Original Article doi: 10.5146/tjpath.2014.01272

CAMTA1 Immunostaining is not Useful in Differentiating Epithelioid Hemangioendothelioma from its Potential Mimickers

CAMTA1 İmmünekspresyonu Epitelioid Hemangioendotelyomun Taklitlerinden Ayrımında Kullanışlı Değildir

Zarifa YUSİFLİ, Kemal KÖSEMEHMETOĞLU

Department of Pathology, Hacettepe University, Faculty of Medicine, ANKARA, TURKEY

ABSTRACT

Objective: Epithelioid hemangioendothelioma is a rare member of vascular tumors of intermediate malignancy. Recently, presence of t(1;3) translocation and WWTR1/CAMTA1 gene fusion, which enhances CAMTA1 expression, are found to be specific to this tumor. We investigated the CAMTA1 immune expression profile of epithelioid hemangioendothelioma and its potential mimickers using a commercially available CAMTA1 antibody.

Material and Method: Standard whole sections from the formalin fixed, paraffin embedded blocks of 12 epithelioid hemangioendotheliomas, 10 angiosarcomas, 9 epithelioid sarcomas, 8 malignant melanomas, 8 signet ring carcinomas, 7 lobular carcinomas of breast, 2 epithelioid mesotheliomas, 2 rhabdoid tumors and 12 miscellaneous hemangiomas were immunostained for anti-CAMTA1 (ab64119, 1:200; Abcam) after pretreatment with citrate pH 6.0 for 20 minutes using Leica Bond detection kit with DAB chromogen. Strong nuclear CAMTA1 expression was scored for its extent as 'negative' (<5% positive), '+1' (5-25% positive), '2+' (25-50% positive) and '3+' (>50% positive).

Results: In 60 out of 70 cases (86%) either 2+ or 3+ strong nuclear staining was seen. Eighty-three % of epithelioid hemangioendotheliomas, 100% of angiosarcomas, 89% of epithelioid sarcomas, 89% of malignant melanomas, 63% of signet ring carcinomas, 71% of lobular carcinomas of breast, 100% of epithelioid mesotheliomas, 50% of rhabdoid tumors and 100% of hemangiomas were stained. Besides neurons, CAMTA1 expression was also observed in squamous epithelium, skin adnexa, breast lobules, prostate glands, bile ducts, colonic mucosa and gastric pits.

Conclusion: Epithelioid hemangioendothelioma, its potential morphological mimickers and other benign or malignant vascular tumors showed strong and diffuse CAMTA1 expression, nullifying the potential use of CAMTA1 immunohistochemistry as an adjunct in the differential diagnosis.

Key Words: CAMTA1 protein, Soft tissue neoplasms, Vascular neoplasms, Immunohistochemistry

ÖZ

Amaç: Epitelioid hemangioendotelyom intermediyer malignite gösteren vasküler tümörlerin ender görülen üyesidir. Yakın zamanda, CAMTA1 ekspresyonunu arttıran t(1;3) translokasyonu ve WWTR1/CAMTA1 gen füzyonu varlığının bu tümör için özgül olduğu bulunmuştur. Piyasada bulunan CAMTA1 antikorunu kullanarak epitelioid hemangioendotelyom ve potansiyel taklitçilerinde CAMTA1 immünekspresyon profilini inceledik.

Gereç ve Yöntem: 12 epitelioid hemangioendotelyom, 10 anjiosarkom, 9 epitelioid sarkom, 8 malign melanom, 8 taşlı yüzük hücreli karsinom, 7 memenin lobüler karsinomu, 2 epitelioid mezotelyoma, 2 rabdoid tümör ve 12 çeşitli hemanjiomun formalin fikse parafine gömülü bloklarından hazırlanan standart tam yüzey kesitler, sitrat pH 6.0 da 20 dakika ön işlemden geçirildikten sonra Leica Bond detection kit ve DAB kromojeni kullanılarak anti-CAMTA1 (ab64119, 1:200; Abcam) ile boyandı. Güçlü nükleer CAMTA1 ekspresyonu yaygınlığa göre negatif (<%5 pozitif), '1+' (%5-25 pozitif), '+2' (%25-50 pozitif) and '+3' (>%50 pozitif) olarak skorlandı.

Bulgular: Yetmiş olgunun 60'ında (%86) +2 veya +3 güçlü nükleer boyanma görülmüştür: epitelioid hemangioendotelyomların %83, anjiosarkomların %100, epitelioid sarkomların %89, malign melanomların %89, taşlı yüzük hücreli karsinomların %63, memenin lobüler karsinomlarının %71, epiteloid mezotelyomaların %100, rabdoid tümörlerin %50 ve hemanjiomların %100'ü boyanmıştır. Nöronların yanı sıra, skuamöz epitel, deri ekleri, meme lobülleri, prostat bezleri, safra kanalları, kolonik mukoza ve gastrik pitlerde CAMTA1 ekspresyonu gözlenmiştir.

