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CD30 EXPRESSION IN MALIGNANT VASCULAR TUMORS AND ITS DIAGNOSTIC AND CLINICAL IMPLICATIONS A: A STUDY OF 146 CASES

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Abstract

Angiosarcoma (AS) is a rare malignant vascular tumor while epithelioid hemangioendothelioma (EHE) is a vascular tumor of low grade malignancy. CD30 is a member of the tumor necrosis factor receptor superfamily, member 8 (TNFRSF8). While expression of CD30 is most commonly associated with lymphoid malignancies or germ cell tumors, occasional angiosarcomas have been reported as CD30-positive. However, there is limited data to evaluate its role definitively in malignant vascular tumors. In this study, we evaluated 91 angiosarcomas (AS), 30 epithelioid hemangioendothelioma (EHE) from various sites, and 25 Kaposi sarcomas (KS). Overall, CD30 was expressed in 31/91 cases (34%) of AS, in 7/30 cases (30%) of EHE but in none of the KS. CD30 was expressed in a membranous staining pattern and positivity in tumor cells varied from focal to diffuse. The positive angiosarcomas included vasoformative more differentiated tumors and also solid, undifferentiated, lymphoma-like examples, one of which was classified as lymphoma prior to the era of immunohistochemistry. The CD30-expression was seen in >50% of tumor cells in a majority of angiosarcomas but only in 7% of EHEs. None of the 55 angiosarcomas studied were immunohistochemically positive for TIA-1 or granzyme B, antigens used as more specific markers for anaplastic large cell lymphoma. Compared with angiosarcoma, normal vascular endothelia of capillaries and muscular vessels showed variable positivity. Among hemangiomas, cavernous and spindle cell hemangiomas showed most frequent endothelial CD30-positivity, whereas in most other hemangiomas, CD30-positivity was scant. In conclusion, CD30 expression occurs in a significant subset of angiosarcomas and EHE and needs to be included in the differential diagnosis with other CD30-positive malignancies to avoid a diagnostic pitfall. It remains to be determined whether patients with strongly CD30-positive angiosarcomas could be candidates for targeted therapy using the recently introduced CD30 antibody drug conjugates.

Keywords

angiosarcoma; epithelioid hemangioendothelioma; CD30; immunohistochemistry; tumor necrosis factor receptor superfamily member 8

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INTRODUCTION

Angiosarcoma (AS) is a rare, usually high-grade malignant vascular endothelial tumor comprising less than 1% of all sarcomas. It most commonly involves skin and less commonly deep soft tissue, breast parenchyma, liver, spleen, and bone.¹⁻³ Histologically angiosarcomas vary from spindled to epithelioid morphology. Poorly differentiated AS with limited vasof ormation and solid growth patterns may simulate other malignancies, such as carcinoma, melanoma, and even lymphoma thus prompting a wide differential diagnosis. Epithelioid hemangioendothelioma (EHE) is a low grade malignant epithelioid vascular tumor primarily involving soft tissue or viscera, especially liver and lung.⁴

CD30 is a 120-kD transmembrane glycoprotein and a member of the tumor necrosis factor receptor superfamily member 8 (TNFRSF8). It interacts with TRAF2 or TRAF5 proteins mediating activation of Nuclear Factor kappa B (NF- κ B). NF- κ B affects multiple signal transduction pathways and plays a central role in cellular proliferation and differentiation.^{5,6}

CD30 is expressed by activated, (but not by resting) lymphoid cells of either T or B-cell origin and among lymphomas in Hodgkin disease, large cell anaplastic lymphoma, some large B-cell lymphomas, and others and is an established diagnostic marker.^{6,7} Variable expression has also been reported in decidual cells, mesothelial cells, myoepithelial cells, NK cells, thymic cells, macrophages, usually with a membranous staining pattern.⁸⁻¹⁰ Embryonal carcinoma is CD30-positive to the degree that this is of potential diagnostic value.¹¹ However, some seminomas can be focally positive.¹² Other potentially positive tumors include melanoma¹³ and nasopharyngeal carcinoma.¹⁴

There are rare instances of variable CD30 expression in benign^{15, 16} and neoplastic^{15, 17-19} endothelial cells. The role of CD30 antigen in malignant vascular tumors has not as yet been clearly defined. Because we and others¹⁷⁻¹⁹ have observed occasional CD30-positivity in angiosarcoma, we undertook this study to clarify the CD30 expression in a large number of malignant vascular tumors including AS, EHE and Kaposi sarcoma to evaluate this potential diagnostic pitfall.

