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Different immunoreactivity of endothelial markers in well and poorly differentiated areas of angiosarcomas

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Abstract This study evaluated the immunohistochemical staining of four endothelial cell markers in well differentiated and poorly differentiated areas of angiosarcomas. Formaldehyde-fixed, paraffin-embedded sections from eight angiosarcomas were studied using the antibodies anti-factor VIII-related antigen (FVIII-RA), *Ulex europaeus* I agglutinin, anti-CD34 (QBEND/10) and anti-CD31 (JC70). The immunostaining of the angiomatous (well differentiated) and solid (poorly differentiated) areas was separately analysed and specificity was evaluated in 20 non-vascular tumours. The antibody anti-CD31 and *Ulex europaeus* were the most sensitive markers staining well differentiated vasoformative structures and poorly differentiated solid areas. Anti-FVIII-RA and anti-CD34 did not stain undifferentiated malignant endothelial cells from solid areas. *Ulex europaeus* and anti-CD34 showed very low specificity; in contrast, none of the non-vascular tumours expressed CD31 or FVIII-RA. JC70 (anti-CD31) appears to be the most useful marker in elucidating the vascular nature of angiosarcomas. Is important to emphasize the lack of specificity of *Ulex europaeus* and the low sensitivity of anti-CD34 and anti-FVIII-RA for poorly differentiated lesions.

Key words Immunohistochemistry · Angiosarcoma · CD31 · CD34 · Factor VIII-related antigen

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Introduction

The histological recognition of malignant vascular neoplasms may cause substantial diagnostic confusion due to their complex microscopic appearance. This ranges from a well differentiated pattern that may simulate a benign haemangioma to an undifferentiated and solid pattern that may be indistinguishable from a carcinoma, malignant melanoma or various types of sarcoma.

Certain cell products, when detected immunohistochemically, point towards endothelial differentiation. Factor VIII-related antigen (FVIII-RA) is actively synthesized by virtually all non-lymphatic endothelial cells [17, 18] and is probably stored and transported by the Weibel-Palade bodies that characterize such cells ultrastructurally [40]. Commercially available monoclonal and polyclonal antibodies with proven specificity for FVIII-RA have been applied successfully to the immunohistochemical analysis of routinely processed tissues. Endothelial cell labelling by these antibodies has been observed in cultured endothelial cells [12, 13] and endothelial cells of non-neoplastic and benign endothelial lesions. However, the immunostaining of angiosarcomas with FVIII-RA is often disappointing [2, 5, 26, 36, 42].

Another antibody commonly used for immunostaining vascular tumours is the *Ulex europaeus* I agglutinin (UEA), a lectin present on the surface of endothelial cells and erythrocytes of blood group 0. A well known characteristic of UEA is its lack of specificity, staining not only vascular tumours, but also many epithelial and non-vascular neoplasms [11, 16, 29].

Recently, antibodies against CD34 and CD31 antigens have been shown to label endothelial cells and vascular tumours in formalin-fixed, paraffin-embedded tissues [10, 30, 35]. The CD34 antigen, also known as human haematopoietic progenitor cell antigen, is a heavily glycosylated transmembrane protein expressed on immature human haematopoietic precursor cells from normal bone marrow, and in a significant number of acute leukaemias [3, 4, 6, 21, 23, 41]. Several studies have demonstrated CD34 antigen expression in vascular endothelium [10,

33], and in endothelial derived tumours [20, 39], especially Kaposi's sarcoma [11, 19, 28, 33, 34].

CD31 is a glycoprotein present in endothelial cells, platelets, granulocytes and monocytes. This molecule is identical to the platelet-endothelial cell adhesion molecule (PECAM-1; [21]). JC70 is an anti-CD31 antibody that marks an epitope resistant to formalin fixation. In routinely processed tissue this antibody stains endothelial cells, scattered plasma cells and megakaryocytes [30].

In this study we compare the immunostaining characteristics and the potential utility of these four endothelial markers in the diagnosis of angiosarcomas. We further analyse the angiomatous (well differentiated) and solid (poorly differentiated) areas of angiosarcomas in search for differences in immunoreactivity.

Materials and methods

Eight angiosarcomas and 20 non-vascular tumours were retrieved from the files of the Department of Pathology of the Hospital Ramon y Cajal, Madrid. The angiosarcomas chosen in this study were from the following organs: spleen (1), liver (1), breast (1), heart (1), bone (1), skin (3). The 20 non-vascular neoplasms included 3 squamous cell carcinomas (2 from the skin and 1 from the lung), 5 leiomyomas, 1 neurofibroma, 1 malignant melanoma, 1 basal cell carcinoma, 3 proliferating trichilemmal tumours, 1 eccrine acrospiroma and 5 trichilemmomas.

