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BRIEF REPORTS

Spindle cell liposarcoma with a *TRIO-TERT* fusion transcript

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Abstract

Conventional well-differentiated, dedifferentiated, and myxoid liposarcomas have long been known to harbor numerous typical genetic alterations that allow for diagnosis of these tumors. These include *MDM2* and *CDK4* amplification in well-differentiated and dedifferentiated liposarcomas as well as FUS-DDIT3 rearrangements in myxoid liposarcoma. More recently, in-frame *TRIO-TERT* fusion genes have been described in a subset of non-translocation-related sarcomas including myxofibrosarcoma, dedifferentiated liposarcoma, undifferentiated pleomorphic sarcoma, pleomorphic rhabdomyosarcoma, and leiomyosarcoma. These genetic rearrangements lead to TERT mRNA expression levels hundreds of times higher than normal, causing increased telomerase activation in these tumors. Herein, we describe an unusual case of a liposarcoma with spindle cell features and a *TRIO-TERT* fusion transcript identified through next-generation sequencing.

Keywords Liposarcoma · *TRIO-TERT* · Fusion transcript

The current classification of spindle cell lipomatous neoplasms is still evolving. In the literature, the term "spindle cell liposarcoma" has been used to describe many tumors including spindled variants of well-differentiated liposarcoma and myxoid liposarcoma, as well as spindle cell lipomas with atypia. The described tumors usually show a population of bland to atypical appearing spindle cells with an admixed population of lipoblasts [1–4]. Few genetic aberrations have been described in these lesions, the most well-known being deletions of *RB1* akin to what is present in spindle cell lipomas [1–3].

By contrast, conventional well-differentiated, dedifferentiated, and myxoid liposarcomas have long been known to harbor numerous typical genetic alterations that underlie their tumorigenesis and serve as diagnostic markers including *MDM2* and *CDK4* amplification as well as *FUS-DDIT3* rearrangements [5].

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Recently, a novel in-frame *TRIO-TERT* fusion has been identified in some non-translocation-related sarcomas [6]. *TRIO* plays an important role in cell adhesion and nuclear signaling pathways and is part of a family of Rho GTPases that act as guanine nucleotide exchange factors [7]. Telomerase (*TERT*) is a ribonucleoprotein complex that is responsible for maintaining telomerase length and extending the life of cells. Various studies have described *TRIO* and *TERT* aberrations in a wide variety of malignancies including bladder, breast, lung, oral cancers, skin cancer, soft tissue sarcomas, and gliomas [8–12]. However, fusion transcripts of *TRIO* and *TERT* have only recently been recognized [13].

More recently, several studies have shown non-translocation-related tumors of mesenchymal origin, including a subset of liposarcomas, to harbor *TRIO-TERT* fusion transcripts. These rearrangements lead to increased expression of *TERT* mRNA expression levels and are thought to drive tumorigenesis by conferring extended life spans upon the malignant cells [7, 12, 13]. Herein, we describe, to our knowledge, the first case of a liposarcoma with spindle cell features found to have a *TRIO-TERT* fusion transcript by next-generation sequencing.

A 69-year-old female with no significant past medical history was referred to our hospital for evaluation and treatment of a left lateral thigh mass identified after minor trauma. Originally thought to be a hematoma by ultrasound, the lesion

continued to grow over the next 3 years and subsequent MRI demonstrated a 6.1 × 6.0 × 2.7 cm enhancing mass centered in the subcutaneous soft tissues of the lateral thigh overlying the posterior vastus lateralis and anterolateral biceps femoris muscles. There were two nodular enhancing components that extended deep to the iliotibial band posterior-medially (Fig. 1a). An image-guided biopsy was performed which showed an atypical myxoid spindle cell neoplasm with no overt features of malignancy. The spindle cells in the biopsy were positive for CD34 and negative for S100, SOX10, GFAP, desmin, STAT6, MDM2, and MUC4. The tumor cells also showed retention of H3K27me3.

