A Novel Specific Genetic Translocation In Epithelioid Hemangioendothelioma, Showing A Fusion Of The Wwtr1 And Camta1 Genes, Supports The Monoclonoality Of Multifocal Epithelioid Hemangioendothelioma.

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Disclosures:

Introduction: We undertook a systematic molecular analysis of a large spectrum of Epithelioid Hemangiendothelioma (EHE), including lesions from various anatomic locations and lesions with different biological potentials. We hypothesized that a better understanding of the molecular signature of vascular tumors may help to refine the present classification system based on immunophenotype alone.

Methods: We retrieved 23 cases of EHE with tissue samples available for molecular analysis from the surgical pathology and consultation files of our institution. In each case, we confirmed the pathologic diagnosis and the histologic grade by reviewing the pathology slides and by immunostaining them for the following endothelial cell markers: CD31, CD34, FLI1, and von Willebrand factor.

The tumors were assessed morphologically for growth pattern, vasoformative nature, epithelioid versus spindle cell composition, cellular pleomorphism, mitotic activity, and necrosis.

For each case, the location of the tumor was recorded, along with the anatomic structures involved. Based on their location, the lesions were classified into 4 groups: bone, soft tissue, intrathorax, and liver.

Because EHE, a low-grade tumor with metastatic potential, is intermediate between epithelioid hemangioma, a benign tumor, and epithelioid angiosarcoma, a high-grade malignant tumor, we included 15 cases of epithelioid hemangioma and 5 cases of epithelioid angiosarcoma to determine if there was any relationship between them. In addition, we included 3 cases of epithelioid sarcoma because this tumor has the same morphologic and immunophenotypic features as EHE.

FISH was performed on paraffin-embedded thick tissue sections using custom-labeled FISH probes, as previously described. Each case was analyzed with 3 probes covering and flanking chromosomes 1p36.3 and 3q25. The rearranged regions of each chromosome were then evaluated using 3 new probes. This process was repeated as much as possible to zoom into the rearranged chromosomal regions.

FISH enabled us to focus on the 200-kb region in which the CAMTA1 and WWTR1 genes are located in chromosomes 1 and 3, respectively. Therefore, we performed reverse transcriptase-polymerase chain reaction (RT-PCR) on the 3 cases of EHE with frozen tissue available using housekeeping primers, as previously described. The RT-PCR products were analyzed by electrophoresis, and the RT-PCR-amplified products were sequenced using the Sanger method.

Finally, we undertook a molecular analysis of 2 multicentric EHEs of the liver, including separate tumor samples from each patient. Our hypothesis is that the identification of an identical WWTR1-CAMTA1 rearrangement in different lesions from each patient could explain the monoclonal origin of EHE.

Results: In this study, we included a total of 17 cases of immunohistochemically confirmed EHE with tissue available for molecular analysis.

Six cases were excluded because of unsuccessful fluorescence in situ hybridization (FISH): 4 cases because of low cellularity and 2 cases because of decalcification. There were 8 women and 9 men, with a median age of 48 years (range, 25 to 68 years). The anatomic distribution of EHE was as follows: 7 cases in soft tissue, 7 in the intrathorax, 2 in the liver, and 1 in bone.

All cases had an identical chromosomal translocation involving chromosomes 1 and 3 [t(1;3)(p36.23;q25.1)].

Immunohistochemically, all tumors were positive for CD31, showing typically strong and diffuse staining, as well as for CD34 and/or Factor VIII or FLI1.

The RT-PCR applied in the 3 tumors with available frozen tissue showed 3 different rearrangements: fragments of exons 8 and 9 of CAMTA1 were fused in-frame to a fragment of exon 2 of WWTR1.

In terms of survival outcome, at follow-up, 9 patients were alive with no evidence of disease, 2 were alive with disease, 4 had died of disease, and 2 were lost to follow-up.

None of the other vascular tumors (13 cases of epithelioid hemangioma, 5 of epithelioid angiosarcoma, and 3 of epithelioid sarcoma) had a WWTR1-CAMTA1 fusion. Two epithelioid hemangiomas of bone were excluded because of unsuccessful FISH
due to decalcification.

In the 2 multicentric EHEs of the liver FISH analysis for the presence of a WWTR1 and CAMTA1 gene rearrangements showed signal abnormalities in both WWTR1 and CAMTA1. Combined results confirmed the translocation t(1;3)(1p36.23;3q25.1) in both EHE cases.

The RT-PCR applied in both cases identified an amplified product in each case, but of two different sizes. However, the size of the rearranged bands from multifocal tumors in each individual patient was identical. RT-PCR amplified two 5′WWTR1-CAMTA13′ variant transcripts from both EHE cases. The 5′WWTR1 showed a consistent breakpoint within intron 3 and intron 4 respectively, while another 2 different breakpoints were seen in exon 9 by 3′CAMTA1. Exon 3 (variant 1) and exon 4 (variant 2) of WWTR1 were fused to CAMTA1 exon 9.

The sequence of the fusion gene confirmed a different WWTR1-CAMTA1 rearrangement in each patient, but an identical WWTR1-CAMTA1 rearrangement from different lesions in each individual patient.

**Discussion:** The classification of epithelioid vascular tumors remains challenging with considerable morphologic overlap spanning across benign to malignant categories. A prior t(1;3)(p36.3;q25) was identified in 2 cases of EHE, however no follow-up studies have been performed to identify the gene fusion or to assess its prevalence in a larger cohort of patients. We undertook a systematic molecular analysis of 17 EHE, characterized by classic morphologic and immunophenotypic features, from various anatomic locations and with different malignant potential. Also included for comparison was a group of epithelioid hemangioma and epithelioid angiosarcoma. FISH positional cloning strategy, spanning the cytogenetically defined regions on chromosomes 1p36.3 and 3q25, confirmed rearrangements in two candidate genes from these loci in all EHE cases tested. Subsequent RT-PCR confirmed the CAMTA1-WWTR1 fusion product in 3 cases. None of the other benign or malignant epithelioid vascular tumors examined showed these abnormalities. CAMTA1 and WWTR1 genes have been previously shown to play important roles in oncogenesis. Our results demonstrate the presence of CAMTA1-WWTR1 fusion in all EHE tested from bone, soft tissue and visceral location (liver, lung) in keeping with a single tumor entity. Thus FISH or RT-PCR analysis for this fusion can serve as a useful molecular diagnostic tool in challenging diagnoses. Like other vascular tumors, EHE can have multifocal presentation in up to 50% of cases. However, whether multifocal EHE represents an unusual pattern of metastasis or multiple separate primary tumors remains to be elucidated. Our identification of WWTR1-CAMTA1 fusion as the genetic hallmark of EHE irrespective of anatomic location was used to clarify this question by comparing the similarity of translocation breakpoints. In fact, we found variability of the fusion transcripts of the t(1;3)(p36;q25) translocation among different patients with EHE. Thus, we undertook a molecular analysis of six samples from two patients with multicentric hepatic EHE to test our hypothesis that the presence of identical breakpoints in WWTR1 and CAMTA1 support the monoclonal nature of multifocal EHE. Using FISH, RT-PCR and subsequent sequencing we confirmed an identical WWTR1-CAMTA1 fusion transcript product from different nodules in each patient. Our results confirm that multifocal EHE are monoclonal and thus representing metastatic implants of the same neoplastic clone rather than a ‘field-effect’ or synchronous occurrence of multiple neoplastic clones.

**Significance:** Level IV

**Acknowledgments:**

**References:**

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