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Correspondence

Diverse clinical presentations of pseudomyogenic hemangioendothelioma associated with *EGFL7::FOSB* fusion: a second case

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To the Editor:

Pseudomyogenic hemangioendothelioma (PHE) is an indolent vascular tumour often presenting with multiple lesions affecting one or more tissue planes, including bone.[1] Although local recurrence is common, these tumours rarely metastasize. Hakar *et al.* reported an unusual case of PHE with widespread metastasis, involving distant tumours in the lung, liver, and brain.[2] The patient in that case carried a novel *EGFL7::FOSB* gene fusion, and the impact of this new fusion on the clinical presentation remained uncertain. This report presents a second case of PHE with the same *EGFL7::FOSB* fusion. However, unlike the previous case, our patient exhibited a rather classic presentation of PHE without evidence of widespread tumours or remote metastasis.

A 35-year-old male patient presented with multiple firm subcutaneous nodules on the outer side of the left shoulder, causing excruciating stabbing pain. Positron emission tomography (PET) and computed tomography (CT) imaging revealed multiple osteolytic lesions at the proximal left humerus, as well as nodules in the surrounding muscles and subcutaneous areas, with increased metabolism of the 18F-fluorodeoxyglucose (FDG) signal (Figure 1A). Magnetic resonance imaging (MRI) confirmed multiple enhanced nodules in the surrounding muscles and showed that the soft-tissue shadow was unevenly enhanced and the bone cortex was destroyed (Figure 1B,C). No lesions were detected in other parts of the body, including the brain, by MRI. The tumour biopsy demonstrated plump spindle cells with abundant eosinophilic cytoplasm arranged in sheets and fascicles, with scattered tumour cells with an epithelioid appearance observed. Some regions displayed a notable neutrophil presence (Figure 2A). The tumour displayed infiltrative margins with adjacent muscles. Immunohistochemical (IHC) analysis showed that the tumour cells expressed CD31, Pan-Cytokeratin (Pan-CK), ERG, and INI-1, but were negative for CD34 (Figure 2B–F). Whole-transcriptome sequencing revealed a rearrangement between *EGFL7* and *FOSB*, with exon 2 of *EGFL7* fused to exon 1 of *FOSB*, resulting in a promoter swap (Figure 3A). A 17-bp fragment from intron 2 of the *EGFL7* was found at the fusion junction. Given the presence of a classical splicing acceptor site "AG" immediately preceding this 17-bp fragment, it is likely that the inclusion of this intronic segment results from alternative RNA splicing. However, no splicing donor site was identified at the end of the intronic fragment. Similarly, there was no splicing acceptor site detected at the 5′ end of the *FOSB* fusion site. These observations suggest that the fusion event occurring at this location is at the DNA level. The deduced DNA breakpoints are as follows (GRCh38/hg38): *EGFL7* gene: chr9:136 667 491 and *FOSB* gene: chr19:45 468 395. The presence of the *EGFL7::FOSB* fusion transcript was further confirmed through a reverse transcription / polymerase chain reaction (RT-PCR) assay followed by Sanger sequencing (Figure 3B,C). A targeted DNA next-generation sequencing (NGS) and a whole genome copy number variation (CNV) and loss of heterozygosity (LOH) assay did not reveal any additional mutations or genomic alterations.

The patient underwent a surgical procedure and simultaneous radiofrequency ablation to remove the nodules. However, 75 days after surgery an asymptomatic nodule emerged near the surgical scar, indicating local recurrence. The patient was treated with Denosumab, an inhibitor of receptor activator of nuclear factor kappa-B ligand (RANKL). Since then, the disease has remained stable, and the patient is able to carry out his daily activities and work normally. The most recent follow-up, 1 year after surgery, showed no signs of disease.

