Journal of Pathology
J Pathol 2014; **232:** 534–540
Published online 29 January 2014 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/path.4322

ORIGINAL PAPER

A novel *SERPINE1–FOSB* fusion gene results in transcriptional up-regulation of *FOSB* in pseudomyogenic haemangioendothelioma

Charles Walther,[1,2*] Johnbosco Tayebwa,[1] Henrik Lilljebjörn,[1] Linda Magnusson,[1] Jenny Nilsson,[1] Fredrik Vult von Steyern,[3] Ingrid Øra,[4] Henryk A Domanski,[2] Thoas Fioretos,[1] Karolin H Nord,[1] Christopher DM Fletcher[5] and Fredrik Mertens[1]

[1] *Department of Clinical Genetics, University and Regional Laboratories, Skåne University Hospital, Lund University, Sweden*
[2] *Department of Pathology, University and Regional Laboratories, Skåne University Hospital, Lund, Sweden*
[3] *Department of Orthopedics, Skåne University Hospital, Lund, Sweden*
[4] *Department of Pediatric Oncology, Skåne University Hospital, Lund, Sweden*
[5] *Department of Pathology, Brigham and Women´s Hospital, Harvard Medical School, Boston, MA, USA*

**Correspondence to: C Walther, Department of Pathology, University and Regional Laboratories, Skåne University Hospital, 221 85 Lund, Sweden. e-mail: charles.walther@med.lu.se*

Abstract

Pseudomyogenic haemangioendothelioma (PHE) is an intermediate malignant vascular soft tissue tumour primarily affecting children and young adults. The molecular basis of this neoplasm is unknown. We here used chromosome banding analysis, fluorescence *in situ* hybridization (FISH), mRNA sequencing, RT–PCR and quantitative real-time PCR on a series of morphologically well-characterized PHEs to show that a balanced translocation, t(7;19)(q22;q13), detected as the sole cytogenetic aberration in two cases, results in fusion of the *SERPINE1* and *FOSB* genes. This translocation has not been observed in any other bone or soft tissue tumour. Interphase FISH on sections from eight additional PHEs identified the same *SERPINE1–FOSB* fusion in all cases. The role of *SERPINE1*, which is highly expressed in vascular cells, in this gene fusion is probably to provide a strong promoter for *FOSB*, which was found to be expressed at higher levels in PHEs than in other soft tissue tumours. *FOSB* encodes a transcription factor belonging to the FOS family of proteins, which, together with members of the JUN family of transcription factors, are major components of the activating protein 1 (AP-1) complex. Further studies are needed to understand the cellular impact of the aberrant expression of the *FOSB* gene, but as the t(7;19) resulting in the *SERPINE1–FOSB* fusion seems to be pathognomonic for PHE, FISH or RT–PCR could be useful for differential diagnostic purposes.

Keywords: pseudomyogenic haemangioendothelioma; gene fusion; SERPINE1; FOSB; RNA sequencing; sarcoma; paediatric

Received 21 October 2013; Revised 3 December 2013; Accepted 20 December 2013

No conflicts of interest were declared.

Introduction

Pseudomyogenic haemangioendothelioma (PHE), also sometimes known as epithelioid sarcoma-like haemangioendothelioma, is a rare soft tissue tumour, predominantly affecting children and young adults, with a male:female ratio of 4.6:1 [1–3]. The tumour is usually situated in the limbs or trunk, may involve skin, subcutis, muscle or (least often) bone, and is multicentric in more than 50% of cases. An unusual feature compared to other soft tissue tumours is that different tissue planes are often involved. The tumour is locally aggressive but distant metastases are rare [2].

The genetics of PHE remains poorly characterized, but a potentially recurrent translocation, t(7;19)(q22;q13), was recently reported by us [4]. Using fluorescence *in situ* hybridization (FISH), the breakpoints in chromosomes 7 and 19 in a case with cytogenetically detected t(7;19) were delineated; however, none of the genes implicated by the FISH results was found to be involved in a gene fusion. The results obtained from metaphase FISH analysis were also used for interphase FISH on nine additional cases from which only tissue sections were available. Among these, only one more case with a potentially unbalanced t(7;19) was found [4]. Since that publication, we have received one additional case of PHE for chromosome analysis. Again, a balanced t(7;19)(q22;q13) was found, prompting further in-depth analysis of the molecular consequences of this translocation. We here present the results obtained through mRNA sequencing, FISH, RT–PCR and quantitative real-time PCR.

