1 A novel *SERPINE1-FOSB* fusion gene in a case of pseudomyogenic

2 hemangioendothelioma results in activation of intact FOSB and the

3 PI3K-AKT-mTOR signaling pathway and responsiveness to sirolimus

4 Abbreviated title: A novel *SERPINE1-FOSB* fusion gene in PHE

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21

22

23 **Abstract**

24 Pseudomyogenic hemangioendothelioma (PHE) is an extremely rare disease that
 25 affects mainly the young and more men than women. PHE are multicentric, locally
 26 aggressive, have low metastatic potential, and affect multiple tissue planes. Genetic
 27 aberrations are frequently detected in PHE and may play important roles in the
 28 occurrence, development, and treatment of this disease. In this study, we report a case
 29 of PHE with a novel *SERPINE1-FOSB* fusion gene. The fusion introduced a strong
 30 promoter near the coding region of *FOSB*, resulting in overexpression of intact FOSB.
 31 Immunohistochemical analysis showed overexpression of pAKT and mTOR in tumor
 32 cells, suggesting activation of the PI3K-AKT-mTOR signaling pathway. The patient
 33 responded well to targeted therapy with sirolimus, an mTOR inhibitor. Our study
 34 correlated dysregulation of a specific signaling pathway and the effectiveness of a
 35 targeted therapy to a specific genetic aberration. This information may be useful for
 36 future investigations of targeted therapeutics and provide a potential predictive
 37 biomarker for therapeutic effectiveness in cases of PHE with this genetic aberration.

38

39 **Keywords**

40 FOSB, PI3K-AKT-mTOR signaling pathway, pseudomyogenic
 41 hemangioendothelioma, *SERPINE1-FOSB* fusion, sirolimus

42

43 **Introduction**

44 Pseudomyogenic hemangioendothelioma (PHE) is an extremely rare disease that was
 45 included in the 2013 WHO classification of soft tissue and bone tumors.¹ This disease
 46 occurs mainly in the young population and affects more men than women. PHE are
 47 typically multicentric, locally aggressive, have low metastatic potential, and affect
 48 multiple tissue planes.^{1, 2}

49 Histologically, PHE is characterized by loose fascicles of spindled and epithelioid
 50 cells, abundant eosinophilic cytoplasm, and vesicular nuclei with distinct nucleoli. In
 51 some cases, neutrophilic inflammatory infiltrates have been noted.^{1, 2}

52 Immunohistochemically, tumor cells are positive for factor VIII, FLI-1, INI-1,
 53 vimentin, MDM2, CDK4, CD31, cytokeratin AE1/AE3, EMA, and P63 and negative
 54 for CD34, S-100, SMA, desmin, MyoD1, and HMB45.^{2, 3} Genetically, chromosomal
 55 rearrangements that result in the fusion of all or part of the *FOSB* coding region with
 56 the promoters of other genes, such as *SERPINE1*, *ACTB*, *WWTR1*, and *CLTC*, have
 57 been frequently identified in PHE, suggesting that activation of *FOSB* plays an
 58 important role in the pathogenesis.^{2, 4-11}

59 In terms of PHE treatment, the multifocal nature and high risk of relapse limit the
 60 efficacy of surgical excision.^{3, 12} Conventional chemotherapy has been administered in
 61 some cases, with variable efficacy. However, intolerable toxic effects have been
 62 noted.¹² In contrast, several targeted drugs, such as sirolimus, everolimus, and
 63 telatinib, have shown efficacy with fewer side effects than chemotherapy.¹²⁻¹⁶

64 Unfortunately, the low incidence of PHE does not allow investigators to perform

65 systematic clinical trials to evaluate the efficacy of a specific treatment, and long-term
66 follow-up data for a specific treatment are limited. Therefore, no systemic therapies
67 have been officially approved for the management of PHE. Thus, in depth knowledge
68 regarding the pathogenesis and treatment and follow-up data for each case of PHE are
69 valuable for understanding and managing this rare disease.

70 Here, we report the diagnosis, genetic examination, treatment, and follow-up of a
71 patient with PHE and discuss the correlation of genetic aberrations with signaling
72 pathway dysregulation and therapeutic effectiveness.