In this report we present the second case of PHE with *EGFL7::FOSB* fusion, showing a different clinical presentation compared to the first case. PHE exhibits variations in tumour size, location, number of lesions, and the involvement of different tissues (dermis, subcutis, muscle, and/or bone).[1,3] While most PHE cases are indolent and recur locally, there have been rare instances of distant metastasis. The biological reasons for these variations remain unknown. So far, six fusion partners for *FOSB* have been identified in PHE (Figure 3D). While all *FOSB* fusion partner genes are expressed in endothelial cells, differences in expression levels have been observed. We explored the GTEx database and found that *EGFL7* is dominantly expressed in vascular

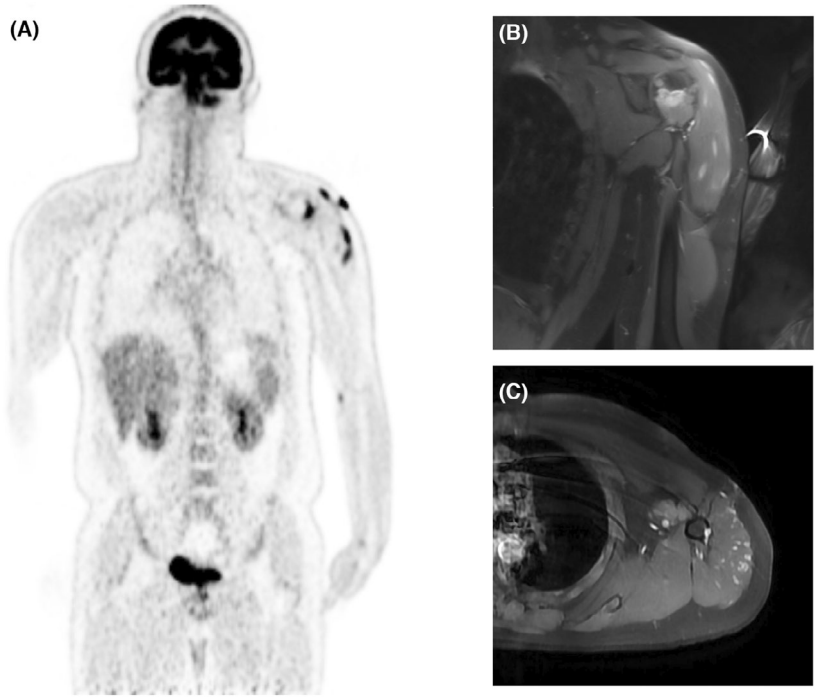

Figure 1. PET-CT imaging showed multifocal FDG-avid lesions in the proximal left humerus and its surrounding intramuscular and subcutaneous tissues (**A**). MRI demonstrated intense and uniform enhancement following gadolinium administration; T1-weighted enhancement and fat suppression, coronal (**B**) and axial orientation (**C**).