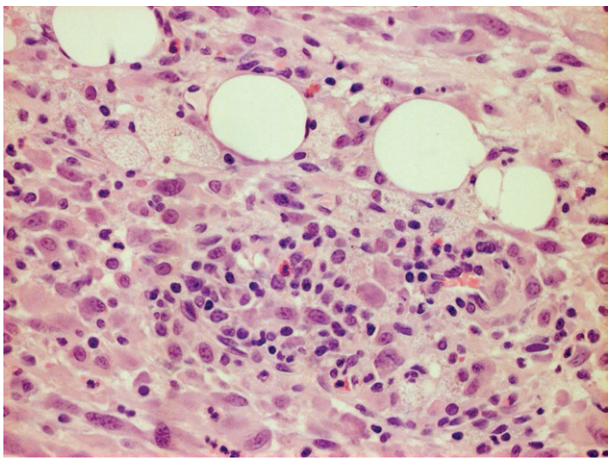

Figure 1. Morphology of pseudomyogenic haemangioendothelioma (case 2). The tumour cells are distributed in a collagenous stroma with scattered neutrophils; magnification = ×40.

Materials and methods

Tumour samples

Fresh tumour biopsies were obtained from two patients. Case 1, a 14 year-old girl with two soft tissue lesions in the chest wall, has been described in detail [4]. Eight months after the initial surgery, a local relapse was detected by MR imaging and surgically removed. She remains disease-free 70 months after the second surgery. Case 2 was a 16 year-old boy with a tumour in the calcaneus bone of the foot. Preoperative needle biopsies were inconclusive but revealed no signs of malignancy, and hence the tumour was treated by curettage for diagnosis. A new operation to achieve free surgical margins was considered too mutilating. Follow-up with X-ray examination indicated skeletal healing and no sign of local recurrence. However, 10 months after diagnosis two new tumours were detected in the inferior ramus of the os ischium and subcutaneously in the abdominal wall.

All samples were obtained after written consent and all studies were approved by the institutional ethical committees.

The histopathological analysis in both cases was entirely in keeping with the diagnosis of PHE. Detailed morphological and immunohistochemical results have been reported for case 1 [4]. The morphology of case 2 is presented in Figure 1. From the consultation files of one of the authors (CDMF), sections from another 10 new cases of PHE, obtained since the original publication [1], were studied by interphase FISH (Table 1). None of these 10 cases was included in the previous FISH study [4].

Cytogenetic and FISH analyses

Cell culture, harvesting and G-banding were performed as described, and the karyotypes were written following the recommendations of the International System for Human Cytogenetic Nomenclature [5,6].

The bacterial artificial chromosome (BAC) and fosmid probes that were used to delineate the breakpoints in 7q22 and 19q13 on metaphase chromosomes were obtained from the BACPAC Resource Center (http:/bacpac.chori.org). These probes, and the additional probes used for interphase FISH, are specified in Table S1 (see supplementary material). Clone preparation, hybridization and analysis were performed as described [4,7]. For interphase FISH on cut sections, at least 95 nuclei from different areas were evaluated in each case. Fusion signals in at least 20% of the nuclei were used as cut-offs for scoring aberrations.

mRNA sequencing and data analysis

mRNA libraries were prepared for sequencing using the Truseq RNA sample preparation kit v 2 (Illumina), according to the manufacturer's protocol. In brief, 200–1000 ng total RNA was enriched for poly-A-tailed RNA, using magnetic oligo-dT beads. The poly-A-tailed enriched RNA was fragmented to a median size of 200 nucleotides by thermal fragmentation at 94°C, 10 s in the 'Elute, Prime, Fragment' buffer. The fragmented RNA was used as templates for cDNA synthesis, using Superscript II reverse transcriptase (Invitrogen). A second DNA strand was produced using DNA polymerase I and RNase H. After end repair and 3′-end adenylation, indexed adapters were ligated to the double-stranded cDNA fragments. The adapter-bound fragments were then enriched using a 15 cycle PCR. Paired-end 101 bp reads were generated from the mRNA libraries, using a HiscanSQ (Illumina).