73 **Materials and methods**

74 **Histological and immunohistochemical analysis**

75 Resected tumor samples were fixed in 10% buffered formalin and embedded in
76 paraffin to prepare 4-mm sections. For histological analysis, the sections were stained
77 with hematoxylin and eosin. Immunohistochemical analysis of cytokeratin AE1/AE3,
78 CD31, ERG, INI-1, BCL-2, Ki67, CD34, D2-40, S-100, SMA, and STAT6 was
79 performed using the BenchMark XT automated slide preparation system (Roche).
80 Immunohistochemical analysis of FOSB, pAKT, and mTOR was performed as
81 follows. The paraffin was removed from the sectioned tumor samples with xylene,
82 and the sections were rehydrated in a graded series of ethanol, followed by
83 heat-induced epitope retrieval in a pressure cooker and blocking of endogenous
84 peroxidase activity. Next, the sections were incubated with primary antibodies
85 overnight at 4 °C and then with a rabbit secondary antibody and finally reacted with 3,
86 3'-diaminobenzidine. The samples were counterstained with hematoxylin. Finally, the

87 slides were analyzed using an E200MV microscope (Nikon).

88 **Detection of the *SERPINE1-FOSB* fusion gene**

89 RNA was extracted from a freshly resected tumor sample using the RNeasy Mini Kit

90 (Qiagen) and reverse transcribed using the RevertAid First Strand cDNA Synthesis

91 Kit (Thermo Fisher) according to the manufacturer's protocol. The cDNA was

92 amplified in a 20-μL reaction containing 1× FastPfu Buffer (TransGen Biotech,

93 Beijing, China), 1 U of TransStart® FastPfu DNA polymerase (TransGen Biotech),

94 2.5 mmol/L Mg²⁺, 0.2 mmol/L of each deoxy-nucleoside triphosphate, and 0.2 μmol/L

95 each forward and reverse primer. Based on a previous study,⁴ an upstream primer

96 targeting *SERPINE1* (5'-AGAGCGCTGTCAAGAAGACC-3') and a downstream

97 primer targeting *FOSB* (5'-GTTCCCGGCATGTCGTAG-3') were used. PCR was

98 performed on a ProFlex PCR System (Thermo Fisher) under the following cycling

99 conditions: 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, 58 °C for 30 s,

100 and 72 °C for 3 min, with a final extension for 5 min at 72 °C.

101 The PCR products were examined by electrophoresis on a 1% agarose gel containing

102 1× GoldView™ Dye (SBSGENE, Shanghai, China) for staining. The recovered PCR

103 products were examined using commercial Sanger sequencing (Sangon, Shanghai,

104 China).

105 **Ethics statement**

106 Signed informed consent was obtained from the patient for the use of his materials

107 and publication of de-identified clinical data, including photographs. The study was

108 approved by the Research Ethics Committee of Zhongshan Hospital Xiamen

109 University.

110 **Results**

111 **Clinical diagnosis of the case**

112 A 61-year-old man with no specific history of disease presented with rapidly
113 progressive multifocal nodules and localized pain in the left hip and left iliac region
114 for more than 2 months. The skin lesions were discontinuous, raised, or subcutaneous,
115 0.3–1.5 cm in diameter, with a smooth surface (Fig. 1A). The physical examination
116 was otherwise normal, and superficial lymph nodes were not enlarged. Magnetic
117 resonance imaging (MRI) revealed multiple lesions in the ilium (Fig. 1C), gluteus
118 medius, gluteus minimus, and subcutaneous region of the left hip (Fig. 1H).

119 As shown in Fig. 2, histopathological examination of a biopsy sample from the left
120 hip revealed that the nodule was in the dermis and subcutaneous tissue and was
121 composed of loose fascicles of spindled and epithelioid cells, with abundant
122 eosinophilic cytoplasm and vesicular nuclei with distinct nucleoli. Neutrophil
123 infiltration and intravascular tumor thrombus were also observed.

124 Immunohistochemical analysis revealed that the biopsy was positive for cytokeratin
125 AE1/AE3, CD31, ERG, INI-1, BCL-2, and Ki67 (15%) (Fig. 3) and negative for
126 CD34, D2-40, S100, SMA, and STAT6 (data not shown).

127 Based on the clinical presentation, histomorphology, and expression of keratins and
128 endothelial markers in the immunohistochemical analysis, the nodules were diagnosed
129 as PHE.

130 **The tumor was responsive to sirolimus**

131 After diagnosis, the patient underwent surgical resection to remove the palpable
 132 nodules and was administered two cycles of anlotinib hydrochloride (10 mg/d) every
 133 2 weeks. During the first cycle of chemotherapy, fresh nodules developed, and pain
 134 remained. During the second cycle of chemotherapy, no evidence of tumor
 135 development was observed, and the pain was alleviated. However, no evident
 136 improvement was noted on follow-up MRI, and chemotherapy was halted due to
 137 drug-induced nosebleeds and periungual sclerosis (Fig. 1D and I). The patient was
 138 then treated with radiotherapy 5 days a week (Monday–Friday) via 6 MV linear
 139 accelerator at a dose of 5000 cGy. Unfortunately, no improvement was noted after 5
 140 weeks of radiotherapy (Fig. 1E and J).