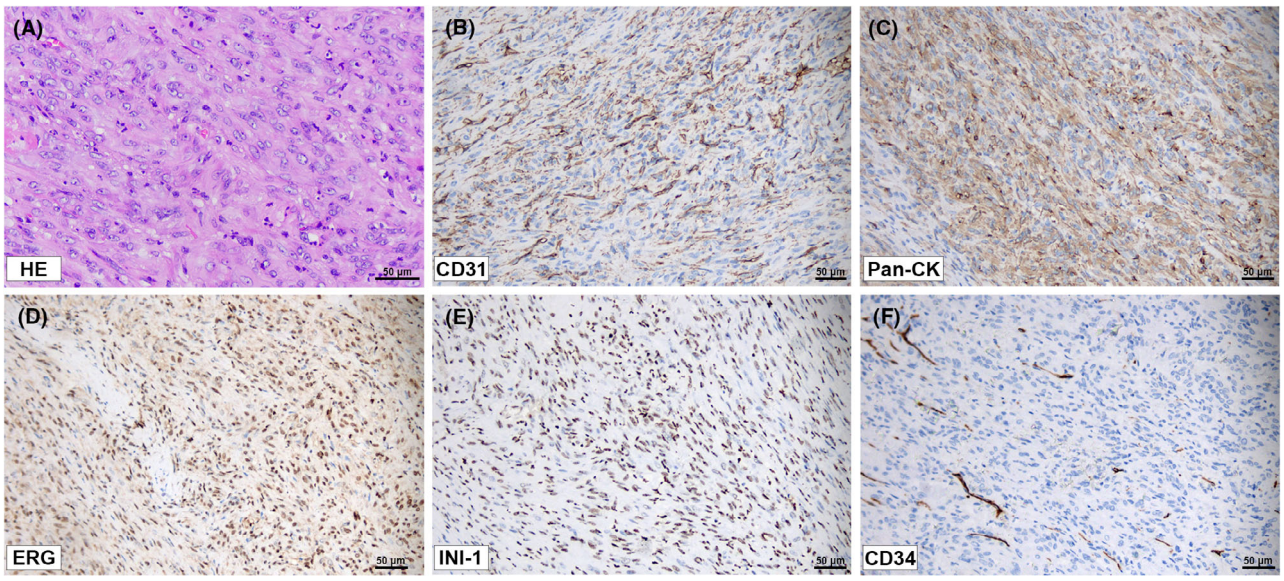

Figure 2. Haematoxylin and eosin (H&E) staining demonstrated spindled and epithelioid tumour cells with neutrophil infiltration (**A**) Immunohistochemistry (IHC) was positive for CD31 (**B**), Pan-CK (**C**), ERG (**D**), and INI-1 (**E**), and negative for CD34 (**F**).

endothelial cells[4] (Figure 3E). Whether the strength of the promoters from various fusion partner genes contributes to some of these clinical variations is an area of interest for further investigation. *EGFL7* has the strongest promoter among all known *FOSB* fusion partners in endothelial cells and may drive higher expression of *FOSB*, which could reconcile with the widely disseminated tumours reported in