To identify candidate fusion transcripts from the sequence data, analyses were performed on gunzipped fastq files, using TopHat v 2.0.7 with the *--fusion-search* and *--bowtie1* options, only allowing for detection of fusions within a minimum distance of 100 000 bp (*--fusion-min-dist* option). UCSC hg19 was used as the human genome reference. The fragment lengths of the mRNA libraries were in the range 100–1000 bp, with a mean of 400 bp, so the mate inner distance (*-r* option) was set to 200, with a standard deviation (*--mate-std-dev* option) of 200. TopHat-fusion-post was run on the output files from TopHat v 2.0.7, allowing for detection of fusions with at least one fusion-spanning read and two fusion-spanning mate-pairs [8].

RT–PCR

Total RNA was extracted from frozen tumour samples, using the reagent RNeasy Lipid tissue kit (Qiagen, Valencia, CA, USA). Reverse transcription and PCR amplifications were performed as described [7,9]. Primers specific for *SERPINE1* and *FOSB* were designed to detect possible fusion transcripts (see supplementary material, Table S2). Transcripts were amplified using an initial denaturation for 2 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 58°C and 3 min at 72°C, and a final extension for 3 min at 72°C. Amplified fragments were purified from agarose gels and directly sequenced using

Table 1. Clinical and genetic data on 12 pseudomyogenic haemangioendotheliomas

Case	Sex*	Age**	Tumour location	Cytogenetics	FISH†	RNA-Seq†	RT–PCR
1	F	14	Chest wall	t(7;19)(q22;q13)	ND	*SERPINE1* nt 100 771 438 *FOSB* nt 45 973 894	*SERPINE1* ex1/*FOSB* ex2
2	M	14	Foot (bone)	t(7;19)(q22;q13)	29%	*SERPINE1* nt 100 770 709 *FOSB* nt 45 971 519	*SERPINE1* ex1/*FOSB* ex1
3	F	41	Calf	ND	42%	ND	ND
4	M	28	Shoulder	ND	80%	ND	ND
5	M	18	Foot (bone)	ND	20%	ND	ND
6	M	21	Hand	ND	28%	ND	ND
7	M	32	Foot	ND	35%	ND	ND
8	M	43	Penis	ND	32%	ND	ND
9	M	18	Thigh	ND	Failure	ND	ND
10	M	35	Spermatic cord	ND	38%	ND	ND
11	F	30	Finger	ND	51%	ND	ND
12	M	19	Foot	ND	Failure	ND	ND

*M, male; F, female.
**Age in years at diagnosis.
†Interphase FISH results on imprint slides (Case 2) or cut sections from paraffin-embedded tumours (cases 3–12). The frequencies with which fusion signals were identified in interphase nuclei are shown.
†nt, nucleotide position according to the GRCh37/hg19 build.

the Big Dye v 1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) on an ABI-3130 genetic analyser (Applied Biosystems). BLASTN software (http://www.ncbi.nlm.nih.gov/blast) was used for the analysis of *SERPINE1* and *FOSB* sequence data.

Quantitative real-time PCR

To assess differences in the expression levels of *FOSB,* TaqMan gene expression assays were performed on RNA from cases 1 and 2 and 13 other soft tissue tumours (one case each of pericytoma, haemangioendothelioma and epithelioid sarcoma, two cases each of benign fibrous histiocytoma and angiosarcoma, and six leiomyosarcomas) serving as controls; *HPRT1* was used as endogenous control. The following TaqMan gene expression assays were used: Hs02800695_ml (*HPRT1*), Hs01547108_gl (*FOSB*; exons 2–3). Quantitative real-time PCR (qRT–PCR) was performed according to the manufacturer's instructions and all reactions were run in triplicate (Applied Biosystems). Calculations were done using the comparative C_T method (ie $\Delta\Delta C_T$ method), using SDS software v 1.3.1.

Results

Cytogenetic findings

As already described, case 1 showed a clonal and consistent translocation, t(7;19)(q22;q13), at diagnosis and at relapse as the sole anomaly [4]. The karyotype in case 2 was 45-46,XY,t(7;19)(q22;q13)/46,XY.

