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BRIEF REPORT

Targeted RNA-sequencing identifies *FBXW4* instead of *MGEA5* as fusion partner of *TGFBR3* in pleomorphic hyalinizing angiectatic tumor

Anne-Laure Rougemont[1] · Margaret Berczy[1] · Nathalie Lin Marq[1] · Thomas A. McKee[1] · Yann Christinat[1]

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Abstract

Pleomorphic hyalinizing angiectatic tumor (PHAT) is a rare mesenchymal tumor of intermediate malignancy. PHAT, and the related hemosiderotic fibrolipomatous tumor, show a recurrent t(1;10)(p22;q24). Fluorescence in situ hybridization (FISH) and BAC (bacterial artificial chromosome) clones have previously identified *TGFBR3* and *MGEA5* as fusion partners. However, targeted RNA-sequencing allowed for the correct identification of *FBXW4* and not *MGEA5* as the fusion partner of *TGFBR3* in a subcutaneous PHAT, a finding further confirmed by RT-PCR. *FBXW4* and *MGEA5* share a common cytogenetic location at 10q24.32, thereby suggesting that the use of less precise technology may have led to inaccurate gene identification. The study of additional cases is however required.

Keywords Soft tissue · Translocation · PHAT · Hemosiderotic fibrolipomatous tumor · Myxoid sarcoma

Introduction

Pleomorphic hyalinizing angiectatic tumors (PHAT) are rare mesenchymal tumors of the superficial soft tissues first described by Smith and colleagues in 1996 [1]. Fewer than 130 cases of PHAT have been reported in the literature [2]. These slow-growing tumors of intermediate malignancy frequently recur locally, but do not metastasize. Early, precursor forms of PHAT are termed hemosiderotic fibrolipomatous tumors, whereas PHAT may also progress to high-grade myxoid sarcoma [3].

PHAT are lobulated infiltrating tumors characterized by groups of irregular ectatic vessels, showing parietal fibrinoid changes, alternating with more solid areas composed of pleomorphic spindled to epithelioid cells with frequent intranuclear inclusions. The mitotic rate is very low, contrasting with the high degree of nuclear atypia [3].

Lambert et al. were first to report a t(1;10)(p22;q24) in an acral myxoinflammatory fibroblastic sarcoma, in the setting of a complex karyotype with further loss of chromosomes 3 and 13 [4]. A recurrent t(1;10)(p22;q24) was further reported in PHAT and in hemosiderotic fibrolipomatous tumors (HFLT), albeit with varying frequencies [5–14]. These molecular findings, together with overlapping morphological features in a subset of the tumors, suggest that all three tumors may represent a spectrum of related neoplasms of varying biological behavior, with metastasizing potential acquired only by the high-grade sarcomas. In a subset of these previous reports, fluorescence in situ hybridization (FISH) using BAC (bacterial artificial chromosome) clones identified *TGFBR3* and *MGEA5* as the putative fusion partners [5–7, 9, 10].

Here, we report a case of PHAT with a t(1:10)(p22;q24.2). The use of targeted RNA-sequencing allowed for the precise characterization of the fusion partners, thereby identifying *FBXW4* instead of *MGEA5* as the fusion partner of *TGFBR3*.

Materials and methods

Clinical and pathological findings

A 45-year-old male had been aware for 2 years of a slow-growing 1.6-cm subcutaneous mass of the medial aspect of the left arm. Recent mild increases in tumor volume and pain were indication for surgery.

✉ Anne-Laure Rougemont
anne-laure.rougemont@hcuge.ch

[1] Division of Clinical P athology, Molecular Pathology Unit, Geneva University Hospitals, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva, Switzerland

Targeted RNA-sequencing

RNA was isolated from formalin-fixed paraffin-embedded (FFPE) tissue using the RNeasy spin-column method (Qiagen). Total RNA was constructed into RNA-sequencing libraries using the Illumina TruSight RNA Fusion Panel Kit (Illumina, San Diego, CA, USA) according to the manufacturer's protocol. The library targeted for 507 fusion-associated genes. cDNA was generated from the cleaved RNA fragments, and paired-end sequencing on an Illumina NextSeq was performed. The raw sequencing data was converted to FASTQ file formats then processed by MapSplice 2.1.5 to detect fusion transcripts.

