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Novel TFG-RET Fusion in Spindle Cell Tumour with S100 and CD34 Co-Expression

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Sir: The classification of spindle cell tumours with monomorphic morphology and lacking a clear immunophenotype remains challenging. Recurrent fusions involving receptor tyrosine or cytoplasmic kinase genes have been described in various spindle cell tumours\(^1\)\(^-\)\(^4\). Among these, spindle cell tumours with monomorphic morphology displaying distinct stromal and perivascular hyalinization and co-expressing S100 and CD34 can be grouped into an entity featuring recurrent fusions involving RAF1, BRAF and NTRK1/2 genes\(^2\). A case with similar morphology showing NCOA4-RET fusion was also described recently\(^5\). In this report, we describe a case occurring in a child in which a novel gene fusion TFG-RET was identified.

A healthy 3-year-old boy presented with a left posterior thigh nodule that had been noted since birth. The lesion was initially flat and erythematous, but as the erythema subsided, the lesion became palpable. Initial ultrasonography showed non-specific features in a 2-cm heterogeneous subcutaneous lesion with vascularity. A follow-up MRI scan 8 months later
showed a well-circumscribed ovoid subcutaneous nodule that had increased to 4 cm in size. A wide excision was performed.

The resected specimen comprised a 4-cm tumour mass that had a homogeneous tan-white cut surface. Histologically, the lesion featured a diffuse to vaguely nodular infiltrate of ovoid to spindle cells with variable cellularity (Figure 1 A). The less cellular areas showed loosely arranged spindle cells in myxoid stroma. Other areas had bands of hyalinized stroma separating the spindle cells (Figure 1 B). The blood vessels showed distinct perivascular hyalinization (Figure 1 C). Some haemangiopericytoma-like vessels were present. There were occasional multinucleated giant cells and scattered infiltrates of lymphoplasmacytic cells. At the periphery, the spindle cells infiltrated adjacent adipose tissue in a reticular pattern (Figure 1 D), reminiscent of lipofibromatosis-like neural tumour. Nuclear atypia was mild. The mitotic count was low. There was no necrosis. The spindle cells were strongly and diffusely immunoreactive for S100 protein (Figure 1 E) and CD99 (cytoplasmic staining). There was patchy reactivity to CD34 (Figure 1 F). The spindle cells were negative for cytokeratin, SOX10, smooth muscle actin, desmin, HMB45, STAT6 and ALK. H3K27me3 was retained.

Next-generation sequencing-based anchored multiplex PCR (Archer® FusionPlex® Solid Tumour Panel) was performed as previously described\(^6\). The novel gene fusion involving exon 8 of the TFG gene and exon 12 of the RET gene was identified. (Figure 2 A).
To confirm this novel gene fusion, we performed reverse transcriptase-PCR (RT-PCR) on RNA extracted from tumour FFPE material using the Reliaprep FFPE RNA extraction kit (Promega, USA) primers flanking the fusion site (forward: 5’ TTT CTG GTC AGC CTC AAC AA; reverse: 3’ CAA ATT CGC CTT CTC G TA G T). This generated a 120 base pair PCR product from samples ran as duplicates. Normal tonsil was used as a negative control. The PCR temperature profile was as follows: cDNA synthesis at 50°C for 15 minutes, initial denaturation at 94°C for 5 minutes, 40 three-step PCR cycles of 94°C for 10 seconds, 60°C for 15 seconds and 72°C for 20 seconds, followed by melting 72°C - 95°C. The PCR products were loaded onto a 3% agarose gel and electrophoresis was performed at 100 V for 40 minutes. The gel was then photographed for visualisation of the bands. Sanger sequencing was performed on the PCR product, confirming the identified gene fusion. In addition, fluorescence in situ hybridization (FISH) showed RET rearrangement in 80% of the cells (Figure 2 B).