FISH

Metaphase FISH analysis using a panel of BAC and fosmid clones delineated the breakpoints at 7q22 and 19q13 in cases 1 and 2. The probes covering the breakpoints were RP11-747G18 for 7q22 and W12-1787G20 for 19q13. Using pools of three Cy3-labelled and four FITC-labelled probes for the breakpoint regions in 7q22 and 19q13, respectively, interphase FISH was performed on cells from case 2 and cut sections from 10 additional paraffin-embedded cases of PHE. Due to the location of the target genes at the telomeric (*SERPINE1*) and centromeric (*FOSB*) ends, respectively, of each pool of probes, cells with fusions should display a single yellow fusion signal on the derivative chromosome 7. This signal pattern was seen in 29% of the cells in case 2 (Figure 2), as well as in 20–80% of the nuclei in the eight informative cases from which only cut sections were available (Table 1).

mRNA sequencing

Several potential fusion transcripts were detected in cases 1 and 2 (see supplementary material, Table S3). By far the most frequently detected transcripts involved the *SERPINE1* and *FOSB* genes; both genes map to the two chromosome bands – 7q22 and 19q13, respectively – implicated by the cytogenetic findings. In case 1 there were 279 spanning reads, 74 spanning mate pairs and 227 spanning mate pairs where one end spanned a fusion, supporting a *FOSB–SERPINE1* fusion. The corresponding figures for case 2 were 260, 70 and 263, respectively. Other detected potential fusion transcripts were considered to be read-through transcripts or other artefacts.

RT–PCR

RT–PCR and subsequent sequencing of amplified products from cases 1 and 2 identified in-frame *SERPINE1–FOSB* fusion transcripts in both cases