Reverse transcription (RT)-PCR for fusion data validation

RT was performed on 500 ng of RNA extracted from the FFPE tumor blocks. Reverse transcription to cDNA was performed using the PrimeScript RT Reagent kit (Takara) with oligo-dT and random 6mers primers. The fusion transcript was amplified using primer pairs specific for the breakpoint regions involving the *TGFBR3* and *FBXW4* genes (*TGFBR3* 5′-TTCTGCTTCATGGTGGATCA-3′ and *FBXW4* 5′-TCCA TTGCTATCAGCCCATT-3′). Amplicons were purified using PeqGOLD gel extraction kit (Peqlab) and sequenced using the Sanger method.

Results

Pathological findings

The tumor showed hyalinized ectatic and irregular vessels, with fibrin deposition and luminal thrombi, surrounded by a densely cellular proliferation of atypical spindle cells (Fig. 1). Despite considerable nuclear atypia, mitotic activity was very scant. Infiltration was seen in the surrounding adipose and fibrous tissue. Immunohistochemical analysis showed diffuse reactivity to CD34, whereas S100 protein and pancytokeratin remained negative.

TGFBR3-FBXW4 fusion

A total of 49.3 million reads were obtained by targeted RNA-sequencing, with an alignment rate of 96.4%. A high confidence *TGFBR3-FBXW4* fusion was identified by MapSplice (chr10:103384502~chr1:92174340). The fusion creates a fusion transcript consisting of exons 1 to 6 of *FBXW4* (NM_022039.3), the donor gene, and exons 14 to 17 of *TGFBR3* (NM_003243.4), the acceptor gene. However, no chimeric protein is created as the exon 14 of *TGFBR3* is out of frame with respect to exon 6 of *FBXW4*. The latter is equivalent to a *FBXW4* frameshift mutation p.Gly280ValfsTer14.

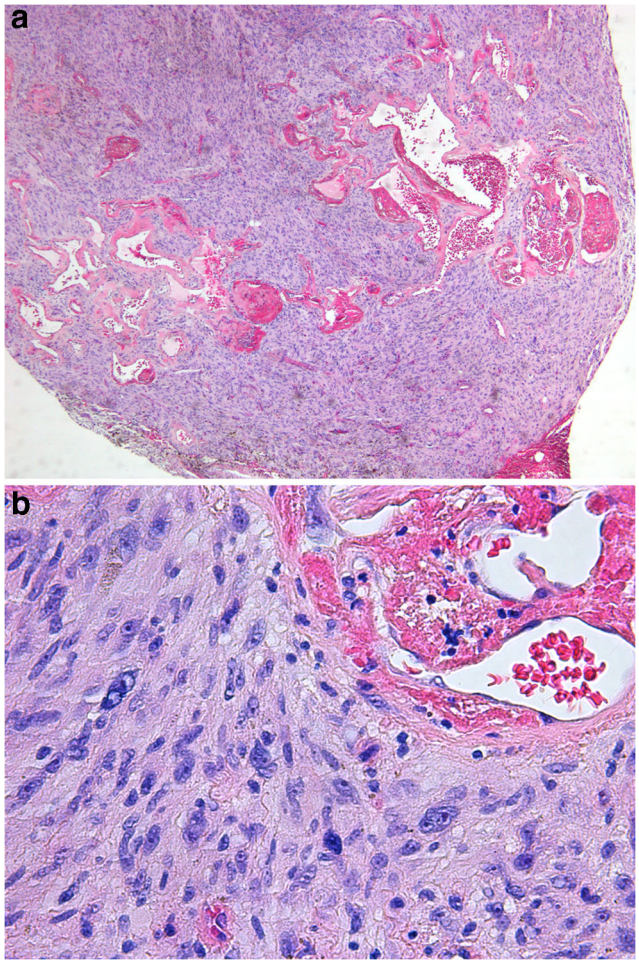

Fig. 1 Histological features. **a** The tumor is composed of ectatic and branched vessels with fibrin deposition in the walls, surrounded by a dense cellular proliferation showing a vaguely fasciculated architecture (hematoxylin and eosin, H&E; original magnification × 40). **b** At higher magnification, a thrombosed vessel (upper right) is surrounded by atypical spindle to oval cells with prominent nucleoli (H&E, × 400)

The RT-PCR reaction using breakpoint-specific primers, followed by Sanger sequencing, confirmed the above-mentioned findings. The molecular findings are summarized in Fig. 2.