Sonuç: Epitelioid hemangioendotelyom, potansiyel morfolojik taklitçileri ve diğer benign ve malign vasküler tümörler güçlü ve yaygın CAMTA1 ekspresyonu göstermiştir ki bu CAMTA1 immünohistokimyasının ayırıcı tanıda yardımcı olarak kullanımını hükümsüz kılmıştır.

Anahtar Sözcükler: CAMTA1 protein, Yumuşak doku neoplazileri, Vasküler neoplaziler, İmmünohistokimya

(Turk Patoloji Derg 2014, 30:159-165)

Received : 24.04.2014 **Accepted :** 03.06.2014

Correspondence: Kemal KÖSEMEHMETOĞLU
Hacettepe Üniversitesi, Tıp Fakültesi, Patoloji Anabilim Dalı,
06100, Sıhhiye, Ankara, TURKEY
E-mail: dokemal@hotmail.com Phone: +90 544 214 70 73

INTRODUCTION

Epithelioid hemangioendothelioma (EHE) is a rare vascular neoplasm described by Weiss and Enzinger in 1982. It affects patients in all ages with slight predominance in females and develops as a painful mass in soft tissue and visceral organs. The tumor typically evolves from the endothelia of a vein and consists of cords of epithelioid endothelial cells with characteristic cytoplasmic vacuoles settled in myxohyaline stroma (1). It was previously regarded as low-grade or "borderline" neoplasm with the ability to metastasize and recur locally, although the occurrence of multicentric presentation and malicious course of visceral (lung or liver) involvement argue against low malignant potential. Therefore, it is reclassified within malignant vascular tumors in 2013 WHO Classification of Bone and Soft Tissue Tumors (2). EHE is still challenging to diagnose, despite the advance of immunohistochemistry, such as CD34, CD31 and Fli1. The diagnosis is usually based solely on the identification of characteristic histological features in order to differentiate it from its potential mimickers, such as epithelial tumors and soft tissue tumors showing epithelial morphology, especially at visceral locations (2).

A novel reciprocal t(1;3)(p36;q23-25) rearrangement was described as a non-random aberration in some case reports of EHE (3, 4). Consequently, Tanas et al. (5) and Errani et al. (6) separately identified the partners of this disease defining gene fusion: WWTR1 (WW domain-containing transcription regulator 1) and CAMTA1 (calmodulin-binding transcription activator 1). WWTR1 is known to interact with DNA binding transcription factors, including those of the Runx family (bone development) in mice and thyroid transcription factor 1 (development of lung) in humans, and to be overexpressed in human breast cancer and papillary thyroid carcinoma. CAMTA1 encodes a transcription factor in all multicellular organisms, conserved in Arabidopsis to humans and is known to be highly encountered in the memory related regions of the human brain. CAMTA1 is almost exclusively expressed within brain, whereas WWTR1 is strongly expressed in endothelium rich organs, such as kidney, lung, liver, and heart (5, 7, 8). WWTR1-CAMTA1 fusion gene product is believed to increase the expression of CAMTA1 in EHE (5). Presence of either WWTR1 or CAMTA1 gene rearrangements is found to be highly sensitive and specific for the diagnosis of EHE (5, 6).

As molecular diagnostic methods, such as reverse transcriptase polymerase chain reaction and fluorescent in situ hybridization (FISH) to detect the EHE-specific WWTR1-CAMTA1 translocation are not yet available in all laboratories, we investigated the CAMTA1 immune expression profile of EHE and its morphological mimickers, using a commercially available CAMTA1 antibody immunohistochemically and its potential diagnostic use in routine pathology.

MATERIAL and METHODS

We collected 12 EHEs and 58 other types of tumors resembling EHE (10 angiosarcomas, 9 epithelioid sarcomas, 8 malignant melanomas, 8 signet ring carcinomas, 7 lobular carcinomas of breast, 2 epithelioid mesotheliomas, 2 rhabdoid tumors and 12 miscellaneous hemangiomas) from the archive. Hemangiomas included 4 capillary hemangiomas, 4 pyogenic granulomas, 2 spindle cell hemangiomas, hobnail hemangioma and epithelioid hemangioma one for (of) each. Three of the angiosarcomas were well differentiated with complex vascular network lined by atypical neoplastic endothelial cells, while the rest were high grade angiosarcomas with epithelioid areas.

Standard whole 4 micron thick sections of total 70 formalin-fixed, paraffin embedded tumors were immunostained for anti-CAMTA1 (ab64119, polyclonal, 1:200; Abcam) after pretreatment with citrate pH 6.0 for 20 minutes at 97 C using Leica Bond detection kit with DAB chromogen. Human hippocampus was used as positive control and only nuclear staining was regarded as positive. The cases showing moderate to strong nuclear CAMTA1 expression were scored for extent of staining as 'negative or 1+' (0-25% of cells positive), '2+' (25-50% of cells positive) and '3+' (>50% of cells positive). All cases showing weak staining were grouped as 'negative or 1+'.