MATERIAL AND METHODS

Angiosarcomas (n = 91), epithelioid hemangioendotheliomas (n = 30), and Kaposi sarcomas (n = 25) were organized into multitumor blocks containing 10-50 cases each. Additionally, 70 hemangiomas or lymphangiomas of different types were also evaluated. All malignant vascular tumors were previously characterized as positive for CD31 (membranous expression) and ERG (nuclear positivity), and HHV8 (Kaposi sarcomas). All cases were evaluated with a mouse monoclonal antibody to CD30 (Leica, NCL-CD30, Bannockburn, IL) in a dilution of 1:50. Results from immunostains previously carried out and reported for claudin-5 from a previous study were available for comparison in selected cases.²⁰ CD30 membrane positivity was scored semiquantitatively as an approximate percentage of positive tumor cells. Immunostaining was performed in a Leica Bond automated immunostainer. Additional immunostains for TIA-1 (1:1000, Immunotech, Marseille, France) and Granzyme B (1:100, Monosan/Sanbio, Mt View, CA) were performed in selected cases. For the latter 2

immunostains, application of the primary antibody (30 min. at room temperature), was preceded by heat-induced epitope retrieval in a citrate buffer, pH 6.0, for 20 min. Detection was performed in a Ventana Benchmark automated immunohistochemistry system (Ventana-Roche, Tucson, AZ). Positive and negative controls were performed with all runs and were verified to show appropriate staining patterns.

RESULTS

In the study cohort of 91 cases of AS, the patients ranged from 10 – 84 years (mean age 56 years) with 46 females, 37 males, and 8 of unknown gender. In the study cohort of 30 cases of EHE, the patients ranged from 17 – 83 years (mean age 51 years) with 18 females, 6 males, and 6 of unknown gender. Most of the 25 Kaposi sarcomas were from elderly patients.

Overall, CD30 was expressed in one-third of angiosarcomas, 31/91 (34%) and in 7/30 (30%) EHE, in a membranous staining pattern (Table 1). CD30 expression varied from focal to diffuse positivity in tumor cells (Fig 1). Figure 2 demonstrates a representative poorly differentiated angiosarcoma with focal CD30 positivity. In general, there was overall uniform intensity of CD30-staining. Twenty percent (18/91) cases of AS and 7% (2/30) EHE had CD30 expression in 50% tumor cells. Expression of CD30 was essentially independent of site or clinical subgroup of AS or EHE (Table 2). All Kaposi sarcomas were negative. Immunostains for TIA-1 and Granzyme B, performed on 55 cases of AS, were negative.

Normal vascular endothelial cells around various vascular lesions typically showed variable CD30-positivity, including endothelial cells of larger vessels with muscular walls, and capillaries. However, in general, CD30-positivity was greatly reduced in hemangiomas, as compared with endothelia of normal vessels around the lesions (Table 3). For example, in lobular capillary endothelia, the capillaries of the lobules were negative, but isolated larger caliber vessels, especially in the lesion periphery (possible “feeding vessels”), showed CD30-positive endothelial cells. The hemangiomas showing most frequent CD30-positivity were cavernous and spindle cell hemangiomas. Figure 3 demonstrates variable CD30 positivity in benign endothelial cells in a capillary hemangioma.

DISCUSSION

Our study demonstrates expression of CD30 in malignant vascular tumors, specifically in angiosarcoma (AS) and epithelioid hemangioendothelioma (EHE). We found that a significant subset (30%) of malignant vascular tumors exhibited strong and diffuse membranous CD30 expression. To our knowledge, this is the first study to date to systematically investigate CD30 immunostaining in a large series of AS, EHE and KS. We report here CD30 expression in approximately one-third (31/91, 34%) cases of AS and in 7/30 (30%) EHE. CD30-positive tumors had no predilection to specific sites or clinical subgroups of AS or EHE (Table 2). Previous to our study, there have been only rare case reports in the literature describing the so-called anomalous expression of CD30 as a

diagnostic pitfall in AS¹⁷⁻¹⁹. Confirming a prior study, we found no CD30 expression in KS¹⁵.

We also encountered CD30 positivity in benign vascular endothelium (Figure 3). CD30 seems to be more consistently present in normal vascular endothelia but absent in many hemangiomas, whereas it is more commonly expressed in cavernous hemangioma and spindle cell hemangioma. Based on these findings, CD30 seems to be lost in many hemangiomas compared with normal endothelia, but is retained or re-acquired in some angiosarcomas and hemangioendotheliomas.

Malignant vascular tumors occur in a variety of primary sites and clinical settings and may exhibit poorly differentiated morphology lacking vasoformation with high grade features and solid growth pattern, thus mimicking carcinoma, melanoma, or lymphoma. Judicious application of multiple endothelial markers in poorly differentiated apparently non-vasoformative malignancies will allow identification some angiosarcomas that could be otherwise confused with other malignancies. However, one has to consider that both CD31 and ERG can be expressed in hematopoietic cells²⁷ so that wide panels of markers including those that are specific to hematopoietic lineage, such as CD43 and CD45 are necessary. On the other hand, CD30 alone should never be used to establish the diagnosis of anaplastic large cell lymphoma, but this diagnosis should be supported by markers such as TIA1 and granzyme-B that are more specific for anaplastic large cell lymphoma and are not expressed in angiosarcoma.