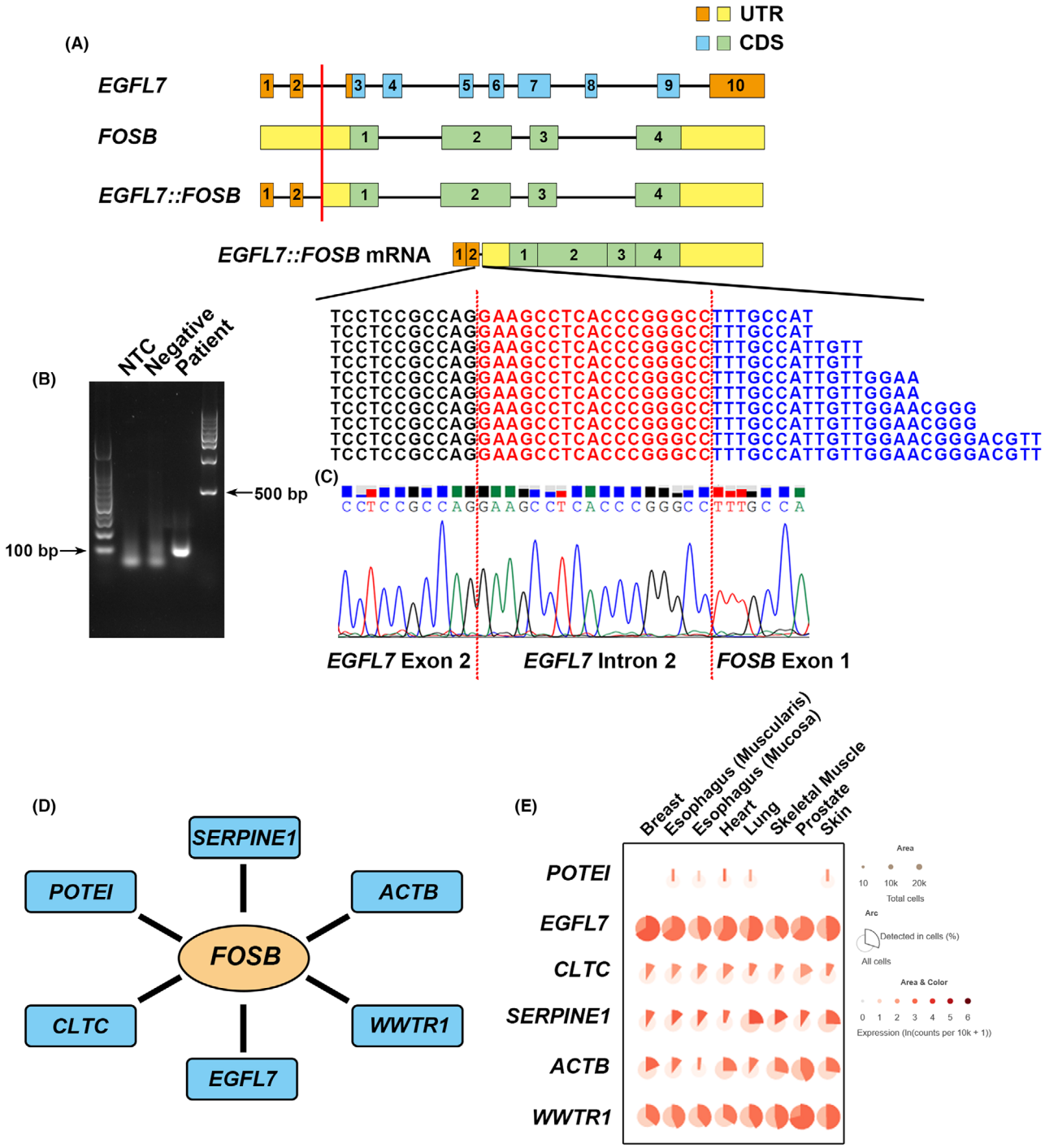

Figure 3. Whole transcriptome sequencing showed a gene fusion between *EGFL7* exon 2 and *FOSB* exon 1, containing the entire coding sequence of *FOSB*. A 17-bp fragment from intron 2 of *EGFL7* were included at the fusion junction by an alternative splicing mechanism (**A**); RT-PCR with primers specific for *EGFL7* and *FOSB* amplified a fusion product at the expected size (**B**); Sanger sequencing of the PCR product confirmed the *EGFL7::FOSB* fusion (**C**). Currently, six fusion partners of *FOSB* are documented in pseudomyogenic hemangioendothelioma (**D**). Relative mRNA expression levels of six known fusion partners in vascular endothelial cells isolated from eight different tissue types (data from GTEx) (**E**).

the first case. However, our case exhibited a typical clinical presentation of PHE with no sign of distant metastasis. Notably, these two *EGFL7::FOSB* cases differ in the breakpoints: our case had an *FOSB* breakpoint in exon 1, resulting in a promoter swap without altering the *FOSB* reading frame, while the other *EGFL7::FOSB* case had a *FOSB* breakpoint in exon 2, leading to both a promoter swap and a chimeric fusion product. A review of previous PHE cases showed *FOSB* breakpoints occurring in either exon 1 or exon 2, with no noticeable difference in phenotypes observed between patients with different breakpoints. This suggests that the oncogenic mechanism of *FOSB* rearrangement likely relies on promoter swap and spatial–temporal deregulation of *FOSB* expression, rather than the chimeric fusion product, which contains all functional domains of FOSB, including the DNA binding domain, Leu-Zip, and C-terminal transactivation domain.[5]

Although additional genomic alterations, in addition to *FOSB* fusion, may potentially influence the clinical course of PHE, limited data are available to analyse this association. *FOSB* fusion was identified as the sole genomic change in our patient's case. Whether rare cases of PHE with distant metastasis involve additional genomic alterations is currently unknown and requires further investigation. Genomic evaluation by targeted DNA NGS in a few PHE cases has shown that *FOSB* rearrangement is the sole genomic alteration.[6] However, the karyotype of PHE revealed an unbalanced translocation involving *FOSB*, suggesting that at least some PHE cases harbour other genomic alterations.[7]

In summary, we report the second case of PHE carrying the *EGFL7::FOSB* fusion, which exhibited a different clinical presentation compared to the first case. It remains to be determined whether the different *FOSB* breakpoints or additional genomic alterations influence the clinical outcome of PHE, including distant metastasis.

Author contributions

Bin Li, Yongbin Hu, Zhiyuan Li and Suya Kang provided clinical data, performed imaging analysis and histopathologic evaluation. Changliang Zhang, Lina Zhao, Nan Chen and Angella Blake contributed to the molecular studies and data analysis. Changliang Zhang and Sheng Xiao prepared the article with support from Bin Li. Bin Li and Sheng Xiao supervised the study.

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Conflict of interest

The authors declare that they have no conflicts of interest to declare for the article.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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