HHS Public Access

Author manuscript

Genes Chromosomes Cancer. Author manuscript; available in PMC 2018 September 01.

Published in final edited form as:

Genes Chromosomes Cancer. 2017 September ; 56(9): 663–667. doi:10.1002/gcc.22470.

TFG-MET fusion in an infantile spindle cell sarcoma with neural features

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Abstract

An increasing number of congenital and infantile sarcomas displaying a primitive, monomorphic spindle cell phenotype have been characterized to harbor recurrent gene fusions, including infantile fibrosarcoma and congenital spindle cell rhabdomyosarcoma. Here we report an unusual spindle cell sarcoma presenting as a large and infiltrative pelvic soft tissue mass in a 4-month-old girl, which revealed a novel *TFG-MET* gene fusion by whole transcriptome RNA sequencing. The tumor resembled the morphology of an infantile fibrosarcoma with both fascicular and patternless growth, however, it expressed strong S100 protein immunoreactivity, while lacking SOX10 staining and retaining H3K27me3 expression. Although this immunoprofile suggested partial neural/neuroectodermal differentiation, overall features were unusual and did not fit into any known tumor types (cellular schwannoma, MPNST), raising the possibility of a novel pathologic entity. The *TFG-MET* gene fusion expands the genetic spectrum implicated in the pathogenesis of congenital spindle cell sarcomas, with yet another example of kinase oncogenic activation through chromosomal translocation. The discovery of this new fusion is significant since the resulting MET activation can potentially be inhibited by targeted therapy, as MET inhibitors are presently available in clinical trials.

Keywords

MET; fusion; infantile; sarcoma; neural

INTRODUCTION

The diagnosis of congenital and infantile sarcomas is frequently challenging due to their rarity and overlapping morphologic features. They typically share a primitive spindle cell phenotype, arranged in intersecting fascicles, with often a non-specific immunoprofile. Molecular genetics can be of critical importance in confirming the diagnosis and excluding

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Conflict of Interest: none

other look-alike benign cellular spindle cell proliferations. For example, the presence of *ETV6-NTRK3* fusion represents the gold-standard for diagnosis of congenital/infantile fibrosarcoma,[1] while the *VGLL2*-related fusions are often associated with congenital spindle cell rhabdomyosarcoma.[2] Tumors lacking these pathognomonic fusions are frequently grouped by default in a wastebasket category of unclassified/undifferentiated spindle cell sarcomas. The use of the latter terminology may imply a high grade malignancy and therefore is often approached with multi-modality therapies, including chemo-radiation. In contrast, most fusion-positive infantile spindle cell sarcomas are associated with a favorable outcome, in which a surgically removed lesion might not require adjuvant therapy, such as chemo or radiation therapy. Furthermore, the lack of a certain diagnostic fusion gene might instead favor an alternative benign diagnosis, such as cellular/atypical infantile myofibroblastic tumors, with features of myofibroblastoma or myofibroma.[3, 4] Another example is the identification of *NTRK1*-related fusions in a group of locally aggressive, S100 protein-positive lipofibromatosis-like neural tumors occurring in children, which allowed distinction from malignant peripheral nerve sheath tumors.[5] In this study we investigate the genetic abnormalities of a clinically aggressive infantile soft tissue tumor with unusual pathologic features, including strong and diffuse S100 protein expression for potential novel gene fusion discovery.

CASE REPORT

A 4 month-old girl presented with a fast growing right-sided paravertebral/ retroperitoneal soft tissue mass, extending from the right kidney to the pelvic area (Fig 1). The tumor infiltrated adjacent structures including sacral bone and encased spinal nerve roots (T2N0M0). The patient underwent surgical removal of the mass, which could only be excised incompletely. The patient subsequently received adjuvant chemotherapy with Ifosfamide, vincristine and actinomycin D, with good clinical response.