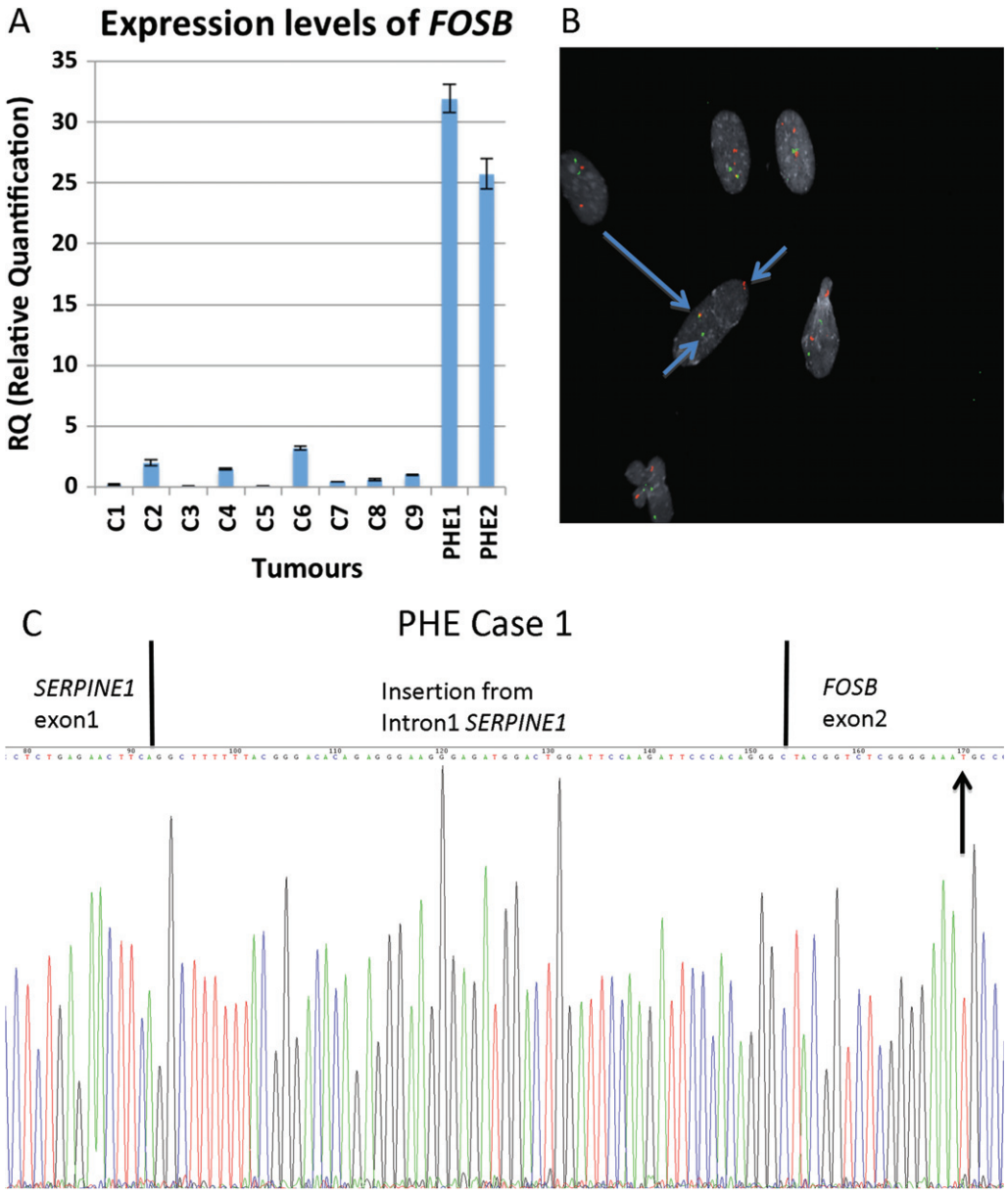

Figure 2. Genetic findings in pseudomyogenic haemangioendothelioma (PHE). (A) Quantitative qRT–PCR results for *FOSB* expression in two PHEs and nine control tumours, showing significantly higher expression levels in PHEs compared to controls. (B) Interphase FISH with BAC probes showing normal red (*SERPINE1*) and green (*FOSB*) signals (short blue arrows) and yellow fusion (*SERPINE1–FOSB*) signal (long blue arrow) in case 2. (C) Chromatogram showing the fusion junction in case 1; 61 nucleotides from intron 1 of *SERPINE1* were inserted at the fusion junction. The translation start codon in *FOSB* is indicated by a black arrow.

(Figure 2). In both tumours the breakpoints in *SERPINE1* were located in the non-coding exon 1. The breakpoint in *FOSB* was located in the beginning of exon 2 in case 1 and in the non-coding exon 1 in case 2. Both cases showed small insertions (61 bp in case 1, 59 bp in case 2) of material from intron 1 of *SERPINE1* at the fusion junction. In case 1, this introduced a new start codon, whereas the original start codon of *FOSB,* which was not present in the fusion in case 1, was retained in the fusion in case 2. The two metastases in case 2 showed the same fusion transcript as the primary tumour in the os calcaneus, verifying that they were metastases (data not shown).

Quantitative real-time PCR

The expression of *FOSB* was significantly higher in the two PHEs (cases 1 and 2) than in the tumours serving as controls (Figure 2; see also supplementary material, Figure S1).

Discussion

Pseudomyogenic haemangioendothelioma is a rare and recently described soft tissue tumour [1]. In the current WHO classification of soft tissue tumours, it is listed as an intermediate malignant, rarely metastasizing,

vascular tumour [2]. We here used various genetic methods to show that PHE consistently displays a *SERPINE1–FOSB* fusion gene, resulting from a translocation between chromosomes 7 and 19, presumably constituting the essential driver mutation in this neoplasm. In a previous study, we had identified by FISH a different breakpoint in chromosome 19, which could now be explained by the existence of several breakpoints in 19q in that case. The previous focus on the wrong target region in 19q, ie an additional breakpoint approximately 1 mb centromeric to *FOSB,* also provides a good explanation for the failed attempts to identify additional fusion-positive cases in the previous publication [4].