On gross examination of the resection specimen, there was a tan-yellow, heterogenous, circumscribed mass measuring 7.9 × 7.8 × 4.5 cm located in the subcutaneous soft tissues with a glistening, gelatinous cut surface (Fig. 1b). Microscopic examination showed a population of bland-appearing spindle-shaped cells growing in short- to medium-sized fascicles embedded in a variably myxoid stroma (Fig. 1c). Rare atypical spindle-to-ovoid cells with increased nuclear size and some hyperchromasia were identified. There was adipocytic differentiation with numerous lipoblasts scattered throughout leading to the diagnosis of a liposarcoma (Fig. 1d). While grossly well circumscribed, microscopically, the lesion showed infiltration into normal fat and fascia (Fig. 1e). There was no necrosis identified, and there was an increased mitotic activity with up to 8 mitoses per 10 high power fields. By immunohistochemistry, the cells were positive for CD34 and negative for desmin, STAT6, MDM2, and CDK4. Rb-1 immunostaining showed positivity within many of the tumor cells which was interpreted as being retained. Fluorescent in situ hybridization studies showed there was no amplification of *MDM2*, and electron microscopic studies confirmed adipocytic differentiation by demonstrating lipid droplets within the fibroblastic-appearing tumor cells. A clinically validated next-generation sequencing (NGS) targeted RNA fusion assay was performed (MGH Solid Fusion Assay v2, using Anchored Multiplex PCR targeting and sequenced on