Grossly, the tumor measured 10 cm and had a fleshy, white multi-nodular cut-surface, with focal hemorrhage. The lesion was not encapsulated and infiltrated adjacent skeletal muscle. Microscopically, the tumor had a lobulated growth, being composed of monomorphic spindle cells arranged mainly in long, intersecting fascicles but was focally patternless (Fig. 2). The cells had a primitive appearance with scant cytoplasm and uniform, ovoid nuclei with open chromatin and inconspicuous nucleoli (Fig. 2). The tumor showed a high mitotic index, with up to 15 MF/10 HPFs. Focal areas of necrosis were noted. The tumor was associated with a prominent vasculature of small to medium-sized vessels. A prominent perivascular cuffing of pericytic smooth muscle cells was also noted, occasionally forming eccentric myoid nodules (Fig. 2). Immunohistochemically, the tumor showed strong and diffuse S100 protein positivity (Fig. 2), while SOX10 was completely negative. Other pertinent negative stains included desmin, myogenin, CK AE1:AE3, and NTRK1. H3K27me3 showed retained nuclear expression. Laminin stain showed a non-specific granular and equivocal pattern. The high mitotic activity was also reflected in the high Ki67-labeling index of up to 40–50%.

Based on these histologic findings and immunprofile, the tumor was difficult to classify. The clinical presentation and morphologic appearance suggested the possibility of an infantile

fibrosarcoma. However, the immunoprofile with strong S100 reactivity was not supportive and suggested instead a neuroectodermal line of differentiation, such as a cellular schwannoma or a malignant peripheral nerve sheath tumor. Thus further molecular studies were undertaken which might hopefully provide insight into further sub-classification. RNA extracted from fresh frozen tissue was subjected to whole transcriptome paired-end RNA sequencing using Illumina platform, as previously described.[5] The data was then analyzed with FusionSeq computational method, which identified a *TFG-MET* fusion candidate (Fig. 3). Results were then validated by Reverse Transcription Polymerase Chain Reaction (RT-PCR) (Fig. 3), which identified the fusion of *TFG* exon 7 with *MET* exon 15. The expression level of MET mRNA showed an intermediate level of overexpression compared to other pediatric myofibroblastic tumors available for comparison on our RNAseq platform (Fig. 3). Further FISH analysis using custom BAC probes from BACPAC sources of Children's Hospital of Oakland Research Institute (CHORI)(Oakland, CA)(http://bacpac.chori.org) showed a break-apart signal in both *TFG* and *MET* genes (Fig. 3). Immunohistochemistry for c-Met (SP44; rabbit monoclonal, Ventana, catalog#790-4430, prediluted) showed diffuse expression with moderate intensity (Fig. 2).

The follow-up available showed that 12 months after initial diagnosis, the child is alive with a stable residual tumor, no neurological sequelae and normal development under metronomic chemotherapy (vincristin, actinomycin D).

DISCUSSION

We applied whole transcriptome sequencing in an unusual infantile spindle cell sarcoma for novel fusion gene discovery in an attempt to better classify this tumor. RNA sequencing has emerged recently as a reliable methodology in identifying new genetic signatures, which have helped in refining the existing classification as well as to identify new pathologic entities based on their unique and recurrent gene signatures. Based on the clinical presentation in an infant and the morphologic appearance with monomorphic spindle cells arranged in intersecting fascicles the main consideration was an infantile fibrosarcoma. However, no *ETV6-NTRK3* gene fusion was detected and immunohistochemically the tumor showed strong S100 protein expression, in the absence of SOX10 staining or loss of H3K27me3. In contrast, cellular schwannomas are consistently positive for SOX10.[6] Cellular (plexiform) schwannomas have been reported in infants and can show worrisome histologic features, including increased cellularity and mitotic activity.[7, 8] Two cytogenetic case reports of congenital plexiform (multinodular) cellular schwannoma revealed a similar trisomy 17, without any other abnormalities.[9, 10] While our case shared overlapping morphologic features with a malignant peripheral nerve sheath tumors (MPNST) including high cellularity, and brisk mitotic activity, it showed strong and diffuse S100 protein reactivity. Instead, most high grade MPNSTs show weak/focal reactivity for S100 protein and loss of H3K27me3 expression.[11, 12] Furthermore, infantile examples of high grade MPNST are exceedingly rare, and in our opinion most of them represent congenital cellular plexiform schwannomas.[7, 8] One additional tumor considered in the differential diagnosis was the recently described S100-positive lipofibromatosis-like neural tumor, which can occur in young children, including infants.[5] Similar to our case, these lesions may show areas of increased cellularity, scattered mitotic activity, and an infiltrative growth within

adjacent adipose tissue. However, they also show CD34 and NTRK1 reactivity and *NTRK1* gene rearrangements. Our case was negative for both NTRK1 overexpression and break-apart *NTRK1* by FISH.