SERPINE1 (also known as *PAI-1*, plasminogen activator inhibitor type 1) encodes a protein that is a member of the serine protease inhibitor family, and that inhibits tissue- and urokinase-type plasminogen activators. These activators convert plasminogen to plasmin, which in turn mediates fibrinolysis and proteolytic degradation of extracellular matrix. SERPINE1 was first identified in endothelial cells, but later studies have shown that the protein is expressed in a variety of cells and tissues [10,11]. It is also highly expressed in many tumours, being implicated in invasion, angiogenesis and metastasis [12,13]. The known high expression of *SERPINE1* in vascular cells and the lack of protein-coding parts from it in the detected gene fusions strongly indicate that its role in the *SERPINE1–FOSB* chimera is to provide a promoter allowing strong expression of *FOSB*. In line with this conclusion, high levels of *FOSB* mRNA expression were seen in the two PHEs compared to other soft tissue tumours (leiomyosarcomas, benign fibrous histiocytomas and vascular tumours). The same tumourigenic mechanism of fusing a powerful regulatory sequence with a potential oncogene has been described in many other neoplasms, the prototypical example being fusions of the *MYC* gene with different immunoglobulin genes in Burkitt's lymphoma [14]. Other soft tissue and bone tumours in which this has been described include lipoblastoma [15], dermatofibrosarcoma protuberans [16] and aneurysmal bone cyst [17].

The *FOSB* gene maps to chromosome band 19q13 and encodes a transcription factor belonging to the FOS family of proteins. FOS proteins and members of the JUN family of transcription factors constitute the major components of the activating protein 1 (AP-1) complex. FOS proteins are unable to form homodimers, and instead heterodimerize with JUN proteins. Such dimers regulate transcription of target genes by preferentially binding to a consensus sequence called the TPA-responsive element in their enhancer and promoter regions [18,19]. FOSB exists in three different isoforms: (a) full-length FOSB consists of 338 amino acids (aa), with a central basic leucine-zipper region and a carboxy-terminal transactivation domain (TAD); (b) the truncated ΔFOSB shares the 237 amino-terminal aa with full-length FOSB, but lacks the carboxy-terminal (TAD); (c) the Δ2ΔFOSB isoform, originating from an alternative translation initiation site in the ΔFOSB transcript, in addition lacks the amino-terminal 78 aa of full-length FOSB. Although much still remains to be elucidated about the relationship between the different isoforms, it seems clear that they have distinct, even opposite, effects on target genes [20–22].

Bearing in mind the central role of the AP-1 transcription factor complex in gene regulation, it is no surprise that the *FOS* genes have been implicated in a variety of physiological and pathological processes, including several malignancies [18]. To our knowledge, however, there is no previous report linking up-regulation of *FOSB* with sarcoma development, but there is quite an extensive literature on the importance of FOSB in the differentiation of mesenchymal stem/progenitor cells [23]. Furthermore, *FOSB* has been reported as the 3′-partner in a gene fusion with the *LMNA* gene in a single case of meningioma. The significance of that fusion, which was one of 19 different gene fusions detected by deep sequencing, is unclear, but it can be noted that the *LMNA–FOSB* gene fusion also included the entire coding region of *FOSB* [24]. Thus, it could be of interest to study the expression of *FOSB* also in other mesenchymal tumours. While the transcriptional up-regulation of *FOSB* through fusion with *SERPINE1* appears unique for PHE – none of more 2300 soft tissue tumours with abnormal karyotypes has displayed the t(7;19) identified in PHE [25] – it seems highly unlikely that increased expression should be restricted to PHE; a wide variety of extra- and intracellular factors leading to increased *FOSB* expression have been identified [18].

Characteristic genetic aberrations have now been described in soft tissue tumours of all lineages, but the subgroup of vascular tumours still remains poorly explored. Next to nothing is known about the underlying genetic changes in benign vascular lesions, and the information on most malignant tumours is at best fragmentary. One exception, however, is epithelioid haemangioendothelioma (EHE) [26]. Recently, two different gene fusions (*WWTR1–CAMTA1* and *YAP1–TFE3*) were detected in EHE [27–29]. The *WWTR1–CAMTA1* fusion, resulting in a chimeric protein, accounts for the vast majority of conventional EHEs, whereas the *YAP1–TFE3* fusion has been detected in EHEs with a slightly different morphology. Similar to the role of *SERPINE1* in the PHE-associated fusion described in the present study, the main role of *YAP1* in the *YAP1–TFE3* fusion seems to be to provide a strong promoter for the 3′-fusion partner. Considering that the *SERPINE1–FOSB* and *YAP1–TFE3* fusions were both detected using next-generation sequencing, it should be worthwhile to study other vascular tumours with similar techniques.

