Human Pathology: Case Reports 14 (2018) 25–32

Contents lists available at ScienceDirect

Human Pathology: Case Reports

journal homepage: www.elsevier.com/locate/ehpc

Case Report

Two cases of *WWTR1-CAMTA-1* fusion-positive epithelioid hemangioendotheliomas with extremely different outcomes

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ARTICLE INFO

Keywords:
Epithelioid hemangioendothelioma
WWTR1-CAMTA1 fusion
CAMTA1 immunohistochemistry
Ossification

ABSTRACT

We report two cases of *WWTR1-CAMTA1* fusion-positive epithelioid hemangioendotheliomas (EHEs) with extremely different clinical behaviors. The first case was a 52-year-old Japanese woman with a complaint of an enlarging right axillary mass. Surgical specimen showed that the epithelioid tumors within the vein had the pathological feature of a cord-like pattern with myxoid or hyaline matrix and the intracytoplasmic lumen with erythrocytes and the immunophenotype of endothelial cells. The final pathological diagnosis was classic EHE. An RT-PCR analysis showed positivity of *WWTR1-CAMTA1* fusion. The patient has now been free of disease for 7 years after surgery. The second case was a 70-year-old Japanese male with a complaint of severe pain of his left thigh. The radiographic findings showed ectopic calcification near the left femur. Surgical specimen showed clusters of malignant epithelioid cells with necrosis and obvious mitoses in soft tissue. They were also shown to be of endothelial origin by immunohistochemistry (IHC). The pathological diagnosis was malignant EHE or epithelioid angiosarcoma with ectopic ossification. An RT-PCR analysis showed the tumor had *WWTR1-CAMTA1* fusion. Thus, the final pathological diagnosis was malignant EHE. Unfortunately, he died 7 months after surgery due to pleural dissemination. Although fusion gene is thought to define one spectrum of specific tumors, our two cases of EHEs with *WWTR1-CAMTA1* fusion showed extremely different outcomes, one is rather benign and the other fatal. Retrospective study showed that both tumors were positive for nuclear CAMTA1 by IHC. Fusion-genes define a unique spectrum of tumors, but even in the tumors within the same spectrum defined by a specific fusion-gene, there are heterogeneities of malignant potentials. Therefore, factors other than fusion-genes may contribute to prognoses.

1. Introduction

Epithelioid hemangioendotheliomas (EHEs) are rare vascular tumors composed of neoplastic endothelial cells with features of chain or cord-like structure or intracytoplasmic lumen in a myxohyaline matrix [1, 2]. EHE was first described by Weiss and Enzinger [3]. They referred to EHE as a vascular tumor often mistaken for a carcinoma. EHEs are thought to be of intermediate malignancy. But Deyrup et al. clinicopathologically divided EHEs into two groups, classic (low-grade) ones and malignant (high-grade) ones [4]. Tumors with > 3 mitotic figures per 50 high power fields (HPFs) or size > 3 cm are thought to be high risk and 5-year-survival of these high-risk patients is 59%, while that of low-risk patents is 100% [4]. So, the clinicopathological differential diagnoses of EHEs include both epithelioid hemangiomas (EHs) at the low-grade end of the spectrum and epithelioid angiosarcomas (EASs) at the high-grade end of the spectrum [5]. However, Deyrup's report was before the discovery of fusion-genes associated with EHEs [4]. WW domain-containing transcription regulator 1 (*WWTR1*)- calmodulin-binding transcription activator 1 (*CAMTA1*) fusion encoded by t(1; 3) (p36; q25) translocation is present in > 90% of EHEs [6, 7]. Furthermore, yes-associated protein 1 (*YAP1*)-transcription factor E3 (*TFE3*) is present in < 10% of EHEs [8]. EHEs with *YAP1-TFE3* fusion were shown to have focally vasoformative features, which is somewhat different from those with *WWTR1-CAMTA1* fusion [8]. A *YAP1-TFE3* unique variant fusion-positive EHE case with solid growth pattern without vasoformation has also been reported [9].

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https://doi.org/10.1016/j.ehpc.2018.07.003
Received 23 April 2018; Received in revised form 8 July 2018; Accepted 11 July 2018

