoint locations within the exons. Given the extent of molecular alterations associated with IFS, next-generation sequencing - based RNA and DNA analysis should be considered in fusion negative cases by FISH and RT-PCR.

One of these alternative molecular abnormalities involves the *BRAF* gene, which encodes a member of RAF family protein kinases, functioning downstream of *RAS* as part of the MAPK signaling pathway and facilitating cell proliferation, survival and transformation [5]. Kao, et al. reported 4 cases of pediatric spindle cell sarcomas with *BRAF* gene rearrangement in 2018. One patient presented with congenital mass in the thigh extending into the pelvis, two patients had pelvic mass and one patient had an extradural tumor with T6-T12 involvement. FISH detected these rearrangements and only the extradural case had further molecular testing confirming *CUX1-BRAF* fusion. Fusion partners in the remaining three cases remained unknown [6]. Rankin, et al. analysed 3633 pediatric tumour samples of extracranial solid tumours, brain tumours and hematological malignancies, of which 221 samples had *BRAF* alterations. In this study, *KIAA1549-BRAF* fusion was detected in three extracranial pediatric sarcoma cases, including cases of embryonal rhabdomyosarcoma, malignant peripheral nerve sheath tumor and sarcoma, NOS [5]. In a recent study of *BRAF*-altered spindle cell neoplasms with IFS-like morphology, Penning, et al. reported one case of *KIAA1549-BRAF* fusion in a three-month-old infant's paraspinal mass [7].

Pediatric tumors with *BRAF* translocation are not limited to sarcomas in soft tissues. It has been known that congenital mesoblastic nephroma (CMN) and IFS share *ETV6-NTRK3* gene fusion [8]. *KIAA1549-BRAF* gene fusion was also recently documented in a mixed CMN to analyze soft tissue tumours in infants [9]. These current findings of *BRAF* gene rearrangement in CMN and IFS, represent additional genetic overlap between the pathogenesis of these two neoplasms.

Table 1: Fusion detection result by the automated fusion caller.

Gene1	Chr1	Pos1	Gene2	Chr2	Pos2	Paired Read	Split Read	Score
Tier I: Fusions with Strong Clinical Significance								
KIAA1549	chr7	138,552,721	BRAF	chr7	140,481,493	1	5	0.596

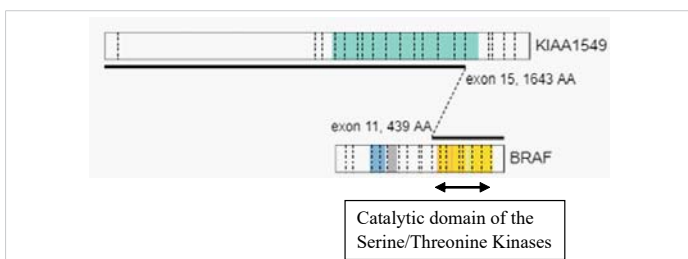


Figure 2: Fusion genomic breakpoints: in-frame fusion between exon 15 of the *KIAA1549* gene (NM_020910) and exon 11 of the *BRAF* gene (NM_004333).



Figure 3: A *KIAA1549/BRAF* gene fusion was identified by targeted RNA sequencing.

Table 2: Oncogenic hotspot SNVs identified by the automated caller.

Gene	Chr	Pos	Depth	Ref	Alt	VAF	Variant type	Population databases	Genetic databases	Somatic databases	Predictive software	Base change	AA change
Tier II: Variants with Potential Clinical Significance													
NF2	chr22	30,070,859	31	C	T	0.13	nonsense	Not reported in gnomAD NR in dbSNP	Not reported in ClinVar.	COSV58526762	FATHMM prediction Pathogenic score 0.96)	r.1375c>u	p.Q459*
Tier III: Variants of Unknown Clinical Significance													
PM2	chr7	6,043,660	24	G	A	0.13	missense variant	Not reporter in gnomAD NR in dbSNP	Not reported in ClinVar.	COSV56220459	FATHMM prediction Pathogenic score 0.9)	r.193c>a	p.L65I

* This sequence change creates a premature translational stop signal (p.Q459*) in the NF2 gene. It is predicted to result in an absent or disrupted protein product.



Similarly, *BRAF*-translocation occurs in a number of low grade pediatric central nervous system tumors [10]. In pediatric low grade gliomas, translocation-related gain of function gene activation leads to an aberrant signalling of the MAPK. Early reports from clinical trials using small molecule inhibitors such as MEK inhibitors (MEKi) appears to be promising [11] in the treatment of pediatric low grade glioma. In light of finding of a similar pathogenetic mechanism in this subtype of IFS in this report, consideration of a similar treatment strategy if this tumor is refractory to chemotherapy may become feasible.

In our case, there was an additional molecular event, involving *NF2* gene, detected. *NF2* encodes a tumor suppressor protein, Merlin (Moesin-ezrin-radixin-like protein). Loss of function mutations or deletions in *NF2* cause neurofibromatosis type 2 (*NF2*), characterized by bilateral vestibular schwannomas. Additionally, patients can develop schwannomas of other cranial and peripheral nerves, as well as meningiomas and ependymomas. *NF2* mutations also occur in spontaneous schwannomas and meningiomas, and other types of cancer, for example primary osteosarcoma, mesothelioma, glioma multiforme, hepato-biliary cancers, melanoma and medullary thyroid carcinoma [12]. There is very limited data available on the role of *NF2* mutation or inactivation in cancer tumour genesis, though it might be relevant for prognosis and future chemotherapeutic approaches.

Conclusion

We report a right thigh mass in an infant with IFS-like morphology, *KIAA1549-BRAF* fusion and oncogenic NF2p.Q459* SNV. This *BRAF* fusion in soft tissue sarcomas is exceedingly rare, and our case is the second one reported in the literature. Considering alternative and less frequent gene arrangements in fusion-negative cases by FISH and initiation of further molecular analysis by TruSight RNA is critically important in pediatric soft tissue sarcomas as these patients might benefit from targeted therapy (e.g.; *BRAF* with MEKi). Further studies of IFS-like tumors with *BRAF* rearrangement are needed to characterize these lesions further and understand if these neoplasms represent a stand-alone pathologic entity with a different biologic behaviour or not. Additionally, *NF2* mutation or inactivation in cancer tumour genesis needs more attention, as it might also be relevant for prognosis and management decisions.

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