Interestingly, mesenchymal tumors with recurrent gene fusions often lack an established histogenesis or a characteristic immunoprofile, thus having an ambiguous line of differentiation. Particularly conflicting is the group of translocation-associated tumors with S100 protein expression in the absence of SOX10 reactivity. One example is ossifying fibromyxoid tumor, a translocation positive neoplasm of unknown line of differentiation, with consistent S100 protein reactivity.[13] Another example is the biphenotypic sinonasal sarcoma, a tumor with dual neural and muscle differentiation, harboring a *PAX3-MAML3* gene fusion in most cases.[14] The so-called 'neural phenotype' was defined on the basis of its S100 protein expression, in the absence of SOX10 staining. Lastly, the most recent example is the lipofibromatosis-like neural tumor with recurrent *NTRK1*-related fusions, in which the neural phenotype was defined based on its consistent S100 protein reactivity, but similarly lacking SOX10 positivity.[5] The significance of S100 positivity alone remains elusive and additional studies are required to establish if this finding is indeed related to neuroectodermal differentiation. Furthermore, these examples illustrate that immunohistochemical results taken out of context might be misleading in the setting of fusion-positive mesenchymal tumors, where there is often no good correlation between immunoprofile and line of differentiation.

Genetic alterations of the *MET* gene, including translocations, amplifications, deletions and point mutations, have been observed in different tumor types, suggesting a potential role for MET activation in oncogenesis. Fusion genes involving *MET* have been first identified through detailed analysis of TCGA data in an effort to define the landscape of kinase fusions across different cancer types.[15] Thus, *MET* fusions involving different gene partners were identified in rare examples of various epithelial malignancies, such as lung, thyroid, kidney, and liver. Among these, an identical *TFG–MET* fusion was detected in a thyroid papillary carcinoma, involving a similar 3' portion of *MET* transcript (exon 15 break). The predicted chimeric protein followed the classic activation paradigm, fusing the amino-terminal dimerization domains to an intact MET kinase domain. More recently, MET fusions have been reported in a subset of pediatric glioblastomas,[16] with similar *MET* breakpoints as ours.

We describe herein a fast growing, locally aggressive infantile spindle cell sarcoma, with a partial neural phenotype defined by strong S100 protein reactivity. Whole transcriptome sequencing detected a *TFG-MET* gene fusion, which resulted in MET overexpression at both mRNA and protein levels. This is the first example of MET protooncogene activation through chromosomal translocation in a sarcoma patient and expands the pathologic spectrum of fusion-positive infantile sarcomas. It remains to be determined if a *MET* fusion-positive genotype translates to a favorable outcome, similar to other infantile/congenital spindle cell sarcomas (i.e. fibrosarcoma, spindle cell rhabdomyosarcoma). Further studies are also needed to establish if the infantile sarcomas with *MET* related fusions represent a stand-alone pathologic entity or else may fit within the infantile fibrosarcoma spectrum despite strong S100 protein reactivity.

Acknowledgments

We are grateful to Dr Christopher D Fletcher, from Brigham & Women's Hospital, Boston, MA, for reviewing the case and providing valuable suggestions on the manuscript. We also want to thank Dr G.A.M. Tytgat from Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands, for providing clinical information.