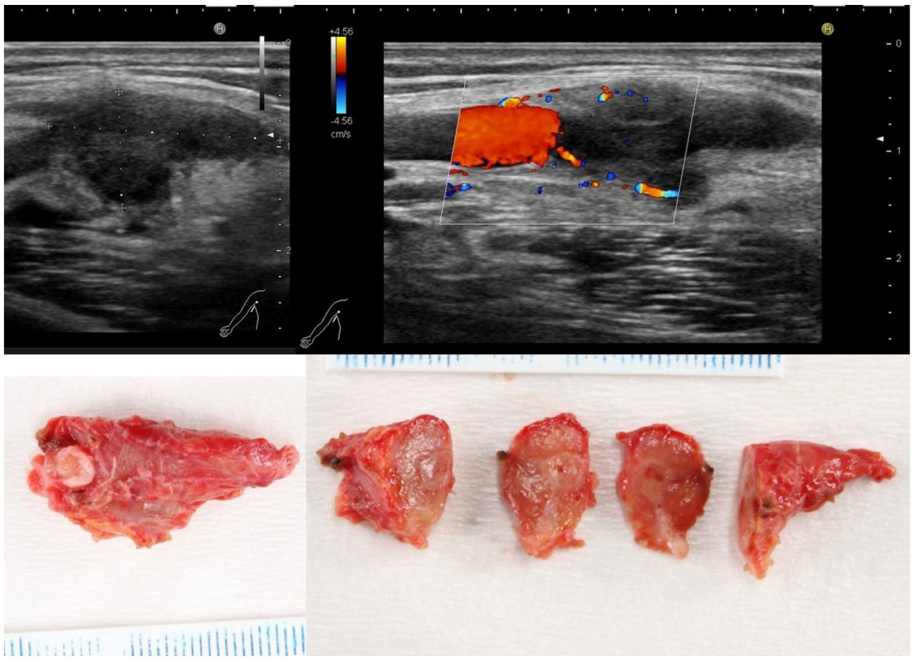

Fig. 1. Ultrasonographic and macroscopic findings of case 1. Ultrasonographic findings are shown in fig. A & B, without and with color Doppler images. The tumor almost obliterates the vessel lumen. Surgical specimen also shows the same findings (Fig. 1C & D). Fig.1C shows the enbloc surgical specimen, while Fig. 1D shows the cut surface of the surgical specimen. The tumor also invades outside the right axillary vein.

Since the discovery of fusion-genes of EHE, EHE has become a spectrum defined by specific fusion-genes. We report two cases of epithelioid endothelial tumors harboring *WWTR1-CAMTA1* fusion. They showed extremely different outcomes, although they were thought to be included in the same spectrum of tumors.

2. Case report

2.1. Case 1

A 52-year-old woman with a complaint of an enlarging right axillary mass visited the Department of Plastic Surgery, Takarazuka City Hospital. The ultrasonographic evaluation before surgery revealed the mass in the right axillary vein. She underwent surgical removal of the mass with intraoperative pathological diagnosis using frozen section. Epithelioid cells were proliferating in and around the vein. Intraoperative diagnosis was not confirmed, suggestive of metastatic carcinoma. For further evaluation, IHCs and genetic testing were performed using surgical materials.

2.2. Case 2

A 70-year-old man with severe pain of the left thigh for 8 months was referred to the Department of Orthopaedic Surgery, Hyogo College of Medicine. The patient showed the radiographic finding of ectopic calcification near the left femur. In the frozen section, malignant epithelioid cells were seen in soft tissue, suggestive of metastatic carcinoma. The biopsy also showed clusters of malignant epithelioid cells with necrosis, and numerous mitoses were proliferating and invading the soft tissue. The slightly vasoformative nature of the tumor cells was suggestive of endothelial origin. Just before the surgical operation, suspected lymph node metastases were revealed by 18-fluorodeoxyglucose-positron emission tomography (18-FDG-PET). Under the pathological diagnosis of malignant endothelial tumor, the amputation of the left lower limb was performed. For further evaluation, IHCs and genetic testing were performed using surgical specimen.

3. Materials and methods

3.1. Ultrasonographic evaluation of case 1

Ultrasonographic evaluation was performed by HI VISION Avius (Hitachi Aloka Medical, ltd, Tokyo, Japan) using a 13 MHz probe with color Doppler.

3.2. Immunohistochemistry (IHC)

Resected tissues were fixed in 10% buffered formalin and embedded in paraffin. Three-micrometer-thick sections were cut and stained with hematoxylin and eosin (H&E). IHC for CD31 (clone JC70A, DAKO, Glostrup, Denmark), Factor VIII-related antigen (polyclonal, DAKO), Ki-67 (MIB-1, DAKO), vimentin (V9, Leica, Wetzlar, Germany), pankeratin (AE1&AE3, Leica), epithelial membrane antigen (EMA) (GP1.4, Leica), CD34 (QBEnd/10, Leica), Fli1 (G146-222, Becton Dickinson, Franklin Lakes, NJ), ERG (EPR3864, Abcam, Cambridge, MA), CAMTA1 (NBP1–93620, Novus Biologicals, Littleton, CO) and TFE3 (P16, sc-5958, Santa Cruz Biotechnology, Santa Cruz, CA) was performed using Bond Polymer Refine Detection (Leica). For ERG and CAMTA1 IHC, pretreatment was performed in Bond Epitope Retrieval Solution 1 (Leica, pH 6.0) for 20 min. For TFE3 IHC, pretreatment was performed in Bond Epitope Retrieval Solution 2 (Leica, pH 9.0) for 20 min. IHC without primary antibodies was performed as negative controls.

3.3. RT-PCR and Sequencing

RT-PCR was performed as described previously [10]. Briefly, total RNA was extracted from formalin fixed, paraffin embedded (FFPE) materials using RNeasy FFPE Kit (Qiagen, Hilden, Germany), and cDNA was synthesized using SuperScript III reverse transcriptase (Thermo Fisher Scientific, Waltham, MA). Then, polymerase chain reaction (PCR) was performed by using MightyAmp DNA polymerase (Takara Bio, Otsu, Japan). PCR condition consisted of one cycle of 98 °C for 2 min, and 30 cycles of 98 °C for 10 s and 62 °C for 1 min. WWTR1-Fex3 (5′- GCTGGGAGATGACCTTCACGGC -3′) and WWTR1-Fex4 (5′- CCGT CAGTTCCACACCAGTGCCTC -3′) were designed as forward primers for WWTR1 exon 3 & 4. CAMTA1-Rex8 (5′- GGCTGGGGCTTGGTCTGGTG -3′) and CAMTA1-Rex9 (5′-GCGAGATGATGCGGTGTTTGGC-3′) were