141 Eventually, targeted therapy with sirolimus, an orally available mTOR inhibitor, was
 142 started (1 mg/day), and oxycodone (50 mg bid) was administered for pain control. At
 143 2-month follow-up, no fresh nodules had developed, and the pain was alleviated (Fig.
 144 1B). Moreover, no drug-induced toxic effects were noted on physical examination.

145 Therefore, the dose of sirolimus was increased to 2 mg/day. Follow-up MRI was
 146 performed at 5 and 20 months after sirolimus treatment, which showed general
 147 improvement of the disease (Fig. 1F, G, K and L), and the largest lesion in the left
 148 gluteus medius had progressively shrunk. Since the pain was alleviated, a reduced
 149 dose of oxycodone (10 mg each night) was sufficient for pain control. Based on the
 150 clinical improvement and lack of side effects, sirolimus therapy and follow-up are
 151 ongoing.

152 **The *SERPINE1-FOSB* fusion gene activates *FOSB* and the EGFR-pAKT-mTOR**

153 **pathway**

154 Since the nodules were diagnosed as PHE and they were responsive to sirolimus, we
 155 investigated the possible pathogenetic and therapeutic mechanisms. We first examined
 156 the nodules for the presence of a *SERPINE1-FOSB* fusion gene. As shown in Fig. 4A,
 157 when the tumor DNA sample was amplified with primers designed for the presence of
 158 a *SERPINE1-FOSB* fusion gene, an amplicon was generated. Sanger sequencing of
 159 the amplicon revealed a novel *SERPINE1-FOSB* fusion gene, in which the
 160 breakpoints of both genes were in non-coding exon 1. Moreover, a 52-bp fragment
 161 from intron 1 of *SERPINE1* was inserted at the fusion junction (Fig. 4B). Since the
 162 coding region of *FOSB* was unaffected, the fusion gene could generate an intact
 163 FOSB protein. Next, we examined the expression levels of FOSB, pAKT, and mTOR
 164 in the tumor. As shown in Fig. 3, strong expression was noted for these targets,
 165 suggesting that the fusion gene activates FOSB and the PI3K-AKT-mTOR signaling
 166 pathway.

167 **Discussion**

168 Genetic aberrations play important roles in the occurrence, development, and
 169 treatment of various human tumors. In PHE, chromosomal rearrangements leading to
 170 the fusion of *FOSB* (the whole or partial coding region) with other genes, such as
 171 *SERPINE1*, *ACTB*, *WWTR1*, and *CLTC*, have been identified.^{2, 4-11} The common
 172 feature of these cases is that the fusion introduces a strong promoter, resulting in
 173 deregulated overexpression of intact, partial, or chimeric FOSB. However, the
 174 pathogenic mechanism by which these genetic aberrations lead to PHE, which is of

value for the development of effective targeted treatments, remains largely unknown.

Recently, van IJendoorn et al.¹⁶ demonstrated that a *SERPINE1-FOSB* fusion-derived chimeric protein could function as an active transcription factor that was not only capable of regulating its own transcription but also upregulating the expression of PDGFRA and FLT1. In their case, the tumors were responsive to telatinib, a drug currently in clinical trials, that can block self-regulation of the fusion gene by specifically affecting PDGFRA, FLT1, and FLT4 signaling and downregulating SERPINE1. However, the presence of different genetic aberrations, such as fusions with different genes or different domains, could alter the pathogenesis as well as the treatment. Therefore, further investigation is required to demonstrate whether this drug is effective in cases with other genetic aberrations such as *SERPINE1-FOSB* fusions with intact or truncated *FOSB* or *ACTB-FOSB* fusions.