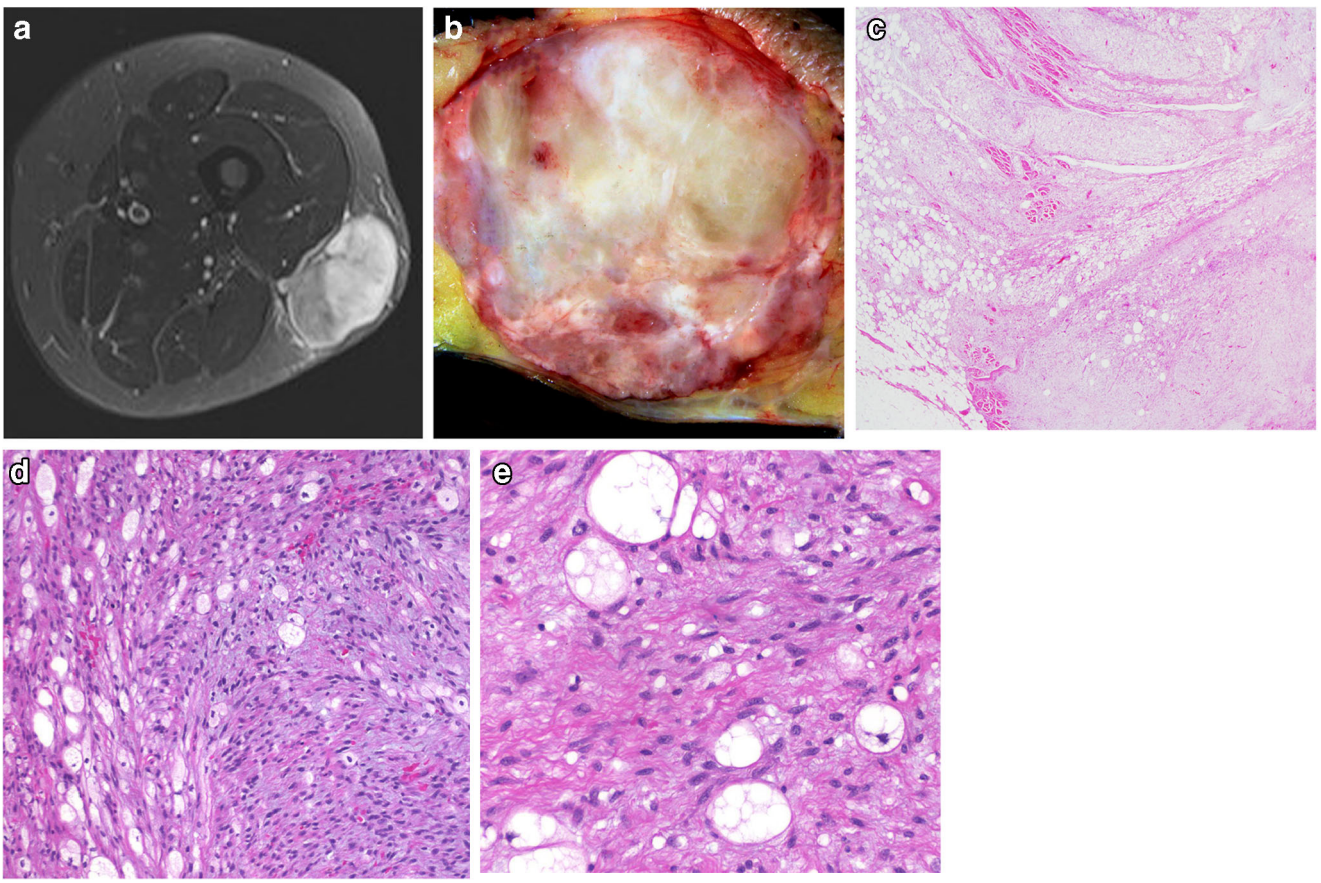

Fig. 1 **a** Axial fat-suppressed T2-weighted image demonstrates a heterogenously hyperintense enhancing subcutaneous soft tissue mass in the lateral thigh which abuts the superficial fascia overlying the posterior vastus lateralis and anterolateral biceps femoris muscles with two nodular enhancing components extending deep to the iliotibial band posterior-medially. **b** A gross photograph showing the cut surface of the subcutaneous, well-circumscribed mass with a tan-yellow glistening/gelatinous cut surface. **c** Low-power magnification showing an infiltrative growth pattern into normal subcutaneous fat and focally around fascial components. **d** Medium power magnification showing bland-appearing spindle-shaped cells embedded in a variably myxoid stroma with interspersed adipocytic components. **e** High-power magnification showing numerous lipoblasts embedded within the fibrous stroma.

an Illumina MiSeq, analysis pipeline version 2.1.0). An in-frame fusion transcript involving *TRIO* Exon33 and *TERT* Exon2 was identified with 184 supporting reads (Fig. 2a–c). No other abnormalities were detected; interestingly, these breakpoints were the same as those previously described [7, 13]. No other *TRIO-TERT* fusion has been identified in any other sample in our laboratory; this includes 8 other lipomatous neoplasms. The diagnosis of a spindle cell liposarcoma with a *TRIO-TERT* rearrangement was thus rendered.

Spindle cell liposarcomas have been described in limited numbers in the literature; while they were originally believed to be variants of atypical lipomatous tumors/well-differentiated liposarcomas, it has now been shown that many lesions classified under this category lack *MDM2* and *CDK4* amplification characteristic of well-differentiated and dedifferentiated liposarcomas [2–4]. In one series, the authors found spindle cell liposarcomas to be positive for CD34 by immunohistochemistry and have *RB1* loss without amplification of *MDM2* or *CDK4*. Unlike benign spindle cell lipomas, the lesions in their series showed atypical lipogenic cells with variations in size and shape and spindled tumor cells with enlarged hyperchromatic nuclei. These lesions were found to occur in the subcutaneous tissues of the extremities, trunk, and head and neck regions and were found to have a generally favorable clinical course with only one local recurrence [2]. Other authors have suggested some of these lesions be reclassified as "fibrosarcoma-like lipomatous tumor" based on their genetic and histologic characteristics [15]. Genetic aberrations including *MDM2* amplification, *RB1* loss, and monosomy 7 have all been used to help guide the