Discussion

In this case study of a subcutaneous PHAT, a targeted next-generation sequencing (NGS) fusion panel successfully identified *FBXW4* as the fusion partner of *TGFBR3*.

The recurrent t(1;10)(p22;q24) reported in HFLT has been observed less frequently in PHAT. The breakpoints map to the *TGFBR3* gene on chromosome arm 1p, and in or near the *MGEA5* locus on chromosome arm 10q [5, 7, 9]. In a series of 10 cases of PHAT, FISH probe sets designed to detect

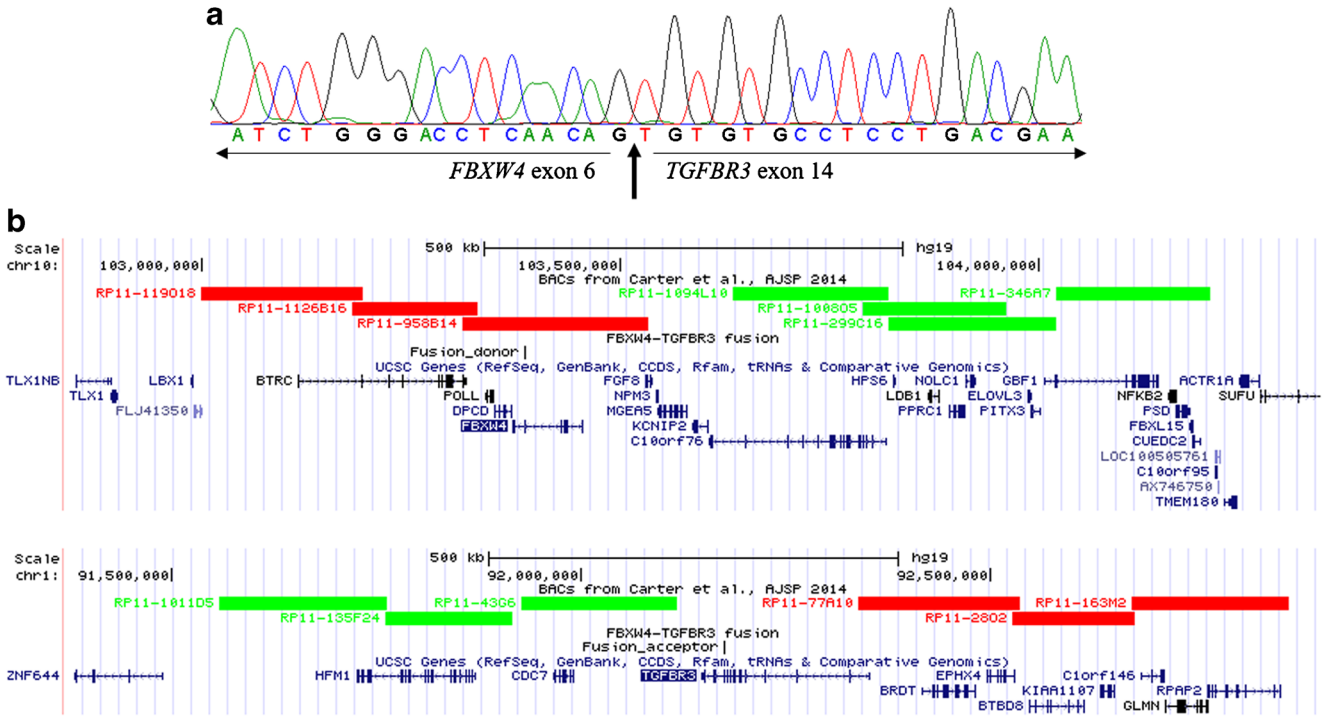

Fig. 2 Genetic analysis of the t(1;10)(p22;q24). **a** Sequencing of the fusion transcript. **b** Cytogenetic location of *MGEA5*, *FBXW4*, and *TGFBR3* mapped to the previously reported BAC clones [6]. Fusion breakpoints are indicated by arrows