Recently, Suurmeijer et al reported a distinct group of low-grade to intermediate-grade spindle cell tumours showing recurrent kinase fusion (RAF1, BRAF, NTRK1)². These tumours feature monomorphic spindle cell with variable cellularity arranged in a patternless fashion, with distinctive stromal keloidal and perivascular hyalinization, and co-expressing S100 and CD34 while being negative for SOX10. Thereafter, another case with similar morphology was reported, harboring NCOA4-RET fusion⁵. With a similar morphology and immunoprofile, it is likely that the current case represents another case with RET fusion. Interestingly, our case also shows occasional multinucleated giant cells which are also present in one BRAF-rearranged and three NTRK-rearranged tumours in the previous cohort by Suurmeijer et al².
The differential diagnoses include infantile fibrosarcoma (IFS), lipofibromatosis-like neural tumour (LPFNT) and inflammatory myofibroblastic tumour (IMT). IFS tends to affect very young children, and the typical location is the thigh. Recently, two RET-rearranged cases with morphology within the spectrum of IFS were described\(^3\). However, in IFS, the spindle cells are usually grouped in a herringbone-pattern which was not present in this case. The presence of distinctive band-like stromal and perivascular hyalinization as well as diffuse S100 and CD34 immunoreactivity is unusual in IFS. Agaram et al. described a novel subset of soft tissue tumours with distinctive lipofibromatosis-like morphology that are also immunoreactive for S100 and CD34\(^1\). In this case, the spindle cells at the periphery of the tumour infiltrate the adjacent adipose tissue, resembling the morphology of LPFNT. The presence of stromal and perivascular hyalinization however was not described in LPFNT. In the series of cases described by Suurmeijer et al, four cases with NTRK rearrangement also show focal areas with reticular infiltration of subcutaneous fat, suggesting that this group of tumours may have focal areas reminiscent of LPFNT\(^2\). The consideration for IMT as a differential diagnosis is due to the presence of focal myxoid area and background sprinkling of lymphocytes. In addition, a case of pulmonary inflammatory myofibroblastic tumour with RET rearrangement has been described\(^4\). However, IMT does not usually co-express S100 and CD34.

\(\text{RET}\) is a receptor tyrosine kinase proto-oncogene that is located at chromosome 10q11.2 which can acquire oncogenic activity through mutation or rearrangement. When activated, it can lead to activation of the RAS-RAF-MAPK cascade, promoting cell survival and proliferation. As \(\text{RAF, BRAF, or NTRK}\) genes are members of the same signaling pathway, oncogenic activation of \(\text{RET}\) gene will have similar effects as activation of the other genes\(^5\). Activating \(\text{RET}\) fusions have been described in papillary thyroid carcinoma and a small
subset of non-small cell lung carcinoma. In mesenchymal lesions, RET gene fusions are rare, but more cases have been described recently as seen in LPFNT, IFS and IMT\textsuperscript{3,4}. The case described by Michal et al had NCOA4-RET gene fusion. In our case, the partner gene was TFG.

To our knowledge, this is the first case documenting the TFG-RET fusion in a spindle cell neoplasm co-expressing CD34 and S100. TFG is located on the short arm of chromosome 3 and encodes a ubiquitously expressed cytoplasmic protein that plays an important role in intracellular trafficking and acts as both an apoptotic suppressor as well as an activator of cells growth. The TFG fusion genes occur mainly through fusion between the 5’ end of TFG and the 3’ end of the associated kinase gene. TFG is ubiquitously and highly expressed across different tissue types, and the presence of a coiled-coil domain allows for constitutive autophosphorylation and oncogenic activation of the fused receptor tyrosine kinase. In this case, the fusion between the TFG gene at exon 8 and the RET gene at exon 12 results in oncogenic activation of RET.

The identification of the TFG-RET chimeric tyrosine kinase in this group of spindle cell tumours co-expressing CD34 and S100 adds RET to a list of oncogenic tyrosine kinases viz. RAF1, BRAF and NTRK1/2 in the pathogenesis of this tumour type. This finding is significant as it suggests the potential efficacy of targeted therapeutic strategies with multikinase inhibitors with anti-RET activity such as sunitinib, sorafenib, and vendatinib.
In summary, we have identified a novel *TFG-RET* fusion in a spindle cell tumour with S100 and CD34 co-expression – a novel, recently described entity. This finding suggests that investigation for the presence of *RET* rearrangement should be considered in spindle cell tumours with similar morphology and immunoprofile.

**FIGURES**

**Figure 1**

(A) H&E stained section (magnification x100) showing spindle cells in a haphazard pattern with variable cellularity and scattered haemangiopericytic-like vessels and perivascular hyalinization. (B) H&E stained section (magnification x200) showing bands of hyalinized stroma, separating the spindle cells. (C) H&E stained section (magnification x200) showing distinct perivascular hyalinization. (D) H&E stained section (magnification x200) showing areas infiltrating adjacent adipose tissue. (E) Immunohistochemical stain for S100 (magnification x200) showing diffuse immunoreactivity. (F) Immunohistochemical stain for CD34 (magnification x200) showing patchy immunoreactivity.

**Figure 2**

(A) Analysis of anchored multiplex PCR result of the Archer® FusionPlex® Solid Tumour Panel showed a *TFG-RET* fusion. Sanger sequencing of the reverse transcriptase-PCR amplicon product revealed the sequence of the *TFG-RET* fusion transcript. (B) FISH using a *RET* break-apart probe shows a positive result with separation of the green and red signals.

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AUTHOR CONTRIBUTIONS

All authors contributed to the generation of ideas and writing of the manuscript.

REFERENCES


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