The tissues were fixed in neutral buffered formalin, embedded in paraffin, and stained with haematoxylin and eosin for routine histological examination. We reviewed all cases, and the original diagnosis was confirmed by standard morphological criteria.

Immunohistochemical studies were performed on 5 μm sections from formalin-fixed, paraffin-embedded specimens. The standard avidin-biotin peroxidase complex system was used. After deparaffination with xylene and dehydration of the tissues with ethanol, endogenous peroxidase activity was blocked with 0.6% hydrogen peroxide in methanol for 10 min. Sections tested for FVIII-RA, CD31 and UEA were treated with 0.001% trypsin in phosphate-buffered saline for 30 min. Each slide was treated sequentially with normal goat serum (Dako Corporation, Carpinteria, Calif., USA), primary antibody at a dilution and from the sources indicated in Table 1, and avidin-biotin-peroxidase complex (Dako). The reaction was visualized by exposure to fresh diaminobenzidine. Appropriate positive and negative controls were run concurrently for each antibody tested.

The immunohistochemical reactions were graded semiquantitatively as follows: negative (–), no detection of endothelial antigen; positive (+), focal endothelial antigen staining observed in less than 25% of the cells; positive (++), uniform endothelial antigen staining observed in more than 25% of the cells. The immunostaining of the angiomatous and solid areas was analysed separately. Angiomatous or vasoformative areas were those areas in which vascular differentiation was microscopically evident. In the solid areas the tumour cells did not form easily recognizable vascular

Table 1 Antibodies used in this study (*M* monoclonal, *P* polyclonal)

Antibodies	Dilution	Source
Anti-factor VIII-related antigen (M)	1:40	Dako
Anti-*Ulex europaeus* I agglutinin (P)	1:200	Dako
QBEND/10 (anti-CD34) (M)	1:100	Janssen R
JC70 (anti-CD31) (M)	1:40	Dako

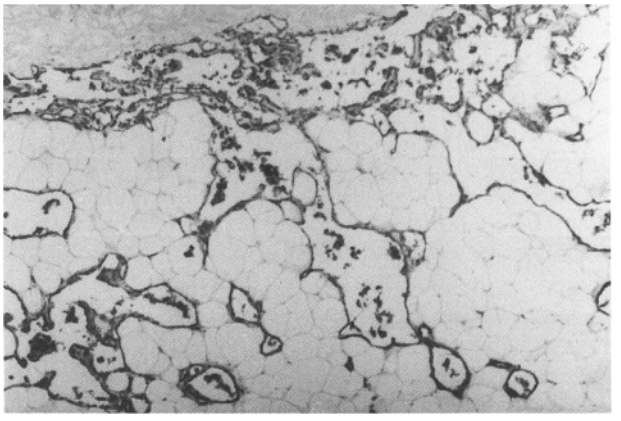

Fig. 1 CD31 immunoreactivity of a well differentiated angiosarcoma from the breast. (×250)

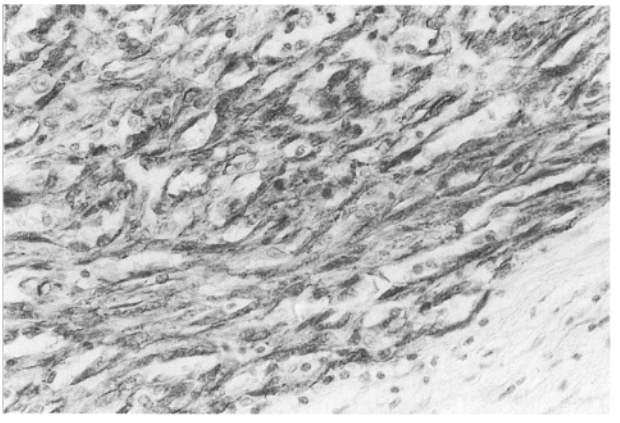

Fig. 2 Intense CD31 immunostaining of tumour cells in a poorly differentiated angiosarcoma of the spleen. (×400)

Table 2 Reactivity of angiosarcomas. *AA* angiomatous (vasoformative) areas, *SA* solid areas, *NP* not present, – no detection of antigen, + focal antigen staining (less than 25% of the cells), ++ uniform antigen staining (more than 25% of the cells)

Case	Site	Factor VIII-related antigen		*Ulex europaeus*		CD34		CD31	
		AA	SA	AA	SA	AA	SA	AA	SA
1	Breast	–	NP	++	NP	++	NP	++	NP
2	Skin	+	–	++	++	+	–	++	++
3	Skin	+	–	++	++	+	–	++	++
4	Skin	+	–	++	++	++	+	++	++
5	Bone	–	–	++	++	–	–	++	++
6	Heart	–	–	++	+	–	–	++	+
7	Liver	++	NP	++	NP	++	NP	++	NP
8	Spleen	–	–	–	–	–	–	++	++