RESULTS

Results are summarized in the Table I. Overall, either 2+ or 3+ strong/moderate nuclear staining were seen in 60 out of 70 cases (86%). Among vascular tumors, 10 cases (83%) of EHEs showed 2-3+ strong CAMTA1 expression (Figures 1A,B, 2A-D). However, CAMTA1 expression was also observed in all cases of angiosarcomas (Figure 3A,B) and hemangiomas, including 4 pyogenic granulomas (Figure 4A,B), 4 capillary hemangiomas, 2 spindle cell hemangiomas, 1 epithelioid hemangioma and 1 hobnail hemangioma. A considerable number of non-vascular neoplasms likely to mimic EHE also demonstrated strong and diffuse CAMTA1 expression: 100% of epithelioid mesotheliomas, 89% of epithelioid sarcomas (Figure 5A,B), 89% of malignant melanomas (Figure 6C and 6F), 71% of lobular carcinomas of breast (Figure 6A and 6D), and 63% of signet ring carcinomas (Figure 6B and 6E), and 50% of extrarenal rhabdoid tumors were CAMTA1 positive.

Table I: Immunohistochemical staining results with CAMTA1 of different tumors

	n	*Negative or 1+	*2+	*3+	Any 2-3+ (%)
Epithelioid hemangioendothelioma	12	2	2	8	83
Angiosarcoma	10	0	3	7	100
Hemangioma	12	0	2	10	100
Epithelioid sarcoma	9	1	3	5	89
Malignant melanoma	8	1	0	7	89
Signet ring carcinoma	8	3	0	5	63
Lobular carcinoma of breast	7	2	0	5	71
Epithelioid mesothelioma	2	0	0	2	100
Rhabdoid tumor	2	1	0	1	50

*CAMTA1 expression was scored for extent of staining as 'negative or 1+' (0-25% of cells positive), '2+' (25 50% of cells positive) and '3+' (>50% of cells positive).

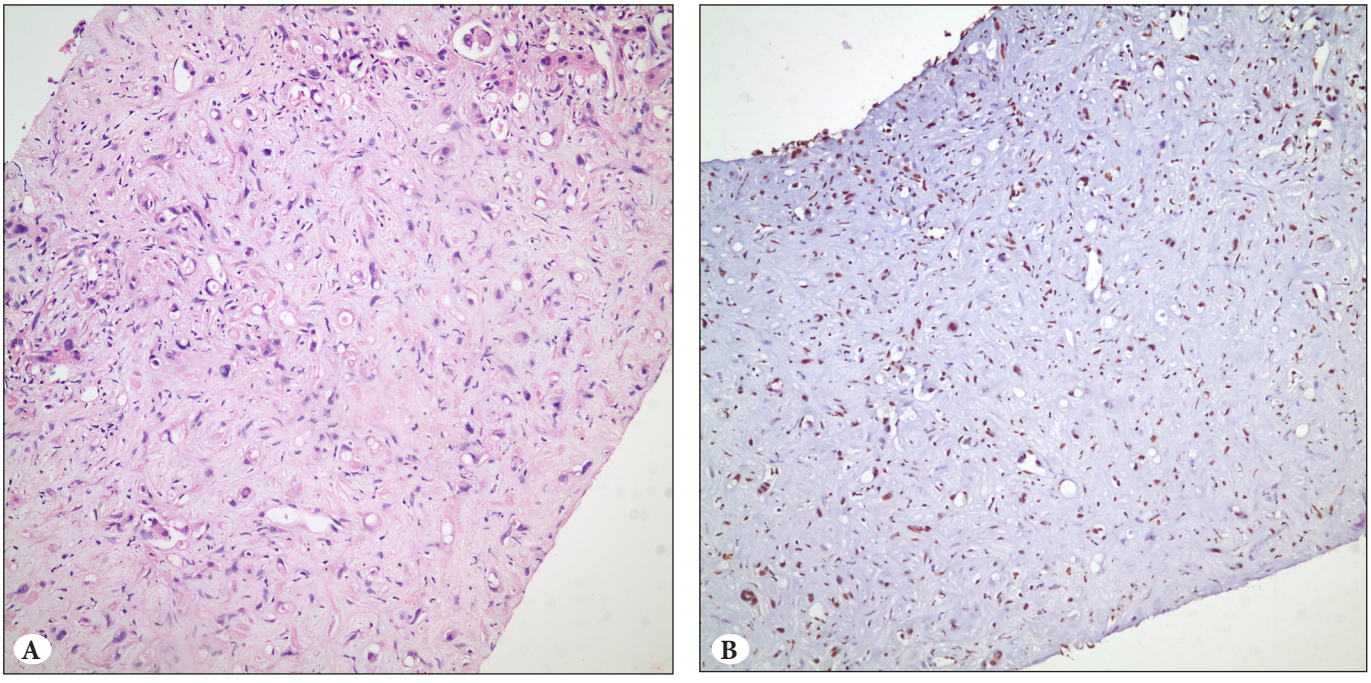

Figure 1: A) Epithelioid hemangioendothelioma of liver (H&E; x200), **B)** Strong CAMTA1 expression was present over 50% of the neoplastic cells (3+) (CAMTA1; x200).