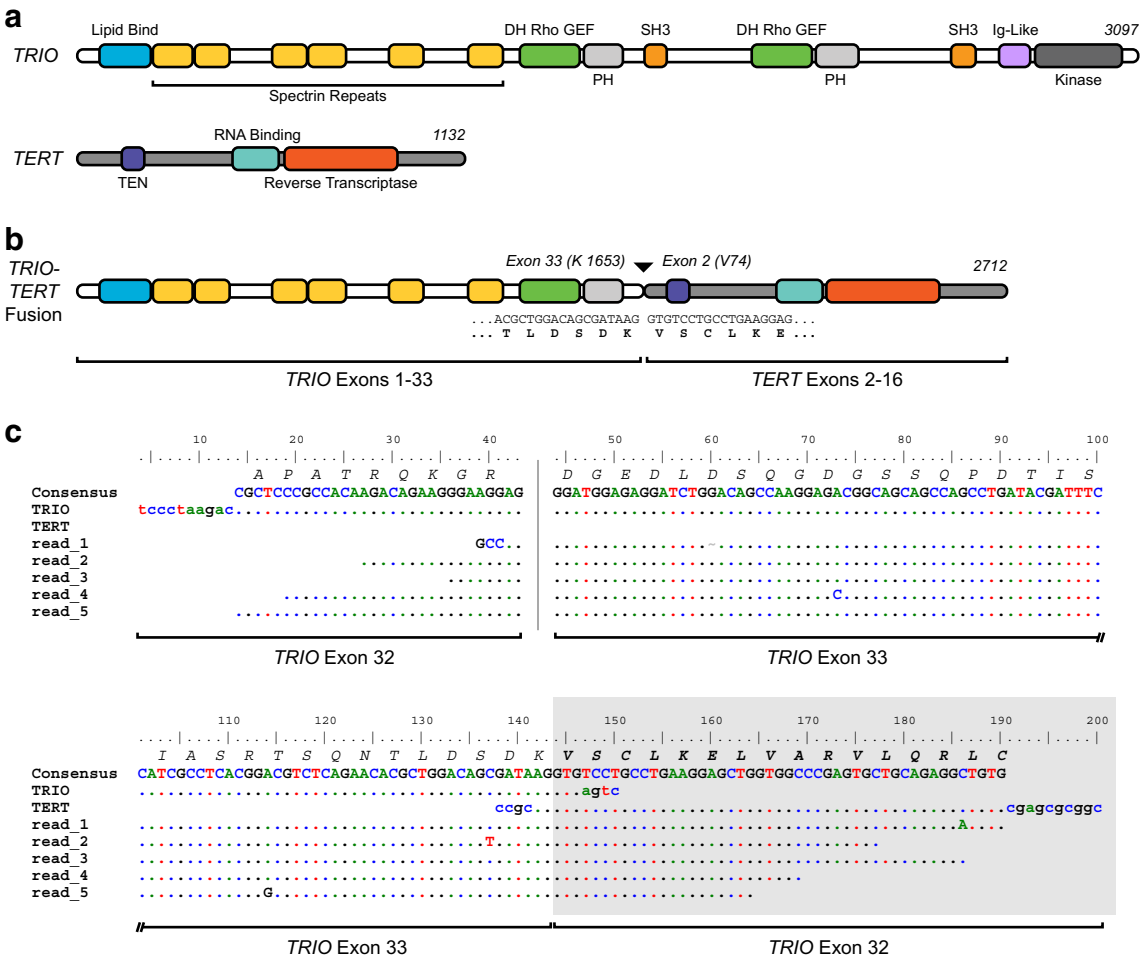

Fig. 2 **a** Protein schematics for *TRIO* (triple functional domain protein, NP_009049.2) and *TERT* (telomerase reverse transcriptase, NP_937983.2). Annotated domains (InterPro) are shown as colored boxes. *TERT* N-terminal domain annotated from Malik et al. [14]. Lipid Bind, CRAL-*TRIO* lipid-binding domain; DH Rho GEF, Dbl homology Rho guanine nucleotide exchange factor; PH Pleckstrin homology; SH3, src homology 3; Ig-Like, immunoglobulin-like; TEN, telomerase essential N-terminal. Proteins are drawn to scale. **b** Predicted *TRIO-TERT* fusion protein joins exons 1–33 of *TRIO* (1653 residues) with exons 2–16 of *TERT*, including all the conserved domains. The RNA and corresponding protein sequences from the fusion reads are shown. **c** Representative RNA sequencing alignment supporting the fusion transcript ("consensus"), with the amino acid sequence shown above in italics. Bases identical to the consensus are marked with a "." and gaps with a "~." There were 184 supporting reads; 5 representative NGS reads are shown. *TRIO* and *TERT* sequences from hg19 are shown, with lowercase bases not matching the consensus

classification of this family of lesions depending on the series examined; however, *TRIO-TERT* fusion transcripts have not yet been described [1–3, 6]. Since *TRIO* rearrangements are not limited to a specific type of histology, it is thought these fusion transcripts may represent secondary chromosomal events caused by these lesions' genetic instability [7, 13]. However, it is worth noting that *TRIO-TERT* fusions have not yet been described in any *RB1*-deleted spindle cell lipomatous tumors, leading us to believe that this may represent a biologically distinct pathway [16].

The lesion herein in did not exhibit any of the previous molecular alterations described in spindle cell lipomatous tumors [1–3]. It remains unclear what significance these findings hold; however, we suggest that the *TRIO-TERT* fusion is likely contributory in some form to oncogenesis. Whether it is sufficient to function as a "driver" oncogene in this neoplasm remains unknown and as other authors have suggested it may occur secondary to an underlying (but yet unidentified) mechanism causing genetic instability. Identifying and molecularly characterizing additional cases of this rare lesion may allow for better classification in the future and permit better delineation of the different categories of spindle cell lipomatous neoplasms.

Author contributions David Suster: primary manuscript author; Martin Taylor: molecular genetics components and Fig. 2; Ivan Chebib: histological images and review of histology; Vikram Deshpande: review of molecular genetics components and Fig. 2; John Mullen: gross photography, gross description, and manuscript review; Miriam Bredella: radiological images and radiology review; G. Petur Nielsen: corresponding author and final review of case report including all images and figures and terminology.

Compliance with ethical standards

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

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