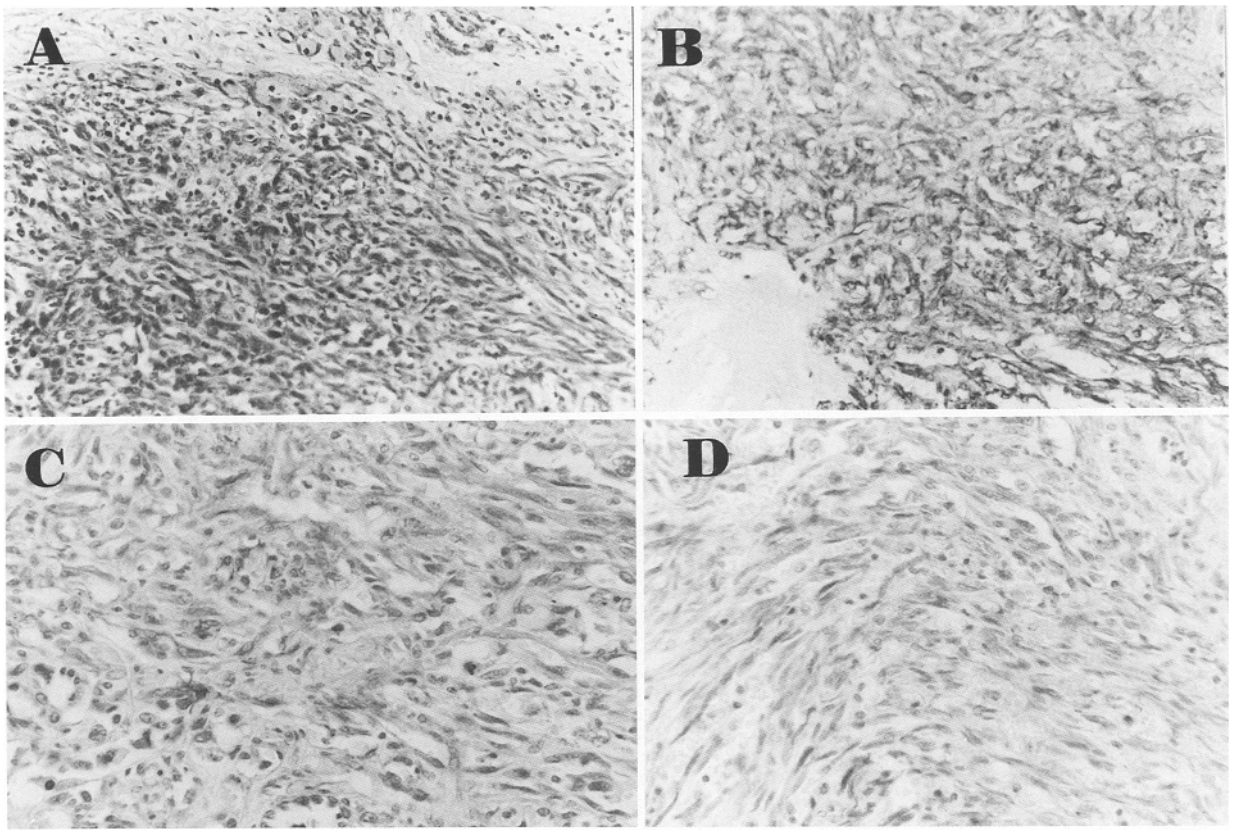

Fig. 3A–D Comparative immunostaining of the solid (poorly differentiated) areas of an angiosarcoma (case 4; ×400). **A** Strong and uniform staining for CD31. **B** Strong and uniform staining for *Ulex europaeus* I agglutinin (UEA). **C** Focal and weak staining for CD34. **D** Negative staining for factor VIII-related antigen

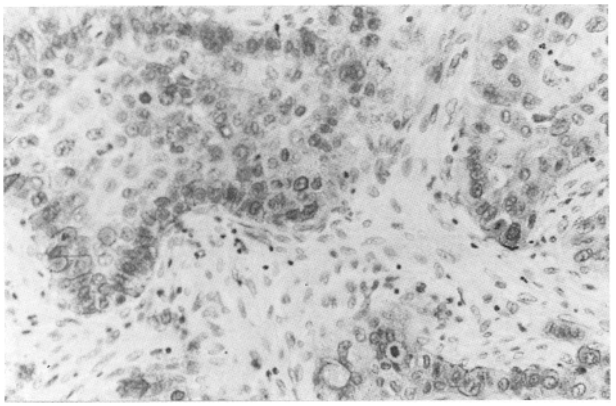

Fig. 4 Squamous cell carcinoma stained for UEA. The positive staining of this epithelial neoplasm reveals the low specificity of UEA for vascular tumours. (×250)

channels. Instead they arranged themselves in compact bundles or sheets of anaplastic cells.