Among non-neoplastic tissues, besides neurons, CAMTA1 expression was also observed in squamous epithelium, skin adnexa, breast lobules, prostate glands, bile ducts, colonic mucosa and gastric pits. Hepatocytes, chorionic trophoblasts, lung, thyroid, smooth and striated muscle fibers and fibroblasts were negative.

DISCUSSION

EHE was recently shown to bear a unique rearrangement involving CAMTA1 on 1p36.23 and WWTR1 on 3q25.1 and it is reported to be restricted to EHE among other vascular neoplasms (5). This fusion in EHE is considered to lead to an aberrant increase in CAMTA1 expression via through a so-called promoter-switch mechanism, driven by the WWTR1 promoter, which is strongly active in endothelial cells (5). Tanas et al. developed a break-apart FISH assay diagnositic for EHE; however, expression of CAMTA1 protein in EHE and neoplasms resembling EHE other than vascular tumors were not investigated (5). This report investigates the immunoexpression profile of CAMTA1 in EHE and its potential mimickers using a commercially available CAMTA1 antibody by routine immunohistochemistry.

CAMTA1 expression was seen in 83% of EHE, which is concordant with the results of Tanas et al. (5) Interestingly, CAMTA1 was also determined in majority of tumors within

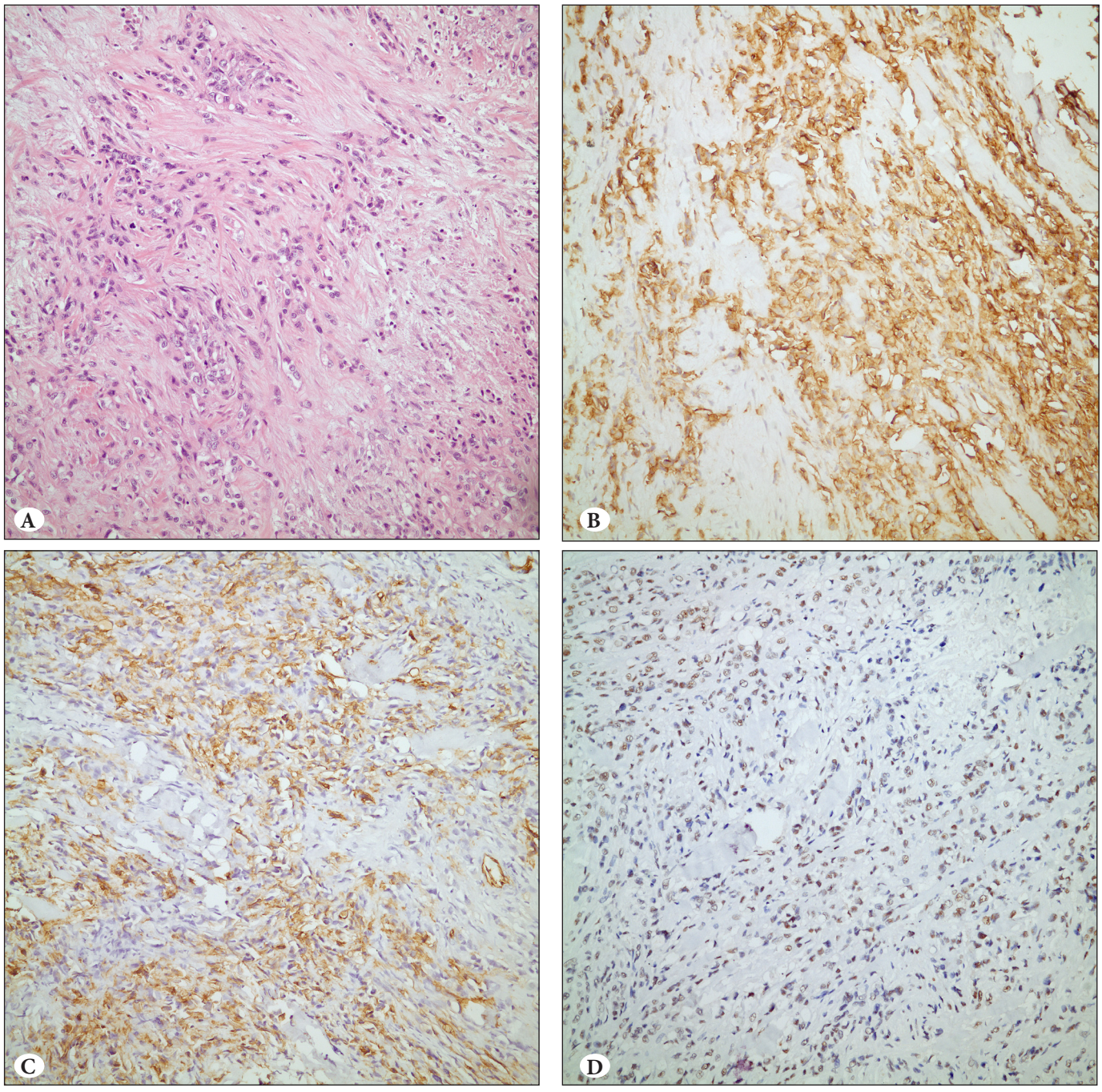

Figure 2: A) Epithelioid hemangioendothelioma arising from paraaortic area (H&E; x200). **B)** CD34 and **C)** CD31 were positive. **D)** Weak CAMTA1 staining seen in 25-50% of neoplastic cells (1+).