While much remains to be investigated with regard to the cellular effects of the detected *SERPINE1–FOSB* fusion, the results of the present study can already be used for clinical purposes. The high frequency of

fusion events in our series of PHEs strongly indicates that RT–PCR and/or FISH could serve as useful diagnostic tools to distinguish PHE from morphological mimics. It would, for instance, be of interest to investigate whether tumours described as epithelioid sarcoma-like haemangioendothelioma [3] harbour the same *SERPINE1–FOSB* gene fusion. RT–PCR could, however, be challenging when using formalin-fixed paraffin-embedded (FFPE) specimens. The fusion transcripts we detected in fresh-frozen PHE samples had different breakpoints, as well as relatively large insertions at the fusion junctions, suggesting that multiple primer combinations must be employed when using FFPE samples for RT–PCR.

Acknowledgements

The technical assistance of Marianne Rissler is gratefully acknowledged. This study was supported by the Swedish Childhood Cancer Foundation.

Author contributions

CW wrote the manuscript and evaluated the data; JT and HL performed the RNA sequencing and data analysis; LM and JN performed the FISH and PCR analyses; FVvS and IO provided the clinical data; HAD provided histopathological information; TF and KN assisted in writing the manuscript and interpreting molecular data; CDMF contributed cases and histopathological expertise; and FM was the principal investigator.