Results

There was great variability in the vascular differentiation of the tumours. Two angiosarcomas (from the liver and breast) were entirely composed of very well differentiated vascular channels exhibiting morphological characteristics of malignancy. Angiosarcomas from the skin and from the heart showed poorly differentiated, sarcomatoid areas, alternating with moderately differentiated areas of atypical spindle cells that formed vascular slits or cavernous vascular spaces. The angiosarcomas from the bone and spleen were poorly differentiated tumours in which the vascular channels were difficult to recognize.

The analysis of the sensitivity of the different antibodies tested is summarized in Table 2. CD31 immunoreactivity was detected in all angiosarcomas, in both well differentiated areas (Fig. 1) and in the poorly differentiated sarcomatous areas (Fig. 2). Similar sensitivity was noted with UEA, except in one case that was negative. In contrast, FVIII-RA stained the cells lining the vascular spaces and slits in only four angiosarcomas, and did not stain the undifferentiated malignant endothelial cells from solid areas. QBEND/10 showed a more consistent immunostaining of the vascular areas but also lacked sensitivity to detect poorly differentiated areas (Fig. 3).

Among the 20 non-vascular tumours analysed, the UEA strongly stained all the squamous cell carcinomas (Fig. 4) and the proliferating trichilemmal tumours, and weakly stained the eccrine acrospiroma. QBEND/10 stained the trichilemmomas, and focal areas of leiomyomas, proliferating trichilemmal tumours, and neurofibroma. None of the non-vascular tumours were stained with anti-CD31 and FVIII-RA.

Discussion

Malignant vascular tumours exhibit great variability in their architectural and cytological differentiation. The vascular nature of undifferentiated angiosarcomas may require the use of immunohistochemical techniques to detect the presence of vascular endothelial antigens. Antibodies to FVIII-RA were the first to be used in this field. Focal and weak anti-FVIII-RA positivity can be detected only in a minority of angiosarcomas [2, 5, 20, 29, 39]. Our results show that this antibody stains mainly the well differentiated vascular areas of the angiosarcomas, while less differentiated areas consistently fail to reveal any positivity.

Positive immunostaining with UEA was noted in all but one of the cases analysed. Similar results have been reported previously [22, 24]. However, the utility of this marker for poorly differentiated malignant neoplasms is limited because it stains a variety of epithelial tumours. UEA positivity has been reported in squamous cell carcinomas [15] and carcinomas of the mammary gland, salivary gland, urinary bladder and colon [16, 29]. We have also noted staining of proliferating trichilemmal tumours and an eccrine acrospiroma. This lack of specificity is probably the reason why UEA has not been considered to be a diagnostic marker for malignant vascular tumours in two recent series [20, 39].

The utility of the antibody anti-CD34 as a marker of malignant vascular tumours is open to question. From our results, the different immunoreactivity of CD34 in well differentiated (positive staining) versus poorly differentiated (weak or negative staining) area of angiosarcomas might explain the contradictory results reported in the literature [38, 39]. Another limiting factor is the lack of specificity of CD34 for vascular neoplasms. CD34 immunostaining can be present in a variety of mesenchymal tumours, including dermatofibrosarcoma protuberans [1], epithelioid sarcoma [39], leiomyoma [1], leiomyosarcoma [31], peripheral nerve sheath tumours [1, 31], neurofibroma [1], clear cell sarcoma [31], malignant fibrous histiocytoma [31], neuroma [1], and follicular tumours [14, 31, 32].

In agreement with previous studies [8, 9, 20, 25, 30] we found that the antibody anti-CD31 is a highly sensitive and specific marker for angiosarcomas. In the comparative study of Kuzu et al. [20], CD31 was found to be superior to FVIII-RA and UEA in labelling angiosarcomas. Furthermore, we noted that the antibody anti-CD31 was the only one that consistently labelled not only the well differentiated vasoformative areas but also the poorly differentiated areas. This makes this antibody especially useful in the evaluation of poorly differentiated malignant tumours, which is one of the important uses of the immunohistochemistry in diagnostic pathology.

In summary, the antibody anti-CD31 appears to be the most reliable marker of angiosarcomas, and should be included in an immunohistochemical panel for evaluating poorly differentiated malignant neoplasms. It is important to be aware of the low sensitivity of FVIII-RA and CD34 for poorly differentiated angiosarcomas, and the lack of specificity of UEA and CD34 for vascular tumours.

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