the differential diagnosis, such as some carcinomas (lobular carcinoma of breast and signet ring carcinomas), sarcomas with epithelioid characteristics (epithelioid sarcomas and rhabdoid tumors), mesothelioma and melanomas, some of which stained even more than EHE. These results are concordant with the report by Rubin and Tanas stating that neither commercial nor home-made CAMTA1 antibody were unable to diagnose EHE specifically (9). Therefore, CAMTA1 immunostaining is not able to replace FISH for CAMTA1 rearrangement as a diagnostic tool for the differential diagnosis of EHE. However, the question for the presence of CAMTA1 rearrangements by FISH in tumors other than vascular neoplasms still remains to be answered.

Besides various neoplasia, we have also demonstrated abundant expression of CAMTA1 protein in various tissues, including gastrointestinal mucosa, squamous and

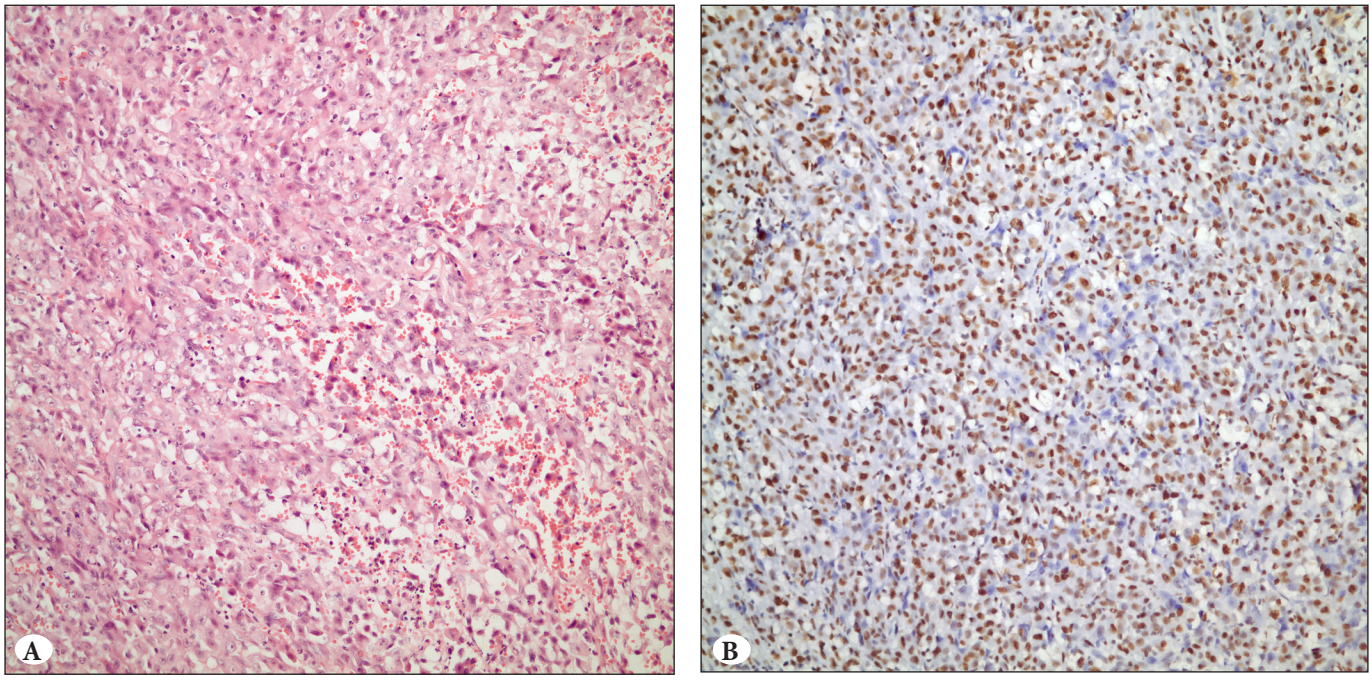

Figure 3: A) Epithelioid angiosarcoma with (H&E; x200), **B)** 3+ CAMTA1 expression.