References

1. Hornick JL, Fletcher CDM. Pseudomyogenic hemangioendothelioma: a distinctive, often multicentric tumor with indolent behavior. *Am J Surg Pathol* 2011; **35:** 190–201.
2. Hornick JL, Mertens F, Fletcher CDM. Pseudomyogenic haemangioendothelioma. In *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone*, Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F (eds). IARC Press: Lyon, 2013; 153–154.
3. Billings SD, Folpe AL, Weiss SW. Epithelioid sarcoma-like hemangioendothelioma. *Am J Surg Pathol* 2003; **27:** 48–57.
4. Trombetta D, Magnusson L, Vult von Steyern F, *et al.* Translocation t(7; 19)(q22; q13) – a recurrent chromosome aberration in pseudomyogenic hemangioendothelioma? *Cancer Genet* 2011; **204:** 211–215.
5. Mandahl N, Heim S, Arheden K, *et al.* Three major cytogenetic subgroups can be identified among chromosomally abnormal solitary lipomas. *Hum Genet* 1988; **79:** 203–208.
6. Shaffer LG, McGowan-Jordan J, Schmid M. *An International System for Human Cytogenetic Nomenclature (2013)*. Karger: Basel, 2013.
7. Jin Y, Möller E, Nord KH, *et al.* Fusion of the *AHRR* and *NCOA2* genes through a recurrent translocation t(5; 8)(p15; q13) in soft tissue angiofibroma results in upregulation of aryl hydrocarbon receptor target genes. *Genes Chromosomes Cancer* 2012; **51:** 510–520.
8. Kim D, Pertea G, Trapnell C, *et al.* TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* 2013; **14:** R36.
9. Panagopoulos I, Mertens F, Domanski HA, *et al.* No *EWS/FLI1* fusion transcripts in giant cell tumors of bone. *Int J Cancer* 2001; **93:** 769–772.
10. Simpson AJ, Booth NA, Bennett B. Distribution of plasminogen activator inhibitor (PAI-1) in tissues. *J Clin Pathol* 1991; **44:** 139–143.
11. Declerck PJ, Gils A. Three decades of research on plasminogen inhibitor-1: a multifaceted serpin. *Semin Thromb Hemost* 2013; **39:** 356–364.
12. Ju H, Lim B, Kim M, *et al. SERPINE1* intron polymorphisms affecting gene expression are associated with diffuse-type gastric cancer susceptibility. *Cancer* 2010; **116:** 4248–4255.
13. Ghosh AK, Vaughan DE. PAI-1 in tissue fibrosis. *J Cell Physiol* 2012; **227:** 493–507.
14. Mitelman F, Johanssson B, Mertens F. The impact of translocations and gene fusions in cancer causation. *Nat Rev Cancer* 2007; **7:** 233–245.
15. Hibbard MK, Kozakewich HP, Dal Cin P, *et al. PLAG1* fusion oncogenes in lipoblastoma. *Cancer Res* 2000; **60:** 4869–4872.
16. Sirvent N, Maire G, Pedeutour F. Genetics of dermatofibrosarcoma protuberans family of tumors: from ring chromosomes to tyrosine kinase inhibitor treatment. *Genes Chromosomes Cancer* 2003; **37:** 1–19.
17. Oliveira AM, Perez-Atayde AR, Dal Cin P, *et al.* Aneurysmal bone cyst variant translocations upregulate *USP6* transcription by promoter swapping with the *ZNF9, COL1A1, TRAP150,* and *OMD* genes. *Oncogene* 2005; **24:** 3419–3426.
18. Milde-Langosch K. The Fos family of transcription factors and their role in tumourigenesis. *Eur J Cancer* 2005; **41:** 2449–2461.
19. Shaulian E. AP-1 – the Jun proteins: oncogenes or tumor suppressors in disguise? *Cell Signal* 2010; **22:** 894–899.
20. Sabatakos G, Sims NA, Chen J, *et al.* Overexpression of ΔFosB transcription factor(s) increases bone formation and inhibits adipogenesis. *Nat Med* 2000; **6:** 985–990.
21. Sabatakos G, Rowe GC, Kveiborg M, *et al.* Doubly truncated FosB isoform (Δ2ΔFosB) induces osteosclerosis in transgenic mice and modulates expression and phosphorylation of Smads in osteoblasts independent of intrinsic AP-1 activity. *J Bone Miner Res* 2008; **23:** 584–595.
22. Ohnishi YN, Sakumi K, Yamazaki K, *et al.* Antagonistic regulation of cell-matrix adhesion by FosB and ΔFosB/Δ2ΔFosB encoded by alternatively spliced forms of fosB transcripts. *Mol Biol Cell* 2008; **19:** 4717–4729.
23. Ramachandran A, Gong EM, Pelton K, *et al.* FosB regulates stretch-induced expression of extracellular matrix proteins in smooth muscle. *Am J Pathol* 2011; **179:** 2977–2989.
24. Brastianos PK, Horowitz PM, Santagata S, *et al.* Genomic sequencing of meningiomas identifies oncogenic *SMO* and *AKT1* mutations. *Nat Genet* 2013; **45:** 285–289.
25. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer 2013, Mitelman F, Johansson B and Mertens F (eds): http://cgap.nci.nih.gov/Chromosomes/Mitelman [accessed 21 November 2013].
26. Weiss SW, Antonescu CR, Bridge JA, *et al.* Epithelioid haemangioendothelioma. In *WHO Classification of Soft Tissue and Bone Tumours*, Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F (eds). IARC Press: Lyon, 2013; 155–156.
27. Errani C, Zhang L, Sung YS, *et al.* A novel *WWTR1–CAMTA1* gene fusion is a consistent abnormality in epithelioid hemangioendothelioma of different anatomic sites. *Genes Chromosomes Cancer* 2011; **50:** 644–653.

28. Tanas MR, Sboner A, Oliveira AM, *et al.* Identification of a disease-defining gene fusion in epithelioid hemangioendothelioma. *Sci Transl Med* 2011; **3:** 98ra82.

29. Antonescu CR, Le Loarer F, Mosquera J-M, *et al.* Novel *YAP1–TFE3* fusion defines a distinct subset of epithelioid hemangioendothelioma. *Genes Chromosomes Cancer* 2013; **52:** 775–784.

SUPPLEMENTARY MATERIAL ON THE INTERNET

The following supplementary material may be found in the online version of this article:

Supplementary materials and methods.

Figure S1. qRT–PCR for *FOSB* expression in two PHEs, two angiosarcomas, one epithelioid sarcoma and one haemangioendothelioma showed significantly higher expression levels in PHEs compared to controls.

Table S1. FISH probes used for gene fusion detection

Table S2. Primers used for RT–PCR and sequencing

Table S3. Results of mRNA sequencing