Supported in part by: P50 CA140146-01 (CRA); P30-CA008748 (CRA); Kristen Ann Carr Foundation (CRA); Cycle for Survival (CRA)

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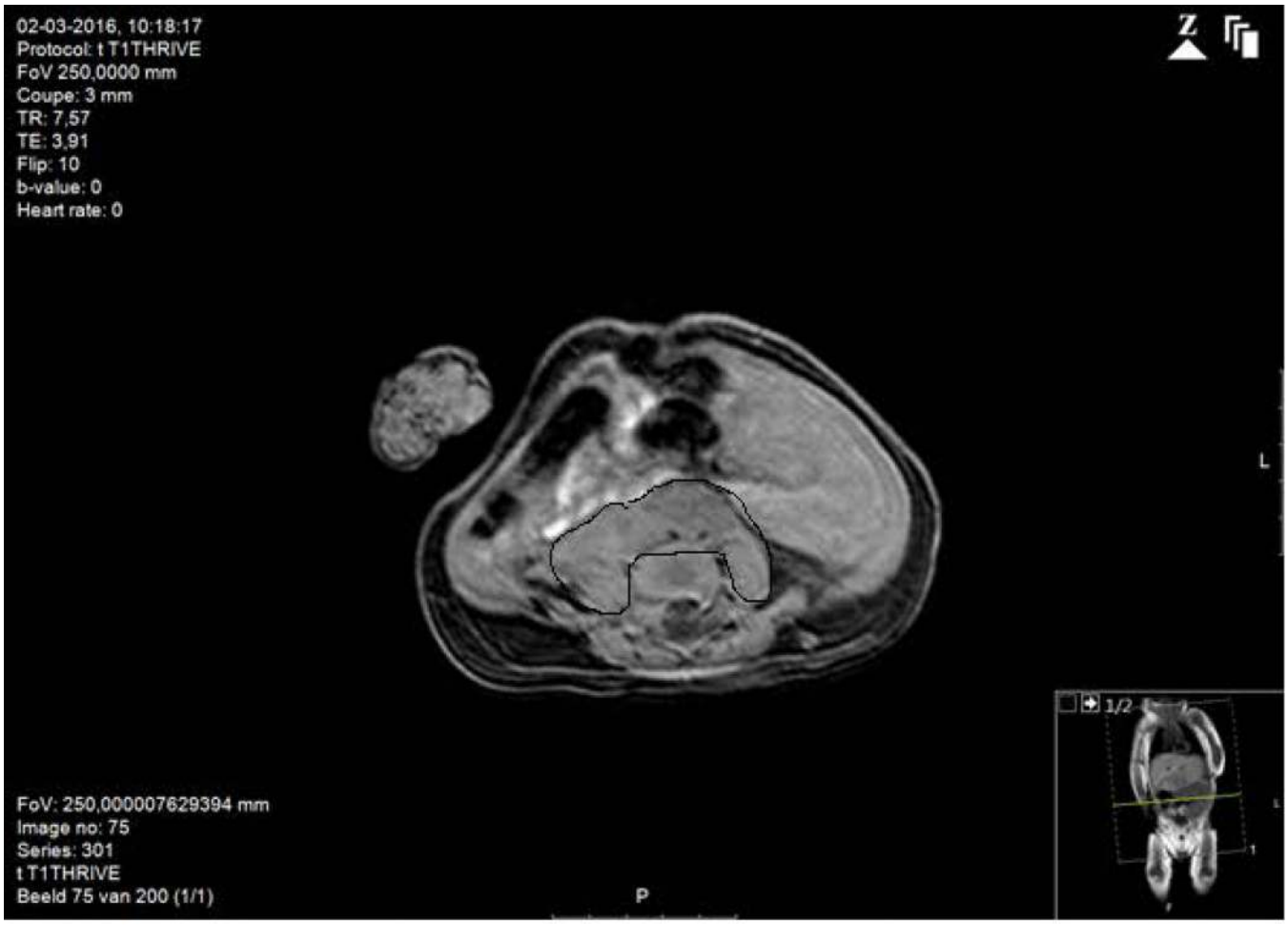

Figure 1. Pre-operative MRI
showing a large infiltrative pelvic mass.