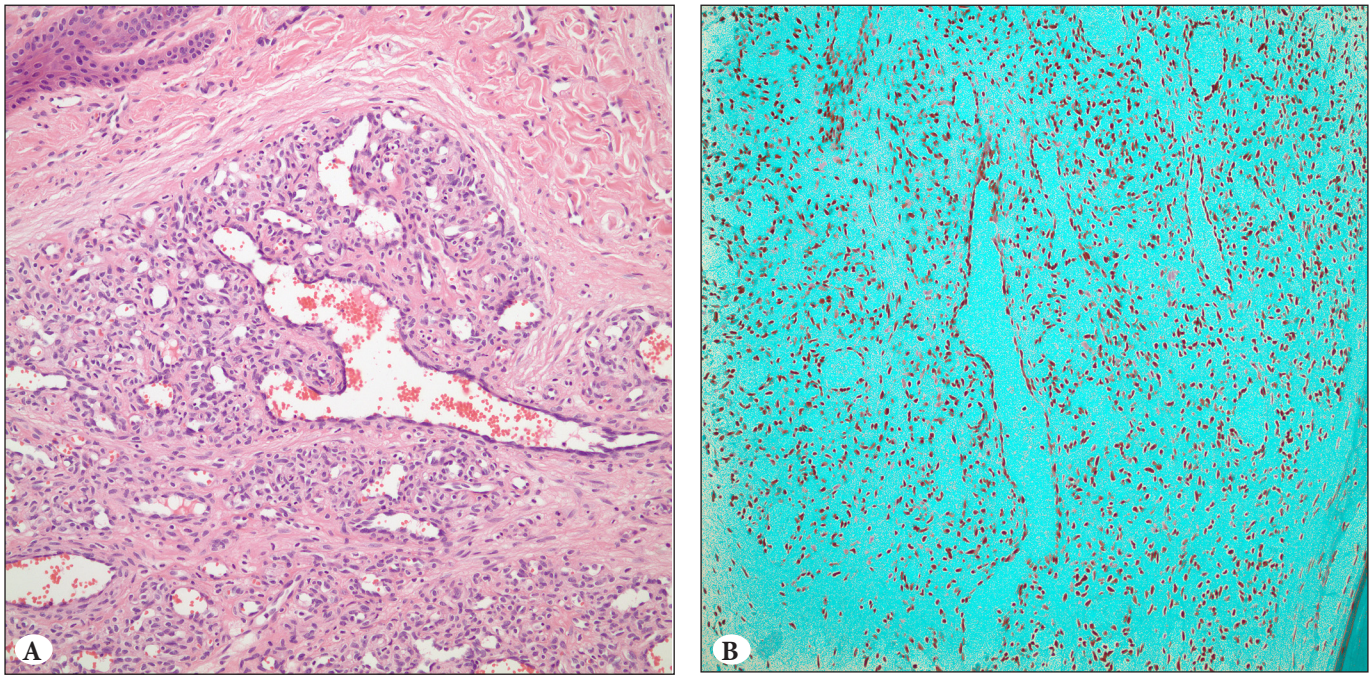

Figure 4: A) Pyogenic granuloma with (H&E; x200), **B)** significant (3+) nuclear CAMTA1 expression.

glandular epithelium. In previous studies, it has been shown that CAMTA1 gene expression is restricted in neurons especially of memory region (10). This concordance may be explained in several ways. First of all, we used a polyclonal synthetic peptide for the residues 1650 to the C-terminus of Human CAMTA1, and this polyclonal antibody may show cross-reaction with other proteins, resulting in non-specific staining. Secondly, very small amounts of CAMTA1 mRNA may be adequate for abundant CAMTA1 protein synthesis. Lastly, as CAMTA1 is proposed to behave like a tumor suppressor gene in normal conditions (11) (on the contrary, function as oncogene in EHE), presence within normal