Similar to a previous case,¹⁵ in our case, mTOR was overexpressed as was pAKT (Fig. 3), suggesting activation of the PI3K-AKT-mTOR signaling pathway, one of the most frequently dysregulated pathways in human cancers that regulates important cellular functions, including metabolism, motility, growth, and proliferation.¹⁷ Dysregulation of this pathway supports the survival, expansion, and dissemination of cancer cells.¹⁷

Genetic alterations in specific oncogenes or tumor suppressor genes, such as *PIK3CA*, *PIK3RI*, *AKT*, *MTOR*, *TSC1*, and *TSC2*, can aberrantly activate this pathway via diverse mechanisms. However, these genetic alterations also provide opportunities for therapeutic targeting of this pathway and potential predictive biomarkers of therapeutic effectiveness.¹⁷ Currently, several distinct classes of drugs targeting the

197 PI3K-AKT-mTOR signaling pathway, including PI3K and AKT inhibitors, as well as
 198 mTOR inhibitors, have been developed and have shown clinical potential.¹⁸ mTOR
 199 inhibitors, such as sirolimus and everolimus, have been effectively used for targeted
 200 therapy of PHE.¹²⁻¹⁵ However, the genetic information for mTOR inhibitor-responsive
 201 cases is limited. In some cases, the genetic information was missing,¹⁴ while in others,
 202 although a translocation or gene fusion was reported, the translational products of the
 203 fusion gene were not described.^{13, 15} Most recently, Bridge et al.⁷ reported a
 204 *CLTC-FOSB* fusion gene resulting in a chimeric protein in a case of PHE, and the
 205 tumor was responsive to sirolimus therapy. In our study, we clearly correlated the
 206 effectiveness of sirolimus therapy with a PHE-related genetic aberration, that is, a
 207 *SERPINE1-FOSB* fusion resulting in overexpression of intact FOSB. This genetic
 208 aberration could be a predictive biomarker for effective sirolimus therapy. FOSB
 209 belongs to the Fos family, which is associated with various cellular functions,
 210 including proliferation, differentiation, and transformation, and it is frequently
 211 involved in the pathogenesis of vascular tumors.¹⁹⁻²² However, the causal relationship
 212 between FOSB overexpression and activation of the PI3K-AKT-mTOR signaling
 213 pathway remains unclear. Nonetheless, the use of combinations of inhibitors targeting
 214 the PI3K-AKT-mTOR signaling pathway in PHE cases is worthwhile, since this
 215 strategy has been shown to be more effective than monotherapy, owing to the
 216 inhibition of compensatory mechanisms involved in intrinsic and adaptive
 217 resistance.^{23, 24} Future investigations of the interconnecting cascades between the
 218 PHE-related genetic aberrations and this signaling pathway will advance our

219 knowledge of PHE and aid in the development of personalized agents with greater
220 target specificity and optimal therapeutic index.

221 In conclusion, we report a novel *SERPINE1-FOSB* fusion gene in a patient with PHE
222 that was responsive to sirolimus therapy. The fusion gene resulted in overexpression
223 of intact FOSB, pAKT, and mTOR, suggesting activation of the PI3K-AKT-mTOR
224 signaling pathway. Our study correlated the dysregulation of a specific signaling
225 pathway and the effectiveness of a specific targeted therapy to a specific genetic
226 aberration, which could promote the investigation of therapeutic targets and provide a
227 predictive biomarker of therapeutic effectiveness for PHE with this type of genetic
228 aberration.

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303

304 **Figure 1. Skin lesions and magnetic resonance imaging of the case.** (A) Multiple
 305 nodules were present at diagnosis. (B) Remission of the lesions was evident after
 306 sirolimus therapy for 2 months. Lesions with high signal intensity in the ilium at
 307 diagnosis (C), after chemotherapy (D), after radiotherapy (E), and after sirolimus
 308 therapy for 5 (F) and 20 months (G), respectively. Lesions with high signal intensity
 309 in the gluteus medius, gluteus minimus, and subcutaneous region of the left hip at
 310 diagnosis (H), after chemotherapy (I), after radiotherapy (J), and after sirolimus
 311 therapy for 5 (K) and 20 months (L), respectively. The size of the largest lesion was
 312 indicated.

313

314

315 **Figure 2. Histopathological examination of a biopsy sample from the left hip.**

316

317 **Figure 3. Positive immunohistochemical staining of a biopsy sample from the left**

318 **hip (400×).**

319

320 **Figure 4. Identification of a *SERPINE1-FOSB* fusion gene.** (A) Amplification of
321 the fusion gene with a specific primer pair. (B) Sanger sequencing of the amplicon
322 showing the structure of the fusion gene. NTC: no template control.

100 ×

200 ×

400 ×

AE1/AE3

CD31

ERG

INI-1

BCL-2

Ki67

FOSB

pAKT

mTOR

A

Sample NTC Ladder bp

B

SERPINE1 exon 1

(-157, NG_013213.1)

SERPINE1 intron 1

(1010-1061, NG_013213.1)

FOSB exon 1

(5406 - , NG_029675.1)