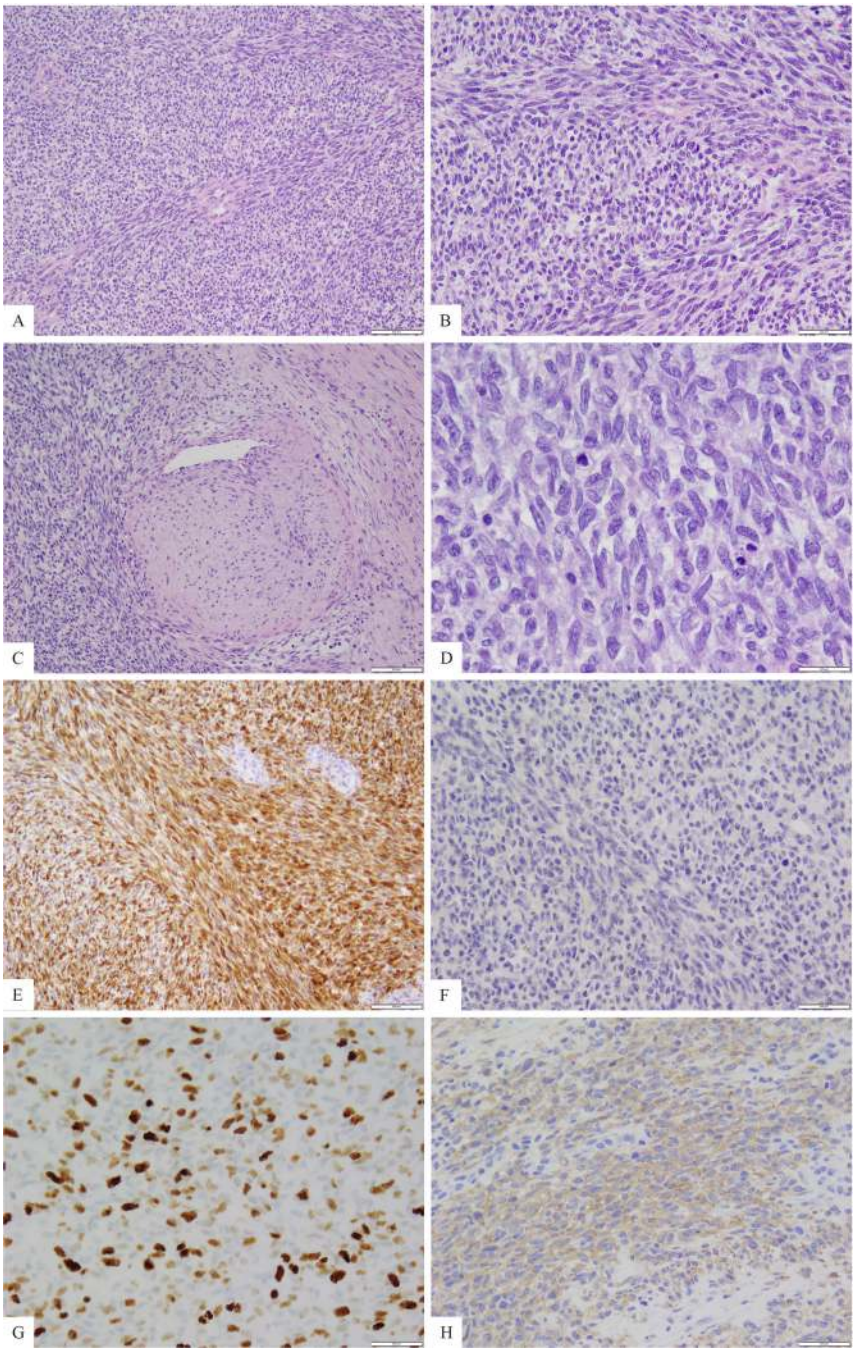

Figure 2. Pathologic features of infantile spindle cell sarcoma with *TFG-MET* fusion

Microscopic features showing a monomorphic spindle cell proliferation arranged in intersecting fascicles (A,B), with prominent perivascular myoid growth (C); the tumor showed a high mitotic activity (D). Immunohistochemical studies show strong and diffuse reactivity for S100 protein (E), while SOX10 is negative (F), a high proliferative Ki67 proliferative index of (G) and diffuse MET expression with moderate intensity (H).

