Novel fusion gene *THBS1::ERBB2* drives a subset of superficial acral fibromyxomas

Dear Editor, Superficial acral fibromyxoma (SAFM), is a painful, solitary, slow-growing, benign soft tissue tumour of the acral regions, typically affecting the hands and feet, for which we have little understanding of the genetic drivers. The two molecular investigations to-date have revealed loss of RB1 by immunohistochemistry (9/10 cases) and fluorescence *in situ* hybridization (7/7 cases), suggesting *RB1* deficiency as a possible driver event of SAFM(1), and an absence of *GNAS1* exon 8 or 9 mutations (0/8 cases), distinguishing SAFM from cellular myxoma(2). As fusion genes are well-established drivers of several types of skin tumours, we collated a large, international, multi-institutional cohort of SAFM (*n*=66), and performed RNA pulldown transcriptome sequencing (RNAseq) to ascertain the presence of fusion genes in this tumour type. Consistent with the clinical presentation of SAFM, the cohort consisted of more males (*n*=38) than females (*n*=28) and the median patient age was 55 ± 14 years(3). The lesions were predominantly located on either the hands (*n*=34), typically the finger (82% of the hand lesions), or feet (*n*=29, with 55% of these cases specifying the toe). Only three lesions were not located on the extremities (two on the elbow and one on the thigh). Sequencing data has been deposited in the European Genome and Phenome Archive (EGA; Accession ID: EGAS00001007737).

First considering the samples with high-quality RNAseq QC metrics (n=41), we identified nine fusion genes, in nine tumours. Seven in-frame fusions (*THBS1::ERBB2, ERBB2::THBS1, THBS1::ALK, NOTCH2::SERBP1, PIR::AP1S2, PPFIBP1::NTRK3, PPP4R3B::CCDC88A*) and two frameshift fusions (*HMGA2::ZNF532, VPS33A::CLIP1*). The *THBS1::ALK* fusion gene has been recurrently reported in inflammatory myofibroblastic tumour of the uterus(4, 5), whereas the other fusion genes are novel. Interestingly, a *THBS1* fusion gene, specifically *THBS1::ADGRF5*, has been recently reported in 89% of acral fibrochondromyxoid tumours – a likely benign mesenchymal neoplasm also arising in the distal extremities(6). Four SAFM cases had two fusion genes present, specifically *THBS1::ERBB2* and *PPP4R3B::CCDC88A* (PR65496a), *THBS1::ERBB2* and *VPS33A::CLIP1* (PR65473a), *PIR::AP1S2* and *HMGA2::ZNF532* (PR65497a), and *THBS1::ERBB2* and *ERBB2::THBS1* (PR64720a).

As *THBS1::ERBB2* was the only fusion gene present in more than one tumour (5/41 cases), we next looked for the presence of *THBS1::ERBB2* in samples with lower quality QC metrics (though still having ≥ 5 split reads supporting the breakpoint; n=25) and identified an additional three tumours. The reciprocal fusion gene was not found in these 25 tumours. Taken

together, the *THBS1::ERBB2* fusion showed a prevalence of 12% (n=8/66 tumours). The presence of the fusion was not associated with sex (male, *n*=3 vs. female, *n*=5; Fisher's Exact test, *p*=0.2689), however, there was a significant association with site, being more prevalent in the feet (*n*=7) than hands (*n*=1; Fisher's Exact test, *p*=0.0195). *THBS1::ERBB2*-positive cases did not show a difference from those without the fusion gene in terms of either age (47 ± 17 vs. 56 ± 14 years, respectively; p=0.7913, two-tailed t-test, after Shapiro=Wilks test assessment), or any defining histopathological features.

Across the eight samples with the *THBS1::ERBB2* fusion gene, there were four different breakpoints (**Fig. 1**). There were three different breakpoints in *THBS1* resulting in the fusion protein either having none or all three of the thrombospondin (TSP) repeat domains. The TSP domains are critical for the function of thrombospondin 1 by acting as binding sites for numerous extracellular matrix ligands(7); however, given not all of the fusions contained these domains suggests they are not essential components of the *THBS1::ERBB2* fusion.

The two different breakpoints in *ERBB2* resulted in the fusion protein either having a partial (*n*=7/8 tumours) or full (*n*=1/8 tumours) protein kinase (PK) domain, which regulates cell growth and survival by autophosphorylation and downstream pathway signalling(8), suggesting the PK domain is an essential part of the *THBS1::ERBB2* fusion. The fusion resulted in aberrant expression of *ERBB2*, as demonstrated by HER2 immunohistochemistry (*ERBB2* encodes HER2) in all eight of the fusion positive tumours, but not in tumours in which we did not detect the fusion (*n*=5; **Fig. 1**).

In summary, our study has identified a novel fusion gene driver, *THBS1::ERBB2*, in a subset of SAFM that was associated with aberrant HER2 expression. Further studies are warranted to assess whether HER2 expression could be a useful diagnostic biomarker for SAFM, and whether HER2-targeted therapies may represent a future therapeutic option for those with recurrent or progressive SAFM lesions driven by this fusion.

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Figure legend

Fig. 1. The *THBS1::ERBB2* fusion gene in superficial acral fibromyxoma (SAFM). Schematic diagram of the chromosomal location and gene structure of the fusion gene partners, *THBS1* and *ERBB2*, followed by the coding sequence (CDS) and PFAM protein domains of the *THBS1::ERBB2* fusion. Across the eight samples with the *THBS1*::*ERBB2* fusion gene, there were four different breakpoints. Upper left panel: chr15:39581924--chr17:39711928 (*n*=2 tumours; representative HER2 immunohistochemistry shown in (a); case PR65473a is a SAFM from the foot of a 46-year old female). Upper right panel: chr15:39583692--chr17:39711928 (*n*=4 tumours, representative HER2 immunohistochemistry shown in (b); case PR64720a is a SAFM from the finger of a 30-year old female). Lower left panel: chr15:39585563--chr17:39711928 (*n*=1, HER2 immunohistochemistry shown in (c); case PR65483a is a SAFM from the toe of a 44-year old female). Lower right panel: chr15:39585563--chr17:39706990 (*n*=1, HER2 immunohistochemistry shown in (d); case PR64722a is a SAFM from the toe of a 66-year old female). Representative HER2 immunohistochemistry for a tumour that did not have the *THBS1*::*ERBB2* fusion gene shown in (e); case PR64723a is a SAFM from the palm of a 57-year old male that did not have the *THBS1*::*ERBB2* fusion gene. Representative haematoxylin and eosin (HE) image of a SAFM shown in (f); case PR65483a. All immunohistochemistry magnifications are x200, and the HE scale bar is 100 μm. Abbreviations: FL, furin-like domain; GF, growth factor receptor IV domain; PL, protein kinase domain; RL, receptor L domain; TSP, thrombospondin domain; VWC, von Willebrand factor type C domain. Full details of all the fusion genes can be accessed at Figshare: 10.6084/m9.figshare.30505703.