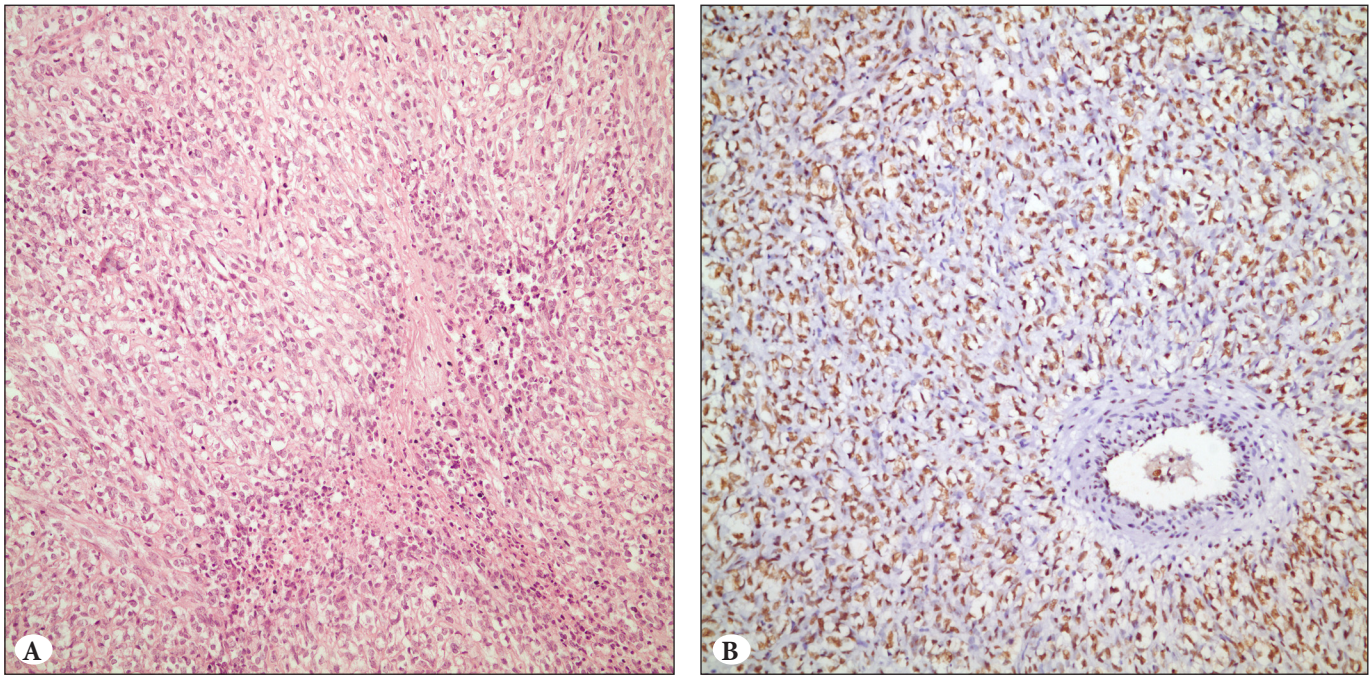

Figure 5: A) Epithelioid sarcoma showing (H&E; x200), **B)** 3+ CAMTA1 positivity.