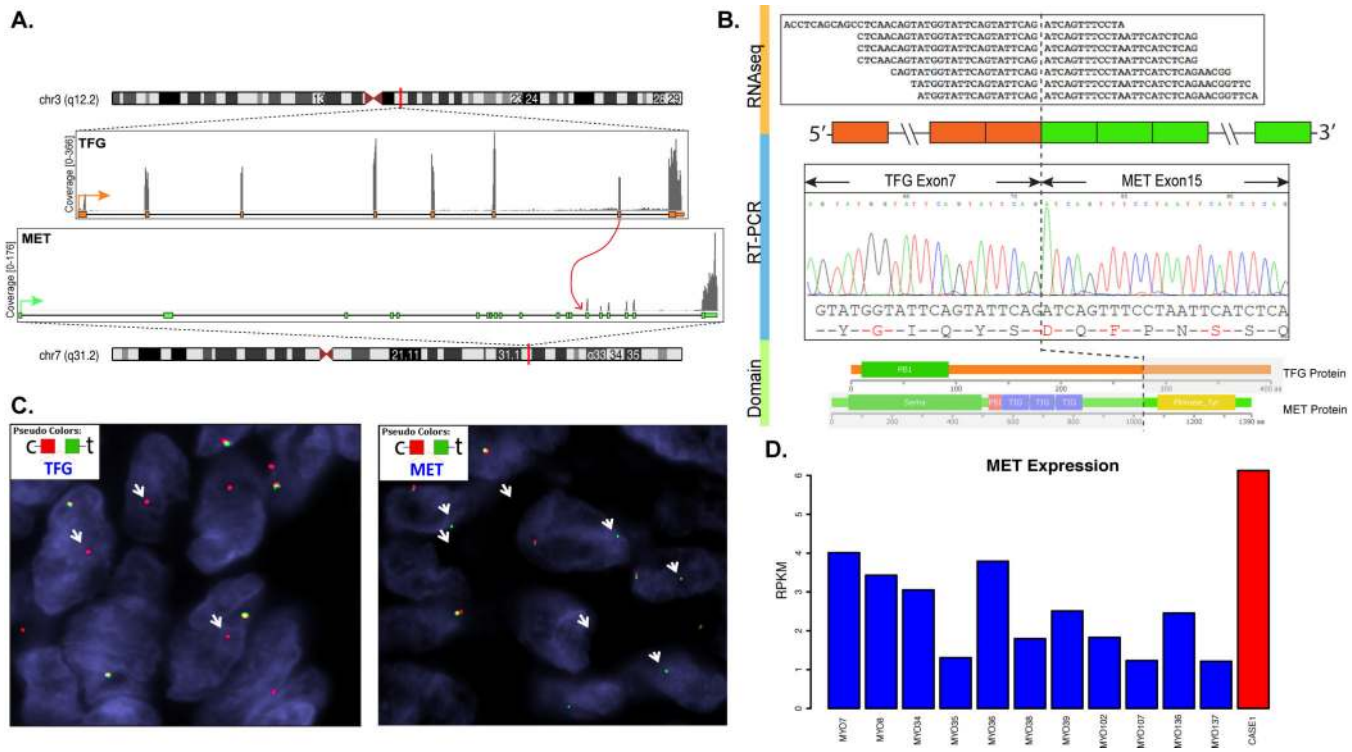

Figure 3. RNA sequencing identifies a novel *TFG-MET* fusion

Diagrammatic representation of *TFG* on 3q12.2 fused to *MET* on 7q31.2 (A). RT-PCR validation of FusionSeq candidate shows the fusion of *TFG* exon 7 to *MET* exon 15, with corresponding projected protein domains retained in the fusion (B). FISH validation showed the presence of an unbalanced rearrangement of both *TFG* (arrow shows centromeric signal, while the telomeric, green, signal of TFG is lost) and *MET* (arrows show the telomeric, green signal, while the centromeric, red, signal of MET is lost) genes (C). Index case showing upregulated *MET* mRNA expression levels (red) compared to other pediatric tumors (myofibromas/myopericytomas, blue) studied on the same RNAseq platform (D).