rearrangement of the *TGFBR3* and *MGEA5* genes identified a rearrangement of one or both these genes in 60% of the cases [6]. The two cases of PHAT analyzed by Zreik et al. both showed rearrangement of the *TGFBR3* gene, with one only tumor showing concurrent *MGEA5* rearrangement [10]. Previous studies by Antonescu et al. [5] and Mohajeri et al. [9] cytogenetic and FISH techniques had failed however to identify a *TGFBR3* or a *MGEA5* rearrangement in the 5 PHAT cases assessed between the two studies.

The different BAC probes designed in the study by Carter et al. [6] and subsequently by Zreik et al. [10] to assess the *MGEA5* gene spanned respectively a 475 Kb region (3′) and a 576 Kb region (5′) on either aspects of the gene. Since the *FBXW4* gene is located less than 90 Kb 3′ from the *MGEA5* gene and within the third BAC probe (Fig. 2b), it is expected that a fusion involving *FBXW4* instead of *MGEA5* will show similar results.

Lending support to an inaccurate gene identification, no chimeric fusion transcript could be identified in previously characterized tumors with a *TGFBR3-MGEA5* rearrangement [5, 7]. The der(10) chromosome was shown to contain the residual 3′ sequences from *TGFBR3* and *MGEA5*, juxtaposed in opposite directions, with loss of the 5′ sequences at mRNA level [7]. The t(1;10) has been shown to result in the overexpression of fibroblast growth factor 8 (FGF8), suggesting that the t(1;10) may alter the transcription of neighboring genes, at a distance from the breakpoint, rather than resulting in the creation of a chimeric fusion transcript [7]. In the case presented here, however, a fusion transcript was identified by RNA-sequencing and was further confirmed by RT-PCR, NGS allowing an accurate characterization of the fusion partners. It would therefore be interesting to re-analyze the previously reported PHAT cases using the primers designed for this case study.

The *FBXW4* gene is a member of the F-box/WD-40 gene family, encoding the FBXW4 protein involved in ubiquitin-mediated degradation. Loss of this ubiquitin ligase complex may play a role in cancer progression [15]. Disruption of the limb apical ectodermal ridge leads to the human condition split hand/foot malformation type 3 (SHFM3, MIM#246560). The critical SHFM3 locus at 10q24 is highly conserved among species, and behaves as an integrated unit. The apical ectodermal ridge signaling is pivotal to vertebrate embryonal limb development, and is under tight and complex regulation notably involving among others both the *FBXW4* and the *FGF8* genes [15].

In conclusion, the use of NGS technology allowed for a refined description of the reported recurrent t(1;10)(p22;q24) in a subcutaneous PHAT, thereby successfully identifying *FBXW4* instead of *MGEA5* as the fusion partner of *TGFBR3*. The close cytogenetic location of the *FBXW4* and *MGEA5* genes and the previous use of less comprehensive techniques suggest initial inaccurate interpretation. The study of further cases of PHAT and HFLT using NGS technology is however required to confirm that *FBXW4* has accurately been identified as the fusion partner of *TGFBR3*.

Author/co-author contributions All authors/co-authors:

• Have substantially contributed to the conception or design of the work (Anne-Laure Rougemont, Thomas A. McKee, and Yann Christinat); or the acquisition, analysis, or interpretation of data for the work (Anne-Laure Rougemont, Margaret Berczy, Nathalie Lin Marq, Thomas A. McKee, and Yann Christinat); AND

• Have drafted the work (Anne-Laure Rougemont, and Yann Christinat) or revised it critically for important intellectual content (Margaret Berczy, Nathalie Lin Marq, and Thomas A. McKee); AND

• Gave final approval of the version to be published (Anne-Laure Rougemont, Margaret Berczy, Nathalie Lin Marq, Thomas A. McKee, and Yann Christinat); AND

• Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (Anne-Laure Rougemont, Margaret Berczy, Nathalie Lin Marq, Thomas A. McKee, and Yann Christinat).

Compliance with ethical standards

The authors/co-authors declare that the study complies with ethical standards.

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

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