Diagnosis of lymphoma versus a malignant vascular tumor, such as AS, has a significant impact on clinical management and prognosis. AS is a much rarer tumor than anaplastic large cell lymphoma and portends a poorer prognosis with higher mortality. While radiation is the mainstay of lymphoma treatment, AS is treated with a combination of surgery and chemotherapy. A large retrospective review of 125 patients with AS found overall 5-year survival was 31%.¹ A prospective study on AS patients found similar survival rates with poorer prognosis for patients with metastatic or recurrent disease at presentation.² A recent study found that the trunk/extremity cutaneous AS had better 5-year overall survival (64.8%) while head and neck cutaneous AS had worst 5-year overall survival (21.5%).³ Metastasis to the lung is most common, and can also involve metastasis to the brain, bone, body cavities. While EHE is a low grade malignant vascular tumor, up to 20 - 30% of EHE can metastasize, most commonly to the liver, bones and lungs.^{4,21}

The role of CD30 in malignant transformation of vascular endothelium is unknown. CD30 is a transmembrane glycoprotein which is a member of the tumor necrosis factor receptor superfamily and affects multiple cytokine pathways. Its expression in malignant vascular endothelium may represent dysregulation of NF κ B and MAP kinase signal transduction in tumorigenesis.²² In the pathogenesis of lymphomas, CD30 signaling reportedly leads to survival of Reed-Sternberg and Hodgkin cells but growth arrest in anaplastic large cell lymphoma, thus suggesting complex, pleiotropic effects.^{22, 23}

Targeted oncologic therapy using naked or conjugated anti-CD30 monoclonal antibodies has recently become available for the treatment of CD30-positive tumors. Brentuximab vedotin,

an antibody-drug-conjugate targeting CD30, received accelerated approval from the FDA in 2011 to treat relapsed Hodgkin lymphoma and anaplastic large cell lymphoma.²⁴⁻²⁶ Further investigation is needed to evaluate if these targeted therapies can be expanded to treat other CD30 positive tumors such as the subset of malignant vascular tumors with CD30 expression. However, it is important to note that variable CD30 expression is present in benign vascular endothelium which may represent a mechanism for potential toxicity using this targeted therapy.

In conclusion, CD30 is expressed in approximately a third of angiosarcomas and epithelioid hemangioendotheliomas and is absent in Kaposi sarcoma. Malignant vascular tumors need to be included in the differential diagnosis of CD30-positive malignancies. Endothelial markers should be added in the evaluation of CD30-positive tumors of uncertain lineage. Prior studies have validated the use of ERG, Prox1, Claudin5 as markers for malignant vascular tumors.^{20, 27, 28} It remains to be determined whether patients with CD30-positive malignant vascular tumors could be candidates in future clinical trials using targeted therapy with monoclonal antibodies to CD30.

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Figure 1. CD30 positive poorly differentiated angiosarcoma with a non-vasoformative pattern is positive for CD31 and ERG. This tumor was also Claudin 5-positive and negative for leukocyte-specific antigens.

Figure 2.

A poorly differentiated angiosarcoma with a lymphoma-like solid pattern shows focal CD30 positivity in addition to expression of vascular endothelial cell markers CD31 and ERG.

Figure 3.
This capillary hemangioma contains CD30-positive endothelial cells.

Table 1

Summary of CD30 expression in angiosarcomas (n = 91) and epithelioid hemangioendotheliomas (EHE), (n = 30)

CD30 expression	Angiosarcoma: n, (%)	EHE: n, (%)
negative	60 (66%)	23 (77%)
positive	31 (34%)	7 (30%)
1 - 10% positive	8 (9%)	1 (3%)
>10% - <50% positive	5 (5%)	4 (13%)
50% positive	18 (20%)	2 (7%)

Table 2

CD30 expression in angiosarcoma by site and subtype.

Angiosarcoma		EHE	
Site (n)	CD30+	Site (n)	CD30+
Cutaneous: face and scalp (17)	6	Arm (4)	0
Other cutaneous (6)	2	Other soft tissue (8)	1
Arm - lymphedema associated Stewart - Treves syndrome (5)	2	Neck (4)	0
Breast, post-radiation (7)	2	Visceral (2)	0
GI tract (7)	3	Bone (1)	1
Lung (8)	4	Metastasis (1)	0
Spleen (4)	1	Other (10)	5
Visceral, other (12)	4		
Body cavities (1)	0		
Other soft tissue (9)	3		
Bone (2)	0		
Metastasis - to lung (3)	2		
Other (10)	2		

Table 3

CD30 expression in endothelia of various benign angiomas. Ranges of percentages of positive cells are shown in parentheses with the estimated median percentage of positive cells.

Hemangioma types		
Cavernous	7/11	(50-100%, median 50%)
Juvenile (Glut-1 positive)	4/5	(3-20%, median 7%)
Lobular capillary *	0/10	
Spindle cell	8/9	(5-50%, median 20%)
Venous hemangioma	1/12	(10%)
Hemangioma, unclassified	5/14	(5-80%, 20%)
Lymphangioma	1/9	(5%)

* Some larger caliber capillaries around the lesions were positive.