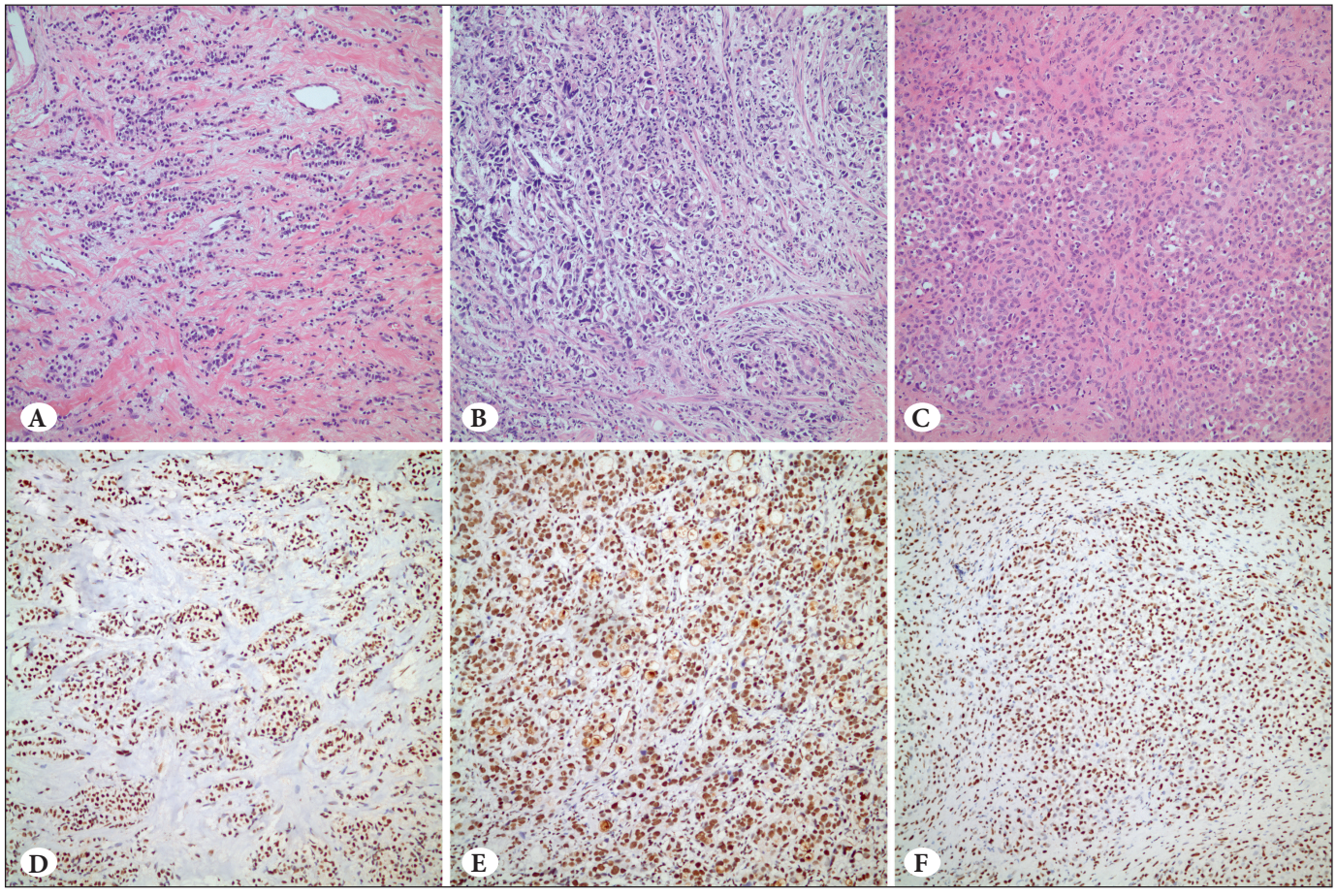

Figure 6: A (H&E; x200) **and D)** Strong and diffuse (3+) CAMTA1 expression in lobular carcinoma of breast, **B** (H&E; x200) **and E)** Signet ring carcinoma, **C** (H&E; x200) **and F)** Malignant melanoma metastatic to lymph node.

tissues is not surprising. Therefore, it may be suggested that CAMTA1 positivity in EHE represents its oncogenic properties, in contrast to other type of tumors and normal tissues, in which CAMTA1 exerts its tumor suppressor function.

In conclusion, expression of CAMTA1 protein was observed not only in EHE and some vascular and non-vascular neoplasia in the differential diagnosis, but also various types of normal tissues other than brain. We have shown that CAMTA1 immunostaining is not useful in differentiating EHE from its potential mimickers.

FUNDING SOURCE

This study was supported by a granted from the Hacettepe University Scientific Research Unit, Grant number: 013D04101001.

REFERENCES

1. Goldblum JR, Folpe AL, Weiss SW, Enzinger FM, Weiss SW. Enzinger and Weiss's soft tissue tumors. 6th ed. Philadelphia, PA: Saunders/Elsevier; 2014.
2. Fletcher CDM, World Health Organization, International Agency for Research on Cancer: WHO classification of tumours of soft tissue and bone. 4th ed. Lyon: IARC Press; 2013.
3. Mendlick MR, Nelson M, Pickering D, Johansson SL, Seemayer TA, Neff JR, Vergara G, Rosenthal H, Bridge JA. Translocation t(1;3)(p36.3;q25) is a nonrandom aberration in epithelioid hemangioendothelioma. Am J Surg Pathol. 2001;25:684-7.
4. Boudousquie AC, Lawce HJ, Sherman R, Olson S, Magenis RE, Corless CL. Complex translocation [7;22] identified in an epithelioid hemangioendothelioma. Cancer Genet Cytogenet. 1996;92:116-21.
5. Tanas MR, Sboner A, Oliveira AM, Erickson-Johnson MR, Hespelt J, Hanwright PJ, Flanagan J, Luo Y, Fenwick K, Natrajan R, Mitsopoulos C, Zvelebil M, Hoch BL, Weiss SW, Debiec-Rychter M, Sciot R, West RB, Lazar AJ, Ashworth A, Reis-Filho JS, Lord CJ, Gerstein MB, Rubin MA, Rubin BP. Identification of a disease-defining gene fusion in epithelioid hemangioendothelioma. Sci Transl Med. 2011;3:98ra82
6. Errani C, Zhang L, Sung YS, Hajdu M, Singer S, Maki RG, Healey JH, Antonescu CR. A novel WWTR1-CAMTA1 gene fusion is a consistent abnormality in epithelioid hemangioendothelioma of different anatomic sites. Genes Chromosomes Cancer. 2011; 50:644-53.
7. Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, Cooke MP, Walker JR, Hogenesch JB. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci USA. 2004; 101:6062-7.
8. Su AI, Cooke MP, Ching KA, Hakak Y, Walker JR, Wiltshire T, Orth AP, Vega RG, Sapinoso LM, Moqrich A, Patapoutian A, Hampton GM, Schultz PG, Hogenesch JB. Large-scale analysis of the human and mouse transcriptomes. Proc Natl Acad Sci USA. 2002;99:4465-70.
9. Rubin BP, Tanas MR. Towards a Proper Diagnosis and Understanding of the Pathogenesis of Epithelioid Hemangioendothelioma. [cited 2014 2/18]; Available from: http://sarcomahelp.org/research/epithelioid-hemangioendothelioma.html.
10. Huentelman MJ, Papassotiropoulos A, Craig DW, Hoerndli FJ, Pearson JV, Huynh KD, Corneveaux J, Hanggi J, Mondadori CR, Buchmann A, Reiman EM, Henke K, de Quervain DJ, Stephan DA. Calmodulin-binding transcription activator 1 (CAMTA1) alleles predispose human episodic memory performance. Hum Mol Genet. 2007; 16:1469-77.
11. Barbashina V, Salazar P, Holland EC, Rosenblum MK, Ladanyi M. Allelic losses at 1p36 and 19q13 in gliomas: Correlation with histologic classification, definition of a 150-kb minimal deleted region on 1p36, and evaluation of CAMTA1 as a candidate tumor suppressor gene. Clin Cancer Res. 2005; 11:1119-28.